UCL SCHOOL OF PHARMACY
BRUNSWICK SQUARE

Novel Applications of at-line Near-infrared Spectroscopy as Process Analytical Technology for Solid Dosage Form Pharmaceutical Analysis

PhD Thesis

Bronwyn Grout

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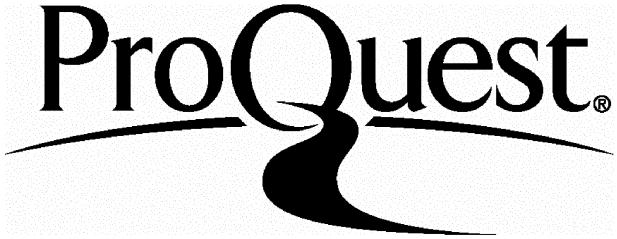

"This thesis describes research conducted in the UCL School of Pharmacy between January 5th 2005 and December 5th 2012 under the supervision of Dr Tony Moffat, Dr Roger Jee, Dr Mire Zloh and Dr Simon Gibbons. I certify that the research described is original and that any parts of the work that have been conducted by collaboration are clearly indicated. I also certify that I have written all the text herein and have clearly indicated by suitable citation any part of this dissertation that has already appeared in publication.

29 November 2012

Signature

Date

To my family

Craig, Emily, Daniel and Thomas

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ABSTRACT

The principal aim of this research was to assess at-line Near Infrared Spectroscopy (NIRS) to support Process Analytical Technology (PAT) applications within solid dosage form manufacturing.

The history of PAT was traced from implementation of process analytical applications prior to the 2003 United States, Food and Drug Administration PAT initiative through to current time. The use of NIRS within the PAT context was reviewed, highlighting two areas in solid dosage manufacturing where further research of at-line NIRS is warranted; material testing and finished dosage form analysis.

Novel applications of at-line NIRS were investigated and developed aligned with the PAT philosophy, to establish an innovative system of analysis that combined chemometrics and spectral analysis with statistical process control (SPC). In particular, various chemometric algorithms were explored to enable rapid monitoring of global spectral quality as well as the quality of specific critical-to-process material attributes within a SPC framework. Novel approaches to within and between batch SPC for tablet quality conformance were also developed including the adaption of distribution profile control charts typically applied to particle size measurement. These were quick to develop with greatly reduced reliance on reference analysis. It provided an opportunity for extensive process monitoring and in-depth process understanding. The work highlighted gaps in currently available chemometric and SPC capabilities within NIR instrument control software and provided insight into a new direction for NIRS analysis in the future.

The new conformance methodology was demonstrated to provide business value and critical science based understanding of the pharmaceutical formulation and processes with successful application of the methodology at a commercial Pfizer facility. This

methodology is in the process of rolling out worldwide. The approach was found to be approachable for plant operators through to quality analysts, and is broadly applicable with the potential to extend beyond the solid dosage form studied.

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LIST OF ABBREVIATIONS

API	Active pharmaceutical ingredient	NV	Nominated value
ANSD	Average absolute normalised spectral distance	PAT	Process Analytical Technology
ASTM	American Society for Testing and Materials	PC	Principal component
CLS	Classical least squares	PCA	Principal component analysis
CoA	Certificate of analysis	PCR	Principal component regression
DA	Discriminant analysis	PLS	Partial least squares
DoE	Design of experiment	PRESS	Predicted error sum of squares
ED	Euclidean distance	QbD	Quality by design
EMA	European Medicines Agency	QC	Quality control
EMEA	European Agency for the Evaluation of Medicinal Products	SD	Spectral distance
FDA	Food and Drug Administration	SE	Standard error
HPLC	High-performance liquid chromatography	SEC	Standard error of calibration
GC	Gas chromatography	SECV	Standard error of cross-validation
GMP	Good Manufacturing Practice	SEE	Standard error of estimation
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use	SEL	Standard error of laboratory
		SEP	Standard error of prediction
		SNV	Standard normal variate
IR	Infrared	SOP	Standard operating procedure
LB	Lower boundary	SPC	Statistical process control
LCL	Lower control limit	TNSD-RR	Total absolute normalised spectral distance over reduced range
LV	Latent variable		
MD	Mahalanobis distance	UB	Upper boundary
MLR	Multiple linear regression	UCL	Upper control limit
MR	Moving range	UV	Ultraviolet
NIR	Near-infrared	VOC	Voice of the Customer
NIRS	Near-infrared spectroscopy	VOP	Voice of the Process

PREFACE

This thesis is organised in chapters with the first chapter providing a high level overview of the Process Analytical Technology (PAT) initiative within the pharmaceutical industry. The chapter closes by identifying the key areas of PAT application which hold the highest value in focusing research.

Chapter two then provides an introduction and overview of the role of near infrared spectroscopy (NIRS) in supporting the PAT initiative. Evaluation of a subset of the available relevant literature demonstrates the value in focusing on at-line applications for material and finished product testing in solid dosage form manufacturing. Chapter three provides background as to the mathematical and statistical theory that underpins NIRS analysis for PAT applications explored in this research.

Chapter four summarises the research conducted on the development of a NIRS conformance methodology for material testing, exploring applications to assess global spectral quality as well as the quality of specific material attributes and associated impact on process and / or product quality.

Chapter five summarises the research conducted on the development of a NIRS conformance methodology for finished solid oral dosage form analysis, focusing on novel approaches for assessment of within and between batch quality during tabletting operations.

Chapter six provides a summary of the research and assesses the criticality of the research outcomes in relationship to closing the gaps in at-line NIRS application to support the PAT philosophy as identified in the first two chapters.

CHAPTER 1 THE PROCESS ANALYTICAL TECHNOLOGY FRAMEWORK

1.1 Introduction

In this initial chapter of the thesis, the author provides an overview of the Process Analytical Technology (PAT) philosophy as it is applied to the pharmaceutical industry. Through this review the critical focus area of scientific research in the area of PAT is identified and is the basis of the research detailed in later chapters of the thesis.

1.2 Process Analytical Technology defined

Chemical and physical analytical testing is the cornerstone of quality assurance of pharmaceutical products, ensuring that the pharmaceutical products that reach the consumer have acceptable quality and efficacy. In the 1990's and early 2000's, many companies had in-house terminology for programs to describe the approach of moving the analytical testing to the sampling location within manufacturing rather than moving the sample to the quality control (QC) testing laboratory in a distant location.

In simplest terms, PAT is the application of analytical instruments and methodologies (including statistical analysis) to the measurement of process and quality attributes at the time, and in the location of, the manufacturing process.

The term PAT was used within Pfizer Inc to describe this work in the late 1990's, with "PAT Analysts" employed at manufacturing sites to develop and implement near infrared (NIR) methods for raw material quality assessment and in-process tablet monitoring.

During 2001 to 2003, the United States Food and Drug Administration (FDA) became interested in PAT and what the application of analysis within the process could provide the Pharmaceutical industry. The FDA defined PAT as:

> *"a system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality."*[1]

The FDA definition was further cemented in the vernacular through the inclusion of the definition verbatim in The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) tripartite guidelines on quality risk management[2] and later on pharmaceutical development.[3]

The FDA advocated that PAT should be focused on gaining understanding of processes, process improvement and ultimately applied to validate and control manufacturing processes. Thus the concept of PAT is broader than simply analytical measurement in itself; rather it has become synonymous with a holistic and strategic application of process analysis within a scientific, risk-based, systems oriented framework.

It was expected that implementation of PAT would provide benefits to the manufacturer through improvements in product development and manufacturing and as a more effective means to demonstrate quality assurance, providing economic business benefits.[4] The regulatory environment was also expected to benefit through more scientific and well understood processes resulting in harmonised and simpler review and auditing.[5]

Before discussing the benefits of PAT and the various modes of PAT application (process knowledge / process design, process monitoring / process analysis and process control), it is worth reviewing the origins and timelines of the PAT initiative.

It is important to stress that PAT is not a new invention of the FDA, rather, industry pursued the initiative well in advance of the FDA involvement. The Wall Street Journal described the earlier work of implementing analytical technologies in the process environment by a cross section of pharmaceutical companies.[6] The review of the range of PAT related NIR spectroscopy (NIRS) applications in Section 2.5 also demonstrates that application of analytics in tune with current PAT philosophies occurred much earlier than the launch and promotion of the term PAT by the FDA. The application of chemometrics and data analysis aligned with the PAT initiative was also stated to occur decades prior to the FDA publication.[7]

The first paper which discussed the FDA's interest in the role of PAT in modernising the pharmaceutical industry was published in 2002.[8] It describes that the FDA first discussed PAT in 2001 with presentations at the FDA Science board meeting in November 2001 culminating in the formation of a subcommittee on PAT in early 2002. Throughout 2002 and 2003, the subcommittee met and devised a draft guidance document on PAT. The vocabulary of PAT became firmly fixed in the pharmaceutical industry following the issue of the draft guidance "Guidance for Industry PAT - A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance"[9] by the FDA in September 2003. This document was later authorised with minimal change and issued in September 2004[1] following a period of industry and academic consultation.

The use of the term PAT allowed focussed discussion between companies in the industry as well as common language for discussions across industry, academia and regulatory bodies.

1.3 Why the pharmaceutical industry needs PAT

The pharmaceutical industry differs from other manufacturing industries in that the product made is consumed by those with compromised health to invoke a physiological effect (primarily the improvement to human health). This has led to the practice of heavily documenting the extensive internal quality assurance testing conducted to ensure that the product released to market is of the highest quality. Regulatory bodies exist to oversee the manufacturing and supply of pharmaceutical products to safeguard the public, and each regulatory body outlines extensive quality assurance requirements in associated guidelines, guidance and / or legal statutes. It is interesting to note that despite this focus on assuring product quality, pharmaceutical manufacturing is said to be behind other manufacturing industries such as the automotive and semiconductor industries.[5] There are high internal quality failure rates (product deviations / rejects), high cost and slow cycle times when compared to other industries. A PricewaterhouseCoopers presentation to the FDA reported that pharmaceutical manufacturing assumes 5-10% of materials are scrapped, while 20% of production costs are spent ensuring quality.[8]

It has been argued that pharmaceutical manufacturing has not changed significantly since the 1950's with a 25 year lag in uptake of new technology.[10] In the past, manufacturers commented that they cannot change due to conservative inflexible regulation and controls placed on the industry by regulatory bodies, while regulatory bodies have responded that they have insufficient information to mitigate the risk of relaxing controls. Applying PAT can reduce the risk by decreasing uncertainty through the provision of process information which was previously unknown.

Process and product knowledge is the key to effective and efficient pharmaceutical manufacturing, and efficient regulatory oversight of the industry. The combination of

process automation and process analytics has been said to provide new mechanisms for process control which can ensure the quality of the final product while providing understanding of the physicochemical phenomena occurring during manufacture of the product.[11] It is hoped by industry that the PAT initiative can reduce product quality problems while increasing the efficiency of manufacturing and quality assurance processes. This would moderate regulatory burdens thus reducing production costs, and in the long run, make pharmaceuticals more affordable.[8]

Review of the multitude of papers published on the PAT initiative[4-6, 8, 10, 12-20] indicates that the primary benefits of PAT include;

- Improved efficiency by managing product variability and improving quality consistency leading to more capable/ robust processes
- Reduced production cycle times by use of PAT measurements and controls
- Reduced rejects, scrap, and reprocessing and prevention of recalls
- Improved customer service, by securing predictable product supply and the potential of real time release
- Increased automation leading to improved operator safety and reduction in human errors
- Increased certainty and confidence in the process robustness
- Continuous quality improvement opportunities within quality risk management program with moderation of associated regulatory burdens
- Positive relationships with regulatory agencies and improving the scientific basis of dialogue between industry and regulatory agencies
- Reduced cost of manufacturing

Thus there is wide agreement that PAT is a key initiative to drive the pharmaceutical manufacturing industry forward; facilitating many improvements to manufacturing that are needed to meet business objectives. It is clear that PAT will lead to benefits to the regulatory agencies, the pharmaceutical companies, patients and to the shareholders. The benefits to shareholders are not often discussed, however it was highlighted in early attention by The Wall street Journal in the pharmaceutical industry's pursuit in PAT.[6] However, most importantly, patients will receive the benefit of PAT through the production of consistent high quality products.

PAT was further embedded in industry culture through the inclusion of PAT concepts in the FDA "Guidance for Industry on Quality Systems Approach to Pharmaceutical cGMP Regulations"[21] and the ICH Harmonised Tripartite Guidelines on Pharmaceutical Development (Q8R2)[3], Quality Risk Management (Q9)[2] and Pharmaceutical Quality System (Q10)[22]. Specifically, PAT is referenced in the ICH documents to gain enhanced understanding of process performance,[3] as a risk control mechanism,[2] as a means for in-process testing[2] and control,[3] and as a support for parametric and real time release.[2] The European Medicines Agency (EMA) launched a PAT team to work with industry on PAT aspects and endorsed PAT through participation in drafting the ICH guidelines. While separate regulatory guidance addressing PAT was not considered necessary in Europe, the EMA (previously known as the European Agency for the Evaluation of Medicinal Products (EMEA)) issued a paper which reflected on the incorporation of PAT into regulatory submissions.[23]

PAT became a key focus area of many symposia, consortiums, conferences and industry organisations from 2003. For example, the American Society for Testing and Materials (ASTM) Committee E55 on Manufacture of Pharmaceutical Products was formed in 2003 to address issues related to process control, design, and performance for the pharmaceutical manufacturing industry and largely focused on the preparation of

consensus standards supporting PAT implementation in the industry (e.g. PAT support of process design[24] and process understanding[25]).

1.4 Modes of integration of PAT applications

The implementation of PAT is typically described utilising three modes of integration; [13, 14, 20]

- at-line - where operators sample from the process and perform analysis at / near to the process stream. The PAT tool is located near to but not integrated into the process.
- on-line - where the PAT tool is integrated into the process with no operator sampling with automated diversion of the sample to the PAT tool making use of components such as sampling loops. Analysis typically occurs faster than at-line methods but at a slower rate than the process equipment operational speed.
- in-line - where the PAT tool is fully integrated (invasively or non-invasively) into the process with no sampling. Analysis occurs at the process equipment operational speed.

At-line analysis has benefits in cost of installation and that the equipment is not dedicated to one piece of process equipment and so can be applied to multiple equipment trains or multiple applications (for example, raw material testing and in-process intermediate monitoring). Process analysers or instrumentation can be easily installed within existing in-process control laboratories or located near production equipment while not impacting the process equipment or being impacted by the process environment (e.g. heat, vibration). The validation of at-line PAT systems is straightforward with no need to review or revalidate process equipment. Implementation

can be done in parallel with routine production without interfering with manufacturing schedules or delaying product commercial release.[16]

The disadvantages of at-line measurement is the lack of ability to apply automated feedback and control, and the smaller sample size that can be analysed due to the need for operators to present the sample to the measurement system. It may be challenging to develop process understanding of the different points or phases within the process for short duration processes as sampling and measuring samples may take too much time to allow sufficient data points to characterise the process.

In-line and on-line integrated application of PAT provide the opportunity for automated control and un-manned analysis at greater sampling frequency and large sample sizes which can provide deep understanding of process phases and pathways, even of short duration, without burdening operators. However, integrated applications have additional cost and engineering requirements to interface the instrumentation with the process, more extensive validation burden (process equipment and product contacting parts) and greater complexity in terms of data, communications and control (e.g. data storage, sophisticated control loops).[16]

When determining the optimum mode of PAT integration, the following aspects should be considered;[13]

- level of desired control (automated, manual, human input for interpretation)
- engineering constraints (plant zoning, restriction on electronics and communication, power, space and access limitations)
- process equipment constraints (pressurised vessels, motion or moving parts)
- environmental constraints (vibrating environment, harsh chemical environment)

- effect of integration on process or equipment validation state (requirement to re-establish equipment/ vessel integrity or revalidate the product).
- information technology constraints (communication to process equipment or plant electronic management and data handling system)
- sampling constraints (representative sampling design, sample/instrument interface, effective sample size, fouling, static/ dynamic measurement)
- PAT equipment restraints (distance of fibre optic cable runs, speed of scan versus speed of sample movement)

1.5 Typical stages of PAT implementation

1.5.1 PAT - Process design and process knowledge

Typically the first application of PAT to a process / product is during process design and process knowledge development. This may occur during research and development for new products or in the commercial phase of a product to gain process understanding or facilitate process redesign. This directly aligns with the overriding goal of PAT to understand the manufacturing process.[26]

The FDA considers a process well understood when:[27]

1) all critical sources of variability are identified and explained;
2) variability is managed by the process; and
3) product quality attributes can be accurately and reliably predicted over the design space established.

Thus, to gain process knowledge and understand the critical steps of the process, PAT may be applied to correlate process and quality attributes, to identify the critical

attributes and to identify the sources of variation in these attributes. A general process assessment may take place to identify the gaps in process knowledge and then PAT applied to specific process steps or attributes.

While gathering process knowledge, PAT can be applied in depth to the process and across multiple process points. This information can be used to devise a 'process signature', which can be defined as the typical process trend / profile or behavior for the attribute under study throughout the process time frame.

Typically the process signature is based on non-quantitative measurements and will be devised on a large sample size sampled throughout the process. The process signature may be formulated with one or more PAT measurement systems and may highlight critical control points or locations of variation in the process, otherwise not identified by the random small scale sampling that occurs for conventional Pharmacopeial analysis. Process signatures are often an ideal way of graphically representing the 'typical process' in all its glory, showing the variation (which may be acceptable or unacceptable) that inherently occurs in the process.

An early application of the process signature approach to gaining process knowledge can be seen in work five years prior to the FDA launch of the PAT initiative where NIR was used to produce a profile of the manufacturing process of an injectible pharmaceutical product.[28]

Different design of experiments (DoEs) can be used during process design, scale up and commercial process development to better understand the interactions of quality and process attributes and the inherent variation in the attributes. Applying PAT at the process design stage (i.e. prior to commercial manufacture), can aid in ensuring a robust efficient process is introduced to the plant[15] and is aligned with the Quality by Design (QbD) principles introduced in the FDA "Guidance for Industry on Quality Systems

Approach to Pharmaceutical cGMP Regulations" in 2006[21] and outlined in the ICH Q8 tripartite guideline on Pharmaceutical Development.[3]

The resulting information gathered from PAT and any other process / product knowledge gained through process assessments and DoEs can be used to define the design space. ICH Guidance on Pharmaceutical Development[3] defines design space as:

> *"The multidimensional combination and interaction of input variables (e.g., material attributes) and process parameters that have been demonstrated to provide assurance of quality."*[3]

The use of PAT can ensure that a more robust, understood process is defined which can cater for variable inputs that are inherent in manufacturing (e.g. variation in materials, environmental factors, ageing of process equipment) while remaining at the set point within the developed design space.[15]

Product marketing and the desire to quickly deliver a new pharmaceutical product to patients often hampers full process design with rapid progress from the clinical stage to commercial manufacture once approval to market is given. Consequently the commercial process is often based on scale up of the laboratory scale process experiments and different DoEs done at early research and development stages rather than in the scale up period. Rapid to implement PAT applications that provide deep process understanding without delaying progress of products through the research and development process are therefore paramount. Though it is beneficial to study potential quality and process attributes prior to commercial manufacturing, understanding the commercial process is often only achieved at the point of validation of the commercial manufacturing process.

During this first stage of implementing PAT (defining and understanding the process) it may be identified that quality attributes previously tested in QC laboratories are not critical to describing the process. Thus redundant, and in some cases laborious and

expensive, quality verification testing of the quality or process parameters can be reduced and the resources targeted on the attributes that are most critical. In some instances, once the process is fully described, regulatory filings can be changed to remove unnecessary registered tests. It must be noted here, that PAT is not implemented with the intention of removing registered tests that are in place to verify or characterise product attributes that relate to product clinical efficacy.

Effective design space and process knowledge can provide opportunities to ensure effective specifications are set for testing of critical attributes from the outset. For example, process and product specific specifications can be applied for raw materials where a material's physicochemical attribute has a critical affect on forward processing, despite the attribute test not being required in Pharmacopeial guidance. This can only occur once full process understanding is achieved.

1.5.2 PAT - Analyzing manufacturing / process monitoring

Once the design space is defined through process knowledge (Section 1.5.1), PAT can be applied for process monitoring of identified critical or variable attributes to demonstrate that processes are within the design space and consistently producing quality product.

If process signatures were devised during the process design and process understanding stage, the same sampling and testing procedures can be applied routinely to assure that the process is continuing to perform according to the understood process signature. If process signatures have not been developed, the process monitoring stage of PAT provides the optimum time to apply PAT to the identified critical process parameters to establish the normal behavior or monitor the typical variation. This is an important step

to proceed through prior to applying specifications and thresholds to the data and moving into the process control stage of PAT (Section 1.5.3).

Often quite a large sample size is used during process monitoring. Real time monitoring provides the opportunity to use the information for rapid identification of any unexpected result or deviation and rapid initiation of root cause assessment followed by an opportunity for deviation rectification. At this stage, feedback is typically manual and requires operator interpretation. This may cause initial production downtimes when unexpected results or deviations occur, particularly as experience is gained in interpretation of PAT data.[8]

As the expertise of the PAT application develops through the process design and understanding stage and the process monitoring stage the PAT data can be used to predict how the process is expected to perform at the next stage in production.

Application of PAT to non-critical process or quality attributes is not value adding as all that will be achieved is the verification of a known stable process rather than satisfying the purpose of PAT to predict product quality attributes though a high degree of process understanding.[27] The FDA views monitoring of non-critical parameters and not using the data for process improvement or control as a deviation from the FDA philosophy of PAT. It should be noted that the FDA interpretation of critical parameters is those that impact product quality or efficacy. PAT may be applied to parameters that critically impact business aspects (e.g. speed of operation) however these applications need not be registered for regulatory review and the inclusion of such applications in filings may detract from the purpose of PAT enabled product and process control.[27]

1.5.3 PAT - Process control / quality assurance and continual process optimisation

As mentioned in Section 1.5.2, the FDA considers the use of PAT data to control the process an integral part of the PAT approach. With the development of sufficient process understanding, it is possible to establish causal and / or predictive relationships between critical parameters and quality attributes such as the incoming raw materials, manufacturing process, in-process materials, and final product quality, which could be used for real time process control.[12, 26]

Ultimately, PAT can be implemented to achieve "real time release" through demonstration in real time throughout manufacturing that all critical processes are understood and controlled to attain the desired quality attributes of the product.[27]

Using feedback and feed forward controls, PAT can be used to provide a mechanism to keep processes within the design space or conforming to established acceptable behaviour. The PAT output can provide the ability to manage, reduce or eliminate identified variability in the process.

Specifications can be built from all the data acquired during the prior two stages of PAT implementation, to assist in rapid interpretation and feedback of the data to make quality decisions. With in-line and on-line PAT approaches the feedback loop can be automated (control loops and communication through software and equipment programmable logic controllers) without interpretation or human intervention.

Continuous process optimisation can occur, where process parameters can be modified within the design space using PAT to verify that the process remains in control. This modifies the view of static processes following the completion of process validation.[12]

1.5.4 PAT - Validation and continuous verification

Concurrent with implementing PAT for monitoring (section 1.5.2) or control (section 1.5.3), PAT information can be utilised to support a change to the product quality validation and verification paradigm. Data generated from PAT can be applied to support validation or may facilitate a reduction in the level of validation testing normally applied. When based on PAT results validation may be achieved in less time, with fewer resources and with a greater degree of quality assurance. Validation and verification is focused on critical parameters with scientific rationale and understanding. This may allow faster scale-up and simpler supply assurance through quicker process validation.[5]

The application of PAT to large sample size, to a large number of process steps or for multiple critical process or quality attributes clearly exceeds the current quality assurance generated by pharmacopeial analysis on a very small sample size at the finished dosage stage.

The use of process knowledge of earlier process stages, followed by process prediction leads to the ability to make informed decisions when process changes occur (for example changes in raw material supply) to ensure the processes continue to operate within the design space. The PAT data rapidly provides confirmation that the process is in control and can thus revolutionise the approach to change control and continuous process improvement. It could thus be proposed that any process improvement or change that produces a process within the approved design space would need no further regulatory review. This is a complete shift from traditional views to process validation and quality assurance with process understanding the foundation.[26]

1.6 Current PAT constraints

With PAT, it could be envisaged that in the future all relevant critical quality and process attributes would be continually monitored, evaluated, and adjusted (within the defined design space) achieving flexible, process quality based endpoints using validated in-process PAT measurements. In doing so, the process would be allowed to cope with and deal with the inherent variability of material and process attributes that can impact the quality of the output. The use of PAT in this way mitigates the risk of process variation to product quality.

However, there are currently several constraints (real or perceived) that need to be overcome to facilitate the industry moving forward with PAT. The constraints listed by Ciurczak in 2003[10] based around the two broad areas of instrumentation and regulation are still constraints nearing ten years after the PAT initiative was launched.[4, 29, 30]

Instrumental issues include the need for; [4, 5, 10, 29]

- Process hardened instrumentation
- Guidance for calibration, validation and instrument standards for process instrumentation
- Ease of interfacing process instrumentation / retrofitting process equipment
- Integrated data-management infrastructure capable of handling large data volumes
- Software compliance and integration with process data management systems
- Guidance on what data must be stored and archived
- Guidance for specifications for large sample size and development of new specifications for attributes with no historical specifications.

Instrumentation issues are rapidly being addressed as technology becomes more advanced and as competition between vendors leads to provision of more robust equipment. The European and United States Pharmacopeias both contain general chapters on NIR spectrometry[31] and spectrophotometry[32] respectively and focus on instrument operation and qualification, particularly reflectance measurement. The inclusion of chapters in pharmacopeia for analytical techniques often used for PAT measurement (e.g. NIRS, Raman) provides a mechanism to provide standardisation and guidance on instrumentation. Industry organisations such as ASTM, International Society for Pharmaceutical Engineering and International Organization for Standardization also provide a mechanism to provide consensus standards on a range of PAT related topics not covered in either regulatory or pharmacopeial guidance. Such industry consensus standards will continue to close some of the instrumentation issues.

Perceived regulatory barriers has been stated to be the largest cause of the pharmaceutical industry lagging in implementing PAT.[14] Though the FDA guidance clearly demonstrated FDA support of PAT, there are still regulatory concerns, including; [5, 10]

- How the current inspection process (regulatory audits) and regulations will change to encompass PAT
- How other regulatory agencies will adopt PAT (compared to FDA and EMA) in regions other than the United States and the EU
- Relief on cost of filing variations on approved products to implement improvements with regulatory impact

In addition to instrumentation and regulatory concerns and constraints, other aspects of PAT that need attention can be grouped under financial considerations and organisational readiness.

Such constraints noted in literature include; [4, 5, 13, 16, 26, 29, 30]

- How to overcome / provide for the need for in-house expertise and training[5] particularly in chemometrics and statistics
- Ensuring appropriate personnel and capital resources as well as project management and leadership to complete projects to sponsor expectations
- How to identify, recruit, and keep the necessary competencies
- How to deal with the culture shock of implementing PAT and changing validation, processing and quality paradigms
- Financial benefits for industry to pursue PAT and the perceived / actual poor return on investment.

The increased focus on science based manufacturing and presence of PAT in conferences, symposium and availability of web based training and webinars is assisting in building in capability of PAT related skill sets in the current and future workforce. Despite this focus, there is continued scope to ensure adequate skills are in graduating students.[4] Retention of PAT capability is another significant challenge, with the experience within Pfizer that those with an aptitude for PAT are often promoted out of PAT roles. Management may assume training is a simple exercise; however there is a greater need for knowledge management of PAT related activities so that when a project manager moves on, a capable person in house has the knowledge and aptitude to maintain and further progress PAT projects.

Vendors may assume that the profits within the pharmaceutical industry are available to individual manufacturing facilities and as such inflate instrumentation and software pricing when selling to the Pharmaceutical industry. The reality is that the manufacturing facilities in the pharmaceutical industry are highly competitive, with the need to recover research and development costs and then compete with the generic

marketplace once product licensing exclusivity has been lost. Within the current global economy every capital purchase within any company is based on the ability to recover the capital investment in a timely manner and such inflated vendor pricing reduces the deployment of PAT.

Areas of concern or constraint (instrumentation, regulatory, financial and organisational capability) need to be addressed and overcome across the industry and within individual companies before PAT can be truly effective, fully integrated and considered routine rather than as a separate initiative.

1.7 Critical review of the current status and future focus of PAT

The benefits of PAT are clearly demonstrated and have been well discussed in the public domain in published journal articles as well as with the prominence of PAT in industry conference agendas since 2003. Though the PAT initiative is nearly a decade old, culture shock and how to ensure management embraces change continues to be a factor in debate on the success of PAT.[4] This is more down to human nature's resistance to change and the longer time needed for PAT competent personnel to reach upper management positions to champion cultural change.

The original drive to implement PAT based on it being the right thing to do, being perceived as an industry leader or to pursue relief from regulatory burden must also translate to business / financial benefits for continued implementation. Often the benefits are stated in soft terms that do not translate well to return on investment calculations and financial benefits. With the economic crises / depression occurring across the globe, pharmaceutical companies have become ever more cost conscious, which can be synonymous with financial conservativeness. Added to this is the greater presence of competing manufacturing in developing nations with cheaper production

and labour costs and narrow margins. As such, investment in significant capital with long term returns is not an industry priority. It is therefore important to focus efforts in developing and implementing PAT on applications that meet the philosophy of the PAT initiative while also meeting constrained business operations. There is a disincentive to implement PAT applications necessitating regulatory filings for marketed products when costly variations are required for separate markets (may be in excess of 100 for globally marketed products). Even when few markets are impacted, applications requiring preapproval may also translate to delayed return on investment depending on which markets are impacted, with some markets taking years to approve PAT related changes.

Review of literature describing historical implementation of PAT indicates that though on-line and in-line PAT analysers deliver greater process knowledge and opportunity for process control and continual process improvement, they do so with disadvantages in cost, time and complexity of installation. Alternatively, at-line applications provide opportunity for rapid installation without integration delays and the ability to apply non-dedicated analysers across multiple process lines and process steps, despite lacking automation or process feedback. Similarly, PAT applications with complex regulatory consequences (necessitate filing) are not aligned with the current financial challenges facing many manufacturing facilities.

The application of PAT in the area of biologics manufacturing is the centre of a recent flurry of publications as the pharmaceutical industry recently gained momentum in developing biologics therapies. Glassey and Rathore provide an excellent summary of recent PAT applications and considerations for biopharmaceutical products[20, 33] Despite this new research area, the pharmaceutical industry continues to be dominated by oral dosage forms. In fact many new biopharmaceuticals target the use of oral dosage form delivery systems to capture the high level of patient compliance seen with oral dosage

forms compared with other delivery systems.[34] It has been reported that tablets account for over 70% of the prescribed medications reaching patients.[35]

Based on the review of the various integration options and stages of PAT implementation as well as the constraints identified from literature review and an understanding of the current pharmaceutical business and economic climate, research in PAT should focus on low cost PAT applications that drive process robustness and efficiency, provide opportunity for monitoring and internal control without regulatory constraints. Focus should also continue on solid dosage form manufacturing as the largest portion of pharmaceutical manufacturing continues to be in this area. At-line applications of PAT focussed on process understanding and monitoring of tabletting processes, are seen as pivotal to address this research focus area and are the subject of this thesis.

CHAPTER 2 NEAR INFRARED SPECTROSCOPY IN PROCESS ANALYTICAL TECHNOLOGY

2.1 Introduction

This chapter describes the role of NIRS to support the PAT philosophy. The background theory of NIRS is touched on and the historical application of NIRS within the PAT framework is reviewed for various units of pharmaceutical operation. Through this review the potential areas for the novel use of NIRS to support at-line implementation of PAT was identified and is the focus of the research described in later chapters of this thesis.

2.2 Desirable attributes of PAT measurement systems

Techniques and tools for process analytics must be rapid to be applicable to test key process and quality attributes of pharmaceutical samples at, or in, the process at equipment operational speeds (real time analysis). They must be able to analyse a sufficiently large sample size to facilitate process understanding and to establish process signatures. PAT techniques must require little sample preparation, and techniques that are non-destructive are highly regarded due to ease of result investigation.

Therefore the desired attributes of PAT measurement techniques are:

- Rapid
- No, or limited, sample preparation
- Non-destructive

As PAT applications are located in the manufacturing plant, it is also necessary that the control system for the PAT technique is able to support a simple operator interface so that the analysis can be performed or monitored by unskilled (non-chemistry trained) plant / factory operators for information feedback and control. Whether a simple interface is available for easy plant operator monitoring and control is largely an instrument software issue and would be of common interest independent of the analytical technique employed.

2.3 Background theory of NIRS

To understand how well suited NIRS is to PAT applications, the theory of NIRS must be examined.

As for mid and far infrared (IR) spectroscopy, NIRS is based on the absorption of energy (in the 780-2500 nm region of the electromagnetic spectrum) by molecular bonds with dipole moments (charge distribution inequality between the atoms) to absorb energy and vibrate.

Molecular vibration can be depicted by the energy diagram based on the Morse function as shown in Figure 1. Molecules largely reside at the ground energy state (v_0) at room temperature and can transition to the higher energy states when the energy matches the energy gap between the energy levels.

The strong fundamental molecular vibrations, which are seen primarily in the mid IR region, occur due to the transition of molecules from the ground energy state (v_0) to the 1^{st} excited energy state (v_1 energy level).

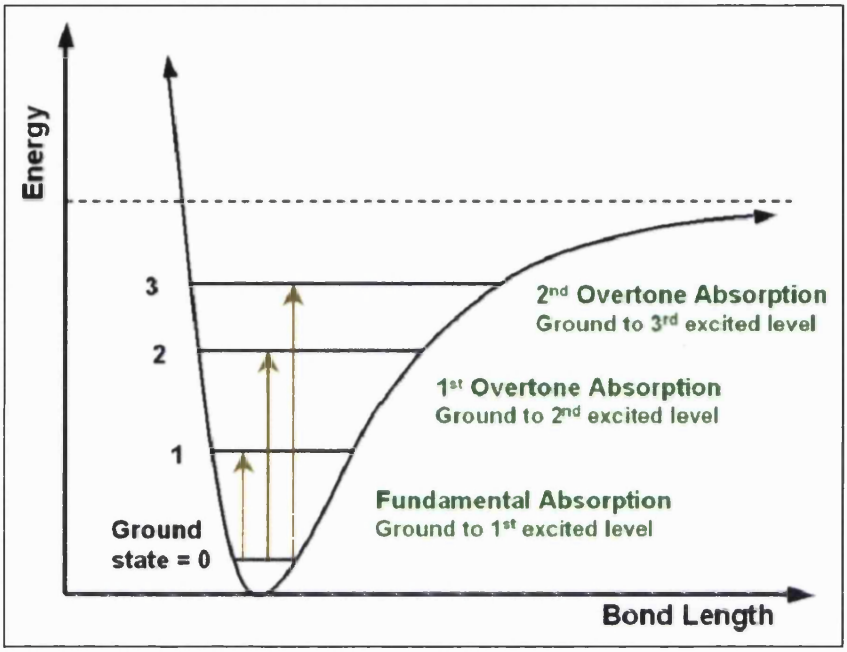

Figure 1: Energy diagram of an anharmonic oscillator

In a perfectly harmonic oscillator, according to Hooke's Law (Equation 1)[36] each energy level is equally spaced and transitions to higher energy levels are not allowed.

$$\text{Frequency}(\bar{\upsilon}) = \frac{1}{2\pi} \times \sqrt{k \frac{m_1 + m_2}{m_1 m_2}}$$

$$\text{Where } k = \text{force constant}$$
$$m = \text{mass of atoms}$$

Equation 1: Hooke's Law

However, molecular vibrations are anharmonic due to intramolecular interactions, and transitions from the ground state to higher energy states can occur with transitions from ν_0 to ν_2 being termed 1st overtones, and ν_0 to ν_3 2nd overtones. Overtone transitions are 100 to 1000 times less likely to occur[36] and are thus much weaker absorptions. These overtones require higher energy and are shifted into the NIR region of the electromagnetic spectrum.

In poly-atomic molecules, multiple fundamental transitions (e.g. bending and stretching of the same bond) can occur simultaneously at the right energy frequency input. As for overtones the higher energy required often causes combination transitions to be seen as weak absorption in the NIR region.

The particular frequency at which molecules absorb energy and vibrate is dependent on the magnitude of the dipole moment, the orientation of the bonds within the molecule (presence of hydrogen bonding, double bonds and steric hindrance reducing the ability of bonds to vibrate) and the anharmonicity of the molecule. These characteristics cause each molecule to uniquely absorb NIR energy, thus creating a NIR spectral fingerprint. Typical NIR absorption bands for organic structures are shown in Figure 2.

Apart from pure liquid pharmaceutical formulations, irradiation of samples with NIR light causes several scattering phenomena by the particles present in the formulation. The different scattering typical with interaction with NIR light is shown in Figure 3.

Specular radiation contains no information on the sample, while the remaining phenomena all contain physical and /or chemical information of the sample. The path length of the NIR light through the sample during sample interaction is affected by the size of the particles through which the light travels; hence both transmission and diffuse reflection spectra contain physical information of the sample. Samples with larger particles will absorb more energy than those with finer particles, causing baseline offsets. The effects of scattering increase as wavelength of light increases causing a curve in the spectral baseline. These scattering phenomena are now well established and the impact is reduced by appropriate mathematical corrections.

Figure 2: Characteristic NIR absorption bands for common organic structures

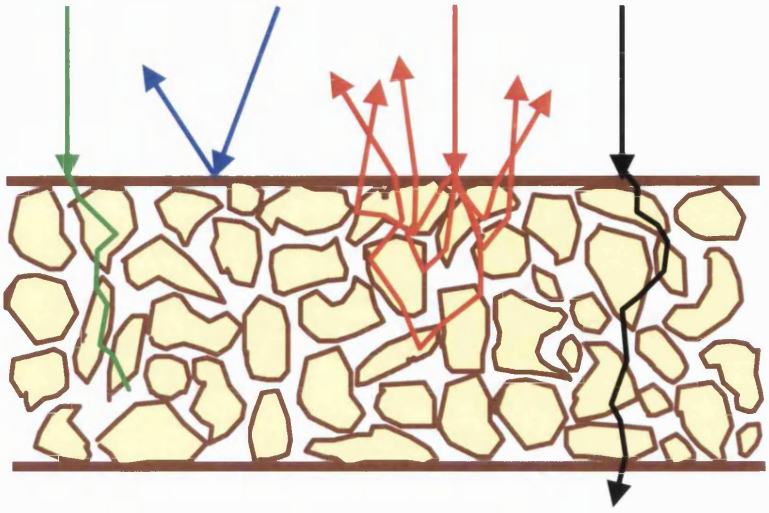

Figure 3: NIR interaction with a particulate sample showing absorption (___), specular reflection (___), diffuse reflection (___) and transmission (___)

NIR measurement occurs in three main modes;

- Transmission: light passes through the sample with the light source on one side of the samples and the detector on the other (black light path in Figure 3).
- Diffuse Reflection: light is shone onto the sample, with shallow penetration into the sample, and is then detected on the same side of the sample as the original light source (red light path in Figure 3).
- Transflectance: light is shone through a sample, hits a reflector and is reflected back through the sample again before being detected on the same side of the sample as the original light source.

In all cases, absorbance values are measured according to the following equation.

$$\text{Absorbance} = \log\frac{1}{T} \text{ or } \log\frac{1}{R}$$

Where T = transmitted light

R = reflected (or transflected) light

Equation 2: Relationship of absorbance to transmission and reflection spectra

2.4 Ability of NIRS to meet desirable attributes for PAT

As the NIR absorption is due to the weaker overtone and combination energy transitions discussed in Section 2.3, the NIR absorbance is much weaker than the fundamental absorbance in the IR region while the frequency of the energy provides excellent sample penetration. This feature allows samples to be analysed without sample dilution typical for IR (e.g. oil mull). The ability of NIRS to analyse neat samples, means that sample preparation is not necessary. Sample presentation for analysis is typically not difficult with two modes usually used - reflectance and transmission. Sample presentation is optimised to ensure the natural path length of the sample (e.g. tablet thickness, liquid cell) is appropriate and normally no sample modification is required.

The ability to analyse samples without modification coupled with weaker absorptions in the NIR region (causing no damage to the molecules) leads to NIRS being a non-invasive, non-destructive and inherently rapid analysis technique well suited to process analysis. The technique is ideal in situations where the sample quantity is limited, as the undamaged sample remains available for further analysis.

Pharmaceutical formulations are typically composed of four to ten components which are primarily complex organic molecules. Therefore, most pharmaceutical components are NIR active. NIR spectra of pharmaceutical products are thus a complex spectral combination of the NIR active components in the formulations. The fingerprinting ability of NIR spectroscopy discussed in Section 2.3 is also reflected in the NIR spectra of pharmaceutical formulations. Though spectra of pharmaceutical formulations are quite complex, once the data are resolved the spectral information provides immense information of the formulation (the excipients as well as the Active Pharmaceutical Ingredient (API)) and also the manufacturing process.

Unlike the majority of analytical techniques, the chemical information of the formulation can also be obtained without losing physical information of the sample. Scattering phenomena, historically seen as a hindrance with NIRS, can be of significant value for process analysis. Scattering information can yield information on the sample particle size (materials or blends), sample density (tablet hardness or powder bulk density), sample thickness (e.g. tablet thickness) and suspension and emulsion characteristics.

Hence, with a single rapid NIR analysis, taking less than one minute, information on the API, key excipients and physical characteristics of the sample can be gained non-destructively without affecting product quality and with minimal sample preparation. NIRS therefore meets the needs for analytical techniques for PAT applications as outlined in Section 2.2.

2.5 Historical applications of near-infrared spectroscopy for PAT

NIRS has been used in the pharmaceutical industry since the late 1980's. However, the early focus of NIRS was on alternate testing for compendial / QC testing. There were limited links to in-process testing or process understanding and instrumentation was largely located within QC testing laboratories. Blanco provides an excellent summary of the use of NIRS in the pharmaceutical industry in the pre-PAT era.[37] However, these applications do not fit within the PAT framework and will not be discussed further in this review.

Though more limited than laboratory based NIRS methods, process linked applications of NIRS pre-date the FDA guidance on PAT. The introduction of the terminology PAT makes identification of process related NIR applications much simpler during literature review on the topic after 2003. In following years, publications were split fairly equally

between papers on how and why PAT is implemented and focus on specific applications. However, as the terminology of PAT became known throughout the pharmaceutical industry, publication of PAT implementation and applications became more popular.

This section reviews a subset of PAT NIRS applications, discussing how the applications have been implemented and whether there has been a discernable change in implementation of PAT related applications following the issue of the draft FDA guidance in September 2003. The review concentrates on solid oral dosage form applications, as the key focus area defined in Section 1.7, including the application of NIRS to input materials through to coating processes of tablets.

2.5.1 Material testing

Variability in physical or chemical properties of excipients and APIs can be a significant factor affecting process robustness and product quality. A number of drug product processes have shown sensitivities to variation in physical or chemical properties of APIs and excipients, even when the materials meet their compendial or registered specifications. Pharmacopeial tests may not focus on physical tests such as particles size distribution and flow, or chemical tests such as moisture content which directly relate to processability. Pharmacopeial tests may also be general for the material with no link to the varied way the material may be used or behave in different drug product manufacturing processes (e.g. therapeutic powder verses direct compression solid oral dosage form) or different formulations (e.g. percent API composition in one product may be 1% by weight and 25% by weight in another).

Compendial testing is performed on a delivery basis, and does not take into account variability that may occur in different containers of the same delivery. This author

collaborated in one such work where despite deliveries passing compendial testing (on averaged results), container variation in starch content impacted the process and the ability of product manufactured from individual drums to meet finished product quality.[38] In this work, NIRS was applied for rapid material testing for starch content in the receival warehouse to aid prediction of product failure from the use of material from individual containers.

NIRS was identified as an important tool for assessing physical and chemical aspects of pharmaceutical materials as early as 1996, when it was established that comparison of NIR spectra can be a rapid tool to material analysis.[39] Applying NIRS to raw materials within the PAT framework must surpass the prior wide application of NIRS for material identification[29, 30, 40-43] and target the application of NIRS to the task of qualifying the material as appropriate for processing.

Material qualification employs various chemometric techniques to assess whether a particular sample is consistent with acceptable material (material meeting the established material specification). Traditional material qualification[29, 42, 44] typically utilises qualitative classification techniques and compares the spectrum of a sample to a static library of acceptable material developed at a single point in time often without linkage to the successful use of the material in manufacturing. This provides limited process information. Roggo[41] equates qualification with distance based chemometric methods, however any classification technique that utilises a library to establish a variability tolerance or acceptability threshold can be applied as a qualitative method. Quantitative methods have also been utilised for material qualification of particular material attributes (e.g. particle size), however thresholds have been typically applied to the output to provide a categorical classification of acceptability.

For material analysis to be a true PAT application it is necessary to begin assessing the relationship between observed material quality and the effect on product quality or on

processing behaviour rather than just relating the output to compendia test replacement or pass / fail binary knowledge of the material. Rather than qualifying that a material is acceptable compared to a reference library it must be established that the library represents a relevant variation that impacts product quality or process performance.

Various papers comment that qualification of materials has the potential to enable prediction of material performance in manufacturing processes.[39, 42, 45, 46] However, a very limited number of publications explore this aspect beyond demonstrating the successful development of a qualification classification approach. Plugge[39] successfully demonstrated the value of qualifying lactose and indicated that his approach could lead to greater understanding of the performance and impact of the lactose in the solid dosage form. This work in 1996 is well aligned with the PAT approach, however, despite much research utilising Plugge's chemometrics approach (Conformity Index) limited application for process understanding has occurred. Rather, the chemometrics has been simply applied as a classification and identification approach. One other example of qualification being put into practice to relate material quality to processing is Lopes' research which demonstrated the prediction of fermentation process success through a four level classification of soy bean flour, linking raw material NIR spectra to processability established in pilot scale experiments.[45] Similarly, Haware's work utilised qualitative methods to characterise lactose and predict tensile strength and other attributes of tablets utilising the material.[46]

The main area where material characterisation by NIR has related material quality to product and quality performance is in polymorph analysis. Though this has largely been in investigational rather than routine application, it is worthy of further discussion as follows in Section 2.5.1.1.

2.5.1.1 Polymorph analysis

Polymorphs (different crystal structures of the same compounds) and compound solvates (often called pseudo-polymorphs) are an area of particular interest due to the significant effect the different forms can have on product efficacy due to differences in physical and chemical properties.[39] Onward processing of the API (e.g. in wet granulation and drying processes) can convert the polymorph form into undesirable forms[47], and conversely the polymorph form of the API can greatly affect the ability of the API to be successfully processed into finished goods due to differences in physical characteristics.

Polymorphs and pseudo-polymorphs have been identified as an ideal application for NIRS as polymorphs exhibit different NIR spectra due to variations in crystal lattice impacting frequencies of vibration.[48] Chieng's recent review provides a summary of the diverse use of NIRS analysis as a PAT application for polymorph analysis complimenting the off-line characterisation with traditional techniques.[49] Polymorphic form of the API within the solid dose preparation matrix has been analysed with the ability to differentiate down to 1.5% of crystalline form of the API in the amorphous formulation.[48] Later work has successfully measured an API to a limit of quantification of 0.8% using a partial least squares regression method.[50] In-line NIRS has been successfully applied to the analysis of phase transitions / polymorph conversion during the wet granulation and crystallisation processes with investigation into the process kinetics. The NIRS methods were shown to provide opportunities for process improvement.[47, 51]

2.5.2 Dryer process monitoring

Drying processes occur in pharmaceutical manufacturing, both in the manufacture of API and in final dosage form manufacturing where wet granulation processes are employed. Traditionally drying processes are conducted for a set time, the dried material sampled, and confirmation that the product is dried sufficiently obtained through off-line moisture analysis (such as Loss on Drying (LOD), Karl Fischer (KF) measurement or mass of condensate from dryer effluent). It is common for material to sit in the drying vessel while waiting for the analytical result and then to require further drying to achieve the desired moisture level. The delays waiting for analysis and the successful application of NIRS for moisture analysis in other industries (utilising the fact that O–H vibrations of water exhibit a large absorption in the NIR region) led NIRS for pharmaceutical drying analysis to be one of the earlier applications explored. Initial focus of the use of NIRS was as direct analytical method replacement of laboratory based KF, gas chromatography (GC) and LOD methods by a quantitative NIRS method. A number of such end-point determination methods were performed and are summarised in Luypaert's review article on NIR applications for moisture analysis.[43]

The traditional approach (KF / GC / LOD), or the use of NIRS as a replacement of such methods within a QC framework, does not provide any information as to the actual drying process. The risk of over-drying the material and the variation in drying from batch to batch, led monitoring of pharmaceutical drying processes to be one of the earliest in-line, in-process applications of NIRS in the pharmaceutical industry.

The application of a NIRS method for the determination of moisture during the drying of a pharmaceutical granulation was reported for a microwave vacuum dryer as early as 1994.[52] This early paper focused on development of a quantitative NIR method as an alternate test to the KF method. However, White also conceded that additional valuable qualitative information about the drying process was provided by monitoring the

moisture curve during the drying process. This is one of the earliest concessions to process trending of analytical data and is directly compatible to the PAT philosophies as espoused by the FDA guidance 10 years later. The application of NIRS to dryer monitoring has been described by various authors since this first successful reported application.[13, 43, 53-56] The majority of published work describes commercial scale applications; however NIRS has also been applied during process development to develop process understanding of the drying process and determining optimum process conditions.[51, 56]

An early in-line application of NIRS for monitoring the fluid bed drying process applied multivariate analysis to project the data into 2-dimensional plots and trend the output to correlate process activities with the NIRS data.[11] This article shows successful PAT use of NIRS in 2000, years prior to the advent of the PAT initiative. This initial application heralded a switch in research to in-line NIRS for real time monitoring and control of critical to quality attributes aligned with PAT. Henningsson's work demonstrated that such an approach enabled improvements in batch-to-batch moisture content consistency leading to favourable yield and quality as well as reduction in process and testing cycle times (leading to an increase in drying capacity of 10%).[16] The development of on-line quantitative methods based on reference chemistry has been largely replaced by applying qualitative trending such as correlating NIR spectral data[51] to process activities without relying on reference chemistry. This approach can provide a much more accurate picture of the drying process and can also overcome building deficiencies in the reference chemistry into the NIRS method (e.g. KF methods may have high error for some analyses due to side reactions or hygroscopic nature of samples and reagents during sample preparation).[53, 57]

The majority of recent papers on drying monitoring focus on in / on-line analysis of fluid bed dryers and agitated pan dryers both of which allow direct product analysis of

the moving mass of drying product. On-line direct analysis of the product is not possible with tray dryers and the only possible option for on-line analysis is exhaust gas measurement (e.g. monitoring the solvent content in dryer effluent)[52, 58] which is an indirect indicator of the product moisture content.

Though at-line NIR analysis for end-point determination does not provide significant process related benefits over traditional moisture analysis techniques, it does provide a mechanism for greater quality assurance and process understanding through the ability to analyse samples of product from various locations in the dryer. The use of at-line NIR for mapping the performance of tray dryers has been overlooked to date with no reports of work in this area. Such mapping would allow measurement across different trays and at different locations and powder depths) providing dryer process understanding and deeper quality assurance of the product. Similarly, enhanced quality assurance and positional mapping across the pan of a fluid bed or agitated pan dyer by at-line NIRS may also be applicable when cost of in-line installation is prohibitive. No work has been reported in this area to date.

2.5.3 Wet granulation and dry roller compaction monitoring

NIRS has been utilised for analysis of wet granulation processes since the late 1990's.[55] PAT is typically applied to the fluidized bed or high shear wet granulation processes of a solid dosage form to monitor the drying process and to identify the optimum end-point. Drying related application of NIRS is described in Section 2.5.2.

Additionally, NIRS has been applied to provide understanding of the mechanism for granule formation[54] through the examination of NIR absorbance as the energetic state of water changed from bound to bulk water. Hydrogen bonding characteristics, related to bulk and bound moisture, vary during the different stages of granule formation.

Räntänen assessed the three key process phases of mixing, spraying and wet massing of a high shear wet granulation process using an in-line qualitative multivariate method showing the utility of NIRS to provide significant chemical and physical process knowledge.[56, 59]

The monitoring of the state of water has been further investigated with a NIRS method that monitored the water state and the conversion of an API to a pseudo-polymorph during the wet granulation process.[47] Applications also utilised the physicochemical nature of NIRS to simultaneously understand the chemical changes in moisture bonding with the physical growth of granules.[60, 61]

Burggraeve's excellent review[62] summarises the history of the use of NIRS for granulation monitoring showing that much work occurred prior to the FDA PAT guidance document being issued, indicating that NIRS was being applied to gain process understanding for wet granulation processes ahead of the FDA initiative.

Roller compaction process is used in combination with milling to moderate particle size (e.g. increase particle size and normalise distribution) and particle properties (e.g. shape). NIRS has been applied as a real-time at-line and in-line non-destructive technique to determine both physical (density, tensile strength and Young's Modulus) and chemical (moisture content and API potency and content uniformity) of the blend compact in a single measurement.[63, 64] All of these parameters impact the efficiency of the next milling step in delivering the particle size desired for onward processing and later blend uniformity of the product blend. The application of NIRS for rapid measurement of roller compaction (dry granulation) enables process optimisation to reduce cycle time and reworks as well as monitoring, reducing and controlling variability of the blend as an input into the next process step. NIRS applied to monitor the particle size distribution following milling has also been applied to verify the

success of the roller compaction and milling process while also reducing process and QC testing cycle time and costs.[63]

2.5.4 Active granule and pellet coating

Though the typical aspect of the granulation process to which PAT is applied is the formation of an API containing granule, it is of note that the growth of granule coating and active pellets has also been studied using NIRS. The active component may be in the granule / pellet core or in the coating. Non-active granule/ pellet coating is often used to control the API release in the finished formulation, enhance the API stability in the product or provide flavour masking.

NIR has also been successfully implemented in the at-line mode to measure the polymer coating process of controlled release granules. The rapid at-line determination of the process endpoint led to an improved cycle time and reduced inventory[16] and was also noted to have potential opportunities for understanding, monitoring and controlling the processes (aligned with PAT philosophy).

The application of in-line NIRS to the granule coating process[65-67] has demonstrated the greater process information gained by applying NIRS and indicates the possible use of NIRS for monitoring and controlling the granule coating process.

Recent work has investigated the use of NIR chemical imaging for the analysis of pellet cores and pellet coating[68] however as yet this has been applied in an investigational mode and the cost and the time to analyse a small sample size will likely result in NIR chemical imaging not being a widely deployable PAT application for the analysis, monitoring and control of granule coating within operations.

2.5.5 Blend homogeneity

Traditionally, solid dosage forms are manufactured by blending together the formulation components for a set blend time established during three validation batches. It is accepted that to produce high quality solid dosage forms, the intermediate blend must be manufactured to a high degree of homogeneity. Traditional testing of pharmaceutical blends by techniques such as high performance liquid chromatography (HPLC) and ultra-violet (UV) spectrophotometry can take longer than the blending process itself and assess the uniformity of the API in sub-samples of the blend alone to describe the blend homogeneity. However, the process-ability of the blend in the next processing step can be greatly affected by excipients such as lubricants or flow agents and quality attributes such as dissolution can be dramatically affected by distribution of excipients such as disintegrants and hydrophobic lubricants.[69,70] Therefore the distribution of key excipients should be determined as part of blend homogeneity testing.

Early laboratory experiments into the use of NIR for pharmaceutical blend mixtures was reported in 1991.[71] In1998, the use of NIRS for blend monitoring was described where the simple variance at wavelengths indicative of the API was used to decrease the use of HPLC assays and provide rapid decisions to forward process.[28] The at-line NIRS analysis of blends across the whole blend matrix (combined NIR absorption of all components) was also first investigated over 10 years ago.[39,72] This early research highlighted the benefit of NIRS to mitigate the extensive assays typically done in QC laboratories during blend validation. The use of NIRS for blend validation rather than just as end-point process monitoring, focused on providing process understanding, aligns well with the PAT philosophy.

The application of NIRS specifically to materials other than the API is more limited but has been discussed with regard to a blending study of magnesium stearate lubricant[69,73]

and starch content in a sucrose starch blend[38] and more recently for minor excipient components in a blend.[70, 74, 75]

The greatest difficulty in measuring the homogeneity of blends is the inherent errors involved in powder sampling using a conventional sampling tool such as a sample thief. Establishing blend homogeneity without the need to extract samples of the blend at all is therefore desired. NIRS lends itself to this application and has been investigated for many years. In-line NIRS also facilitates analysis of large sample size with no loss in product yield from sampling. Larger and more representative sample size is of interest and the industry and regulatory groups continue to debate stratified sampling and the minimum number of samples needed to describe the homogeneity of a blend with the FDA guidance on stratified sampling remaining in draft since 2003.[76]

In in-line NIRS applications, the NIRS instrumentation is typically interfaced with the blenders through fibre optic probes and measurements are taken frequently throughout blending. The earliest in-line applications of NIRS to blend analysis was reported at the end of 1995[77] and early 1996.[78] Both papers utilised variance analysis and trend charts to monitor blend matrix homogeneity during the blending process. Other papers since have described similar statistical process control (SPC) approaches (variance analysis, trend charting, Hotelling's T^2 test) coupled with chemometric data interpretation tools such as spectral matching, PCA and regression methods.[74, 75, 79-84] It has been found that the blending curve and blending end-point varies between batches, which highlights the benefit of applying PAT to each batch as ongoing quality verification.[82, 84]

Much research predates the issue of the FDA guidance document in 2004. This demonstrates again that companies were already actively pursuing PAT for key pharmaceutical applications before the FDA promotion of PAT. Recent work has emphasised the value of NIRS in PAT applications to support quality by design efforts.[84] Papers by Puchert[80] and De Beer[13] provide good reviews of the diverse NIRS

applications for blend analysis, while Blanco's review describes the various qualitative and quantitative approaches to data analysis, particularly for end-point determination.[85]

NIR chemical imaging, an area of recent growth, was also investigated prior to PAT becoming well established in the industry.[86, 87] Recent work on NIRS imaging[29, 80, 88] continued the focus on process understanding on the microscopic level distribution of components within the blend matrix. This provides an incredible degree of information on both the spatial and chemical composition of the blend and has assessed correlation of NIR imaging results to later processing and finished product quality. The key limitations with NIR imaging being deployed within the production environment are; the sample area and resolution vs. the time of analysis, cost and ability to interface within the production environment (dusty, vibrating, etc.). Instrument and technology development continues to work towards real time NIR chemical imaging while competition in the market is starting to reduce prices. Within the current economic climate and instrument capabilities, NIR imaging is beyond scope for wide deployment to support process monitoring and control.

Though in-line analysis is the preferred mode of NIR analysis, some blenders (such as stationary ribbon blenders, or rotating screw cone blenders) do not facilitate in-line or on-line blend analysis. In such cases it may be possible to measure product at blender discharge however spatial understanding of the blend uniformity within the blender is lost. Thus at-line blend testing with sampling will continue to be required in some form and will facilitate PAT application of NIRS.

2.5.6 Chemical attribute monitoring of solid dosage form

Historical application of NIRS for intact tablet analysis has concentrated on the quantitative API potency prediction followed by content uniformity determination.[89-101] Development of these quantitative methods has been applied primarily as a tool to decrease the cycle time through the laboratories, exploiting the advantages of being fast and non-destructive. These methods are not typically applied to gain process understanding and are well summarised in Lupaert's review.[43]

A significant change in NIRS approach was demonstrated by Plugge and Van Der Vlies in 1993, where qualitative systems were used to provide semi-quantitative predictions and quality assurance.[102] Plugge introduced the Conformity Index algorithm as a measure of the "degree of conformity" of a batch with samples of standard quality. Axon et al. appreciated the potential of transmission NIRS for the qualitative content uniformity assessment of drug substance in intact tablets using a simple univariate qualitative approach.[28] However, since this initial investigation of qualitative methods of NIR for tablet analysis in the early 1990's, little further work has been reported in applying qualitative analysis in the area of in-process monitoring of larger sample size during tabletting manufacturing.

Reich mentioned qualitative NIR tablet methods (applying "conformity" testing) in passing during her review article on pharmaceutical applications of NIRS.[42] However there was no detail and literature is dominated by quantitative methods. The primary arena that qualitative analysis is currently applied to tablet analysis is in tablet identification[103-105] and counterfeit analysis[106-108] rather than analysis of the tablet components within a manufacturing setting.

There is limited reference to semi-quantitative methods for tablet analysis chemistry[103] and for quantitative methods developed without reference chemistry.[109,110] De

Maesschalck's work utilised a semi-quantitative approach coupling regression with discriminant analysis, however, this was then applied as an identification method within the research and development arena to differentiate different dosage strengths of clinical trial samples rather than in commercial tabletting operations.[103] Blanco's reference free approach was to enable calibrations built on pure component powder mixtures prepared gravimetrically in a laboratory setting to be corrected for spectral differences when applied to production samples. This approach, though rapid to develop, may lead to ongoing update of the representative process residual spectrum as process variations occur over time. Blanco does not comment on how this approach would be validated within a commercial facility and whether the limited calibration set would be robust to the typical process variability that occurs in the life of a product, NIR instrument and process equipment.[109, 110] Shi and Li's work investigated the use of qualitative or low reference methods to assist in rapid screening of clinical trials tablets, however no discussion was given on the extension of the approach in the commercial setting.[111, 112]

A limited number of papers do discuss the virtue of applying NIRS to larger sample sizes[28, 113-118] to gain understanding of the tabletting process and provide heightened quality assurance. The applications for this purpose have been minimal due to the continued lack of direction of the application of appropriate specifications for large sample sizes (large *n*).[119] Applying NIRS at-line for process monitoring rather than as product release testing does provide a mechanism for gaining the process understanding and inherent quality improvements while staying clear of the debate on large *n*, however this appears to have been overlooked and no papers were identified discussing tablet component trending.

NIR chemical imaging has been applied to process understanding of tablets and capsules and investigation into various quality issues from as early as 2002[86] and continues to be an area of research.[29, 87, 120, 121] More recently NIR chemical imaging has

been applied to counterfeit analysis.[122] As described in Section 2.5.5, the cost, time and small sample size limits chemical imaging analysis being deployed as a routine PAT application for finished solid dosage form component analysis.

Several on-line NIR analysers are commercially available, (e.g. Bruker Tandem) however the cost is quite prohibitive and success is very dependent on the ability to automatically deliver the tablets to the analyser in a consistent manner (requiring expensive robotics). The use of such on-line systems has not been published widely for tablet testing[112] and has not been reported for capsules.

Colon Soto reported the first use of in-line NIRS at the exit of the tablet press for component quantitation.[115] This allowed a much larger sample size to be analysed, however the accuracy was in excess of 4% at the centre of the calibration range and an in-house conveyer system was required. Karande furthered this work through the use of in-line NIR inserted in the tablet press directly after the tablets are ejected from the dyes. However this work reported poor accuracy with the prediction error as high as 17.4% at the target API content.[114]

No other work has been reported with in-line NIRS of intact tablets. An alternative to in-line tablet analysis, identified by Reich in 2005,[42] is described by Liu[123] with the use of blend analysis from within the tablet press at the point of compression. This is proposed as an alternate approach to content uniformity testing and follows in-line analysis methodologies as for blend analysis in Section 2.5.5.

With the limitations of in-line and on-line analysis, at-line analysis is the primary mode of PAT aligned NIRS applications for large sample size analysis of tablets and capsules. Within this focus, qualitative approaches are an area of opportunity for research given the limited process focused application to date.

2.5.7 Physical attributes monitoring of solid dosage forms

NIRS has been investigated to gain understanding on product attributes other than API potency prediction and content uniformity of solid dosage form (tablets and capsules), with considerable interest in dissolution, tablet disintegration and hardness characteristics.

One of the earliest papers on in-process assessment of physical attributes of solid dosage forms discussed the use of NIRS for predicting dissolution times and tablet hardness.[113] Though this 1995 work focused on developing quantitative methods for assessing the physical attributes, the authors acknowledged that pattern recognition methods were valuable and could be applied within manufacturing for qualitative classification of samples and that the developed quantitative methods could be applied on-line for process control.[113] The application of NIRS for tablet hardness analysis was further investigated with the comparison of the use of simple statistical techniques to the more complex chemometrics / multivariate regression analyses. This work correlated the absorption shifts seen due to changes in the effective pathlength of NIR light through tablets compressed under different tabletting pressures to the tablet hardness.[116] The application to tablet hardness was extended to assess tablet porosity as well as hardness[117] and has also applied NIRS to dissolution prediction at different compression forces[118] (though in this later paper there is distinct deviation from linearity observed in the linearity graphs at low dissolved API content).

Hattori's recent work investigated the ability of NIRS to study the ingress of water, porosity and the relationship to dissolution of intact tablets.[124]

Much of the research has remained focused on development of quantitative methods despite challenges in developing calibration samples over the range of the attribute under investigation. Reich indicated that qualitative approaches might prove more

practical;[42] however limited research has been conducted on qualitative methods. Also though it is inferred that higher sample numbers can be tested, there is no published targeted discussion on the possibility of applying the method for real-time process monitoring or control.

2.5.8 Capsule and tablet coating

The capsule material used to encase the pharmaceutical blend in capsule finished dosage form has significant impact on quality attributes such as dissolution and in the end product efficacy. NIRS has been utilised for understanding the capsule characteristics for both hard and soft gelatine capsules. At-line NIRS has been employed for bulk capsule characterisation (such as moisture)[125] synonymous to analysis of raw materials discussed in Section 2.5.1. Additionally, at-line NIRS has been applied to assess capsule changes once filled with product (e.g. cross-linking) summarised well by Reich.[42] Investigation of capsule characteristics in an on-line mode during encapsulation has not been reported, however this is feasible within the encapsulation and packaging process prior to blister closure, which would provide a means to assess variability in capsule characteristics.

Tablets are coated for aesthetic purposes (e.g. appealing marketable colour), to align with regulations (e.g. country regulations on colour of particular therapeutic products), to provide flavour masking or to control active component release (e.g. dissolution rate of sustained release product). Coating materials are typically insoluble, slowly dissolving or erodible (for release control), readily soluble coating (for colour and appearance), sugar coating (for flavour masking) and less often active component coating.

NIRS has been successfully applied to gain process understanding of coating processes for tabletted products and is commonly applied to assess the coating thickness[53, 113, 126, 127] and prediction of dissolution.[128] As early as 1995, NIRS was applied to assessing film coating thickness of film coated tablets and predicting dissolution times.[113] This author collaborated on one such application where reflectance NIRS was used with quantitative models to correlate spectral features to coating components in an extended release product to gain understanding of coating effectiveness.[53] This work also describes the extension of analysis from coating thickness to gaining additional process knowledge through understanding the relationship of spectral features to the API release rate. Valuable knowledge was gained despite the developed method showing insufficient correlation for quantitation. Other research has focused on the use of at-line NIRS to monitor the curing end point process to understand changes occurring in the coating structure.[129]

Direct on-line analysis of the pan coated product during the process is hampered by the hostile mechanics of operation (heated environment tumbling product with atomised sprayed coating material). As such, limited work has been conducted with the NIR instrument deployed in an in-line mode. Recently in-line applications at commercial scale has been reported by Möltgen[127] and Gendre[128, 130] indicating a new focus of NIRS for pan coating monitoring.

NIR chemical imaging has been applied to process understanding of tablet coating and related quality issues.[29, 127] As described previously, chemical imaging analysis is not currently suitable for routine PAT application for coating analysis.

2.6 Scientific research gap analysis of NIRS for PAT - research aims and objectives

NIRS has been widely investigated and applied within the pharmaceutical industry. The application of NIRS for PAT applications predates the introduction of the PAT framework by the FDA, however the publication of the FDA guidance has mobilised interest across the industry and ensured PAT terminology is embedded in the pharmaceutical manufacturing culture.

Despite considerable research and application of PAT across the various areas of pharmaceutical manufacturing for solid oral dosage forms, several gaps of application of NIRS to support PAT exist. In particular, the typical approach of developing qualitative identification methods for materials with no extension beyond replacing traditional identification tests yields little process understanding and does not facilitate the development of causal relationships between materials and process behaviours. Also, development of alternate quantification methods with time consuming reference chemistry may not be suited to the need for rapid deployment within processing areas, flexibility or ease of update and may not provide process information necessary to gain the desired process understanding or provide process monitoring or control. There is a gap in the application of simple and rapid to implement NIR applications and methods which are aligned to PAT by providing a mechanism for process understanding and quality assurance. Research in this area will add value to the pharmaceutical industry by closing this gap and will also respond to the scientific need for mechanisms of NIR analysis not reliant on time consuming and potentially error laden reference chemistry. Thus research should focus on spectral information and novel uses of chemometric algorithms combined with SPC.

After review of the pros and cons of at-line versus on-line mode of NIRS as well as the previous applications in literature, the aspect of NIRS measurement with greatest potential scope for impactful research was determined to be at-line analysis. The critical drivers for this focus were the ease of integration and interfacing, ability to multipurpose the instrumentation (thus offsetting capital investment) and the wide availability of at-line systems in the industry. Thus the research has global applicability for even the most cost conscious locations in the global pharmaceutical manufacturing environment.

The ideal deployment of PAT would assess critical attributes and parameters at each unit of operation throughout solid dosage form manufacture as each process step impacts the next. Following a review of published application of NIRS to solid dosage production across the breadth of pharmaceutical manufacturing, from materials and intermediate processing through to end stage processing described in section 2.5, it was identified that the most value would be derived from focusing research on gaps in the application of at-line NIRS to support PAT at raw material and tablet component analysis. The raw material process step is common to all oral dosage forms whether granulated or direct compression, while tabletting was found to be the dominant finished dosage form in the marketplace, and each of these areas were identified with research gaps.[35]

Figure 4 shows a flow chart mapping the typical solid dose manufacturing process, highlighting the two research focus areas; materials and tabletting analysis. Sections 2.6.1 and 2.6.2 describe the aims and objectives of the two research focus areas, while Chapter 4 and Chapter 5 describe the research in detail.

2.6.1 Aims and objectives – research in materials analysis

Following the scientific research gap analysis and review of published work, the first research focus aim was to investigate and develop novel applications of NIRS for at-line analysis of materials aligned with PAT.

The objective was to develop an approach that rapidly assesses the material quality as a whole and for particular material attributes of interest through the application of rapid to develop qualitative NIRS methods and the use of SPC techniques to enhance the assessment of material quality through deeper interrogation of material attributes and the linkage to both product quality and process behaviour. The developed methodology was required to be approachable for scientifically untrained warehouse operators to allow rapid analysis at the point of material receipt.

2.6.2 Aims and objectives – research in tabletting analysis

The aim for the second research focus area was to investigate and develop novel NIRS application to facilitate at-line process monitoring throughout tabletting for a key tablet component of interest aligned with PAT.

The objective was to develop an approach with rapid to develop qualitative or semi-quantitative methods that did not rely on significant reference chemistry and couple the methods with SPC techniques to provide a means for in-depth understanding of the tabletting process. The developed methodology was required to be approachable for scientifically untrained tablet press operators to allow at-line monitoring and potential manual feedback as the process occurs.

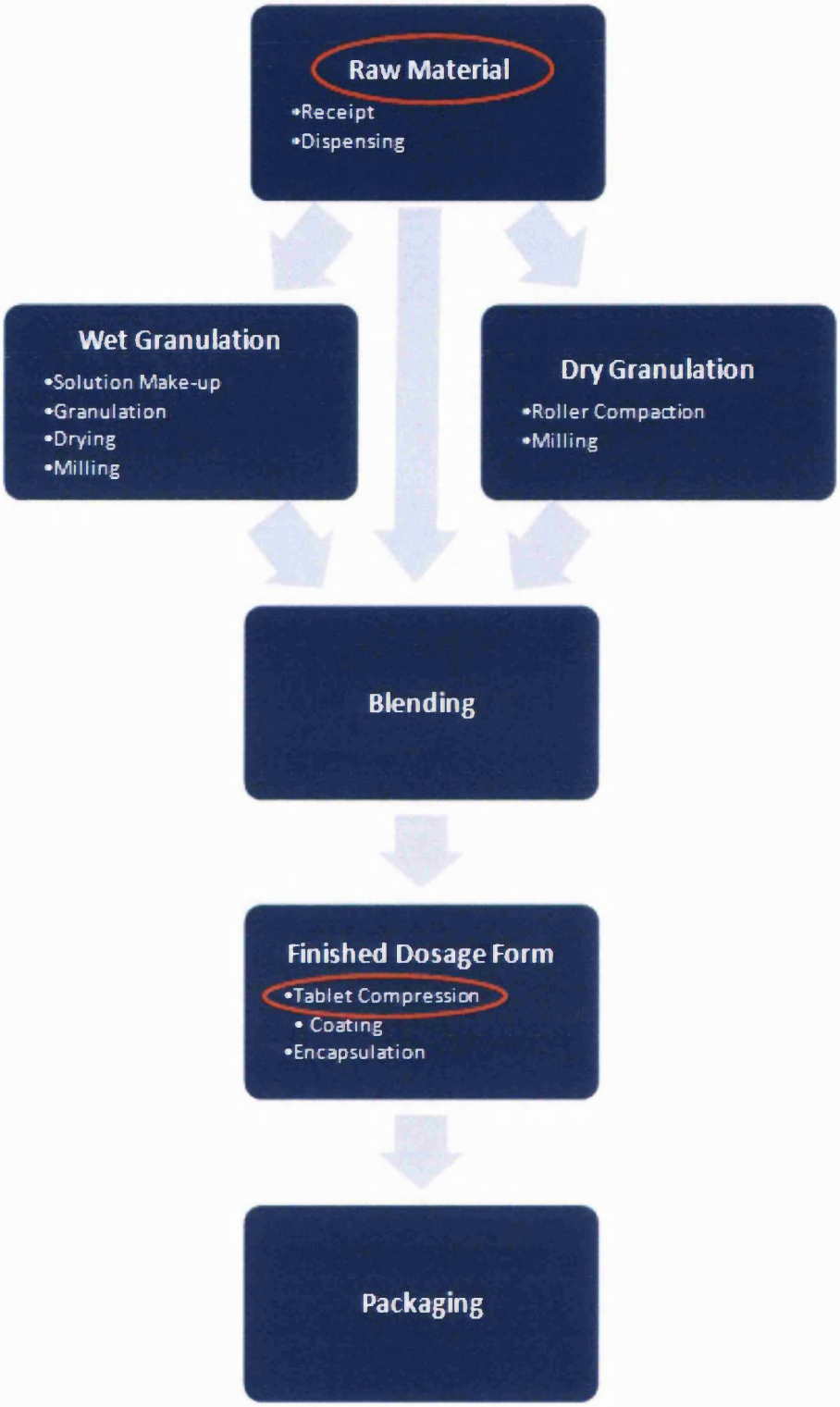

Figure 4: Flowchart of a typical solid dose manufacturing process with areas of research focus circled in red

CHAPTER 3 CHEMOMETRICS AND STATISTICS FOR NIRS PAT APPLICATIONS

3.1 Introduction

To successfully utilise the complex NIRS spectral data for PAT applications, data mathematics treatments, multivariate analysis and statistical analysis techniques are applied to extract the important and relevant sample information.[131] Pomerantsev and Rajalahti provide good summaries of the use of chemometrics in PAT since 1993.[7, 131] The common techniques applied, and in particular those employed in the PAT applications researched and described in Chapter 4 to Chapter 5, are summarised in this chapter.

3.2 Data treatments

Spectroscopic data such as that from NIR measurement systems is a complex data array, whereby each sample measurement comprises n individual results where n is the number of data points across the wavelength or wavenumber range of the measurement. As such, the NIR spectrum is a curve joining these individual measurement results. As mentioned in Section 2.3, the NIR spectrum includes the analytical response of the sample (absorbance of energy by the sample) as well as a component due to specular radiation (physical effects). The spectrum will also contain a component of instrumental noise.

Data treatments are mathematical corrections of the data to correct for components in the data not related to the sample measured (such as instrument noise) or to the analyte of interest (such as pathlength effects). Common data treatments are described in the following sections.

3.2.1 Smoothing

Smoothing acts to join data points in the NIR spectrum in a fluid curve across the range of frequencies analysed (range is typically quoted as wavelength (nm) or wavenumber (cm^{-1}) depending on software) and remove instrument electronic noise. Smoothing is an essential step before other data treatments to ensure that small peaks caused by joining individual data points or electronic noise are not erroneously assigned an analytical source. Smoothing is a common technique used across analytical chemistry and not a data treatment unique to NIRS. Convention is to apply the lowest smoothing treatment to remove the random noise in the signal without inadvertently removing small peaks that do relate to the measured sample. A common smoothing technique is the moving block mean across n data points, described in the equation below. Figure 5 shows the effect of applying smoothing to NIR Spectra.

$$\text{Corrected } A_x = \frac{\sum_{i=\left(x-\frac{n-1}{2}\right)}^{\left(x+\frac{n-1}{2}\right)} A_i}{n}$$

Where A_x = absorbance value at frequency x

n = number of data points applied for smoothing

Equation 3: Typical smoothing calculation

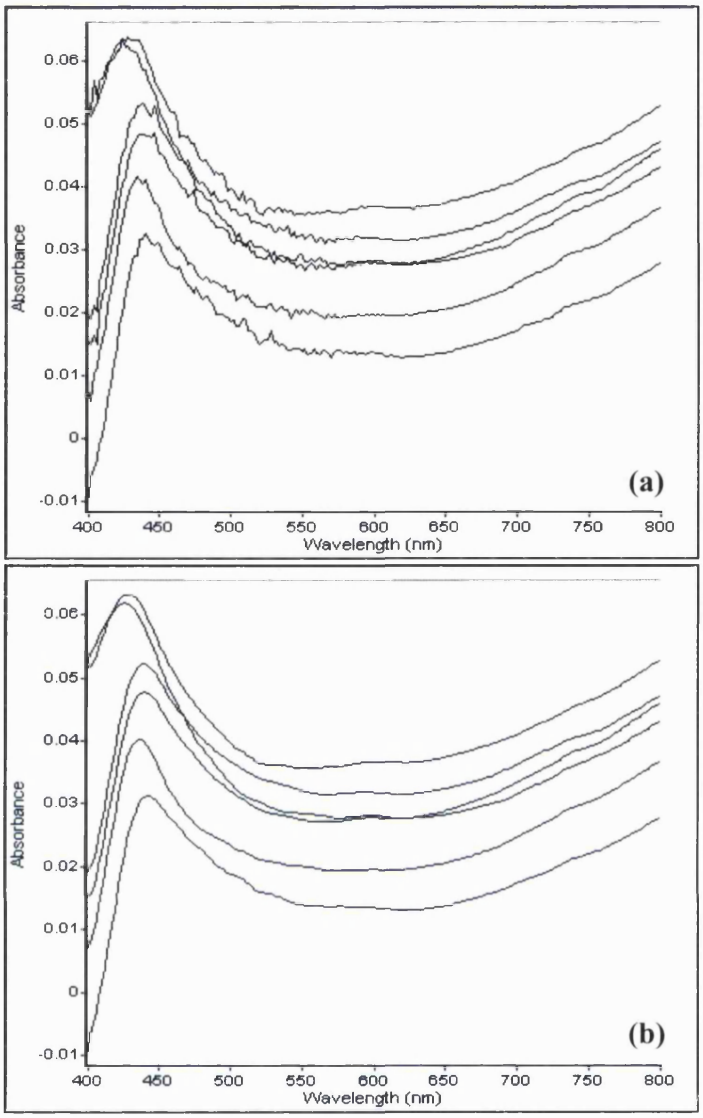

Figure 5: Zoomed image of spectra before (a) and after (b) smoothing

3.2.2 Scatter correction and normalisation

Scatter correction and baseline normalisation is a useful mathematical treatment for NIR spectra of particle based samples. As discussed in Section 2.3 and illustrated in Figure 3, NIR spectra of particulate samples includes contributions from specular reflection and scattering interactions of the light with the particles. Scattering effects are largely due to the size of the particles approaching the wavelength of the NIR radiation. The smaller the particles in a sample the more light is reflected from the particle surface, and

less absorption occurs compared to coarser material. The result of this effect is baseline shifts.

Scatter effects are more significant in transmission measurement where a larger portion of the sample is measured and is also compounded by pathlength effects. Variation in the thickness of samples directly impacts the light emitting from the sample and reaching the detector, also contributing baseline offsets.

Particulate scatter effects also vary across the NIR wavelength range with more effects occurring at the IR end of the electromagnetic spectrum at lower energy and lower penetration. The varying nature of scattering across the wavelength range causes the NIR spectra to have a curved baseline.

Scatter correction and normalisation are a range of techniques used to correct for baseline and physical effects. The simplest scatter correction technique is applying a linear absorbance correction value across the wavelength range. These techniques do not take into account the varying impact of the effects across the wavelength range and can result in treated spectra containing baseline offsets at portions of the range (e.g. at extremes of the spectra when a centre point correction is applied).

Normalisation techniques are more effective in correcting the baseline offset across the entire spectrum by accounting for the variation in the range of absorbance. The Standard Normal Variate (SNV) pre-treatment described in Equation 4 is one such normalisation technique. SNV centres the spectrum by subtracting the average absorbance value of the spectrum from every point in the spectrum, then divides each point by the standard deviation calculated from the Y-axis value of every point in the spectrum. Each corrected spectrum will have a mean absorbance of zero and standard deviation of absorbance intensities across the wavelength range of one. Figure 6 shows the success of SNV normalisation in removing varying baseline offsets in NIR spectra.

$$\text{Corrected } A_x = \frac{A_x - \frac{\sum_{i=1}^{n} A_i}{n}}{S}$$

Where A_x = absorbance value at frequency x

n = number of data points in the spectrum

S = standard deviation of n absorbance values

Equation 4: Standard Normal Variate calculation

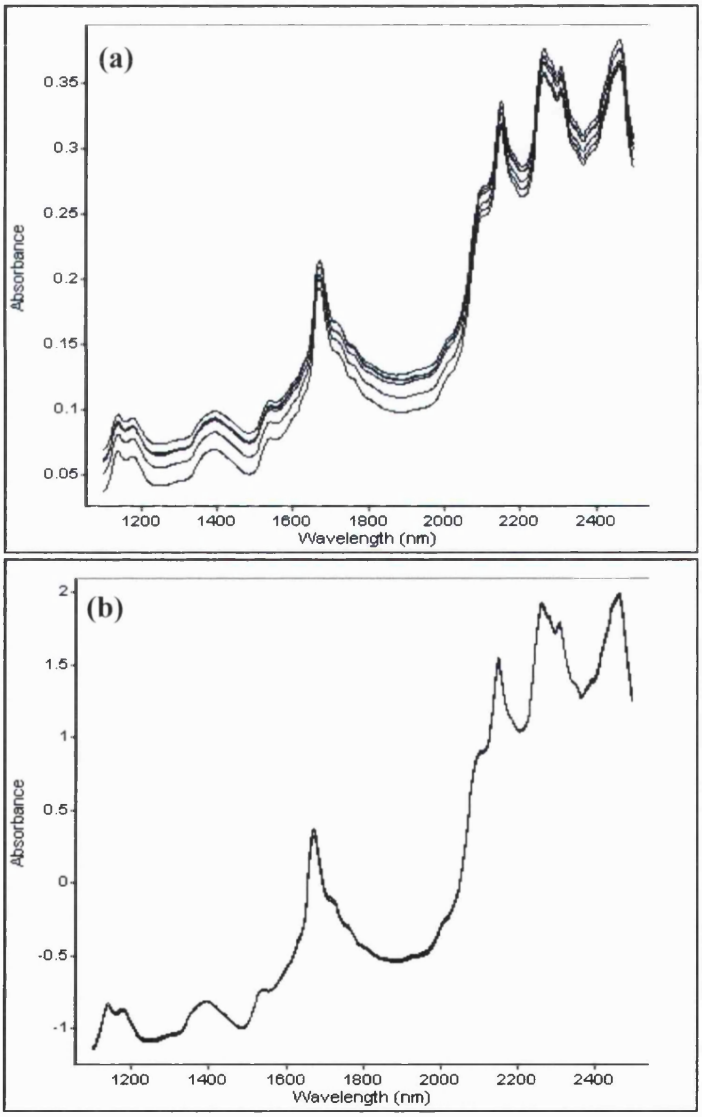

Figure 6: Smoothed raw spectra before (a) and after (b) SNV normalisation

3.2.3 Derivatives

As discussed in Section 3.2.2, the NIR spectrum has a curved baseline. This can lead to challenges in resolving analyte absorptions from this curve. In addition, the NIR spectrum is composed of complex broad, overlapping overtones and combination absorbance bands from multiple components and/or functional groups. Derivatives are a mathematical treatment which remove curved baselines, account for residual physical effects and resolve broad peaks and improve fine structure.

The 1st derivative spectrum is derived from a moving block slope calculation across a set number of data points. The peaks in the 1st derivative spectrum reflect changes to the slope of the NIR spectrum. Each raw NIR spectrum peak will thus result in two peaks in the 1st derivative spectrum

The 2nd derivative spectral treatment repeats the moving block slope calculation on the 1st derivative spectra and the resulting 2nd derivative spectrum represents the rate of change of slope in the original NIR spectrum. Figure 7 provides a depiction of the derivative mathematical treatment for a single absorption peak showing the relationship of the derivative to the original raw spectrum. The 2nd derivative is often preferred as the peak absorbance can be related directly to the known absorbance bands of organic structures while 1st derivative peak absorbance values are shifted.

Figure 8 shows the ability of derivatives to extract and emphasise spectral features for the same data set as shown in Figure 6. Also note that as the order of derivatives increase, the noise increases which can impact method sensitivity. This is illustrated in Figure 6 and in Figure 8, where the absorbance range for the raw spectra in Figure 6 reduces with each derivative treatment shown in Figure 8. The selection of the appropriate derivative treatment for a given data set should be determined balancing the enhancement of the spectral features with the reduction in signal to noise ratio.

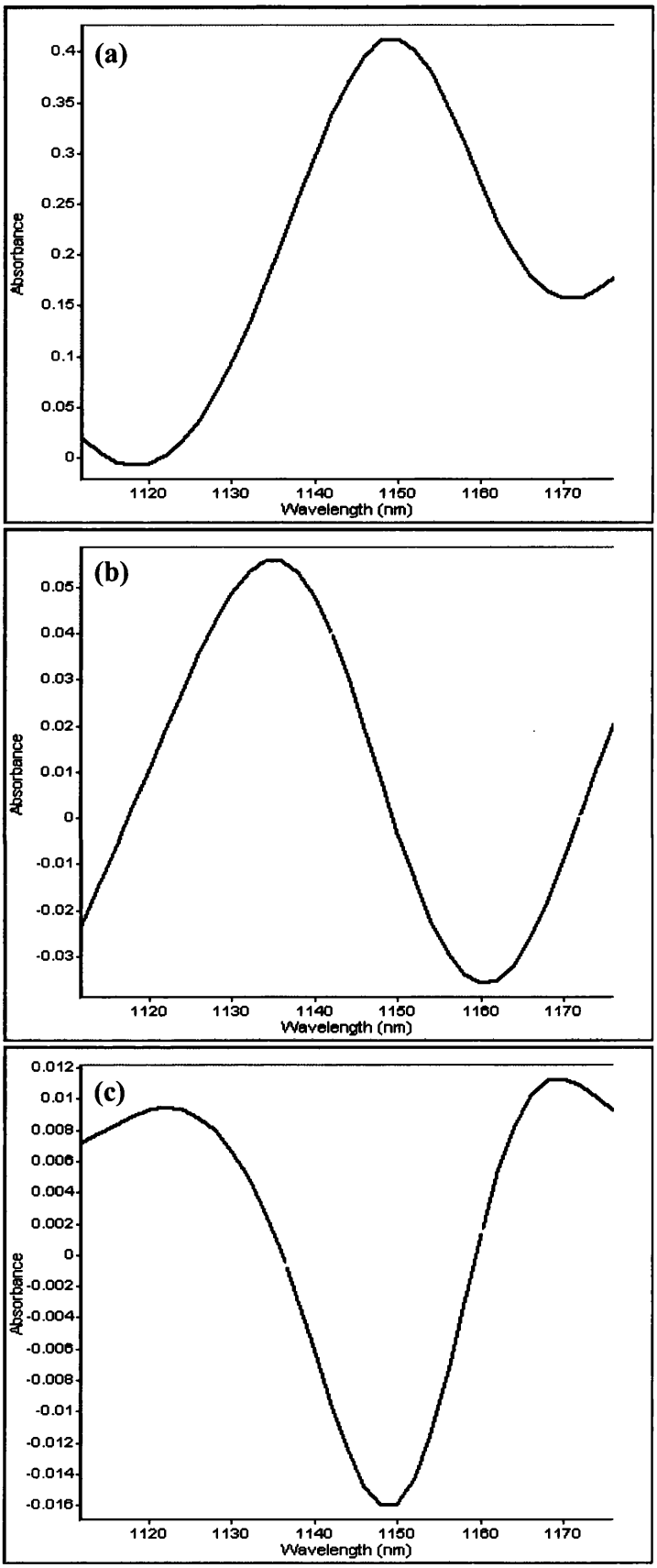

Figure 7: Representation of the derivative mathematical treatment with (a) raw spectrum, (b) 1st derivative spectrum and (c) 2nd derivative spectrum

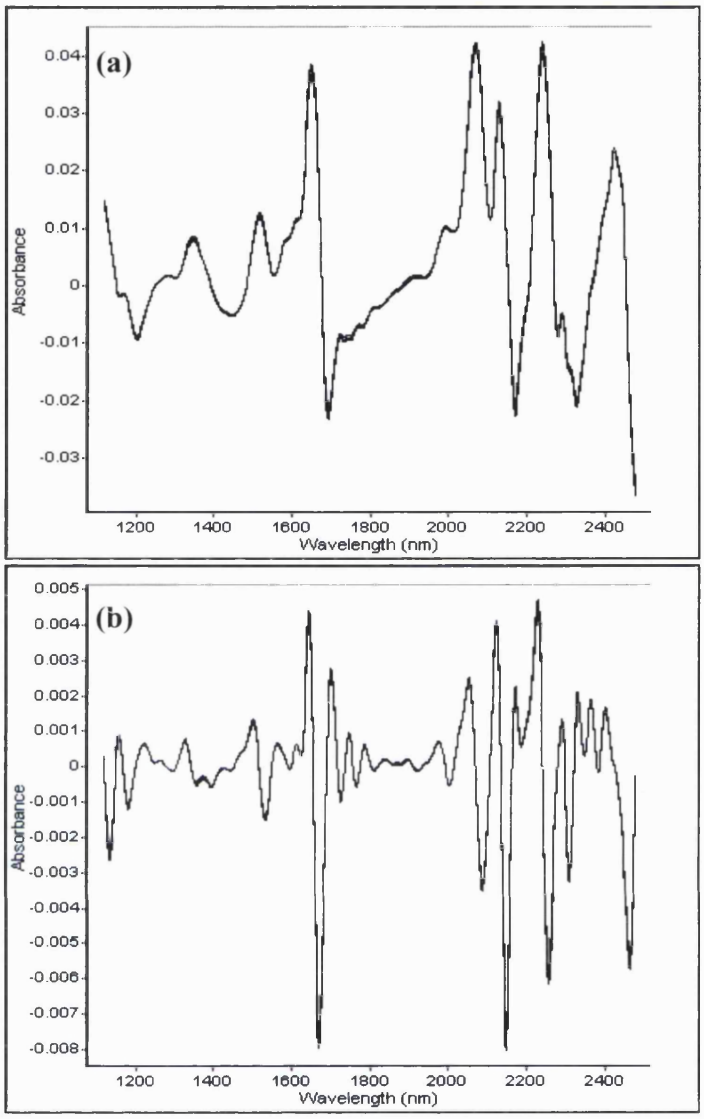

Figure 8: Smooth normalised spectra with (a) 1st derivative and (c) 2nd derivative treatment

3.3 Chemometrics and multivariate analysis

The term Chemometrics was founded in the early 1970's and though many definitions exist, in simplest terms, chemometrics can be defined as:

> *"the application of mathematical and statistical method to chemical measurements."*[132]

In particular, chemometrics is applied to extract information from complex multivariate measurement data sets, to reduce complexity of data (often termed data compression) understand the relationships and interdependence of the multiple variables and to

improve performance of analytical methods generated from the complex chemical measurement data.

As mentioned previously in Section 2.3, the NIR spectrum is composed of an array of individual absorbance values across the frequency range of the measurement system. At each data point in the range, the absorbance has contributions from many different sources, the analyte of interest, the product matrix, instrument noise, environmental factors (e.g. humidity, vibration), and sample handling effects (preparation and interface with the measurement system). Chemometrics is commonly used to interrogate the multivariate array of data in the NIR spectrum and draw out the important elements that relate to the analyte of interest.

In terms of NIR data analysis there are three main types of chemometrics applied; classification analysis, regression analysis and discriminant analysis (which utilises regression to enable classification analysis).

3.3.1 Classification analysis

Classification analysis in NIRS is the use of chemometrics to analyse the NIR spectra and produce a categorical determination, often pass or fail. This is most commonly applied within the pharmaceutical industry for identification determination (e.g. raw material identification).

Various chemometric algorithms are applied to the NIR spectrum as a means of comparison to an established reference spectrum or reference spectral dataset (often termed reference library). Several techniques dominate the literature and are discussed in this section.

3.3.1.1 Correlation

Correlation determines how well the sample spectrum overlays with the reference spectrum (or average spectrum if a reference library is used). Correlation between two variables is defined in statistical terms as the covariance of the variables divided by the product of the respective standard deviations as shown in Equation 5. For correlation models used in classification chemometrics, the x variable is the sample spectrum while the y variable is the reference spectrum (or average spectrum if a reference library is used).

The correlation calculation is analogous to simple linear regression of the absorbance values of the sample spectrum at each frequency against the paired absorbance value of the reference. The resulting correlation coefficient (r) provides the output of the analysis, though often software will quote the r^2 value in place of r. An example is shown in Figure 9.

$$\text{Correlation (r)} = \frac{\sum_{i=1}^{n}(x_i - \bar{x}) \cdot (y_i - \bar{y})}{\sqrt{\sum_{i=1}^{n}(x_i - \bar{x})^2 \cdot \sum_{i=1}^{n}(y_i - \bar{y})^2}}$$

Where x_i = absorbance value at frequency i for the sample

y_i = absorbance value at frequency i for the reference

n = number of data points in the spectrum

Equation 5: Correlation (r) calculation

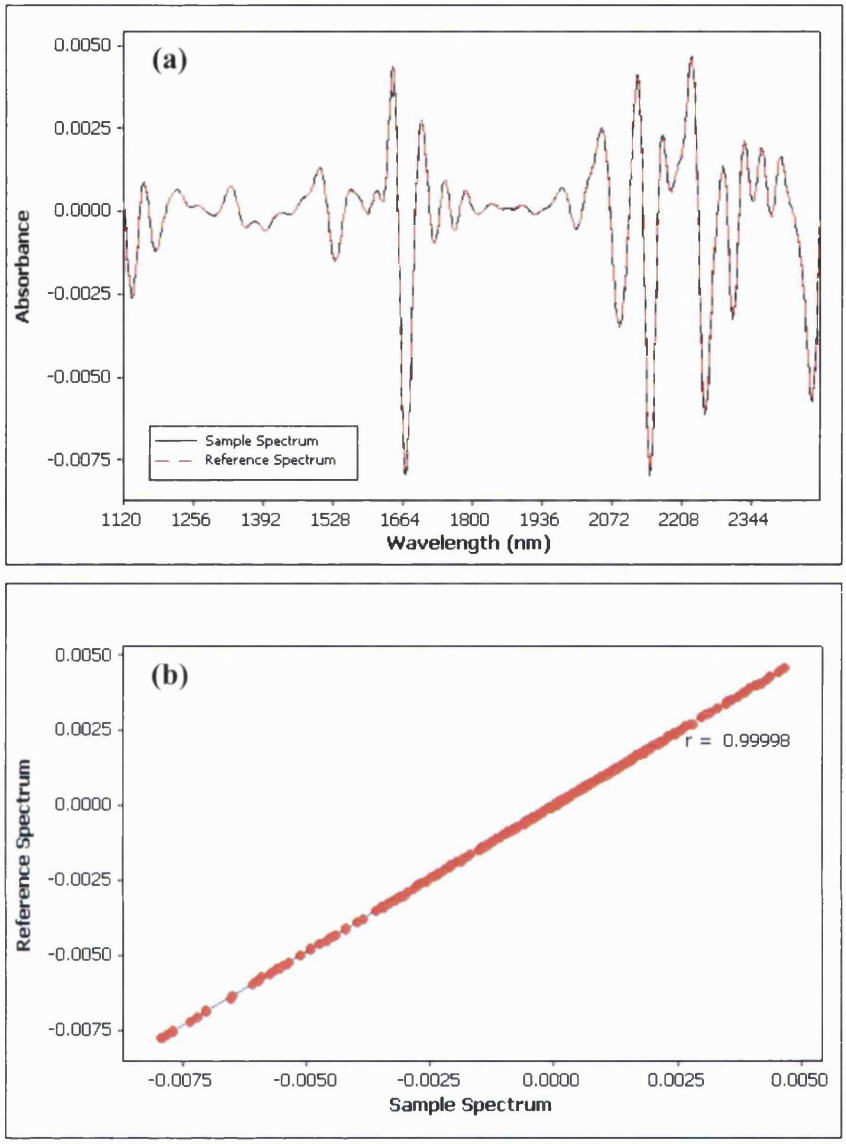

Figure 9: Sample and reference 2nd derivative spectra (a) with resulting regression plot and associated correlation coefficient (b)

3.3.1.2 Spectral distance and Euclidean distance

Spectral distance (SD) can be considered as the array of differences between two spectra across *n* wavelengths. This is a common measurement between the reference spectrum (or average reference spectrum of a reference library) and a sample spectrum.

Different software packages provide various statistics based on the SD metric, these include:

- Total absolute SD - The sum of the absolute values of the SD across the spectra (equivalent to the area under the curve) as shown in the equation below:

$$\text{Total absolute SD} = \sum_{i=1}^{n} \left| A_{ref_i} - A_{s_i} \right|$$

Where A_{ref_i} = absorbance values for the reference spectrum at point i

A_{s_i} = absorbance values for the sample spectrum at point i

n = number of data points in the spectrum range

Equation 6: Total absolute spectral distance calculation

- Average absolute SD - The mean of the absolute values of the SD across the spectra as shown in the equation below:

$$\text{Average absolute SD} = \frac{\sum_{i=1}^{n} \left| A_{ref_i} - A_{s_i} \right|}{n}$$

Where A_{ref_i} = absorbance values for the reference spectrum at point i

A_{s_i} = absorbance values for the sample spectrum at point i

n = number of data points in the spectrum range

Equation 7: Average absolute spectral distance calculation

- Maximum absolute SD - The maximum absolute SD value at any frequency across the spectra (termed Distance Score in some vendor software).

- Euclidean distance (ED) – the square root of the sum of the squared SD across the measurement range as shown in the equation overleaf:

$$ED = \sqrt{\sum_{i}^{n} \left(A_{ref_i} - A_{s_i}\right)^2}$$

Where A_{ref_i} = absorbance values for the reference spectrum at point i

A_{s_i} = absorbance values for the sample spectrum at point i

n = number of data points in the spectrum range

Equation 8: Euclidian distance calculation

The total and average SD or ED statistic is preferred for global spectral qualification over maximum SD as these statistics encompass the results across the entire spectral range.

For the above calculations, each wavelength is given equal weighting in the calculation and does not take into account the magnitude of the absorbance at the wavelength or whether a particular wavelength is expected to have greater variation. The result is that SD measurements will emphasise when a sample varies at a peak of stronger absorbance. Smaller peaks which may be of more interest in a particular analysis will have less impact on the results.

3.3.1.3 Normalised SD

Normalising the SD accounts for the different variance in the different variables (in this case wavelength) by dividing the SD at each wavelength by the standard deviation calculated at each point for the reference library. The four metrics in 3.3.1.2 can be calculated on the normalised SD values.

Some chemometrics software packages may term the display of normalised SD against wavelength the "Conformity Index Plot". This visual display can be useful when diagnosing differences or identifying wavelengths of significant variation.

3.3.1.4 Principal component analysis

NIR spectra have contributions from many different sources: the analyte of interest, the product matrix, instrument noise, environmental factors, and sample handling. NIR spectra also incorporate significant co-linearity where the analyte or product matrix absorbs at several places across the spectrum. Where the analyte absorption increases, matrix absorptions inversely decrease through dilution effects. This means that some of the variables (wavelength absorbance) can be written as approximate linear functions of others. Additionally, the NIR spectrum is composed of many overlapping bands of absorbance from each component in the matrix; therefore any given variable may have contributions from multiple components besides the analyte of interest.

Principal Component Analysis (PCA) solves this issue by combining variables that vary in a related way together into a principal component (PC). A reference library is used to establish the PCs. Each NIR spectrum is decomposed into a set of PCs (variation spectra or loadings) with a corresponding constant scaling factor (PC score). The first PC will account for the greatest sources of correlated spectral variation, with the subsequent PCs explaining lesser sources of variation. The set of PCs could be used to reconstruct the spectrum of a sample by multiplying each PC by the corresponding PC score and adding the results together until the new spectrum closely matches the original spectrum. Each sample would have a different set of PC scores since each sample will be at least subtly different. The portion of the spectrum not explained by the number of PCs chosen for the analysis (the spectral residual) is often assumed to be noise. However, it is best described as un-modelled variation.

$$\text{Spectrum} = \sum_{i=1}^{p} (PC_i \times Score_i) + RS$$

Where PC_i = principal component loading matrix for the ith principal component

$Score_i$ = score values for the ith principal component

p = number of principal components

RS = residual unexplained spectral variation matrix

Equation 9: Equation relating principal components to sample spectrum

PCA can be used to reduce noise from the spectra ('clean' the spectra), provide simplified visualisation of the NIR data and can also be utilised for classification analysis. Figure 10 shows a pictorial representation of PCA for an example with four sample spectra. Figure 11 illustrates the PC Scores plot for the example in Figure 10 showing the scores for the second PC versus the scores for the third PC to demonstrate visualisation and simple classification for a set of 12 samples.

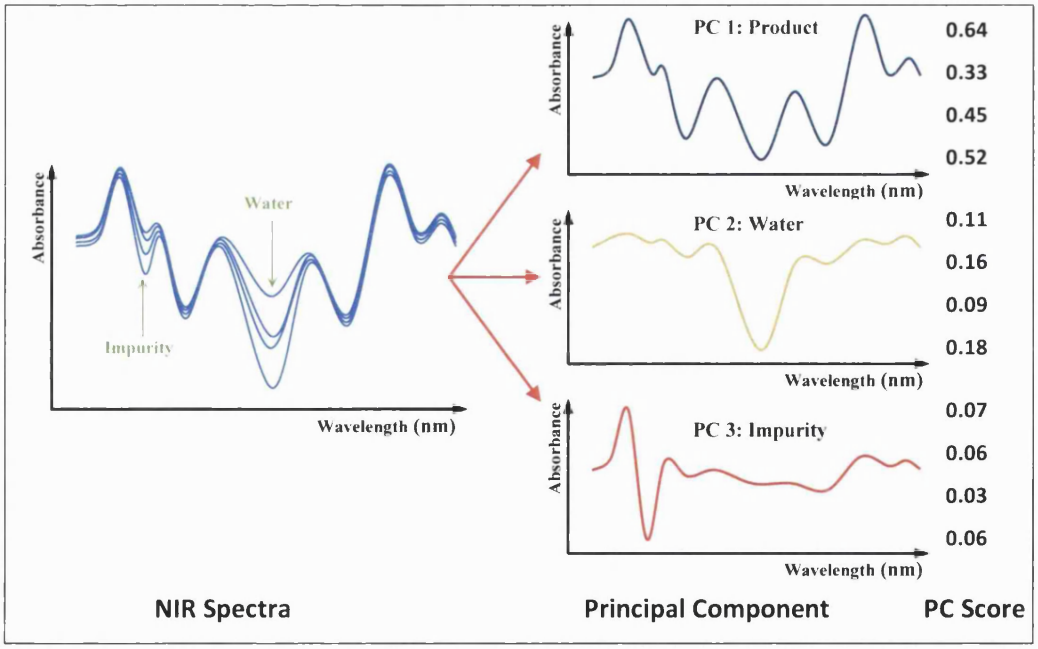

Figure 10: Representation of deconstruction of four NIR spectra into PCs and PC scores.

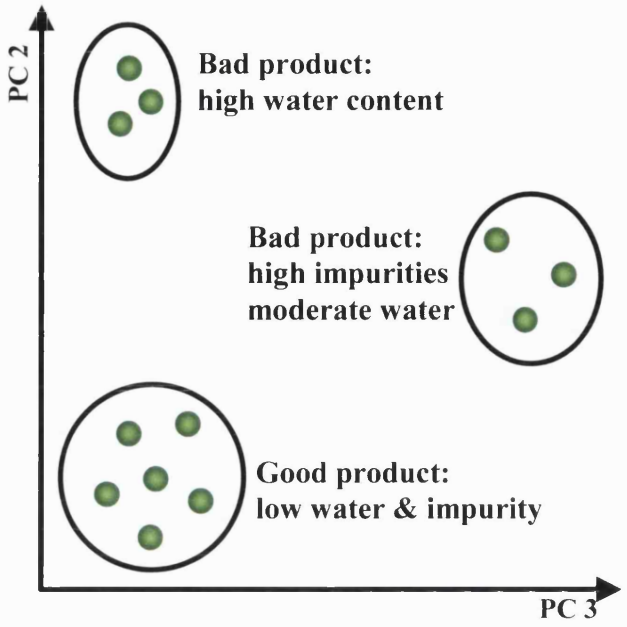

Figure 11: Representation of PC scores plot showing PC2 against PC3.

The simplest form of classification can be performed on one or more PCs through applying a threshold on acceptable score values. Alternatively, the ED and normalised ED can be calculated on the PC score variables. Note that the normalised ED in PC space is termed Mahalanobis distance (MD). The modified equations for ED in PC space and MD are shown in Equation 10 and Equation 11.

$$ED = \sqrt{\sum_{i}^{p} \left(Score_{s_i} - Score_{ref_i} \right)^2}$$

Where $Score_{ref_i}$ = score values for the mean reference spectrum for PC i

$Score_{s_i}$ = score values for the sample spectrum for PC i

p = number of principal components in the analysis

Equation 10: Euclidian distance calculation in PC Space

$$MD = \sqrt{\sum_{i}^{p} \left(\frac{Score_{s_i} - Score_{ref_i}}{S_i} \right)^2}$$

Where $Score_{ref_i}$ = score values for the mean reference spectrum for PC i

$Score_{s_i}$ = score values for the sample spectrum for PC i

p = number of principal components in the analysis

S_i = standard deviation of Score values for PC i for reference library

Equation 11: Mahalanobis distance calculation

When the PCA analysis describes one population, the centre of the population will be at the origin. Thus the equations for ED and MD are simplified and become a direct function of the PC scores (the term $Score_{refi}$ becomes zero). When PCA analysis describes multiple populations, ED and MD can be calculated between a sample spectrum and the center of each population. When a threshold is applied, the test sample can be classified as within one or more of the reference populations.

Typically MD is used in this application to take into account the variability in the population in a given PC.

3.3.2 Regression analysis

Regression algorithms provide a method of correlating spectral data to the analyte concentration of interest. Four types of regression analysis are widely available in NIR software packages. These are discussed briefly in this section in increasing order of complexity.

3.3.2.1 Classical least squares (linear) regression

In methods such as classical least squares (CLS) regression, a single point in the spectrum is correlated against the reference method to produce an equation that relates analyte concentration to NIR absorbance at that point (Beer-Lambert Law). CLS regression is widely utilised in analytical chemistry and is appropriate for NIR analysis of simple matrices such as solutions (e.g. Figure 12).

$$y = m_i\, x_i + b$$

Where y = analyte concentration
x_i = absorbance value at frequency i for the sample
m_i = regression coefficient (slope) at point i
b = residual unmodelled component

Equation 12: Equation describing linear regression

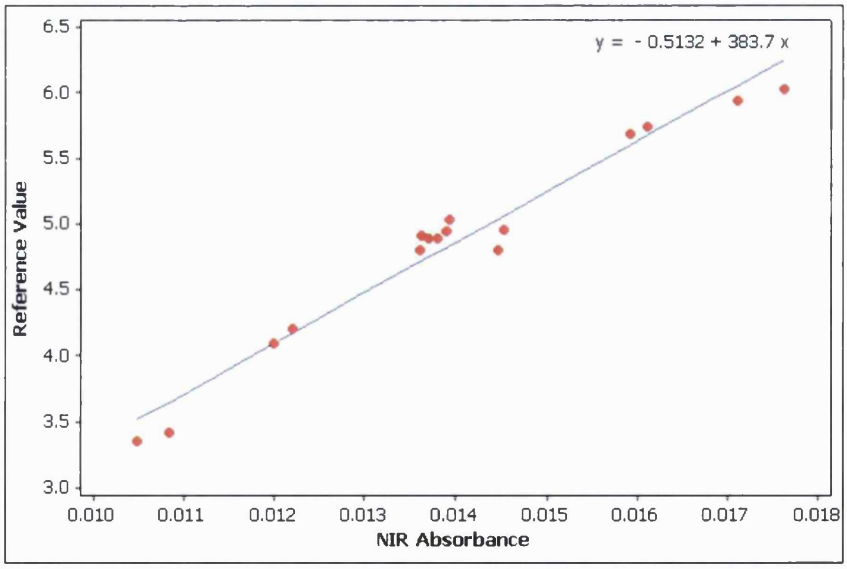

Figure 12: Example linear regression plot and associated regression equation

3.3.2.2 Multiple linear regression

Co-linearity and co-absorption as described in section 3.3.1.4 contribute interference when calibrating NIR spectral data. Multiple Linear Regression (MLR) is a variant on linear regression in which two or more points in the NIR spectrum are correlated against the reference method to produce an equation with less impact of interfering co-linearity and co-absorption. The primary wavelength should correspond to a known absorption for the analyte of interest. The additional (helping) terms are typically chosen to represent inverse correlations (for example matrix components that are diluted as the analyte concentration increases) and/or areas in the spectrum with absorbance features not related to the analyte of interest (for example physical effects such as particle size or moisture absorption). As such, MLR is a simple improvement on CLS to account for co-linearity and co-absorption. The MLR regression equation can be written:

$$y = m_i x_i + m_j x_j + b$$

Where y = analyte concentration
x_i = NIR absorbance at wavelength i
m_i = regression coefficient (slope) at point i
x_j = NIR absorbance at wavelength j
m_j = regression coefficient (slope) at point j
b = residual unmodelled component

Equation 13: Equation describing MLR for two terms

3.3.2.3 Principal Component Regression

Principal component regression (PCR) extends on MLR, however in place of the set of different linear variables (i.e. wavelengths), multiple PCs are used as regression terms. This is achieved in a two step process whereby a PCA is performed on the dataset to determine the PCs to include followed by regression of these variables against the reference values.

The selection of the PCs is solely based on the spectral variance in the data set and thus it is possible to include terms in the regression equation that explain spectral variations that have no relation to the analyte of interest and may lead to poor prediction performance and reduced specificity compared to other regression techniques. PCR is useful for simple matrices where spectral variation of the analyte of interest is dominant in the high order PCs and for such systems where wavelengths are highly correlated as the use of PCs take into account the underlying collinear relationships within the data. New samples are projected in the same PC space and scores calculated before prediction based on the established regression equation.

$$y = \sum_{i}^{p} m_i \, Score_{s_i} + b$$

Where y = analyte concentration
$Score_{s_i}$ = score values for the sample spectrum for PC i
p = number of principal components in the analysis
m_i = regression coefficient (slope) at PC i
b = residual unmodelled component

Equation 14: Equation describing principal component regression

3.3.2.4 Partial Least Squares Regression

Partial least squares (PLS) regression builds on PCR; however the relationship of the spectral variance to the analyte of interest is incorporated in the initial PCA. The analyte adjusted or (analyte weighted) PCs are often termed latent variables (LVs) rather than PCs to differentiate from pure spectral variance PCs. Once the LVs are determined, regression of these variables against the analyte reference values is performed.

$$y = \sum_{i}^{p} m_i \, Score_{s_i} + b$$

Where y = analyte concentration
$Score_{s_i}$ = score values for the sample spectrum for LV i
p = number of Latent Variables in the analysis
m_i = regression coefficient (slope) for LV i
b = residual unmodelled component)

Equation 15: Equation describing partial least squares regression

Note that some NIR vendor or chemometrics software now also provides PLS2 capabilities which allows for regression with multiple analytes of interest (multiple 'y's).

3.3.3 Discriminant analysis

Discriminant analysis (DA) is a modification of the regression techniques where the y values used in the regression are assigned to categorise populations in the data to predetermined classification groups. For DA with two classes, one class is assigned the value "1" (often applied to 'acceptable' samples) and the other "0" (often applied to denote 'unacceptable' samples).

All four regression techniques described in 3.3.2 can be utilised for DA with two classes. When there are more than two classes of interest CLS, MLR, PCR and PLS should be applied in series with one model created for each class. Additionally, NIR vendor or chemometrics software packages that provide PLS2 capabilities allow PLS2-DA to be used to create one model that incorporates all classes, with the multiple 'y's representing the different classes.

3.4 Statistics and statistical process control

Statistical techniques are used to assess the performance characteristics of the developed methods as well as to develop mechanisms for process monitoring and control.

3.4.1 Statistics used to assess method accuracy

The most common statistical parameters applied to chemometric regression methods to indicate method performance are correlation coefficient (r), the squared correlation coefficient (r^2) and the standard error (SE).

The correlation coefficient and the squared correlation coefficient are applied to chemometric regression methods to indicate the closeness of fit of a particular relationship (e.g. linear fit).

The correlation equation shown in Equation 5 can be applied directly to the predicted and reference values. This is reproduced and modified to represent the r^2 statistic in Equation 16.

$$r^2 = \frac{\left(\sum_{i=1}^{c}\left(y_{p_i} - \bar{y}_p\right)\cdot\left(y_{r_i} - \bar{y}_r\right)\right)^2}{\sum_{i=1}^{c}\left(y_{p_i} - \bar{y}_p\right)^2 \cdot \sum_{i=1}^{c}\left(y_{r_i} - \bar{y}_r\right)^2}$$

Where y_{p_i} = predicted value for case i

y_{r_i} = reference value for case i

$\bar{y}_p$ = mean predicted value

$\bar{y}_r$ = mean reference value

c = number of cases

Equation 16: Calculation of r^2 regression statistic

Standard error is applied to chemometric regression methods to indicate the method accuracy and relates the error in prediction compared to the established true value (typically referred to as the reference value).

SE can be calculated for the estimated values for the calibration sample set (termed SEC or SEE depending on software), cross validation results (termed SECV), prediction results of validation data sets (termed SEP) as well as laboratory reference results (SEL).

$$SE = \sqrt{\frac{\sum_{i=1}^{c}\left(y_{p_i} - y_{r_i}\right)^2}{c-1}}$$

Where y_{p_i} = predicted value for case i

y_{r_i} = reference value for case i

c = number of cases

Equation 17: Equation for calculation of Standard Error

Note that some vendor software will utilise c rather than c-1 as the denominator in the SE calculation. For data sets with large sample size (e.g. over 30 samples) which is typical for chemometric regression methods, the impact of this calculation variation is negligible. However, it is important to check the calculation used before comparing values across different vendor software.

3.4.2 Student's– *t*-test – a test for outliers and equivalence of means

The one sample *t*-test is used to determine whether a value is statistically different from the rest of the values in a univariate data set and can be used as an outlier test. A paired *t*-test is used to demonstrate whether a subset of results (sample population) belongs to the global population and is often used for regression performance assessment to demonstrate equivalence between the output of the NIR prediction and a reference method. In both cases, the *t*-test is performed by calculating the t_{crit} and determining the probability that the t_{crit} falls within the Student's t distribution (given a particular confidence – typically 95% confidence).

$$t_{crit} = \frac{|\bar{y} - y_i|}{\frac{(S_y)}{\sqrt{c}}}$$

Where $\bar{y}$ = mean of individual values
y_i = individual value for case i
c = number of cases

Equation 18: Equation for calculation of t_{crit} for one sample *t*-test

$$t_{crit} = \frac{\left|\overline{y_D} - y_{D_i}\right|}{\frac{(S_D)}{\sqrt{c}}}$$

Where $\overline{y_D}$ = mean of $(y_{p_i} - y_{r_i})$ for c

$y_{D_i} = (y_{p_i} - y_{r_i})$ for case i

S_D = standard deviation of $(y_{p_i} - y_{r_i})$ for c

y_{p_i} = predicted value for case i

y_{r_i} = reference value for case i

c = number of cases

Equation 19: Equation for calculation of t_{crit} for Paired *t*-test

3.4.3 F-test and ANOVA – analysis (equivalence) of variance

The F-test is used to determine if the variation in two univariate data sets are equivalent. the F-test is performed by calculating the f_{crit} and determining the probability that the f_{crit} falls within the F-distribution (given the degrees of freedom (n - 1) and a particular confidence – typically 95%).

ANOVA (Analysis of Variance) extends the F-test by taking into account variance contributions from groupings within the data sets and is applied to assess the significance of variation within and between data sets. It is a multivariate F-test used on a small number of variables. The most common uses of ANOVA are intermediate precision assessments and inter-laboratory studies where contributions to the method variance due to different measurement conditions (different analyst, different day, different measurement process or different instrument) are assessed.

$$f_{crit} = \frac{(S_{c_1})^2}{(S_{c_2})^2}$$

Where S_{c_1} = standard deviation of values for first condition

S_{c_2} = standard deviation of values for second condition

Equation 20: Equation for calculation of f_{crit} for an F-test or ANOVA

3.4.4 Multivariate quality statistics – Hotelling's T^2 and Q residual statistic

Hotelling's T^2 and Q residual statistics are used to assess whether a value is statistically different from the rest of the population in a multivariate data set. The T^2 value is synonymous to a Student's *t*-test in multidimensional space rather than univariate space and can be applied as a multivariate outlier test. The T^2 statistic assesses how close to the population mean an individual sample (or the mean sample of a second sample population) lies by comparing the vectors in relationship to the covariance matrix for the multiple variables modelled in the multivariate data set.

The Q residual assesses the component of the sample measurement (or the mean sample of a second sample population) that is not explained by the multiple variables modelled in the multivariate data set.

As with the univariate Students *t*-test, the T^2 and Q residual statistics are calculated and the probability that the values fall within the expected distribution (given a particular confidence – typically 95% confidence) is determined. Vendor software and multivariate and PAT texts contain details of the equations.[29, 133, 134]

3.4.5 Normality

Many statistical techniques (e.g. Student's *t*-test) are based on the assumption that data are randomly distributed about the mean value with the frequency distribution forming a 'normal' bell shaped curve. When such a state exists, the data are said to have a normal or Gaussian distribution. Once normality is established, the data can be assessed based on established characteristics of the normal distribution (e.g. 68%, 95% and 99.7% of data will fall within ±1, ±2 and ±3 standard deviations of the mean respectively).

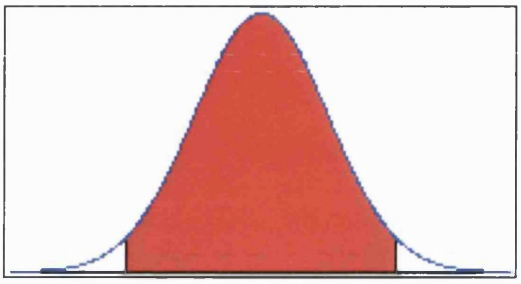

Figure 13: Normality distribution curve with shading representing 95% of the data (within two standard deviations from the mean)

Various tests exist for assessing normality (e.g. Anderson-Darling, Shapiro-Wilk test)[135, 136] mainly based on either correlation of the sample population distribution to that of a Gaussian distribution or comparison of the cumulative empirical distribution function. Different tests have different power or sensitivity to non normal behaviour such as kurtosis or skewness and different capability to assess different sample sizes. There is much speculation as to the best technique to use with methods based on correlation said to be more powerful with limited sample number compared to empirical cumulative distribution function tests.[135] The choice is often governed by the test availability within statistical software. In all cases, a metric is calculated to assess the probability that the values fall within the expected normal distribution (given a particular confidence – typically 95% confidence).

When data do not follow a normal distribution, the data may be mathematically converted (e.g. through logarithmic or polynomial transforms) to a normal distribution so that standard statistical techniques may be performed.

3.4.6 Statistical process control

Statistical process control (SPC) charts were introduced by Shewhart[137] in the 1930's as a means to provide a tool to monitor and control variation in industrial processes. The basis of the approach is that a highly capable and controlled process should follow a normal distribution and deviations from this normal distribution are caused by systematic noise and/or special cause events causing shifts or increased variance. The use of a graphical control chart aids in the identification of deviations from normal Gaussian behaviour and provides opportunity for process quality improvement.

The typical SPC charts take the form of data displayed in time sequenced order across the x-axis with the measured values plotted on the y-axis centred about the process mean and with control lines set at the appropriate confidence interval (most often ±3 standard deviations of the mean).

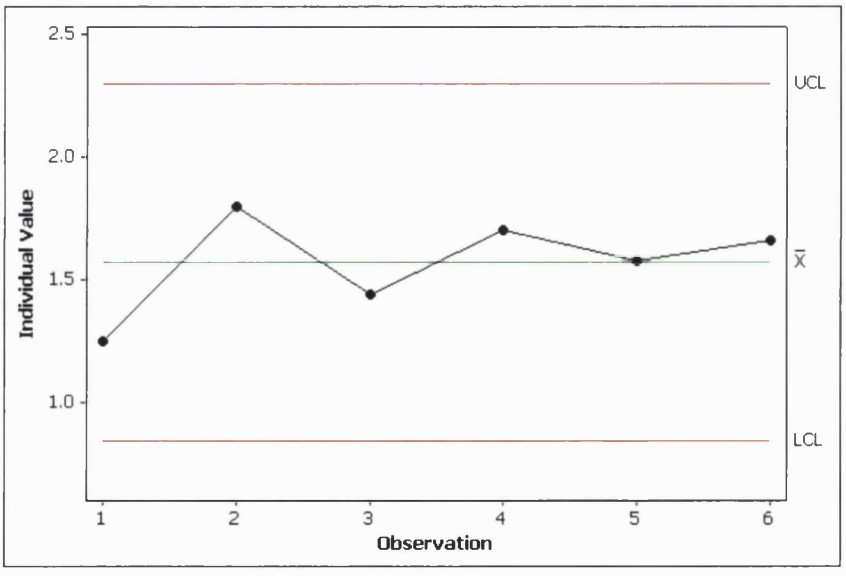

Figure 14: Example of a Shewhart SPC chart with upper and lower control limits (___) shown at three standard deviations from the mean (___)

In modern SPC software, the identification of deviations from statistically expected behaviour is automated, with the software interrogating the data to identify systematic and special cause effects underlying the randomly distributed data. Patterns such as consecutive runs, oscillations, shifts and other trends will be flagged automatically for the reviewer once parameters are set for such pattern recognition. In some cases, control limits are of less interest than the presence of unexpected patterns and some SPC charts will not display control limits (e.g. Run charts in Minitab statistics software).

The typical Shewhart SPC chart assumes that the data set is of one population, data is of a continuous nature (not categorical or truncated) and that the population is of normal Gaussian distribution. If these assumptions are not met, tests for probability and statistical significance will lead to incorrect conclusions, although there may still be merit in the visualisation of the data and the identification of trends. Alternatively, data can be mathematically transformed to meet the normality assumption or non-parametric tests and charts can be used (beyond the scope of this discussion).

3.4.7 Multivariate statistical process control

SPC is historically performed in a univariate manner, with one chart per measured variable. When variables are inter-related, this may lead to incorrect assumptions of control and unexpected poor quality outcomes or incorrect presumptions of control (e.g. individual SPC charts for human height and age, which are clearly related variables, may not identify the special cause of a person with a growth hormone disorder).

For systems with correlated variables, multivariate SPC (MSPC) should be applied.[138] The most common MSPC charts are based on Hotelling's T^2 and Q statistics with the upper and lower control limits based on the desired confidence interval.

Unlike conventional SPC charts, significant understanding is needed of the variables that are modelled in the underlying multivariate space when a result is identified as not conforming to the established control limits. As yet, MSPC is not widely deployed in the pharmaceutical industry for process control. MSPC charts are more often employed for graphically representing the performance of a given static multivariate analysis event (e.g. a specific PCA analysis[79]) , model suitability and optimisation[139] or for model monitoring[140] /verification before applying a quantitative model (verifying that a new sample is within the model space and within quantitative method scope). As such this may be considered a multivariate model statistic chart rather than true MSPC where the intention is to provide a mechanism for monitoring and control. Few examples have been published to date of MSPC applied to pharmaceutical manufacturing to enable monitoring and control.[18, 80, 141]

3.5 Review of software capability for NIRS PAT applications

Within a pharmaceutical manufacturing operation, it is typical to employ vendor software to control PAT analyzers assuming the software is Good Manufacturing Practice (GMP) compliant. As such, method development and routine operation of NIR equipment for PAT applications rely on functionality within vendor software. Often vendor software is sadly lacking, with only a small selection of the various chemometric and statistical techniques described in Section 3.3 and Section 3.4 available within any given vendor offering.

In some cases, advanced chemometric functionality is available within development modules of the software (which proves useful during investigation, data mining and method development). However, the same chemometric algorithms are then not available in a routine operation mode. In an effort to provide easy user interface and

reduce human error, some vendor software has reverted to a 'black box' where a user with no chemometric knowledge can follow a wizard and develop and implement a chemometric model. This may be enough when developing simple identification methods or traditional quantitative methods for samples with simple matrices. However, such lack of routine functionality inhibits the deployment of new and diverse applications which will be required to deliver the software capability to support PAT applications of NIRS and also prevents the use of the chemometrics tool to derive deep process understanding.

As part of this research, this author worked with one vendor to attempt to extend the chemometric and statistical functionality to the user interface. This effort was ultimately unsuccessful with functionality added in off-line analysis mode but not easily operated in real time by production operators or requiring significant investment (e.g. through the use of Microsoft SQL server database or macros that are difficult to validate to GMP requirements). It was felt that the inability to deliver the desired improvements may point to a lack of understanding of pharmaceutical specific requirements for software and data by vendors traditionally focused on other industries. There was limited uptake of the added functionality by Pfizer manufacturing sites, which was in turn misinterpreted by the vendor as lack of widespread interest in the functionality across Pfizer. The vendor was reluctant to invest further effort on increasing functionality until there was significant industry pressure that would translate to sufficient customers willing to pay to upgrade functionality. Despite Pfizer being a significant customer, the vendor felt the increased functionality was sufficient for the industry interest. This was not felt to be vendor specific, rather it is the sentiment from many vendors, and prevents innovative pursuit of improved chemometrics and statistics for PAT applications.

Cross industry effort pursued the concept of a common PAT software platform that can be deployed across all PAT applications within a factory to manage the operation of analysers, application of process models and management of PAT data.[142] Several vendors now have such software available (e.g. SiPAT from Siemens). However, despite this effort, implementation of common PAT software platforms has been found to be largely cost prohibitive in the industry setting for established products and facilities.

The outcome is that the application of PAT based applications of NIRS involves a compromise in what software functionality is available combined with willingness to utilise multiple software to perform advanced chemometrics and SPC within the GMP controlled environment.

The impact of software capability will be further discussed with regard to the specific research described in Chapter 4 and Chapter 5.

CHAPTER 4 APPLICATION OF AT-LINE NIRS FOR PAT

APPLICATIONS IN SOLID DOSAGE PHARMACEUTICAL PRODUCTION – MATERIAL ANALYSIS

4.1 Introduction

The first of the identified areas of research was the investigation of novel ways to apply at-line NIRS for PAT applications in the pharmaceutical industry for material analysis.

As discussed in section 2.5.1, NIRS has been widely employed for material identification and various qualification techniques have also been developed. Typically, material qualification by NIRS employs chemometric classification techniques to assess whether a particular sample is consistent with acceptable material (defined in a reference library). Within the pharmaceutical industry it is not common to utilise adaptive chemometrics techniques such as neural networks (where each sample analysed is added to a continuously growing reference library) due to real or perceived regulatory hurdles of method validation. Instead, material qualification methods compare new samples to a static reference library established when the method was developed with little consideration to the linkage of the reference spectra to changing process behaviour. Additionally, the continuous nature of the chemometrics data output is largely ignored once the classification is determined. It is challenging to derive any process information from a simple categorical classification. For material analysis to be a true PAT application, it is necessary to begin assessing the relationship between observed material quality and the effect on product quality or the effect on process behaviour. NIR material conformance is a term coined for this purpose to incorporate the material qualification chemometric output with SPC (through historical trending) to

provide a means to gain greater understanding and a measure of process behaviour prediction (aligned with PAT philosophy).

Section 4.2 explores the identified gap of the application of NIRS for assessing global quality (whole spectra) of materials by trending the continuous chemometric output against historical data to gain process understanding and predict process behaviour of the material in the forward process.

Once process understanding is established, a particular material attribute may be identified as critical to the process or product quality. Section 4.3 reviews the application of the NIR material conformance approach to a target material attribute rather than the entire spectrum of a sample. The material attribute of interest may be traditionally tested to define material quality in QC laboratories or may be a material attribute specific to forward processing and as such provide additional process insight.

This research demonstrates the ability of rapid NIR analysis of materials through NIR Material Conformance approach to assess global quality and critical material attributes that impact product quality and process effectiveness.

4.2 Global material quality conformance

The development and application of a global material quality conformance method is described in this section demonstrating the value of this research. Amlodipine besylate (structure shown in Figure 15) was selected as the target material as it was the API in Norvasc® tablets, the highest volume and highest value product for the manufacturing facility in which the work was undertaken. Prior to this research, NIR material identification was implemented at the site. As such, spectral data for each delivery (and each container within the delivery) was available to undertake the work without additional NIR analysis.

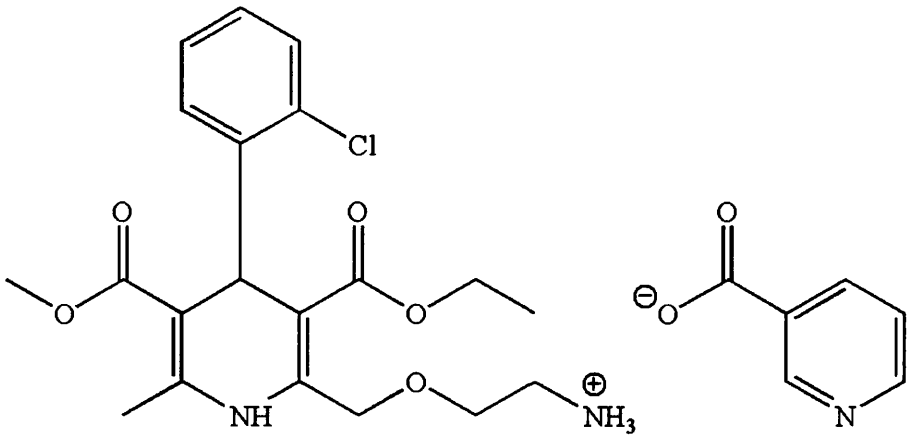

Figure 15: Chemical structure of amlodipine besylate

4.2.1 Materials and methods

4.2.1.1 Design of analysis

A reference library was needed to provide flexibility in which qualification algorithms were investigated. A minimum number of five deliveries were required to develop a robust reference library. To ensure a suitable degree of natural variability of the material was represented in the reference library, additional deliveries were obtained and a subset of these samples chosen to be included in the reference library. To avoid spectral redundancy, single spectra for each delivery were utilised for the reference library (spectra from the first container of each delivery). Various algorithms were explored to select the most appropriate modelling approach for the global quality conformance method.

To ensure robust development of the SPC approach, it was determined that a minimum of five deliveries independent of the reference library would be required. Individual spectra for each container in the deliveries were required to develop the SPC approach so that it was applicable to the real life scenario of assessing the conformance of every container in a delivery. This was important as the dosage strength of the amlodipine besylate in the Norvasc® finished product is low (approximately 3.45% weight / weight

basis) and only one to two containers of API are utilised in any given batch of Norvasc® tablets, hence individual container conformance was essential.

4.2.1.2 Samples

One representative spectrum from each of nine deliveries of amlodipine besylate ((R,S)3-ethyl-5-methyl-2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate besylate) were reviewed and six spectra were selected to establish the reference library. Seven further deliveries were utilised to establish (five deliveries) and verify (two deliveries) the SPC charts (varying number of spectra per delivery based on the number of containers delivered). A selection of six subsequent deliveries of the material was then reviewed and the suitability of the approach demonstrated as two deliveries were found to show non-conformance.

All samples were prepared for NIRS analysis by placing the material in SUN Sri 4 mL borosilicate glass shell vials (part number: 500 070) to a fill depth of 1 cm and compressing with uniform pressure using a stainless steel weighted cylinder (1 cm diameter and 5 cm length).

4.2.1.3 NIR apparatus and software

NIR reflectance spectra were measured using a FOSS NIRSystems 6500 Series II spectrophotometer (FOSS NIRSystems Inc., Silver Spring, MD, USA) configured with vial module with Si (400-1098 nm) and PbS (1100-2498 nm) detectors. The spectrophotometer was controlled by DeLight software, version 2.3b and D2NIRS software, version 1.2a (DSquared Development, La-grande, OR, USA).

NIR spectra were measured for each vial of material over the wavelength range of 400-2498 nm at 2 nm intervals. Each recorded spectrum was the average of 32

individual scans (a total of 35 s scan time per vial) and recorded with respect to a Spectralon® reference (LabSphere Inc, North Sutton, NH, USA). The analyses were conducted at typical laboratory temperature/ humidity environments of 20 to 25 °C / 60% relative humidity using the FOSS vial module aperture plate and holder.

DeLight version 2.3b with DMentia 1.1b software (DSquared Development, La-grande, OR, USA) and Microsoft® Excel, version 9 (Microsoft® Corporation) were utilised for chemometric model development and predictions, while Minitab® 16 version.1.16 (Minitab Inc, State College, PA, USA) was utilised for statistical evaluation and SPC charts.

4.2.2 Global material quality conformance method development

4.2.2.1 Spectra pre-treatment

It is Pfizer general practice to apply the gentlest spectral pre-processing to remove noise and reduce specular reflection without masking or hiding spectral features that may be useful in the qualification of the material or introducing artefacts not related to the sample analysed. For the measurement system utilised, five point smoothing is the minimum smoothing option applicable. SNV is also the Pfizer preferred normalisation treatment. Figure 16 demonstrates the effect of the selected pre-treatments, while each pre-treatment and the corresponding effects are described in Table 1.

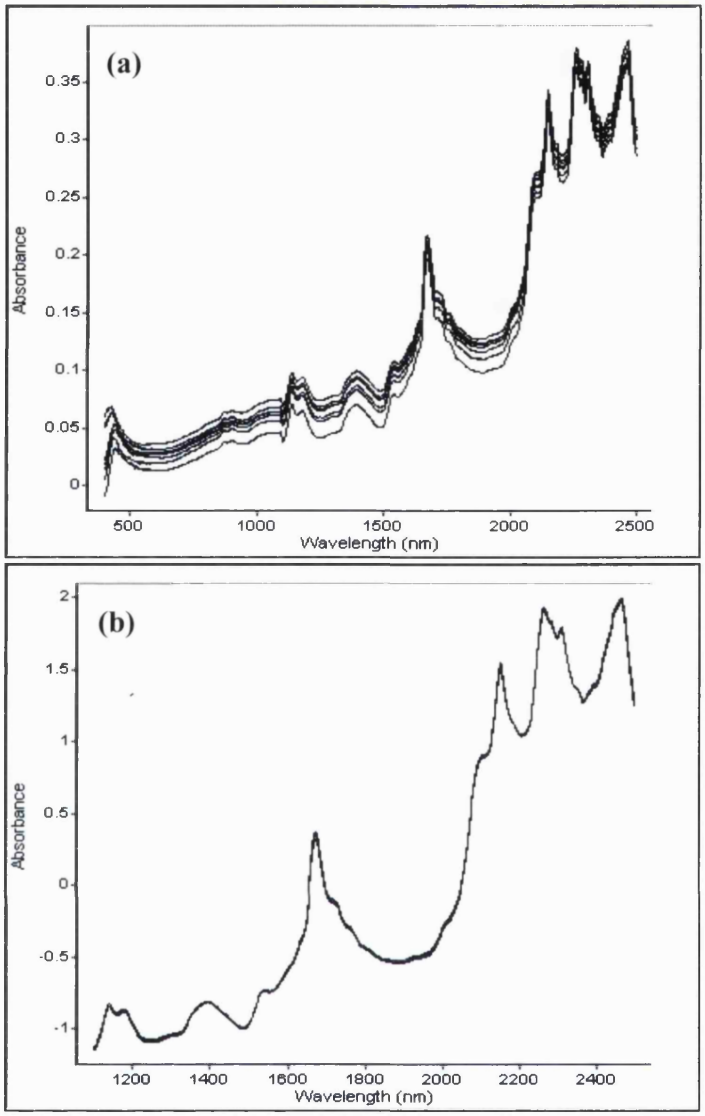

Figure 16: Raw NIR spectra of ten amlodipine besylate deliveries across; (a) full wavelength range and (b) reduced wavelength range with five point smoothing and SNV baseline correction

Table 1: Optimisation of data pre-processing treatments

Extract 1100–2498 nm	Remove visual region and focus on region available from PbS detector to prevent impact from detector switch over.
Five point smoothing	Provide continuous curve through the data points
SNV normalisation	Remove baseline, path length & multiplicative scatter effects

Derivatives are commonly applied to further remove slope effects and enhance spectral peak features. The derivative and gap (number of data points) recommended by FOSS NIRSystems Inc for material analysis is 2nd derivative with 10 points. To assess the impact on spectral features, the 2nd derivative spectra using five point and 10 point gaps were compared. Figure 17 shows the spectra of the two treatments and demonstrates that the ten point gap derivative treatment gives acceptable reduction of noise and simplifies the spectra (e.g. no unresolved saddle peaks) without loss of valuable spectral features.

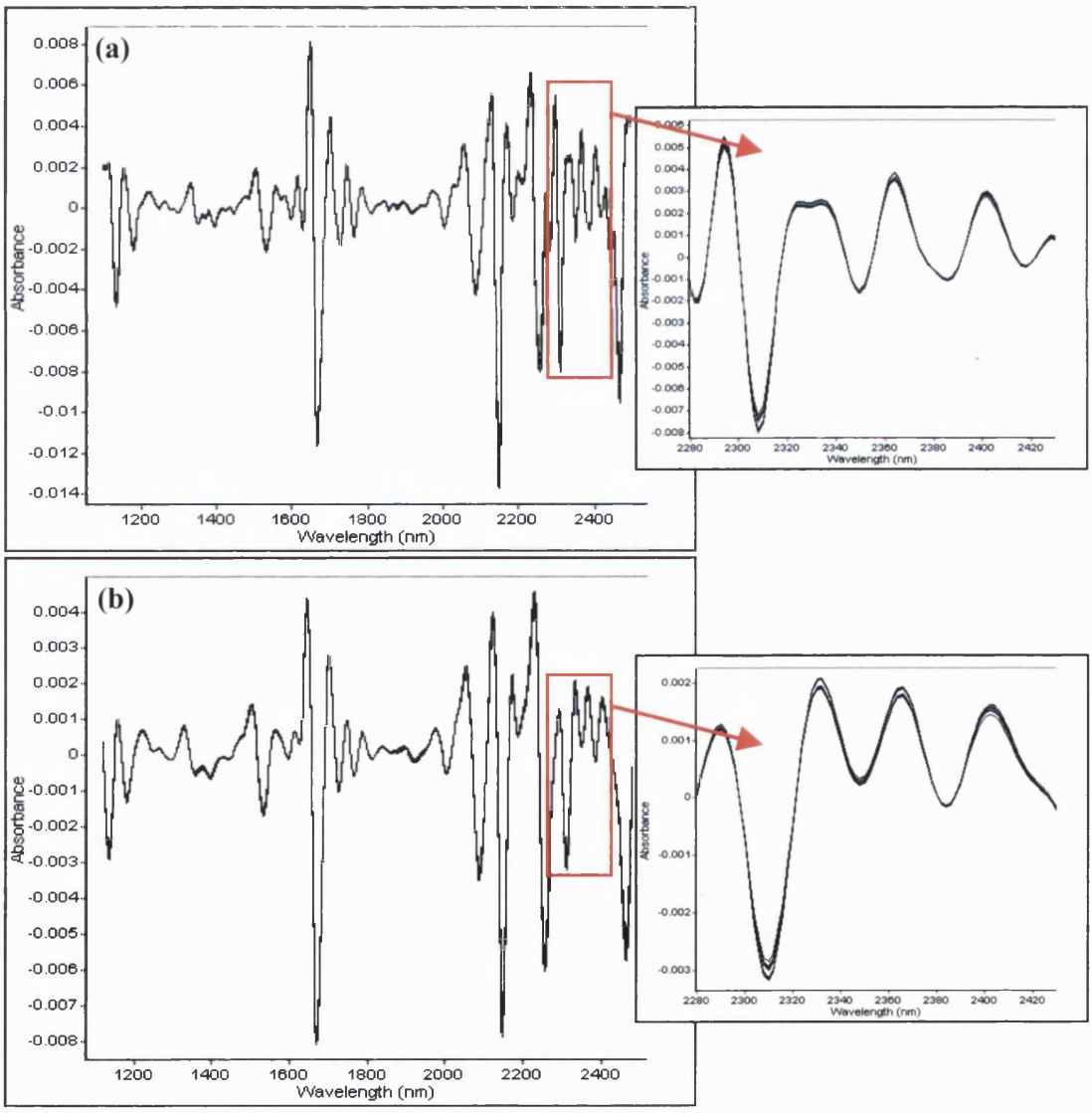

Figure 17: Full range and zoomed 2nd derivative spectra of amlodipine besylate with (a) five point derivative gap and (b) ten point derivative gap

4.2.2.2 Model development

PCA was used in addition to visual inspection of the pre-treated data to select the six deliveries that represented the range of natural variation in amlodipine besylate. Spectra from the deliveries at the extremes of the PCA scores plots were chosen for the reference library. Two deliveries were removed, one that was centred on the PCA plot (hence contributing redundant variation) and the other which was located near another point in the first two PCs (contributing similar variation to the nearby point). On closer review it was found that these two points were sequential deliveries received at the manufacturing site and were from the same material supplier manufacturing lot.

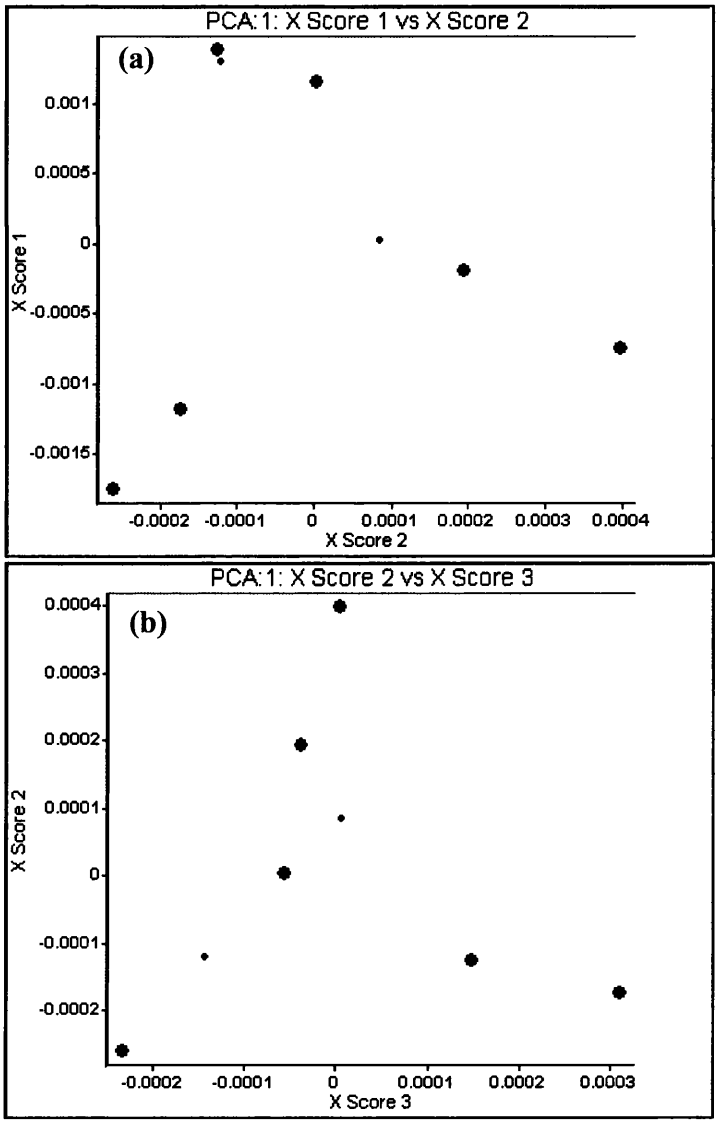

Figure 18: PCA scores plot for the eight deliveries for (a) PCs 1 & 2 and (b) PCs 2 & 3 where bold points are those assigned to the reference library

Global material quality conformance can best be achieved with chemometrics techniques that assess the entire NIR spectrum. Three such techniques were assessed in parallel: correlation, average absolute normalised SD (termed ANSD hereafter), and PCA-MD (refer to Section 3.3.1 for a description of these techniques).

Correlation, normalised SD and PCA-MD models were developed in DMentia software using default thresholds of 0.99, 3 and 3 respectively and three PCs for the PCA-MD model (explaining 88.6% of variation). The models were then applied in DeLight software to predict correlation, normalised SD and PCA-MD results for the reference library. ANSD values were then calculated from the normalised absolute SD value across the full wavelength range. Table 2 shows the prediction results.

Table 2: Predicted global material quality conformance statistics for each chemometric technique

	Correlation	ANSD (Absorbance)	PCA-MD
Reference library 1	0.99998	0.51015	1.9708
Reference library 2	0.99998	0.64204	1.2095
Reference library 3	0.99994	0.84962	1.4621
Reference library 4	0.99995	0.51577	1.6842
Reference library 5	0.99999	0.23911	0.79455
Reference library 6	0.99996	0.71290	2.0119

The reference library data were analysed in Minitab and charts of the individual values (I-charts) generated to represent the data graphically. These charts are shown in Figure 19 with the limits set at three standard deviations (___) from the mean value (___). The I-chart for correlation uses an upper boundary limit of 1.0 and the ANSD a lower boundary limit of 0.0 as the calculated control limits were beyond the allowed values for correlation (1.0) and ANSD (0.0).

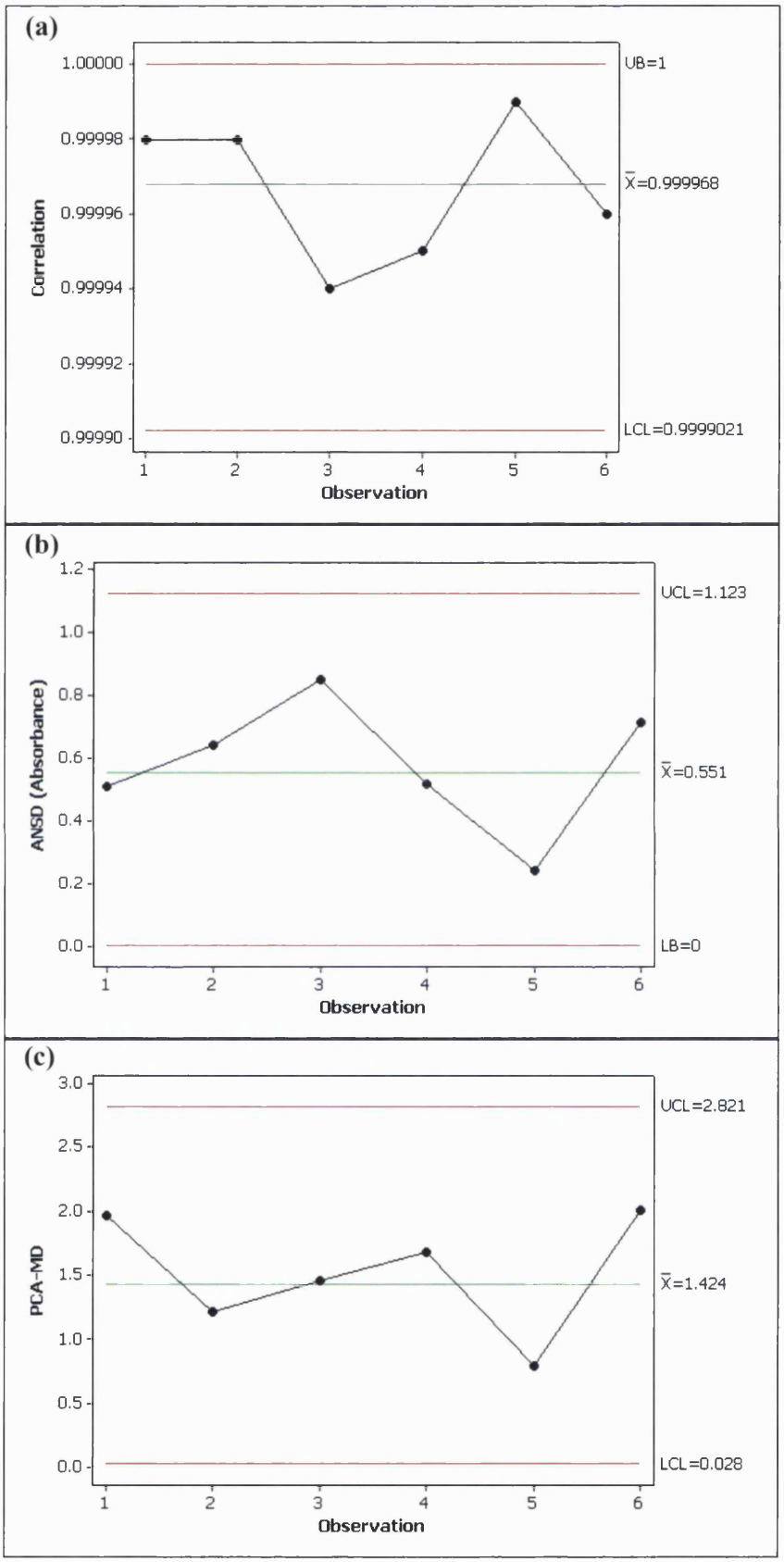

Figure 19: I-chart of conformance prediction results for the reference library: (a) correlation, (b) ANSD and (c) PCA-MD

4.2.2.3 Establishment of the conformance SPC charts

Once the conformance models have been created, SPC charts must be established that will monitor results over time. This ensures that, though the metric is calculated against a static model, deliveries can be compared to historically acceptable deliveries over time and trends towards non-conformance can be identified prior to failure against the static model. Five deliveries of varying number of containers were used to establish the SPC charts with a further two deliveries used to verify the SPC chart before implementation into the manufacturing environment. Though future deliveries may have any number of containers; it must be assumed that the historical deliveries are representative of both the typical number of containers as well as the typical variation within the delivery. As individual and/or combinations of containers may be used in production, both an individual container historical plot as well as an overall delivery summary historical plot is valuable.

An individual value SPC chart (I-chart) is used to represent the typical quality of individual containers in a given delivery with the control limits set by the individual results from the five historical deliveries. The individual container SPC chart also graphically represents the variability within containers of a delivery. As delivery acceptance decisions are based on the overall delivery result, a SPC chart for overall delivery average data was needed. An X-bar chart in Minitab was considered to be the ideal control chart to represent the overall quality of a delivery. However, the X-bar chart function in Minitab adjusts the control limits based on the sample size of the subgroups leading to a confusing control chart for operators to interpret when the number of containers may change for each delivery. For ease of implementation, average results were calculated for each delivery from conformance metric outputs for each container and an overall delivery control chart developed using the I-chart function on the averaged values.

Shewhart[137] control charts are based on the normal gaussian distribution and it is important to verify that the conformance data are normally distributed prior to developing the SPC charts. The Graphical Summary function in Minitab was used to represent the data and assess normality for each conformance metric (Appendix 1 on page 293). No evidence of non-normality was observed (p>0.05) at the 95% confidence level. Normality p-values for each set of data are shown in Table 3. Note that output for overall delivery metrics are an estimate only as the number of data points is small, reducing the power of the normality tests.[135]

Table 3: Summary of the normality assessment (p-value) of the global material quality conformance prediction results for the historical data set

	Correlation	ANSD	PCA-MD
Individual Container	0.335	0.420	0.573
Overall Delivery	0.696	0.572	0.847

The SPC charts for the three metrics studied are shown in Figure 20 and Figure 21. These SPC charts show both the five historical deliveries used to establish the control chart limits as well as the two verification deliveries. The mean and standard deviations applied in establishing the control charts are shown in Table 4 on page 116. Control limits (___) were established at three standard deviations from the mean (___), except for correlation individual and overall delivery charts which used an upper boundary of 1.0 and the PCA-MD individual and overall delivery charts which used a lower boundary of 0.0. Boundary limits were used in these cases as the control limits calculated were beyond the allowed values for correlation (1.0) and PCA-MD (0.0).

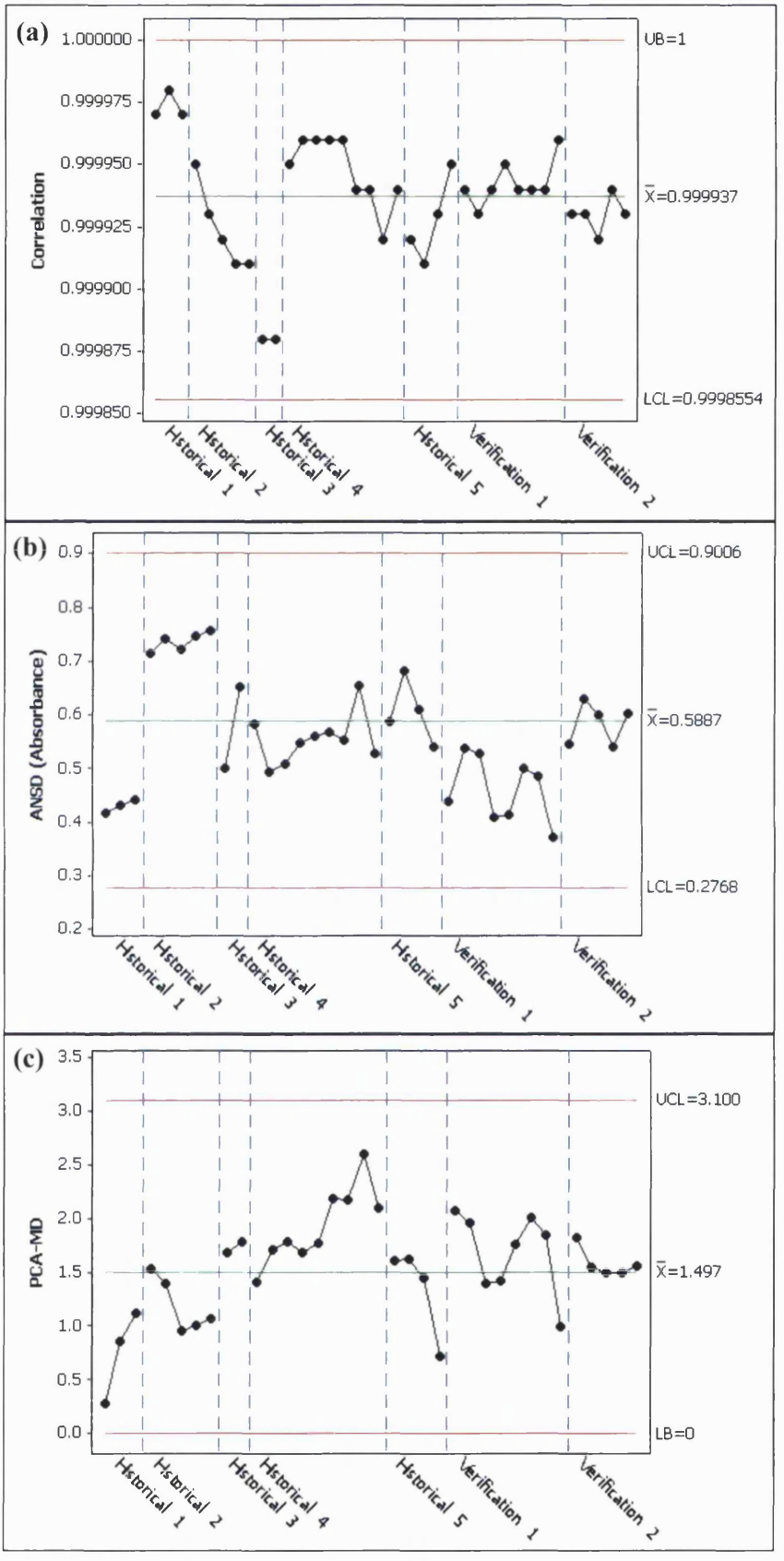

Figure 20: Individual container SPC charts for historical and verification deliveries: (a) correlation, (b) ANSD and (c) PCA-MD

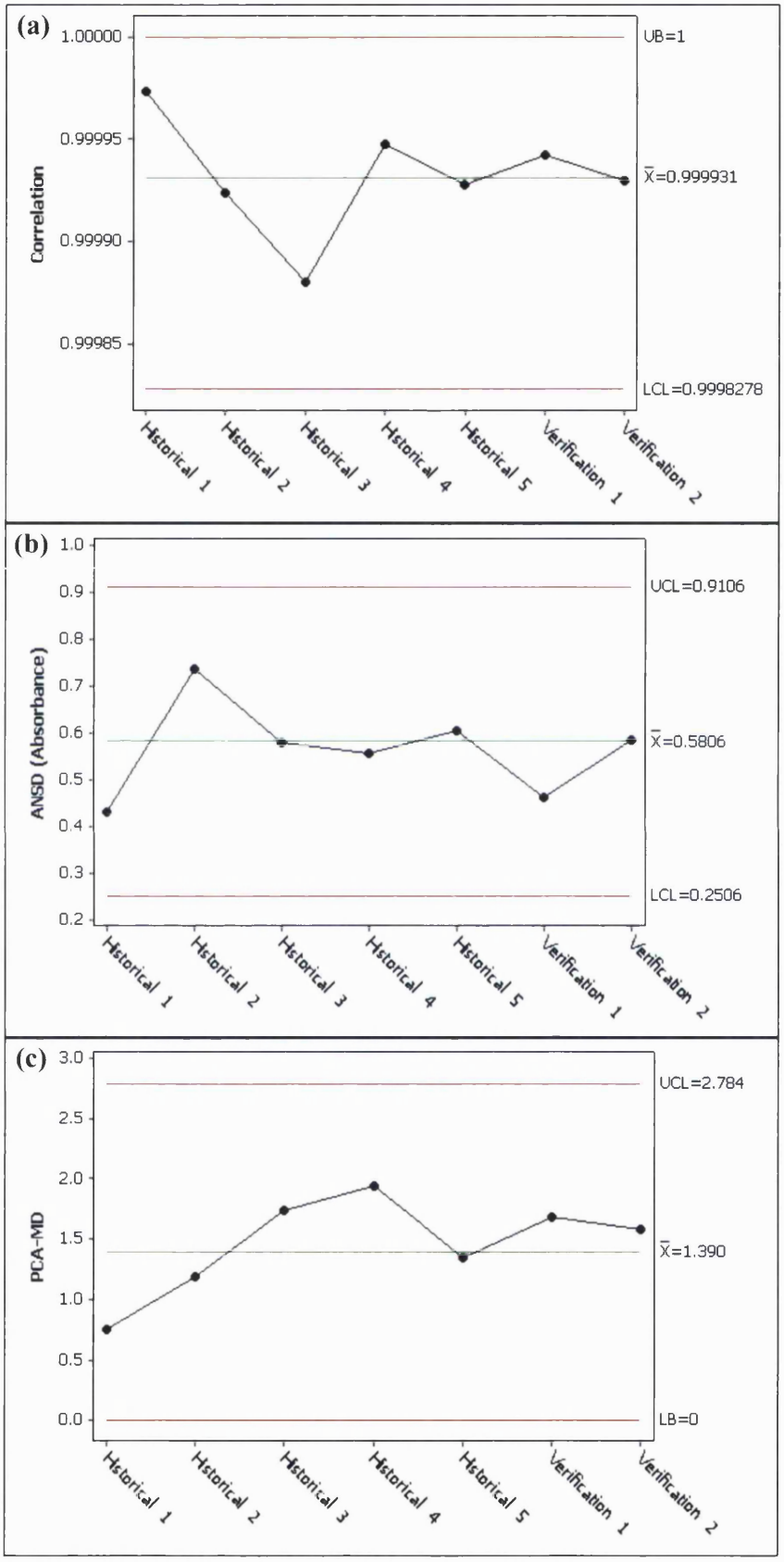

Figure 21: Overall delivery SPC charts for historical and verification deliveries: (a) correlation, (b) ANSD and (c) PCA-MD

Table 4: Parameters used to generate historical SPC charts for global material quality conformance

		Correlation	ANSD (Absorbance)	PCA-MD
Mean	Individual Container	0.99994	0.58873	1.4973
	Overall Delivery	0.99993	0.58061	1.3902
Standard Deviation	Individual Container	0.0000272	0.10397	0.53436
	Overall Delivery	0.0000344	0.11002	0.46461

4.2.2.4 Implementation of the conformance method in manufacturing

The developed global material quality conformance methods were applied to a selection of subsequent deliveries of amlodipine besylate (including two deliveries with non-conformance) to demonstrate the suitability of the approach. The resulting SPC charts for these deliveries are shown in Figure 22 and Figure 23 with non-conforming points shown in red.

The first delivery is identified as out of conformance in the correlation and ANSD SPC charts while the fifth delivery is identified as out of conformance on all three SPC charts.

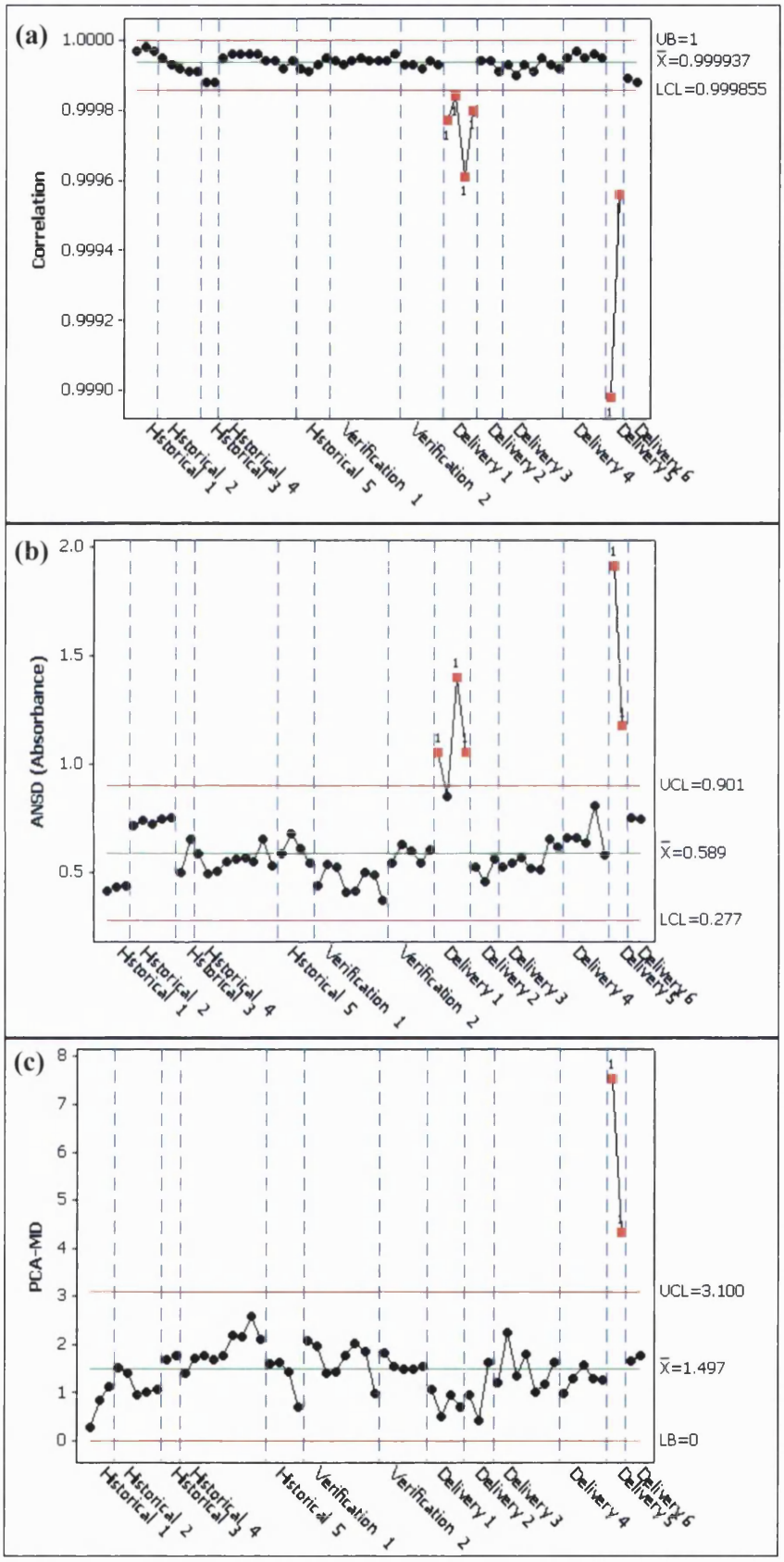

Figure 22: Individual container SPC charts for historical, verification and subsequent deliveries: (a) correlation, (b) ANSD and (c) PCA-MD with non-conforming containers in red

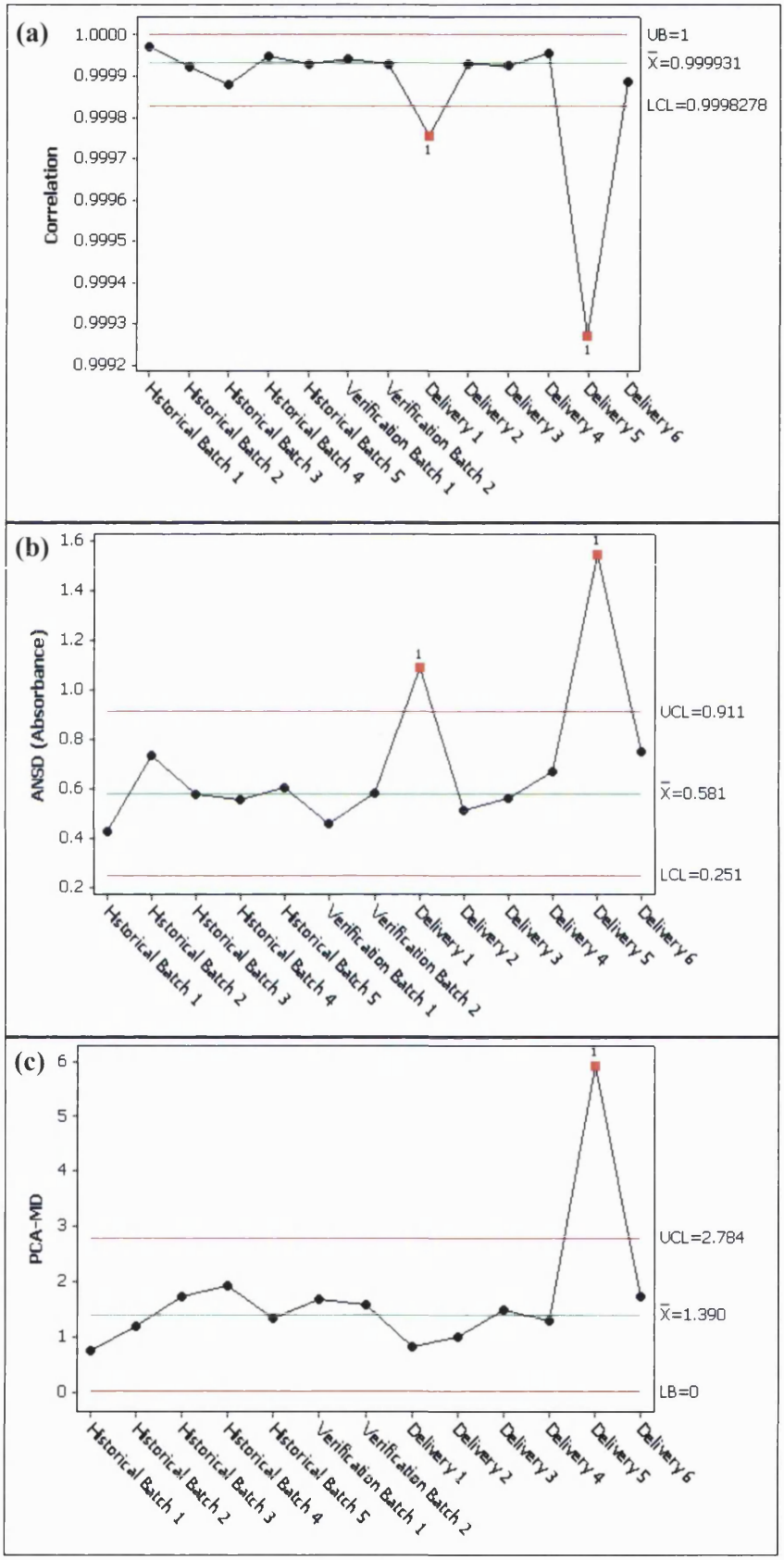

Figure 23: Overall delivery SPC charts for historical, verification and subsequent deliveries: (a) correlation, (b) ANSD and (c) PCA-MD with non-conforming deliveries in red

Correlation methods are based on similarity of the test spectrum to the mean spectrum of the reference library without taking into account variation within the reference library about the mean spectrum. The ANSD method does take into account the variability in the reference library across the wavelength range and reduces overweighting of the result to wavelengths of high natural variation. PCA-MD also accounts for variance of acceptable deliveries in the reference library and also reduces the impact of variation not identified as the major sources in the PCs included in the algorithm.

The fact that PCA-MD found the first delivery to be in conformance while correlation and ANSD indicated the delivery was out of conformance indicates that the variation causing the non-conformance in the first delivery is occurring in later PCs and not in the first three PCs used to build the PCA-MD model. The fifth delivery, however, must have a variation to the reference library within the first three PCs to be identified as out of conformance.

The first step in investigating a deviation in conformance is to review the spectra of the nonconforming deliveries with respect to both the reference library and recent conforming historical deliveries. Figure 24 shows the raw spectra overlay.

The first container of the fifth delivery has notably less raw spectral absorbance at higher wavelength than those in the reference library and other delivery samples indicating that the amlodipine besylate in this container is of finer particle size. This was confirmed by observation by the warehouse operators that sampled and analysed the delivery.

The spectra from the containers in the first delivery and the second container of the fifth delivery fall within the range of the raw spectra of the reference library and the spectra from containers in the conforming deliveries.

The variation in particle size for the first container of the fifth delivery is not the source of non-conformance as the mathematical pre-treatments remove physical effects prior to applying the conformance algorithms. Figure 25 shows the 2nd derivative spectra overlay.

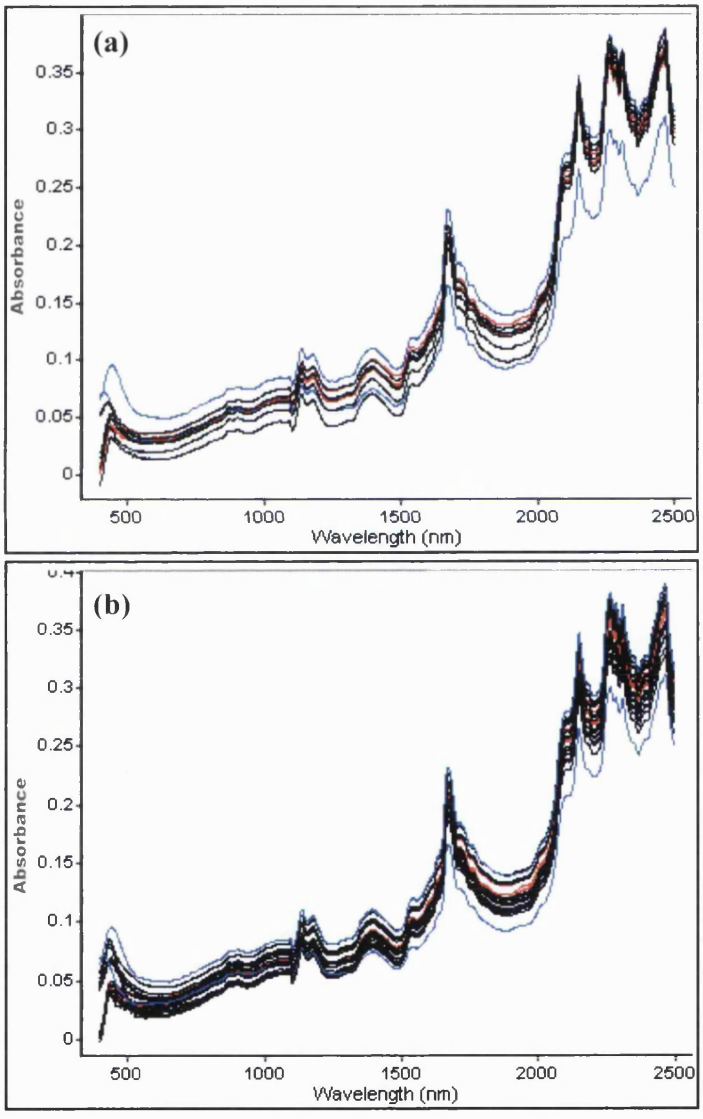

Figure 24: Raw spectra of individual containers from deliveries 1 (___) and 5 (___) overlaid with spectra from: (a) reference library (___) and (b) conforming deliveries 2, 3, 4 & 6 (___)

Review of the 2nd derivative spectra clearly shows a region of variation for the first delivery at 1900–2100 nm (circled in Figure 25) while the fifth delivery shows less obvious variation. It can be challenging to visually review NIR 2nd derivative spectra due to the complexity of the spectra. The use of investigational tools related to the conformance method algorithms can aid in understanding the source of the non-conformance.

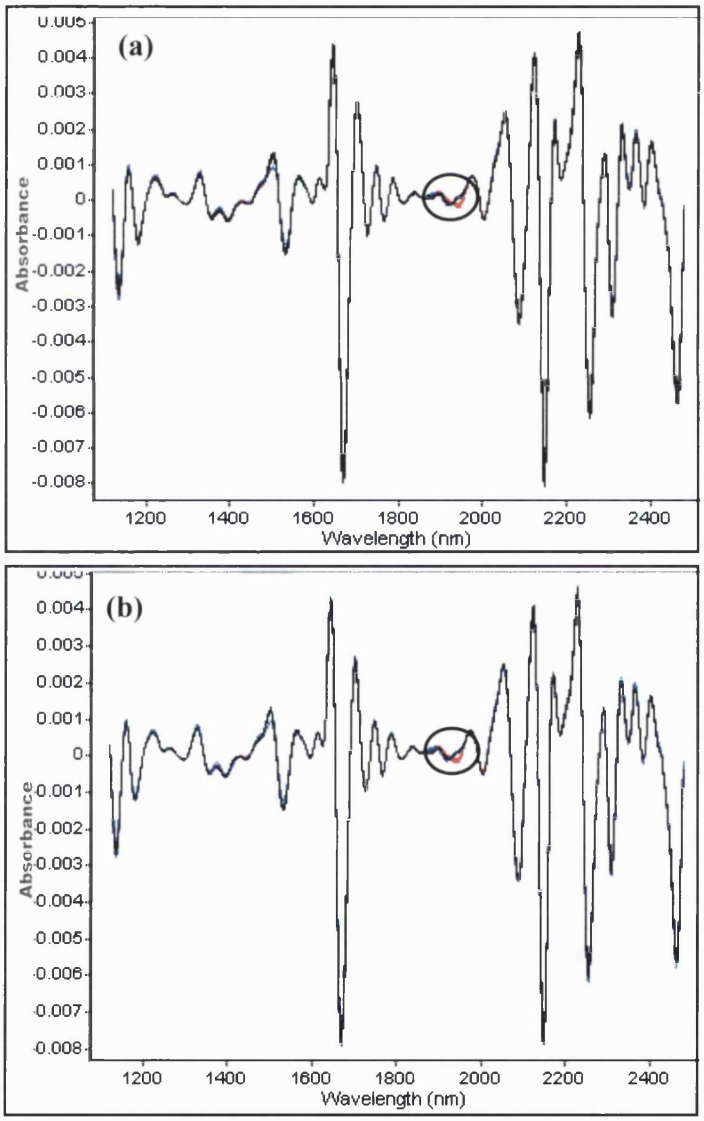

Figure 25: 2nd derivative spectra of individual containers from deliveries 1 (___) & 5 (___) overlaid with spectra from: (a) reference library (___) and (b) conforming deliveries 2, 3, 4 & 6 (___)

To investigate the source of the non-conformance in the correlation conformance method, correlation plots for the sample spectra against the reference mean spectrum (Figure 26 and Figure 27) and residual (sample – reference mean spectrum) plotted at each wavelength (Figure 29 and Figure 30) for the first and fifth deliveries were reviewed. The plots were generated within Minitab as DeLight software did not provide any mechanism to review the cause of correlation deviation. The first containers of the second and third deliveries were also reviewed to indicate the plots appearance for conforming deliveries (Figure 28 and Figure 31). Note that the scale is identical for all residual plots shown in Figure 29 to Figure 31 to aid in comparison.

The first delivery showed a similar scatter about the line of best fit compared to the conforming deliveries with a small pocket of data points above the line, circled in Figure 26. This observation is consistent with the residual plot where the magnitude of the residuals at the majority of the range are similar to conforming batches apart from two wavelength locations, at 1944 nm and 2078 nm. This observation is consistent for all containers in the delivery. Variation at these wavelengths is characteristic of a change in absorption by O-H structures (refer to Figure 2) and points to moisture as the source of the variation. Smaller correlated variations occurring at 1446nm also relate to O-H 1st overtone absorption support this hypothesis. The fifth delivery shows slightly increased scatter about the line of best fit compared to the conforming deliveries indicating variation across the range. The second container also shows a small pocket of data points above the line shown circled in Figure 27.

The residuals plot shows markedly greater variation in the fifth delivery compared to conforming deliveries. Additionally, the first container of the fifth delivery shows high residuals at 1132 nm, 1660 nm and 2154 nm and the second container of the fifth delivery high residuals at 1136 nm, 1502 nm, 1530 nm and 1668 nm which are characteristic of changes in absorption from C-H and N-H functional groups (refer to

Figure 2). Both functional groups are present within amlodipine besylate (refer to Figure 15) and are also commonly seen in solvents used in API manufacture.

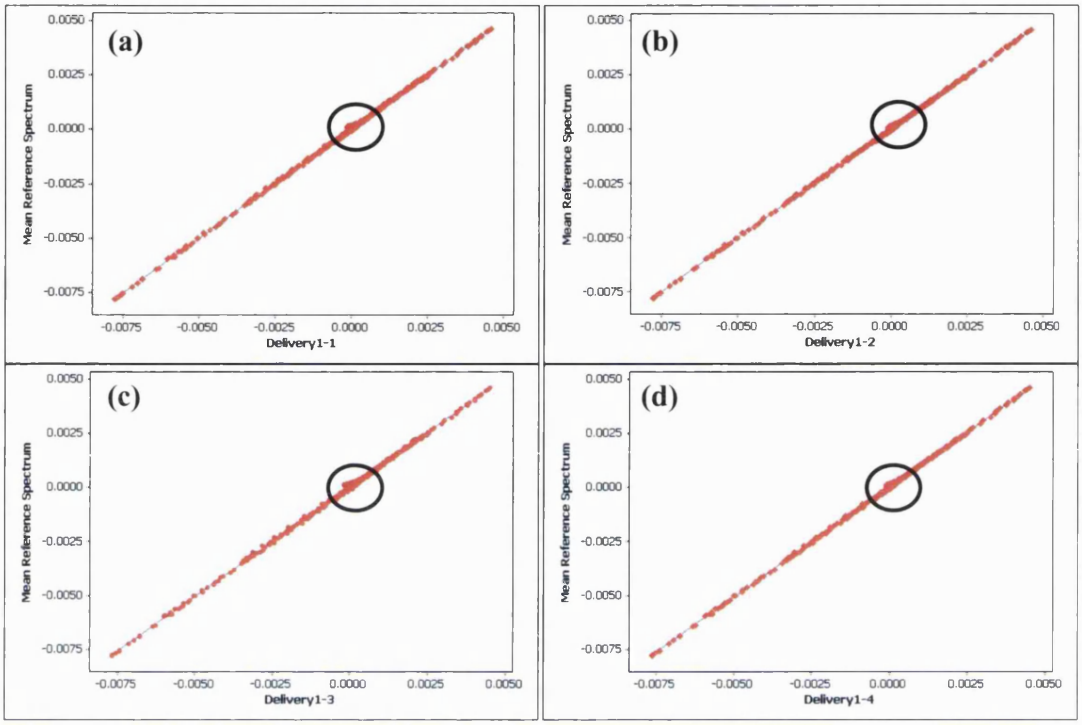

Figure 26: Correlation plots for delivery 1 (a) container 1 to (d) container 4

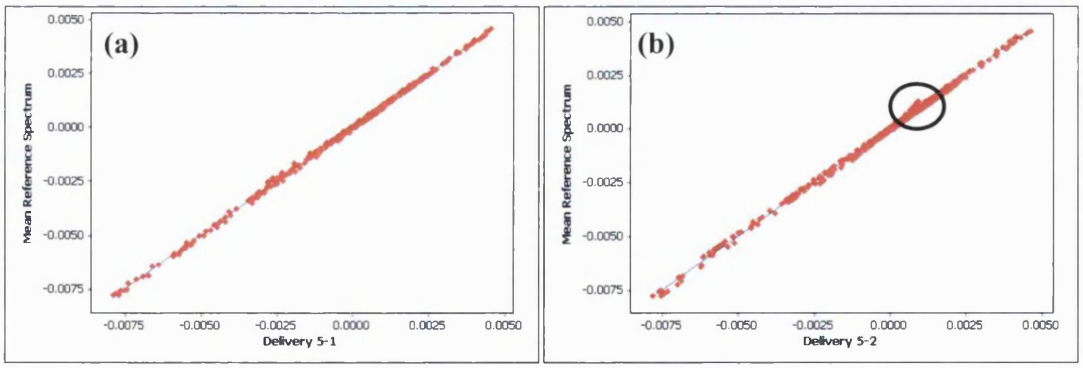

Figure 27: Correlation plots for delivery 5 (a) container 1 and (b) container 2

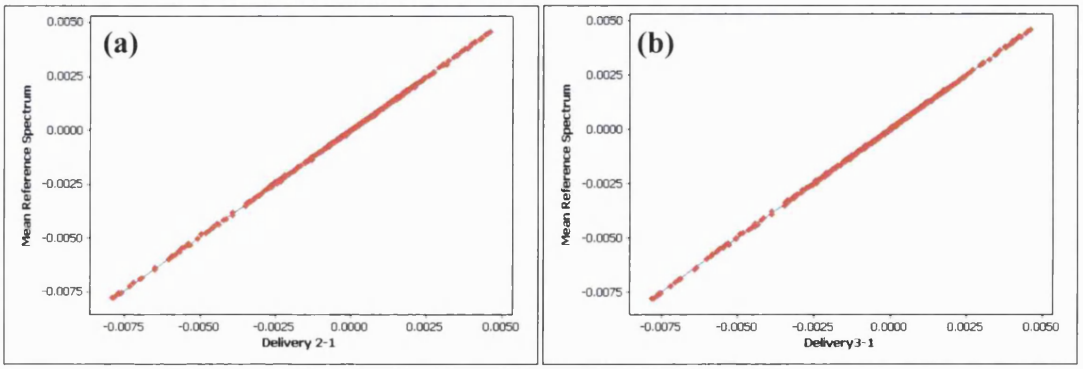

Figure 28: Correlation plots for 1st container from (a) delivery 2 and (b) delivery 3

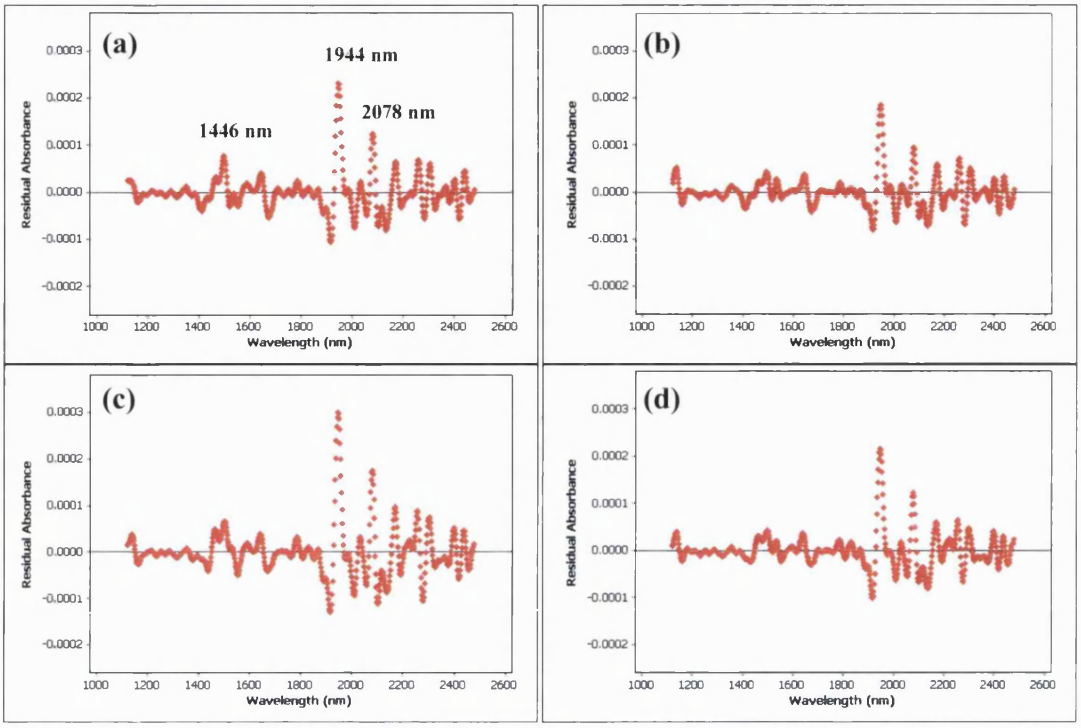

Figure 29: Residual plots for delivery 1 (a) container 1 to (d) container 4

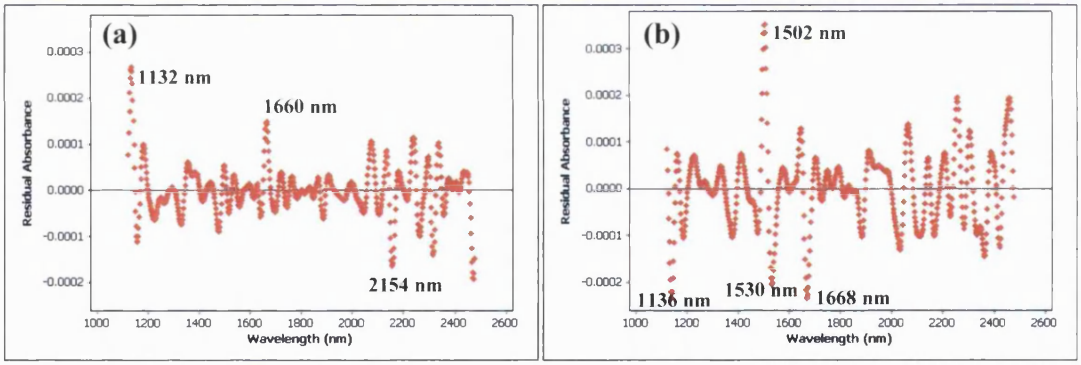

Figure 30: Residual plots for delivery 5 (a) container 1 and (b) container 2

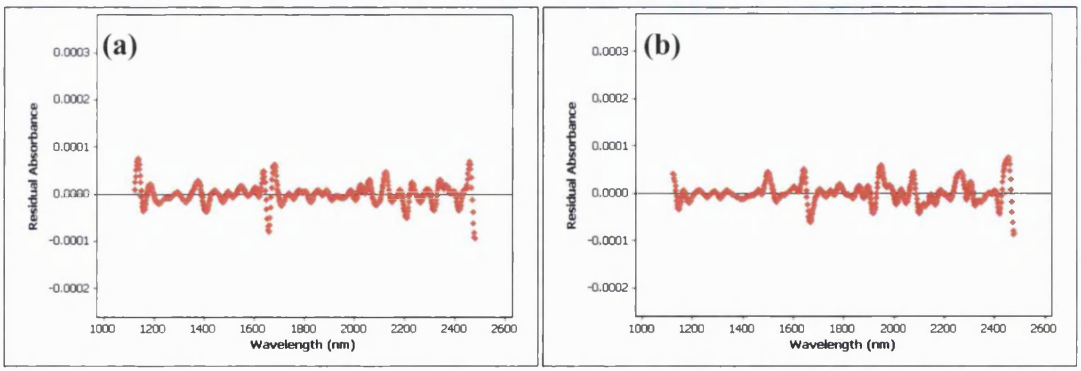

Figure 31: Residual plots for 1st container from (a) delivery 2 and (b) delivery 3

To investigate the source of the non-conformance in ANSD results for the two non-conforming deliveries, the conformity index plot (absolute normalised SD against wavelength) available within the DeLight software was reviewed (Figure 32).

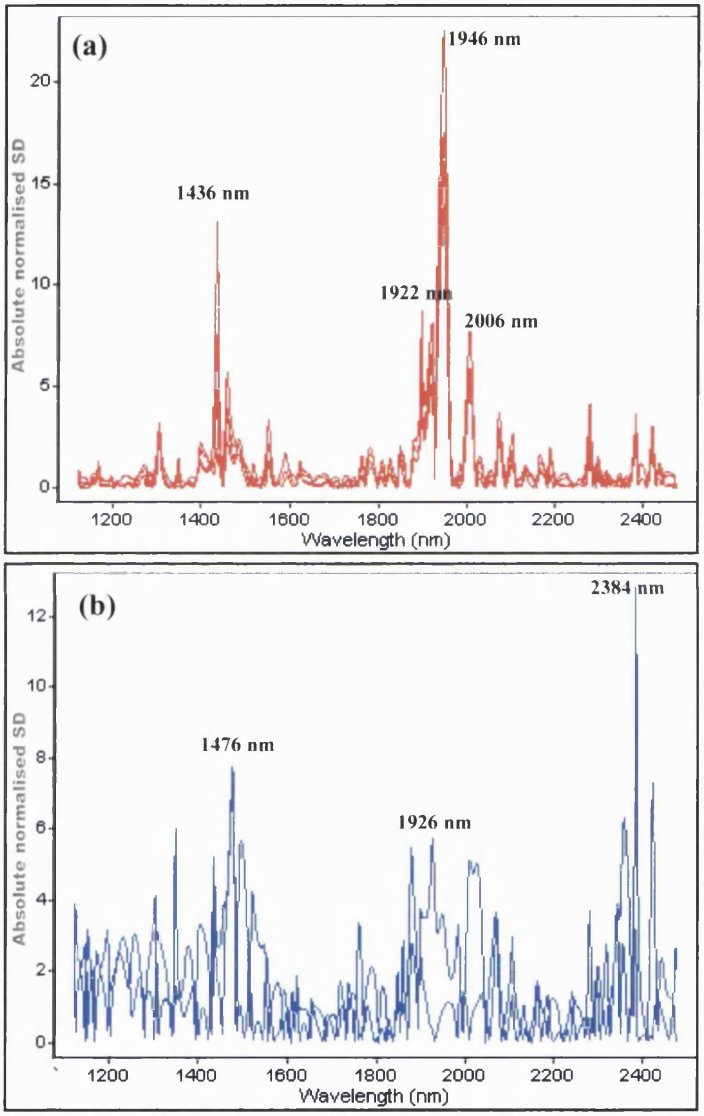

Figure 32: Conformity index plots for (a) delivery 1 and (b) delivery 5

The first delivery shows very high absolute normalised SD at 1946 nm and 1436 nm and moderately high values at 1922 nm and 2006 nm with low values elsewhere in the range. This is characteristic of changes in O-H absorption (refer to Figure 2) indicative of moisture variation. The fifth delivery shows higher absolute normalised SD at

wavelengths across the range, particularly 1430–1520 nm, 1880–2030 nm and 2350–2425 nm. These regions are characteristic of O-H and C-H functional group absorption present in amlodipine besylate (refer to Figure 15). The general increase in absolute normalised SD across the range may be due to residual physical effects; however it may also indicate changes within the crystal lattice of amlodipine besylate affecting the absorption of all functional groups present in the molecule.

Note that there are differences in the wavelengths of variation for the correlation method compared to the ANSD method. Normalising the data based on the reference library reduces the impact of smaller peaks and at those wavelengths already known to vary, as established in the reference library.

To investigate the source of the non-conformance identified in the PCA-MD conformance method for the fifth delivery, PCA analysis was performed to review the projection of the 2nd derivative spectra for this delivery in PCA space alongside the reference library.

From the PCA scores plots shown in Figure 33, the first container of the fifth delivery separates significantly from the reference library on the 2nd PC axis and moderately on the 3rd PC axis, while the second container separates significantly from the reference library on the 3rd PC axis.

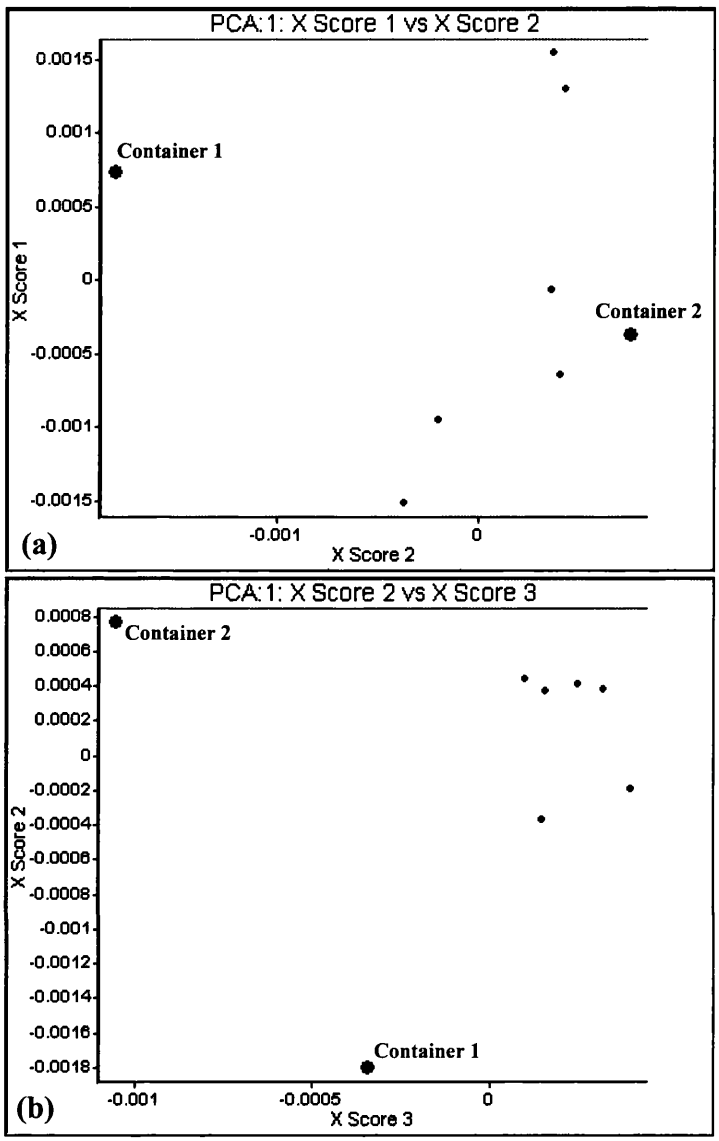

Figure 33: PCA scores scatter plot of delivery 5 and reference library spectra, (a) PCs 1 vs. 2 and (b) PCs 2 vs. 3

Review of the loadings for the 2nd and 3rd PCs in Figure 34 shows that the first container varies to the reference library at 1136 nm, 1502 nm, 2256 nm and 2464 nm while the second container varies at 1128 nm, 1496 nm and 2156 nm. These regions are characteristic of changes in absorption from C-H and N-H functional groups (refer to Figure 2) present within amlodipine besylate (refer to Figure 15) and also commonly seen in solvents used in API manufacture.

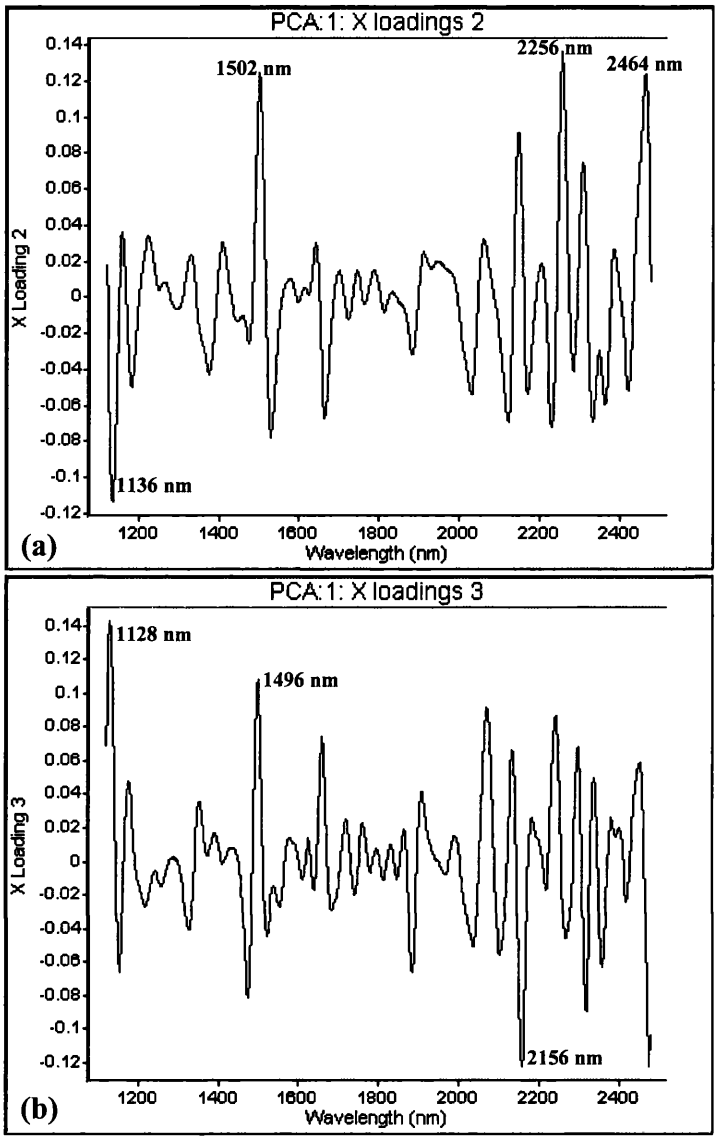

Figure 34: PCA Loadings for (a) 2nd PC and (b) 3rd PC for delivery 5 and reference library spectra

It is of interest that the first delivery was not seen to be out of conformance by the PCA-MD conformance method. This is likely due to the spectral variation in this delivery occurring at wavelength regions not incorporated in the three PCs used to create the conformance algorithm. To confirm this hypothesis, PCA analysis was performed to review the projection of the 2nd derivative spectra for this delivery in PCA space alongside the reference library.

From the PCA scores plots shown in Figure 35, the first delivery separates distinctly from the reference library on the 2nd PC axis. The loadings plot for the 2nd PC (Figure 36) indicates that the main source of variation within this delivery compared to the reference library is occurring at 1946 nm which is the characteristic O-H absorption associated with moisture.

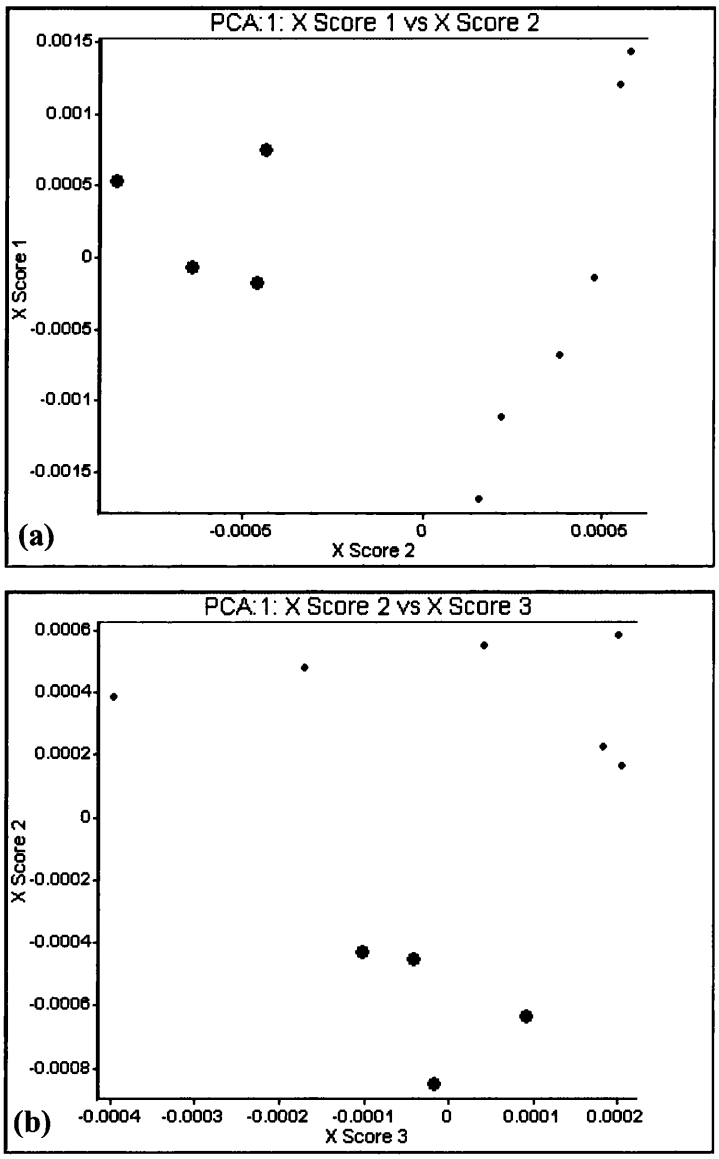

Figure 35: Scores scatter plot of delivery 1 (bold) and reference library spectra, (a) PC 1 vs. 2 and (b) PC 2 vs. 3

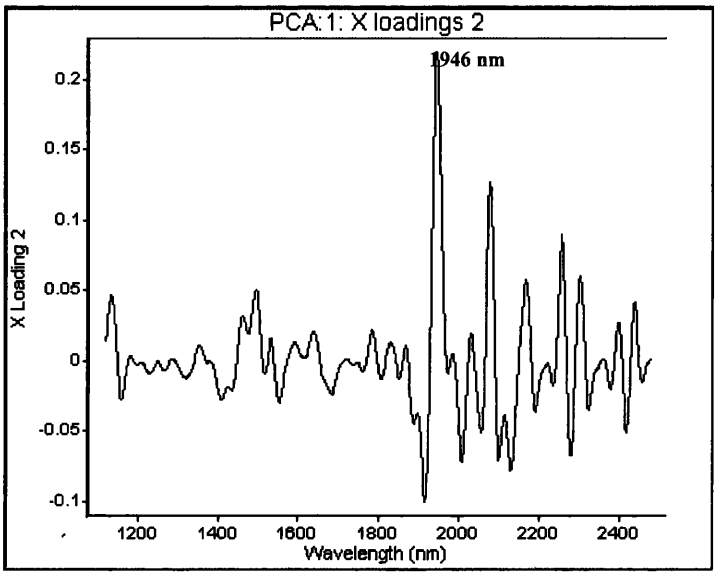

Figure 36: Loadings for PC2 for PCA analysis of delivery 1 and reference library spectra

Examination of the loadings plots for the PCs used in the PCA-MD conformance method (Figure 37) confirms that this region is not represented in the three PCs used in the conformance model. Figure 37 highlights this by overlaying the three PCs and focusing the wavelength axis to the region about the absorbance of interest (note the y-axis for plots in Figure 37 are to same scale for ease of comparison). Thus variation in absorbance in this region will not impact the PCA-MD value and will not trigger a non-conformance.

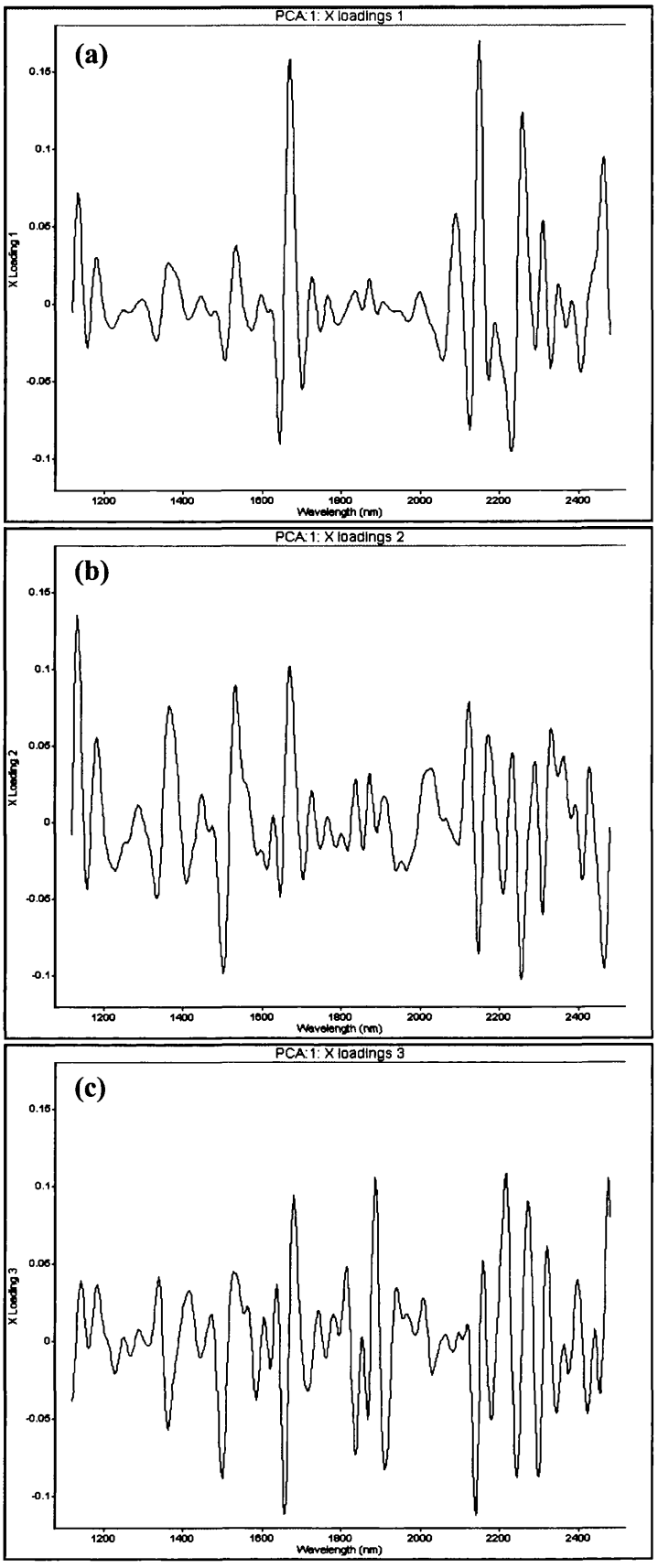

Figure 37: Loadings for (a) PC 1, (b) PC 2 and (c) PC 3 of the three PC loadings of the reference library

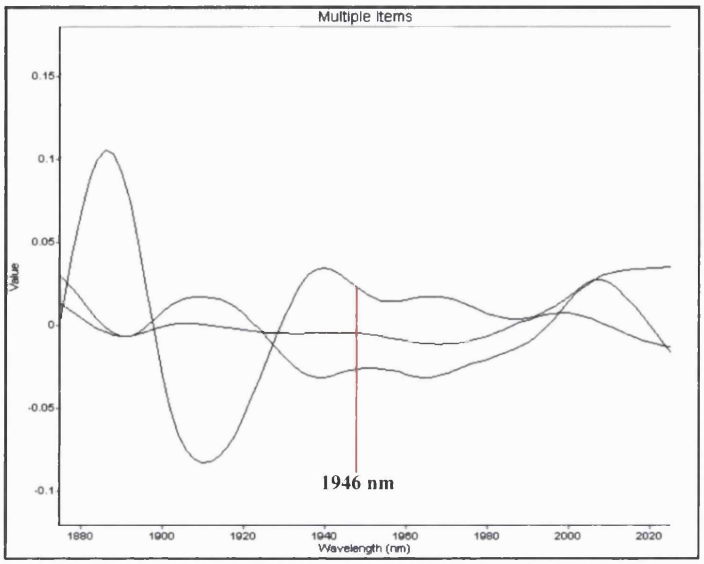

Figure 38: Zoomed overlay of the three PC loadings of the reference library

4.2.3 Global material quality conformance method discussion

4.2.3.1 Impact of non-conformance to the product and process

The results of the NIR global material quality conformance for the first and fifth deliveries triggered close review of the material certificate of analysis (CoA) and ensured that both deliveries received full QC testing. No registered QC test failed for either delivery. However, moisture content was confirmed to be out of trend for the first delivery (towards upper specification limit). As Norvasc® tablets are a low dose direct compression product (3.45% drug loading on weight/ weight basis), risk assessment determined that the higher moisture content API would not contribute significant moisture to the formulation and would have low impact to finished product quality and stability. The use of the material was observed closely in production to establish whether this non-conformance would impact processability (e.g. impact flow characteristics or change the stickiness of the blend during tabletting). It was determined that the non-conformance had no impact on product or process. Though this non-conformance could be classified as a false negative, the occurrence provided

opportunity to gain greater insight and understanding of the formulation and processing design space that would otherwise not have been available. As such, this excursion from normal trend provided an opportunity to gain process understanding and knowledge and demonstrates the fit of the global material quality conformance approach with the PAT initiative. With this variation understood and not found to impact quality or conformance, the global material quality conformance method would be able to be updated to include the variation if desired to prevent future false negative non-conformance.

Despite the fifth delivery not being found to fail or to be out of trend on any QC test, at dispensing, the 2nd container was found to have become caked during storage (entire contents of container was one solid mass). This container was thus rejected and not utilised in production. Close observation of the use of the first container in production showed significant impact to processing with the product blend sticking considerably to the tablet press, extending processing time two-fold and impacting the product quality through poor clarity of the tablet embossing. Further QC testing as part of an extensive investigation as to the processing and material deviation continued to find no deviation on the registered quality tests. However, additional tests targeted to understand the observed changes to the NIR N-H absorption, found a higher level of amine based residual solvent content than typical (though well within acceptable residual solvent levels). It is hypothesised that incorporation of the residual solvent into the crystal lattice (solvate) is the source of the noted variation in the C-H functional groups absorption and the root cause of the sticking behaviour.

This work shows very clearly the significant impact that global material quality conformance methods can have in understanding processes and supporting investigation of process variations. At the time of receipt in the warehouse, rapid analysis of the

material by NIR can raise awareness of potential issues and assist in causative knowledge and deeper understanding of formulation and process understanding.

4.2.3.2 Conformance method selection for continued implementation

In a real world scenario, three conformance algorithms would not be selected for parallel implementation. Review of all three conformance methods within this research provided insight into the capabilities of the three approaches to suit practical implementation. Section 4.2.2.4 demonstrated that both correlation and ANSD were able to differentiate non-conforming deliveries of amlodipine besylate while PCA-MD was only able to identify one of the non-conforming deliveries. This is due to the variation in the first delivery occurring in regions of the spectra not modelled in the PC space. This demonstrates the importance in understanding the ability and limitations of the various techniques.

PCA based quality conformance algorithms are limited to identifying variation in only those wavelengths incorporated in the PCs of the model and risk false positive conformance. If a conforming delivery is then found to be involved in process or quality difficulties, the NIR analyst has the opportunity to revise the algorithm to ensure the PCA model is sensitive to the variation and correct the false positive conformance. In the outcomes described in 4.2.3.1, the first delivery was not found to impact process or quality. However, if the PCA-MD model had been the only method utilised, the opportunity to gain process insight and broaden understanding of the design space and influence of moisture on the process and product would have been missed.

PCA-MD is best applied for materials with extensive understanding of material characteristics where there is confidence that the PCA model will truly represent expected variation in the material. It may not be the best choice initially when building

process understanding. Another challenge is that vendor software may not facilitate the use of algorithms in PCA space for quality prediction. Often vendor software provides PCA for investigation rather than prediction. Where it is available, higher analyst understanding is needed to interpret the output.

Correlation based conformance methods are very simple to develop and require limited knowledge of sources of variation in the material. Correlation may risk emphasising variation at wavelengths with known and acceptable variability and may risk false negative conformance. When such false negative conformance occurs, the NIR analyst has the opportunity to revise the algorithm to ensure that the mean reference spectrum truly represents the mean absorbance. However, the variability or magnitude of absorbance peaks is not accounted for in the algorithm. In the outcomes described in 4.2.3.1, the first delivery was found to be out of correlation conformance due to large variation at peaks associated with moisture; however this was not found to impact process or quality. Thus, the non-conformance for this delivery may be considered a false negative result. If the variation causing the non-conformance is an isolated incident, no update may be necessary. However, flagging subsequent deliveries exhibiting the same non-conforming variation would consume unnecessary time and effort documenting that the variation has no impact and may potentially delay production and supply of pharmaceutical product to customers. As such, if a repeat incident was expected, update of the correlation global material quality conformance method would be recommended.

Correlation is a very simple approach and all typical vendor software packages provide this algorithm. However, as vendors expect this algorithm to be applied in a pass / fail approach, there is typically no capability in the software to readily investigate the cause of non-conformance. As was seen in this research, data must be exported to external software to investigate the root cause of any non-conformance identified.

ANSD appears to be a good compromise in balancing the risk of false positive and negative conformance. When a false result occurs, incorporating the un-modelled variation is more effective than with correlation as the variation will be built into the standard deviation at deviating wavelengths as well as the mean spectrum. Similarly to correlation, the non-conformance of the first delivery may be seen as a false negative conformance and may indicate the need to update the global material quality conformance method if subsequent deliveries are found to contain the same variation.

Not all vendor software provides flexibility in the quality metrics available for normalised SD. Some vendors do not provide the means to develop normalised SD methods at all, while others report only the maximum value at any wavelength in the region. In such cases, the resulting conformance SPC would be built on results at different wavelengths for each sample analysed which is of little statistical and predictive value. If normalised SD data are not available, data would need to be exported to external software for further calculations which would not suit the manufacturing (or material warehouse) environment.

In general, trending of results is not available in vendor software and SPC charts need to be developed external to vendor software and is independent of the conformance method algorithm chosen.

For the manufacturing facility where this work was conducted, both correlation and ANSD approaches were pursued. ANSD was the preferred option however, as the site already used a correlation model for material identification purposes, it was determined that both global material quality conformance methods would be implemented.

The DeLight software utilised at the site allowed simple implementation of the methods in the facility. This software has the capability to reproduce the individual container I-chart including control limits to allow the operator to monitor the results in real time.

As each delivery is saved as an individual data file and variable averaging is not a capability of DeLight (to address the variable number of containers in each delivery), it was not possible to reproduce the overall delivery SPC charts. As such, following data acquisition and model prediction, data were exported to external software for SPC trending and review by Quality Assurance personnel. The manufacturing staff selected Excel to perform the SPC trending due to operator familiarity with Excel compared to Minitab. Standard operating procedures (SOPs) were developed and warehouse operators trained to conduct the NIR analysis and the initial trend chart review. It was found that the conformance methodology was extremely approachable to warehouse operators who appreciated the visual nature and real time feebdack on their work. The warehouse operators felt an empowerment and enhanced engagement that offset the slight increase in workload to perform the NIR analysis. Warehouse operators were able to immediately raise a concern to the Quality Assurance department when a non-conformance occurred. Quality Assurance personnel had increased information on the material quality to enable decisions on material investigations. Quality Assurance personnel received additional training on reviewing the output of global quality conformance methods, however an NIR trained analyst was generally required to perform deviation investigation and root cause assessments. The implementation of conformance methodology was seen as very successful by warehouse operators, Quality Assurance personnel and site management.

Figure 39 shows the user interface that operators were able to use to monitor the global material quality conformance in real time during material receipt testing.

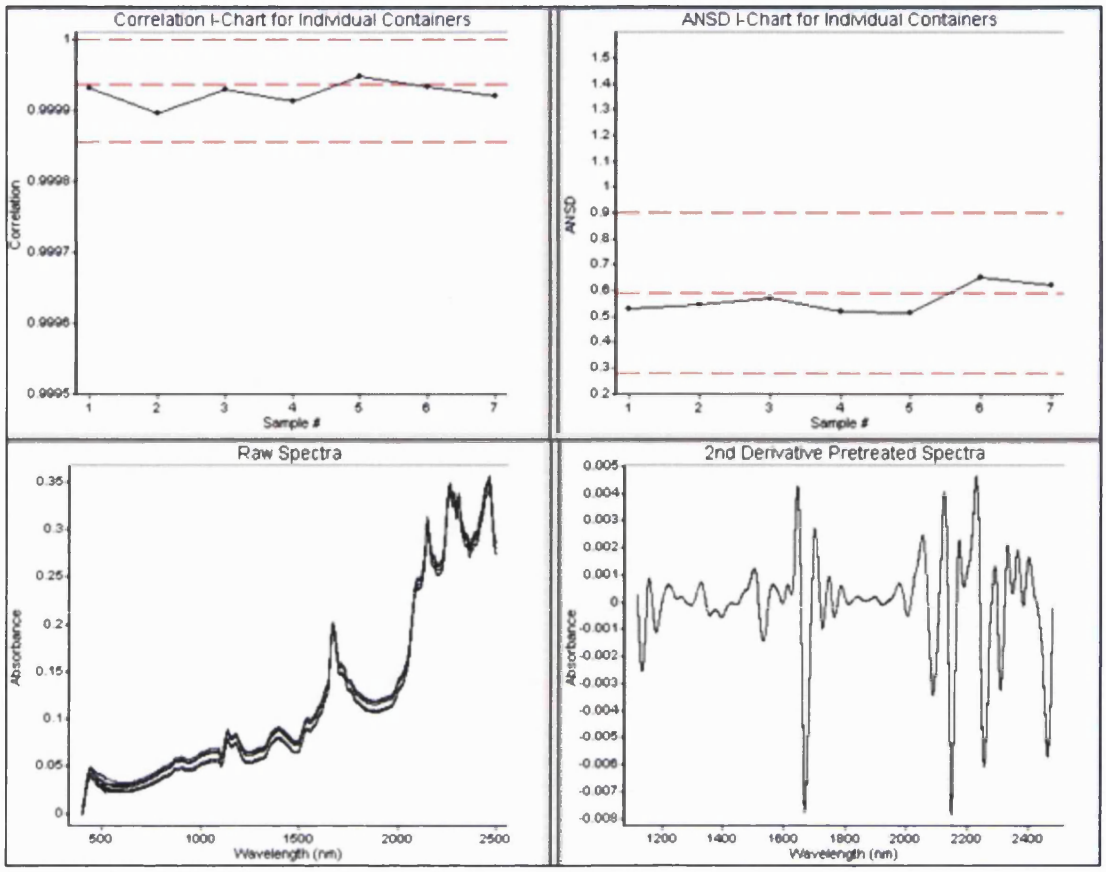

Figure 39: Operator interface for developed global material quality conformance for amlodipine besylate with DeLight software

4.2.4 Summary - criticality of research

This research investigated the use of continuous data output from three qualification algorithms (correlation, ANSD and PCA-MD) and SPC charting to create a Global Material Quality Conformance approach. Typically, material qualification algorithm output is used simply as a categorical classification technique and the extended use of the data with SPC has not been previously reported.

Three global material quality conformance methods were developed and applied in parallel to commercial deliveries of amlodipine besylate at a Pfizer facility and the value of the work was demonstrated in the ability to identify non-conforming deliveries as described in 4.2.3.1. The benefits and limitations to the three different methods were

compared and commentary was provided as to the availability of vendor software to easily implement such approaches.

Global material quality conformance methods were demonstrated to provide an opportunity to gain greater insight in to the design space of product formulations and processes and enabled rapid identification of material variation with the potential to greatly impact the pharmaceutical manufacturing process and/ or product quality. The approach also facilitated rapid root cause determination of material variation that impacted product and process.

4.3 Material attribute quality conformance

The development and application of a material attribute quality conformance method is described in this section to extend on the approach and value described in Section 4.2.

Material attribute quality conformance is applicable when a specific aspect of a material's quality is identified as benefiting from monitoring and control. Unlike global material quality conformance methods, material attribute quality conformance methods are not sensitive to variation from aspects of the material other than the target attribute. Chemometric algorithms are chosen that narrow the focus to only those spectral features in the NIR spectra that relate to the attribute of interest. Following on from the research conducted in Section 4.2, amlodipine besylate was selected as an appropriate research subject with the amine based residual solvent content as the target attribute. Following exhaustive quality testing and investigation, it had been determined that the amine based residual solvent had no impact on product quality and stability other than issues with appearance. The significant challenge for the manufacturing site was the impact of processing the material with high amine based residual solvent content in the tabletting operations. Though the quality of the product reaching the customer was preserved, the

supply of this vital medicine was impacted by the manufacturing difficulties of using the material and the uncertainty as to the extent of processing difficulties encountered lot to lot. Global investigation of this issue showed that deliveries of API with high amine based residual solvent caused problems at many sites of manufacture with the severity of impact related to the processing equipment factors (e.g. brand of tablet press, speed of compression and compression tooling finish). Processing issues could be mitigated through process interventions such as slowing press operation and regularly cleaning and polishing press punches and dyes of sticking material throughout processing.

The high impact of the amine based residual solvent in amlodipine besylate indicated that this material attribute was an excellent candidate for the material attribute quality conformance method research.

4.3.1 Materials and Methods

4.3.1.1 Design of Analysis

The reference library established for the global material quality conformance methods researched in Section 4.2 was the basis of developing the material attribute quality conformance method. The spectra in the global material quality conformance method reference library provided the range of amlodipine besylate deliveries with acceptable low amine based residual solvent content. For ease of description, this reference library is termed "acceptable reference library" for the remainder of Section 4.3.

Single spectra for two further deliveries with high amine based residual solvent content and unique manufacturer's lot were required to provide flexibility in investigation of appropriate chemometrics algorithms. These spectra were used as either negative challenge samples (for method algorithm optimisation) or for extension of the

acceptable reference library to represent unacceptable material depending on the algorithm chosen. For ease of discussion, the reference library including the two high amine residual solvent spectra is termed "extended reference library" for the remainder of Section 4.3. Various algorithms were explored to select the most appropriate modelling approach for the material attribute quality conformance method.

To ensure robust development of the SPC approach, it was determined that a minimum of five deliveries with acceptable level of amine based residual solvent content independent of the acceptable reference library would be required. An individual spectrum for each container in the deliveries was required to develop the SPC approach so that it was applicable to the real life scenario of assessing the conformance of every container in a delivery. As mentioned in Section 4.2.1.1, this is important as the dosage strength of amlodipine besylate in the Norvasc® finished dosage form is low (approximately 3.45% weight / weight basis) and only one to two containers of API are utilised in any given batch of Norvasc® tablets.

4.3.1.2 Samples

The deliveries from the two different manufacturers' lots with unacceptably high amine based residual solvent content were identified as those with high deviation in the global material quality conformance method SPC charts and confirmed as having severe impact on processing. One representative spectrum (from the first container in the delivery) from each of the two deliveries was selected and added to the acceptable reference library to create the extended reference library.

The seven deliveries with acceptable amine residual solvent content immediately prior to the first instance of a delivery with high amine based residual solvent content were selected to establish (five deliveries) and verify (two deliveries) the SPC charts (varying

number of spectra per delivery based on the number of containers delivered). A third delivery with unacceptably high amine content was selected (independent of those in the extended reference library) and included in the verification set.

Six subsequent deliveries were then reviewed and the suitability of the approach demonstrated through the inclusion of deliveries with non-conformance.

All samples were prepared for NIRS analysis by placing the material in SUN Sri 4 mL borosilicate glass Shell vial (part number: 500 070) to a fill depth of 1 cm and compressing with uniform pressure using a stainless steel weighted cylinder (1 cm diameter and 5 cm length).

4.3.1.3 NIR Apparatus and software

NIR reflectance spectra were measured as outlined in Section 4.2.1.3.

DeLight version 2.3b with DMentia 1.1b software (DSquared Development, La-grande, OR, USA) and Microsoft® Excel, version 9 (Microsoft® Corporation) were utilised for chemometric model development and predictions, while Minitab® 16 version.1.16 (Minitab Inc, State College, PA, USA) was utilised for statistical evaluation and SPC chart development.

4.3.2 Material attribute quality conformance method development

4.3.2.1 Spectra pre-treatment

The pre-treatment optimised in 4.2.2.1 was applied to all spectra for the material attribute quality conformance method development.

4.3.2.2 Model development

Material attribute quality conformance can best be achieved with chemometric techniques that assess the spectral features directly related to the target attribute. Whole region techniques explored in Section 4.2 are not suited. The simplest approach is to identify an individual wavelength that directly relates to the attribute of interest. However, the complex overlapping nature of peak absorptions in NIRS makes it challenging to identify a single wavelength at which absorption is due solely to one matrix component free of other interference (from other matrix components or physical effects). As such, multiple wavelength techniques are better suited. Rather than performing ratios or other manual mathematical manipulations of absorbance at multiple wavelengths, MLR analysis (refer to Section 0) with DA (refer to Section 3.3.3) can be developed in most vendor software. The MLR-DA output can then be trended to represent where on the continuum of acceptable to unacceptable future deliveries fall.

Normalised SD metrics (refer to Section 3.3.1.3) over a narrow range can be applied once a suitable range is identified that is specific to the target material attribute. Care needs to be taken to ensure that the region is not sensitive to variations not related to the attribute of interest.

PCA based methods can be applied after training the PCA on what variation is of interest. If the variation between good and acceptable lots is described wholly within the first PC, a simple threshold applied to the PC score values may be suitable. Where variation between acceptable and unacceptable material requires further PCs, PCA-MD can be used. However, it should be noted that the distance is calculated from the centre point of the distribution in the reference set (between the acceptable and unacceptable data). Weighting the reference set towards the acceptable lots can ensure the variation of interest is captured within high order PCs without shifting the centre of the distribution

too far towards the unacceptable lots. Care should be taken in understanding any other sources of variation contributing to the PCs used in the model to avoid false non-conformance from other spurious causes.

PCR-DA or PLS-DA (refer to Section 3.3.3) can also be applied and the output trended for future samples. The main advantage of PLS-DA is that the variation in the reference data (in this case class category) directs the compression of the data, while PCR-DA relies solely on spectral variation in the data compression into PCs.

Three techniques were assessed in parallel, MLR-DA, total normalised absolute SD over reduced range (termed TNSD-RR hereafter), and PLS-DA. Note that the use of PCA scores thresholds was of interest however prediction of PCA scores from a stored PCA model is not available within DeLight software.

The normalised SD model developed in DMentia for the global material quality conformance method described in Section 4.2.2.2 was utilised for the basis of the TNSD-RR method. Note that for the TNSD-RR conformance method approach, the extended reference library is not used to develop the model, rather it is utilised to optimise the method. To determine the reduced range to use for the TNSD-RR method, the 2nd derivative spectra of the extended reference library was reviewed. Regions of distinct variation between acceptable and unacceptable amine based residual solvent are easily visually identified, in particular between 1446–1556 nm and 1900–2100 nm (circled in Figure 40). These regions relate to the characteristic regions of 1st overtone and combination absorption related to O-H and N-H functional groups (refer to Figure 2) in the amlodipine besylate and amine based solvent. Impact to the O-H absorptions may relate to the solvent itself or the impact of the solvate on the O-H bonds of the API.

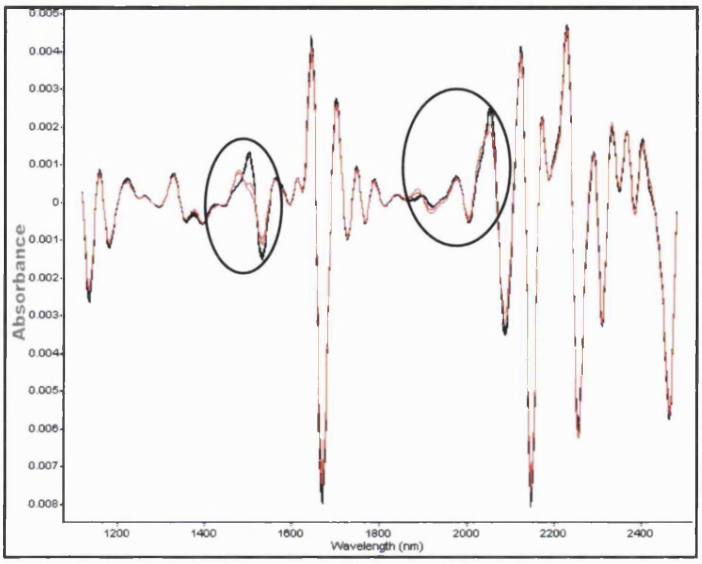

Figure 40: 2nd derivative NIR spectra of the extended reference library with high amine based residual solvent content spectra in red

TNSD-RR for the extended reference library was calculated from the normalised SD value across the range 1446-1556 nm in the DeLight software. The TNSD-RR results (shown in Table 5) were analysed in Minitab and represented graphically through the development of a chart of the individual values (I-chart) with the control limits (___) set at three standard deviations from the mean (___) for the six acceptable deliveries. The resulting I-chart for TNSD-RR (shown in Figure 41) uses a lower boundary limit of 0.0, as the calculated control limit was beyond the allowed values of 0.0. Note that the two deliveries in the extended reference library with high amine based residual solvent content are marked in red with a "1" superscript. This is the notation used in Minitab to indicate data points that fail the control test of being within three standard deviations from the mean.

Table 5: Predicted material attribute quality conformance statistics for TNSD-RR

	TNSD-RR (Absorbance)
Reference library 1	19.47
Reference library 2	46.43
Reference library 3	44.99
Reference library 4	41.30
Reference library 5	18.09
Reference library 6	23.51
Reference library High Amine 1	608.8
Reference library High Amine 2	489.7

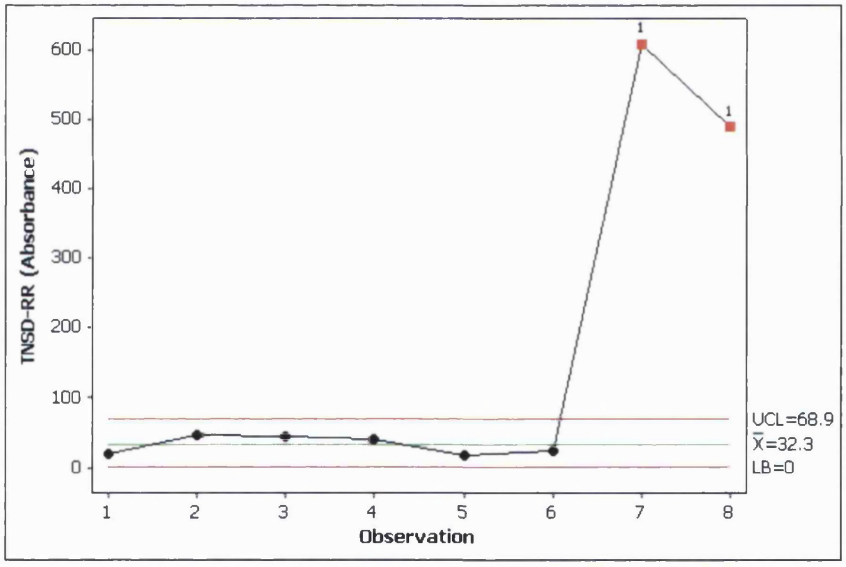

Figure 41: I-chart of predicted TNSD-RR results for the extended reference library

The MLR-DA model was developed in DMentia software. A plot of correlation against wavelength (Figure 42) assisted in the selection of the primary wavelength (wavelength in a region of high correlation to the acceptability classification) and a secondary wavelength (low correlating wavelength utilised to stabilise the MLR model against unrelated variation).

Closer examination of Figure 42 identified strong positive correlation (r>0.95) between 1490–1512 nm and 2050–2072 nm (circled in black) and strong inverse correlation (r<-0.95) between 1452–1484 nm and 2008–2036 nm (circled in red). These regions included the regions identified visually when selecting the reduced range for the TNSD-RR method.

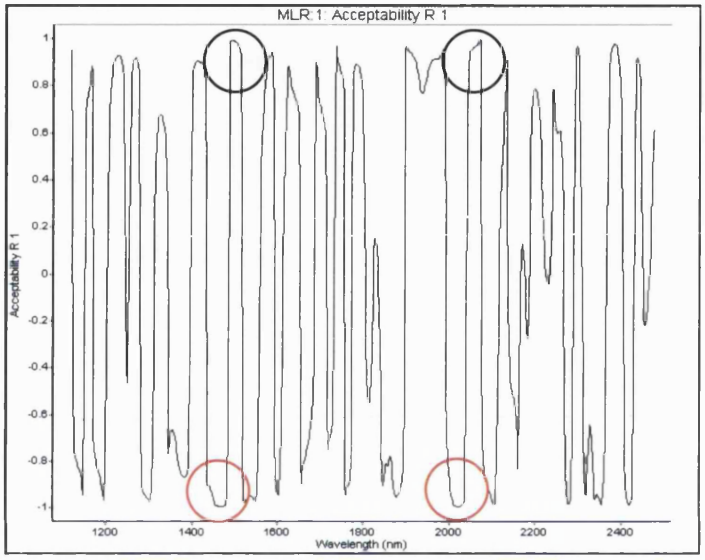

Figure 42: Correlation of acceptability class for 2nd derivative spectra of the extended reference library

Examination of the positive correlation regions showed absolute maximum correlation at 1502 nm and 2052 nm, while the inverse correlation region showed absolute maximum correlation at 1478 nm and 2028 nm. Of these highly correlating peaks, the peak at 1502 nm is centred more within the correlating region and would thus provide a more stable MLR model. A MLR-DA model was developed with 1502 nm as the primary wavelength and with 1614 nm selected by the DMentia software as the secondary wavelength.

Figure 43 shows the 2nd derivative spectra focused into the wavelength region of these peaks to illustrate the suitability of the wavelength choices. Both wavelengths are at peak maxima and will therefore be stable to slight wavelength shifts for the life of the

conformance method. There is also clear discrimination between the acceptability classes in the extended reference library at 1502 nm and no correlation at 1614 nm indicating this is an excellent secondary wavelength choice to stabilise the MLR-DA model.

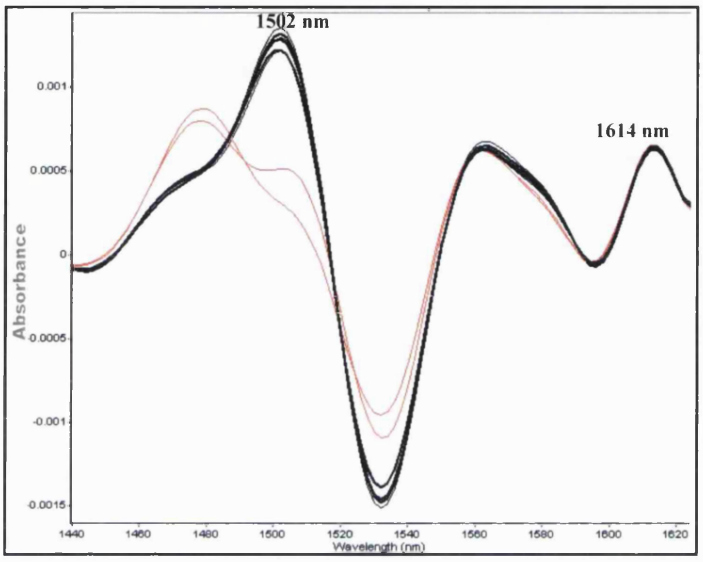

Figure 43: Zoomed 2nd derivative spectra of the extended reference library with high amine based residual solvent content spectra in red annotated with the MLR-DA model wavelengths

The MLR-DA model statistics calculated as described in Section 3.4.1 are shown in Table 6 and demonstrate the model is very capable of relating the correlation of acceptability class (r^2 >0.99) with low error (SEE of 0.03556 representing a 3.556% error in estimating acceptable deliveries).

Table 6: Model statistics for the MLR-DA model

r2	0.9949
SEE (class)	0.03556
slope	0.9661
Intercept (class)	0.02539

The MLR-DA results for the extended reference library predicted in DeLight (shown in Table 7) were analysed in Minitab and represented graphically through the development of a chart of the individual values (I-chart) with the limits (___) set at three standard deviations from the mean (___) for the six acceptable deliveries. The resulting I-chart for MLR-DA is shown in Figure 44. Note that the two deliveries in the Extended Reference Library with high amine based residual solvent content are marked in red with a "1" superscript, the notation used in Minitab to indicate data points that fail the control test of being within three standard deviations from the mean.

Table 7: Predicted MLR-DA material attribute quality conformance results for the extended reference library

	MLR-DA (class)
Reference library 1	1.032
Reference library 2	0.9680
Reference library 3	1.023
Reference library 4	0.9711
Reference library 5	0.9571
Reference library 6	0.9986
Reference library High Amine 1	-0.008790
Reference library High Amine 2	0.05956

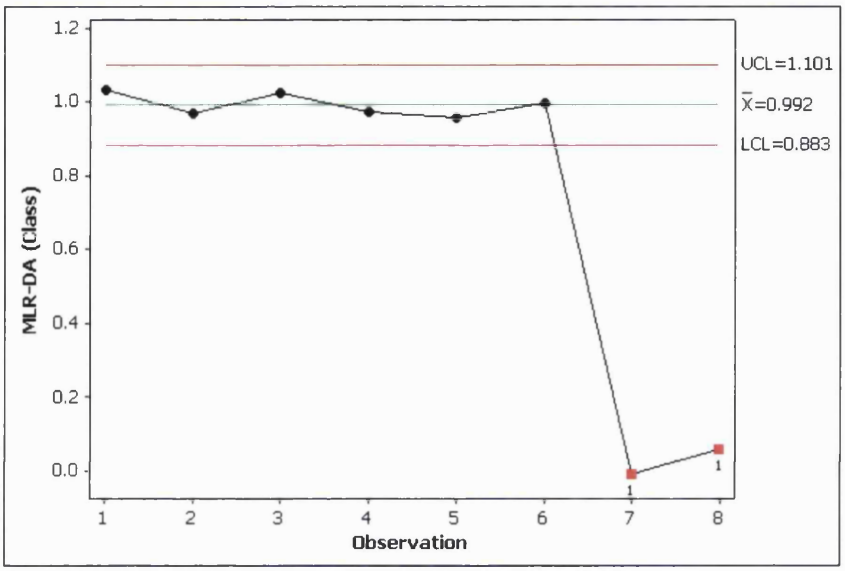

Figure 44: I-chart of predicted MLR-DA class results for the extended reference library

To assess the capability of the MLR-DA model to predict satisfactorily for samples independent of those used in the model, deliveries external to the extended reference library were predicted with the model in DeLight and the prediction statistics reviewed. Two deliveries of varying number of containers with acceptable amine based residual solvent content and one delivery of unacceptable amine based residual solvent content were used to verify the model. The predicted results and the residual between the predicted and expected class are shown in Table 8.

The SEP for the MLR-DA was calculated according to Equation 17 and found to be 0.03682 (representing a 3.682% error in estimating acceptable deliveries) and yielded a SEP: SEE ratio of 1.04. This close agreement in errors indicates that the MLR-DA model is very capable when predicting new deliveries.

Table 8: Predicted MLR-DA material attribute quality conformance for verification deliveries

	Predicted Class	Expected Class	Residual
Verification 1	0.9824	1	0.0176
Verification 1	0.9823	1	0.0177
Verification 2	0.9717	1	0.0283
Verification 2	1.020	1	-0.0200
Verification 2	1.023	1	-0.0230
Verification 2	1.022	1	-0.0220
Verification 2	1.042	1	-0.0420
Verification 2	0.9758	1	0.0242
Verification 3	0.04227	0	-0.04227
Verification 3	-0.05751	0	0.05751
Verification 3	-0.05762	0	0.05762

PLS-DA models were developed over the full wavelength range in DMentia software. Review of the PLS scores plots in Figure 45, shows that the deliveries with the acceptable and unacceptable amine based residual solvent content separate predominantly along the first LV axis. The axes in the plots in Figure 45 are to the same scale showing the relative magnitude of the variation explained in each LV.

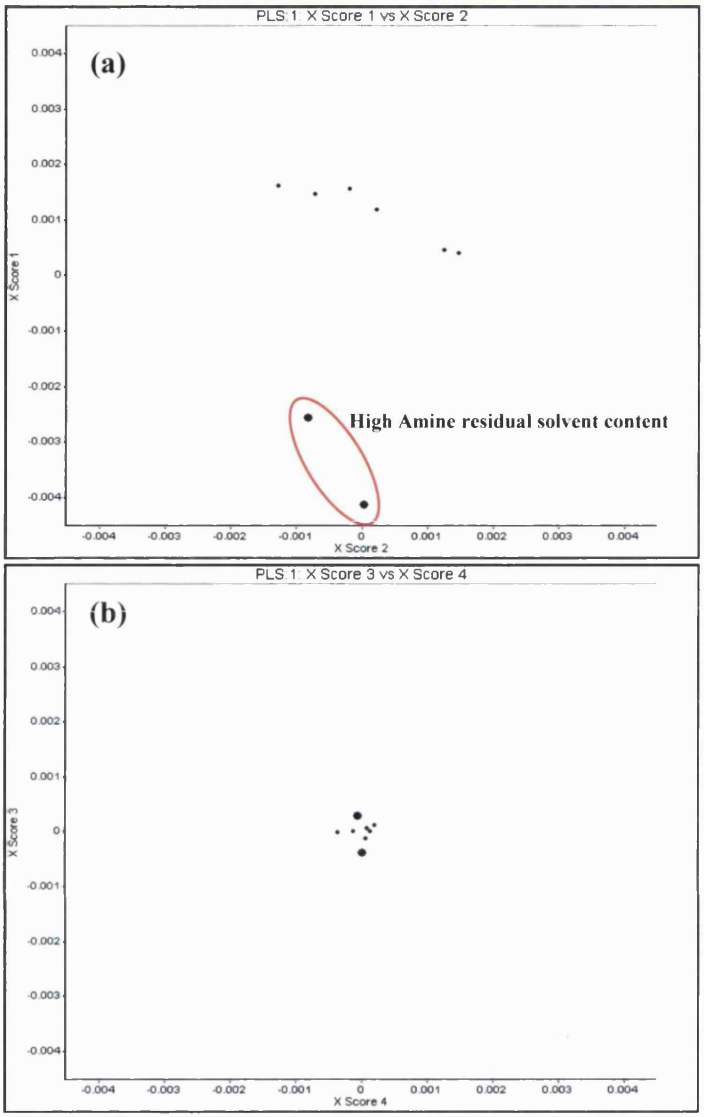

Figure 45: LV scores plots for the extended reference library (a) LVs 1 vs. 2 and (b) LVs 3 vs. 4

Examination of the weighted loadings of the PLS models in Figure 46 shows that the first LV includes the wavelengths at the peaks identified in the MLR-DA to be highly correlated to the acceptability of amine based residual solvent content. The wavelengths of variation included in the 2nd and 3rd LV were not in specific regions identified with high correlation.

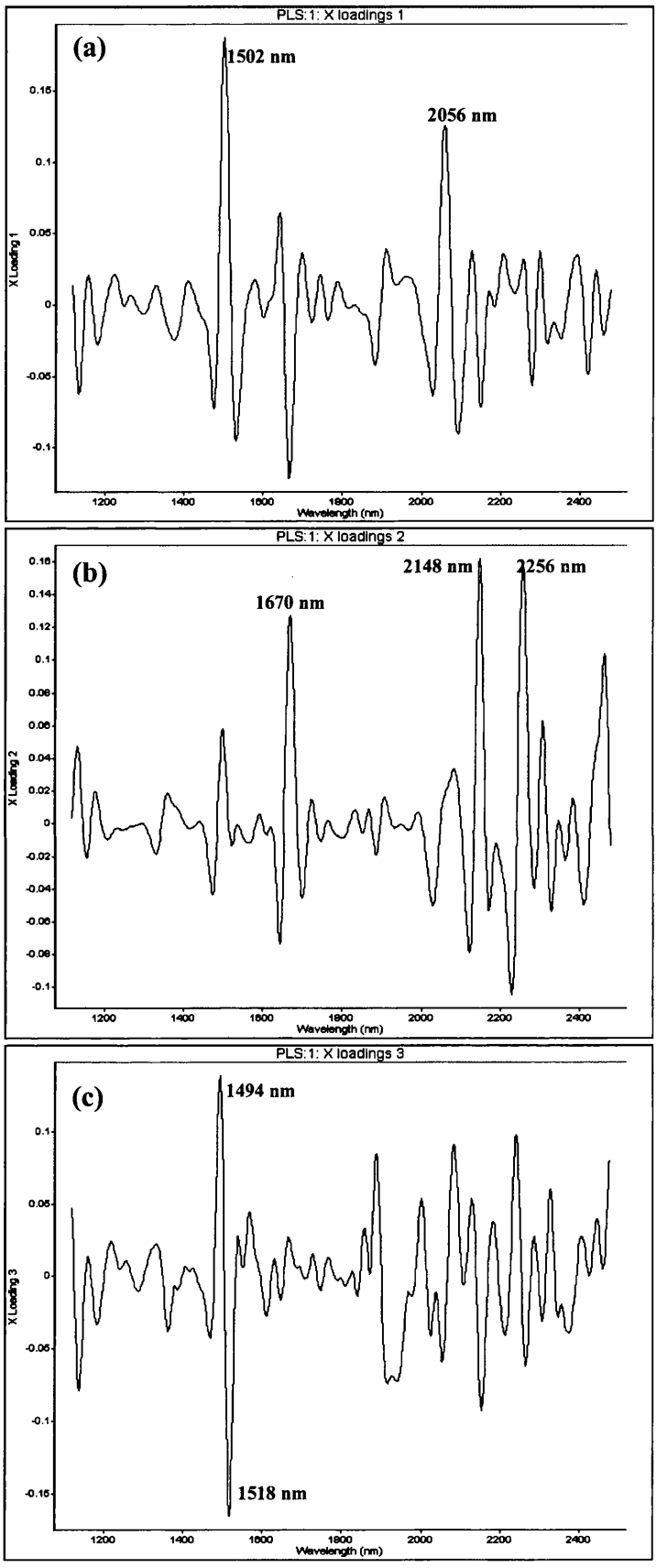

Figure 46: Weighted loading for the PLS-DA model of extended reference library (a) LV 1, (b) LV 2 and (c) LV 3

To better understand the variations included in the second LV, a plot of the residual spectra after the removal of the variation included in the 1st LV was examined (Figure 47). Closer inspection identified that the largest variation remaining in the residual spectra that would be modelled in the 2nd LV is related to differences of two samples within the extended reference library (second and third reference deliveries).

Examination of the residual spectra at the wavelengths identified in the PLS-DA loading for the 2nd LV, showed that at 1670 nm and 2148 nm, the unacceptable lots (red spectra in Figure 47) group with the second and third reference deliveries (bold black spectra in Figure 47) away from the remaining four reference deliveries.

The 2nd LV may then stabilise the PLS-DA model through appropriately weighting the structured variation not associated with amine based residual solvent content.

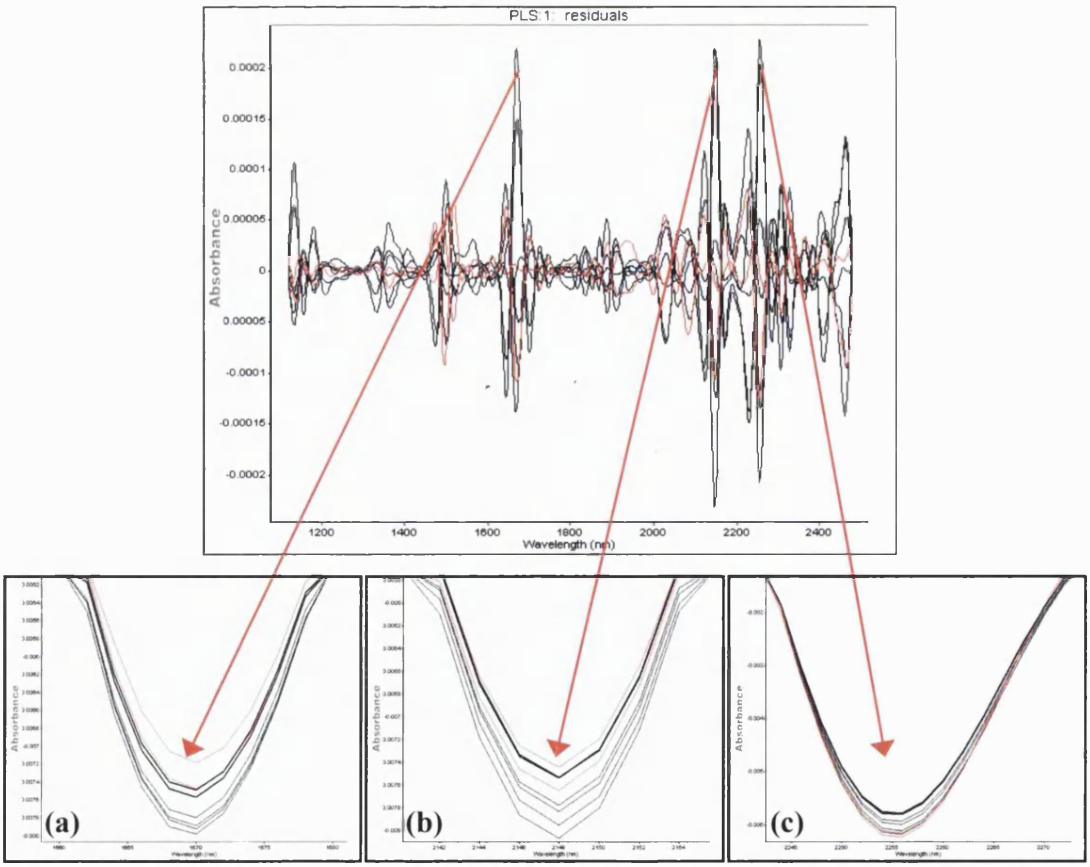

Figure 47: Residual spectra after removal of spectral variation accounted for in LV 1, with associated 2nd derivative spectra at identified wavelengths of LV 2; (a) 1670 nm, (b) 2148 nm and (c) 2256 nm

To better understand the variation included in the 3rd LV, a plot of the residual spectra after the removal of the variation from the first two LVs was reviewed (Figure 48). It is worth noting that the scale of the residual spectra is an order of magnitude smaller than the residual after only one LV is removed (Figure 47) and the residual spectra appears less structured (more random). The areas of highest residual relate to differences between the two unacceptable amine based residual solvent content deliveries. Closer examination of the residual spectra at wavelength regions identified in the PLS-DA loading for the 3rd LV, shows that at 1494 nm and 1518 nm the residuals do not relate to specific peaks in 2nd derivative spectra rather at points of inflection.

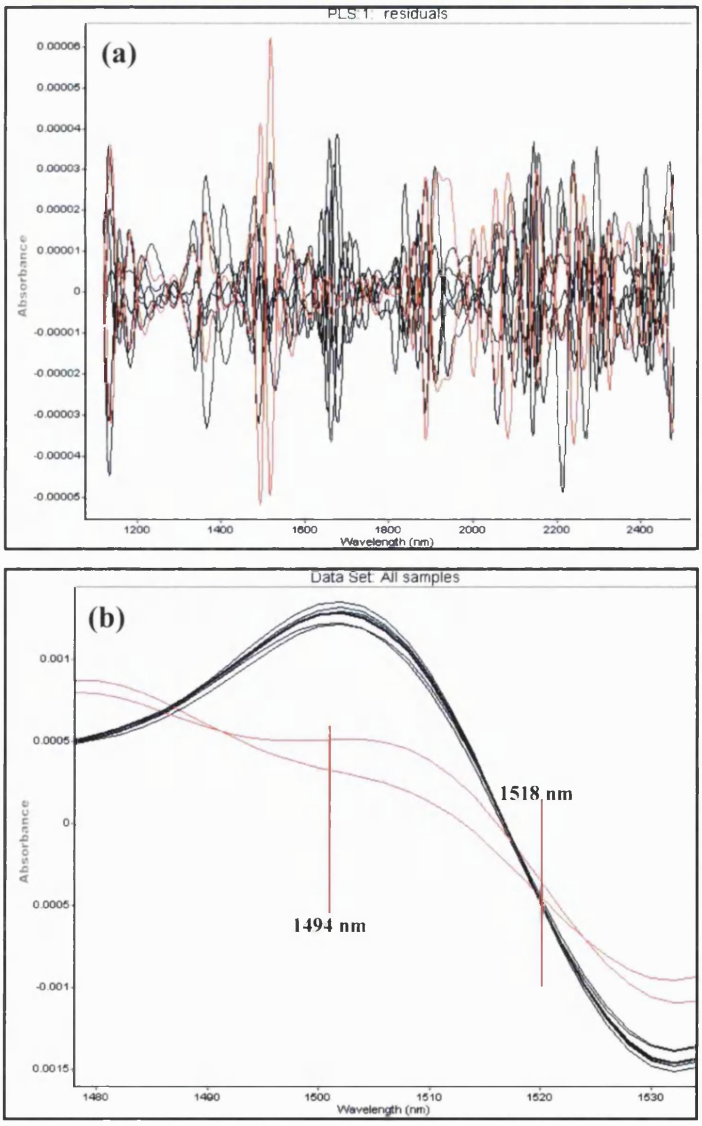

Figure 48: (a) Residual spectra after first two PLS LVs with (b) associated 2nd derivative spectra at identified wavelengths of LV 3

Predicted Error Sum of Squares (PRESS) was calculated for five LVs with leave-one-out cross validation to assist in selecting the optimum number of LVs to use in the model. As expected, Figure 49 shows a continued improvement in the SEE as an increasing number of LVs are included in the model. This relates to the model being better able to describe the extended reference library spectra as more variation is included. However, the SECV shows an improvement as the 2nd LV is included and then increasing error as additional LVs are included in the model. This indicates that the model may be modelling noise and unrelated variation in the data contributed by individual reference library samples, destabilising the model's ability to accurately predict the acceptability classification. Observation of the variation removed in both the x-axis (spectral variance) and y-axis (acceptability variance) as each LV is included in the model, shows a steep increase with the inclusion of the 2nd LV (to approximately 85% for each axis) followed by a slower incline.

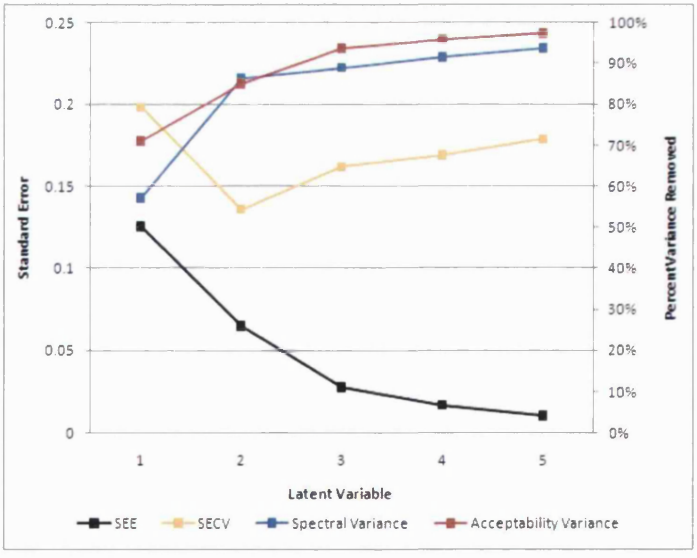

Figure 49: Standard error (class) and percent variance for PLS-DA models developed with varying number of LVs

Review of the scores, the loadings and the PRESS output indicates that two LVs may be optimal, balancing an improved error without over fitting and including noise and unrelated variation. As the PRESS was performed on limited data (8 spectra) it was worth also assessing a PLS-DA model with three LVs to see whether the additional y-axis variation included (reaching 93.6%) leads to sufficient improvement in prediction to offset the risk of adding noise and increasing error.

The PLS-DA model statistics for the two models are shown in Table 9 and indicate that the PLS-DA model with three LVs is more capable of relating the correlation of acceptability class (higher r^2) with lower error. Also note the three LV model compares well with the MLR-DA model statistics described in Table 6.

Table 9: PLS-DA model statistics

	Two LVs	Three LVs
r2	0.9774	0.9959
SEE (class)	0.06958	0.02949
slope	0.9774	0.9959
Intercept (class)	0.01694	0.003044

To assess the capability of the two PLS-DA models to perform satisfactorily for samples independent of those used in the models, deliveries external to the extended reference library were predicted with the models in DeLight and the prediction statistics reviewed. The three deliveries used to assess the MLR-DA model performance were utilised to verify the performance of PLS-DA models developed on both two and three LVs. The predicted results and the residual between the predicted and expected class are shown in Table 10.

Table 10: Predicted material attribute quality conformance for verification deliveries using the two developed PLS-DA models

		Two LVs		Three LVs	
	Expected Class	**Predicted Class**	**Residual**	**Predicted Class**	**Residual**
Verification 1	1	1.070	-0.070	0.9965	0.0035
Verification 1	1	1.043	-0.043	0.9666	0.0334
Verification 2	1	0.8830	0.1170	0.9566	0.0434
Verification 2	1	0.9111	0.0889	1.010	-0.010
Verification 2	1	0.9157	0.0843	0.9953	0.0047
Verification 2	1	0.9154	0.0846	0.9896	0.0104
Verification 2	1	0.9211	0.0789	1.008	-0.008
Verification 2	1	0.8728	0.1272	0.9312	0.0688
Verification 3	0	-0.08713	0.08713	-0.01932	0.01932
Verification 3	0	-0.1406	0.1406	-0.1007	0.1007
Verification 3	0	-0.1299	0.1299	-0.09285	0.09285

The SEP for the PLS-DA predictions were calculated according to Equation 17. The two LV PLS-DA model had a SEP of 0.1045 yielding a SEP:SEE ratio of 1.50 while the three LV model had a SEP of 0.05213 yielding a SEP:SEE ratio of 1.77. As with the Extended Reference Set, the two LVs model SEP is almost twice that of the three LVs model. The SEP: SEE ratio gives an indication of the future capability of PLS models to predict unknown samples. A rule of thumb often used in the pharmaceutical industry (noted in the 2003 EMEA guidance on NIR[143]) is that SEP: SEE ratio greater than 1.4 may indicate potential over-fitting of the model (inclusion of reference specific variation or noise) and may indicate a risk to long term robustness. The required accuracy for a two class DA model is not as stringent compared to the conventional use of PLS for quantitative analysis. Thus it is more useful to note that although the two LV model may be more robust for the prediction of future deliveries (lower SEP: SEE ratio)

this slight improvement is offset by the significant increase in the error in the two LV model compared to the three LV model. Thus, the three LV PLS-DA model was selected for continued development of the material attribute quality conformance method.

The final PLS-DA prediction results for the extended reference library predicted in DeLight (shown in Table 11) were analysed in Minitab and represented graphically with a chart of the individual values (I-charts) with the limits (___) set at three standard deviations from the mean (___) for the six acceptable deliveries. The resulting I-chart for MLR-DA is shown in Figure 50.

Note that the two deliveries in the extended reference library with high amine based residual solvent content are marked in red with a "1" superscript, the notation used in Minitab to indicate data points that fail the control test of being within three standard deviations from the mean.

Table 11: Final predicted material attribute quality conformance for the extended reference library using the final three LV PLS-DA model

	PLS-DA (Class)
Reference library 1	0.9901
Reference library 2	0.9545
Reference library 3	1.011
Reference library 4	1.046
Reference library 5	1.025
Reference library 6	0.9678
Reference library High Amine 1	0.001380
Reference library High Amine 2	0.004710

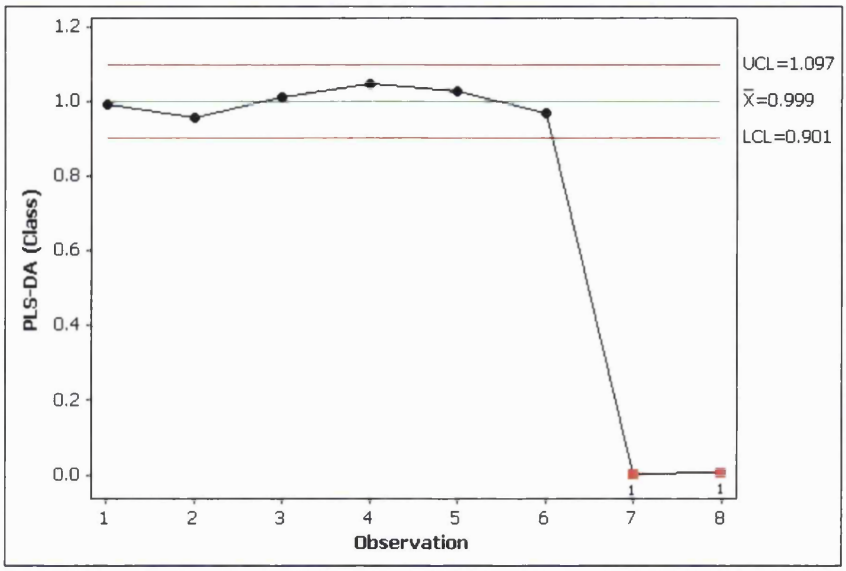

Figure 50: I-chart of predicted PLS-DA results for the extended reference library with points with high amine based residual solvent in red

4.3.2.3 Establishment of the conformance control charts

Once the conformance models have been created, SPC charts must be established that will monitor results over time. This ensures that, although the metric is calculated against a static model, deliveries can be compared to historically acceptable deliveries over time and trends towards non-conformance can be identified prior to failure against the static model. Five historical deliveries of varying number of containers were used to establish the control charts for the material attribute quality conformance methods. These five deliveries were all known to process well and contained typical acceptable amine based residual solvent content. Three deliveries (those used to verify the MLR-DA and PLS-DA model SEPs in Table 8 and Table 10) were then used to verify the material attribute quality conformance SPC charts to demonstrate the suitability of the approach prior to implementation into the manufacturing facility. As was discussed in 4.2.2.3, individual and/ or combinations of containers may be used in production, and both an individual container historical chart as well as an overall delivery chart is valuable.

Shewhart[137] control charts are based on the normal Gaussian distribution and it is important to verify that the conformance data is normally distributed. The Graphical Summary function in Minitab was used to represent the data and assess normality for each conformance metric (Appendix 2 on page 295). No evidence of non-normality was observed (p>0.05) at the 95% confidence level. Normality p-value for each set of data is shown in Table 12. Note that output for overall delivery metrics is an estimate only as the number of data points is small, reducing the power of the normality tests.[135]

Table 12: Summary of the normality assessment (p-value) of the material attribute quality conformance prediction results for the historical data

		TNSD-RR	MLR-DA	PLS-DA
p-value	**Individual Container**	0.439	0.248	0.569
	Overall Delivery	0.369	0.407	0.433

An individual SPC chart (I-chart) was developed to represent the typical quality of individual containers in a given delivery with the control limits set by the individual results from the five historical deliveries. Average results were calculated for each delivery and overall delivery SPC charts developed using the I-chart Minitab function. The mean and standard deviations applied in establishing the control charts are shown in Table 13. Control limits (___) were established at three standard deviations from the mean (___) value.

Table 13: Parameters used to generate the SPC charts for material attribute quality conformance

		TNSD-RR (Absorbance)	MLR-DA (Class)	PLS-DA (Class)
Mean	Individual Container	57.54	1.034	0.9504
	Overall Delivery	54.82	1.027	0.9527
Standard Deviation	Individual Container	16.25	0.04690	0.04531
	Overall Delivery	11.17	0.04850	0.03113

The SPC charts for the three material attribute quality conformance models studied are shown in Figure 51 and Figure 52. These SPC charts show both the five historical deliveries used to establish the control chart limits as well as the three verification deliveries and demonstrates that the SPC charts easily distinguish deliveries with unacceptable amine based residual solvent content.

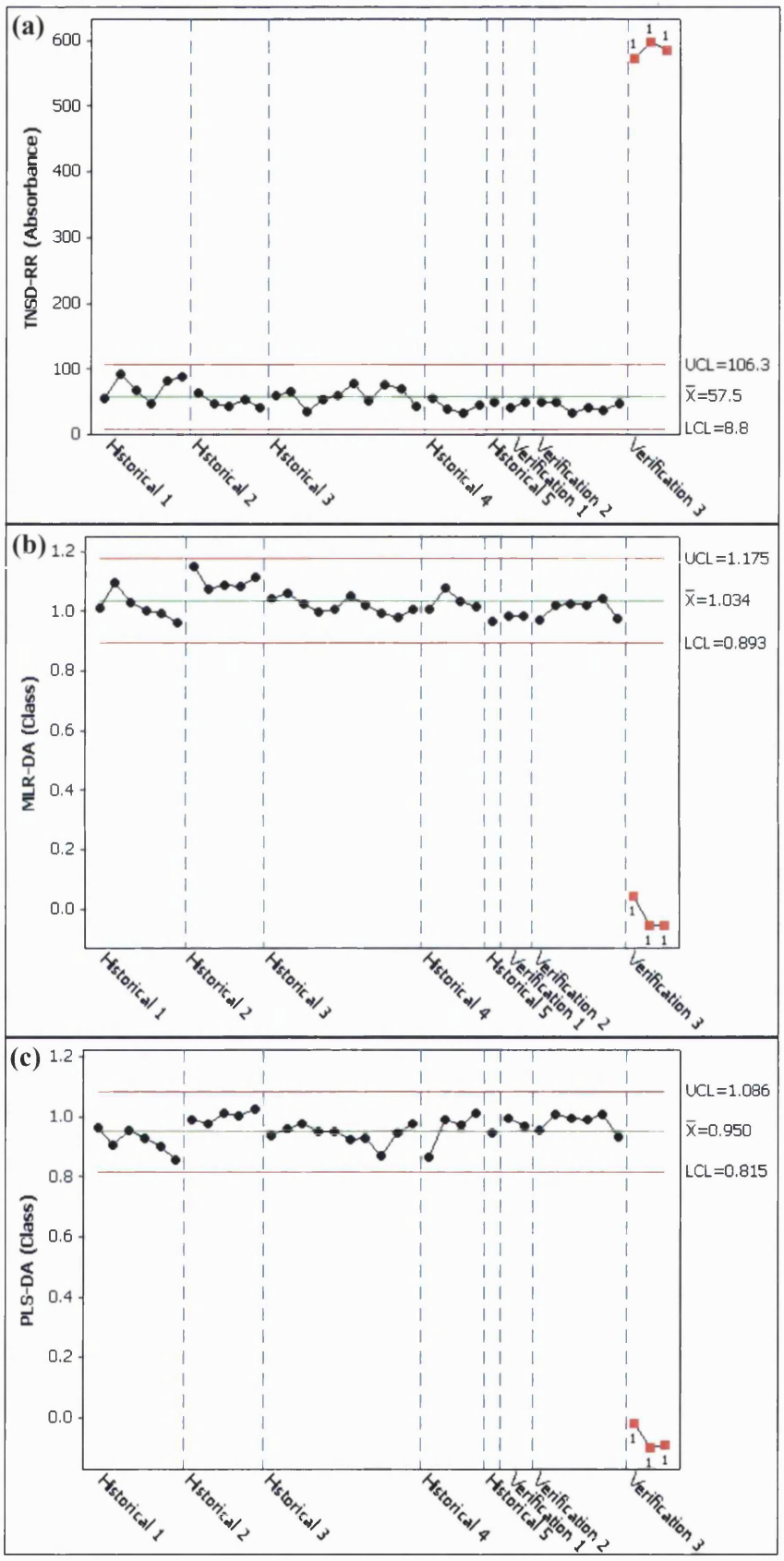

Figure 51: Individual container SPC charts for historical and verification deliveries: (a) TNSD-RR, (b) MLR-DA and (c) PLS-DA with containers with high amine based residual solvent in red

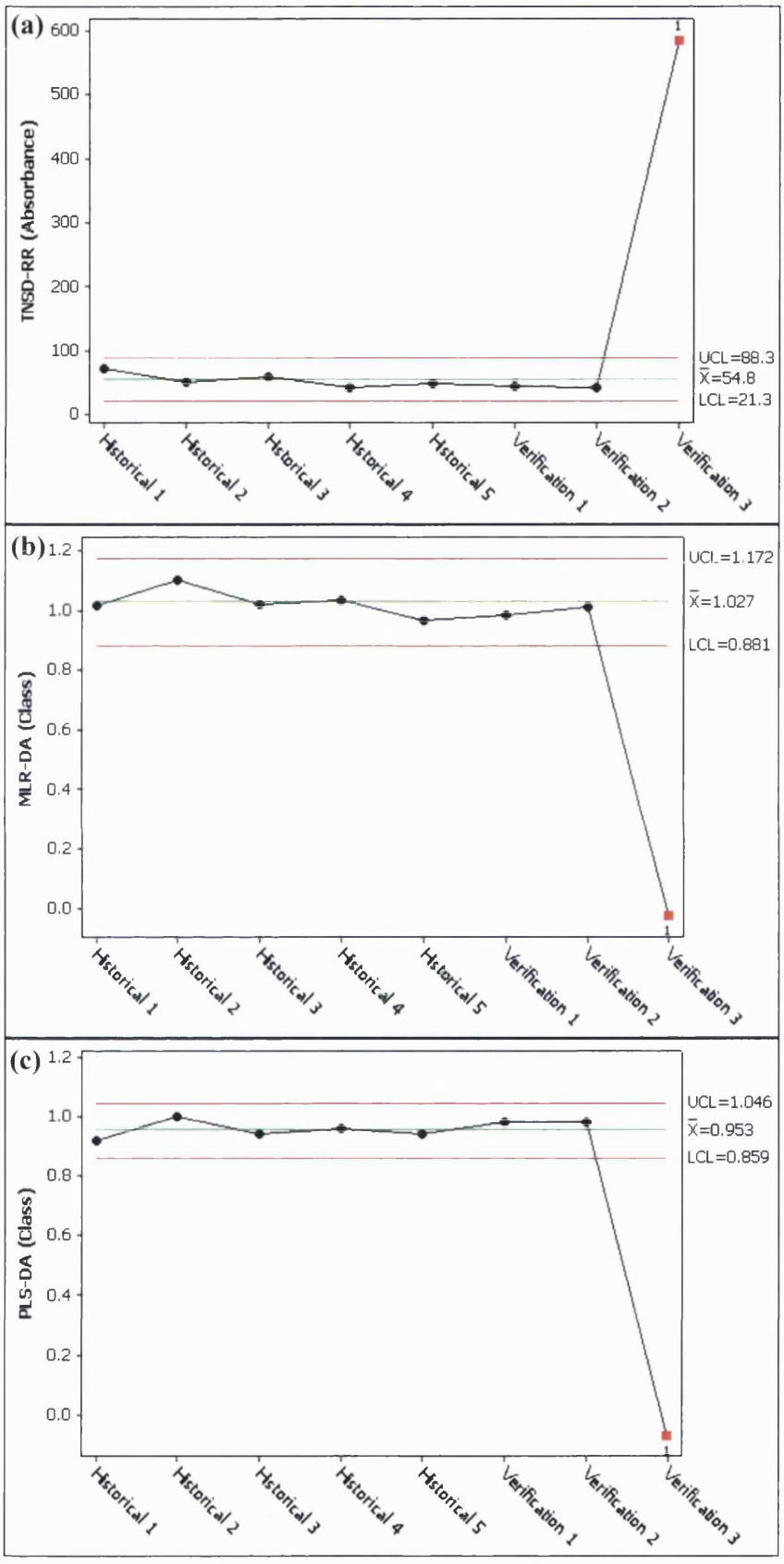

Figure 52: Overall delivery SPC charts for historical and verification deliveries: (a) TNSD-RR, (b) MLR-DA and (c) PLS-DA with deliveries with high amine based residual solvent in red

4.3.2.4 Implementation of the developed method in manufacturing

The developed conformance methods were applied to six subsequent deliveries. The material attribute quality conformance SPC charts showing the historical, verification and subsequent deliveries are shown in Figure 53 and Figure 54. Note that the second delivery is the same delivery as that denoted as the fifth delivery in the global quality conformance method research detailed in 4.2.2.4.

The MLR-DA SPC chart indicated both containers of the second delivery were out of conformance, while only container two showed non-conformance for the TNSD-RR and PLS-DA SPC charts. The fourth delivery was identified as just out of conformance for TNSD-RR, yet was seen as conforming in both MLR-DA and PLS-DA conformance SPC charts.

The fifth and sixth deliveries were both out of conformance for all three methods with the sixth delivery showing the most extreme non-conformance.

The first step in investigating a deviation in conformance is to compare the spectra of the non-conforming containers and deliveries to the acceptable reference library and recent conforming deliveries. Figure 55 shows the raw spectra overlay showing that the fourth delivery has increased absorbance at higher wavelength indicating the material is coarser and more scattering than the acceptable reference library and the other conforming deliveries. Meanwhile, the fifth and sixth deliveries and first container of the second delivery are finer (less absorbing). Container one of the second delivery appears the finest of all samples while container two sits at the coarser end of the range of particle size in the acceptable reference library and conforming deliveries. The difference in particle size of the second delivery was confirmed by appearance during sampling as noted in 4.2.2.4.

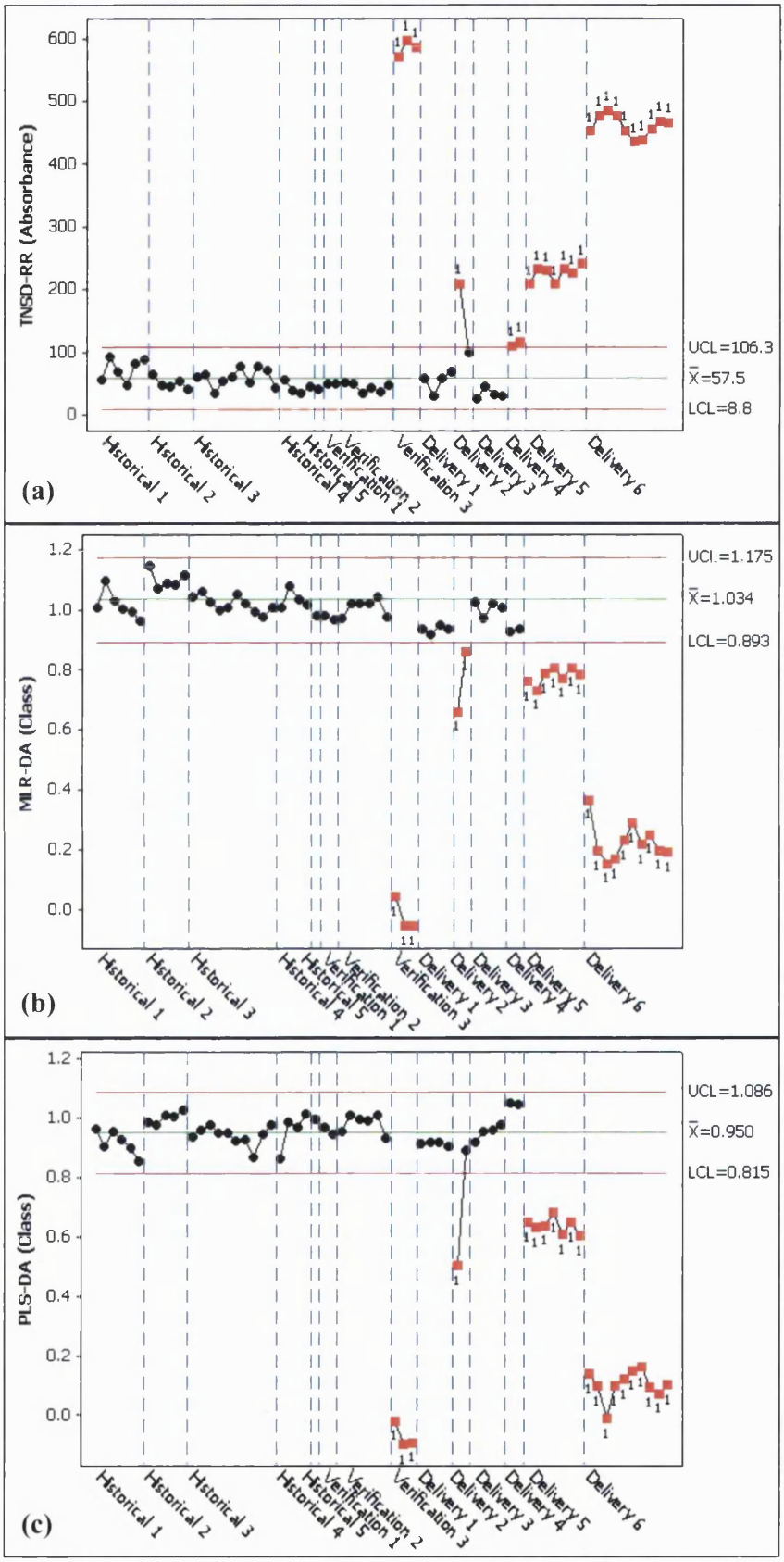

Figure 53: Individual container SPC charts for historical, verification and subsequent deliveries: (a) TNSD-RR, (b) MLR-DA and (c) PLS-DA with containers with high amine based residual solvent in red

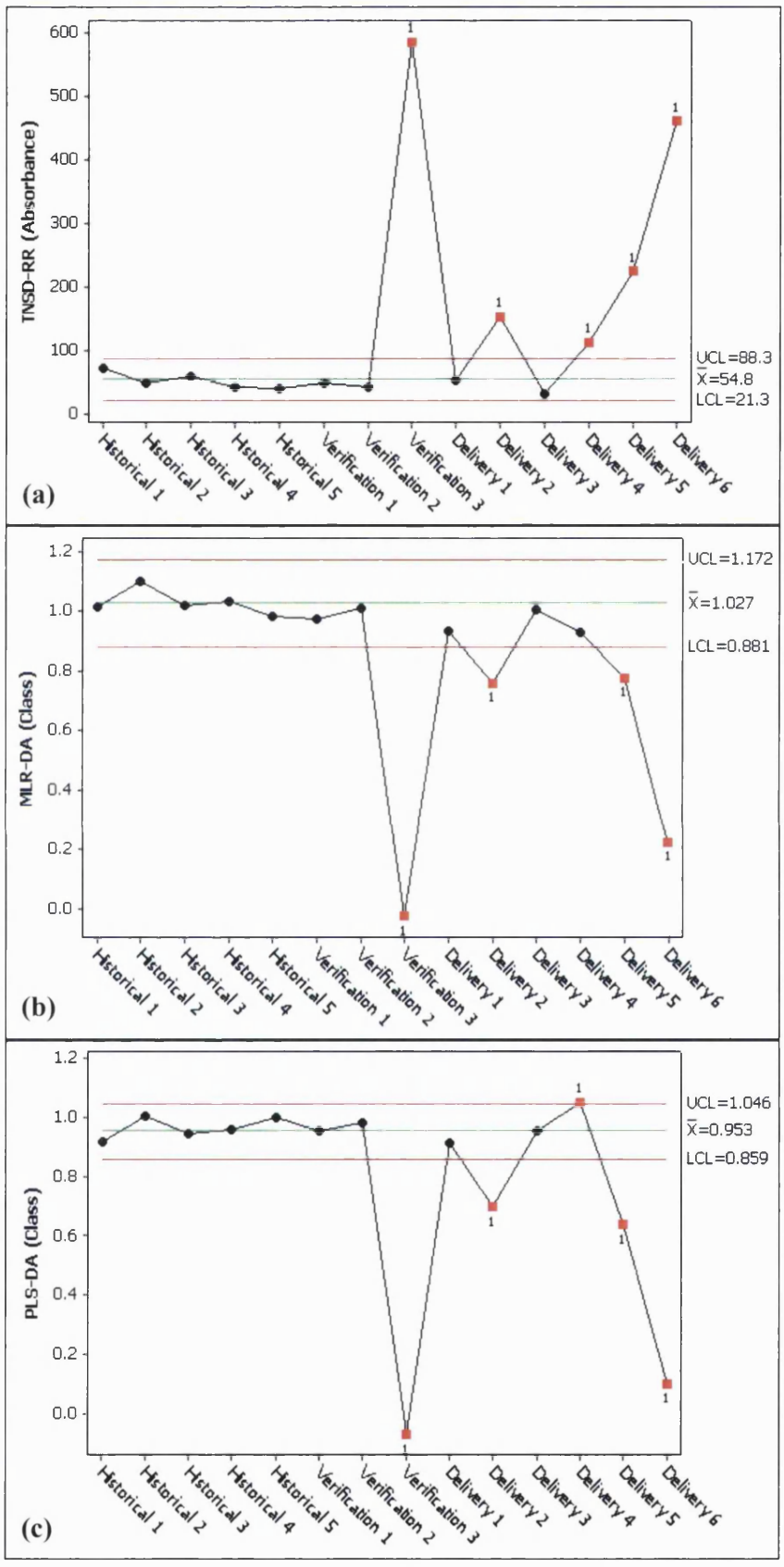

Figure 54: Overall delivery SPC charts for historical, verification and subsequent deliveries: (a) TNSD-RR, (b) MLR-DA and (c) PLS-DA with deliveries with high amine based residual solvent in red

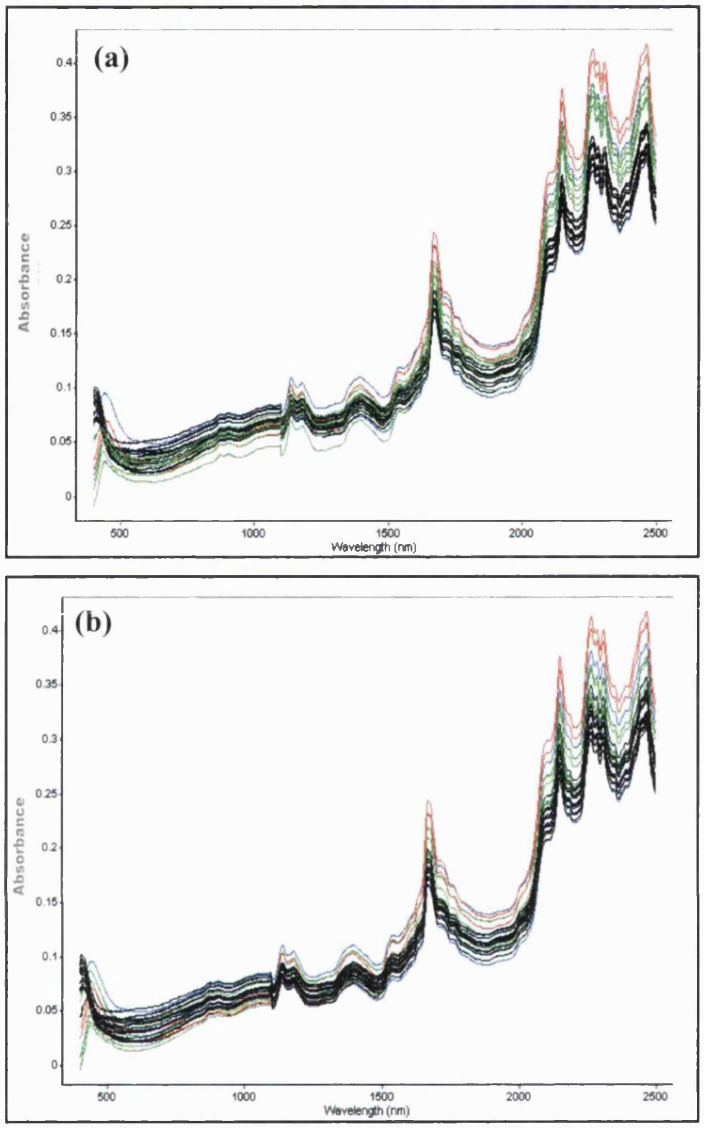

Figure 55: Raw spectra of individual containers for deliveries 2 (___), 4 (___) 5 & 6 (___) overlaid with (a) acceptable reference library (___) and (b) deliveries 1 & 3 (___)

Figure 56 shows the 2nd derivative spectra overlay indicating the regions of most difference are between 1440–1560 nm and 1990–2110 nm (circled in Figure 56) which are the regions identified to be related to the amine based residual solvent content.

Figure 57 contains an expansion of the circled regions in Figure 56 to allow closer examination of the variations occurring in these wavelength regions.

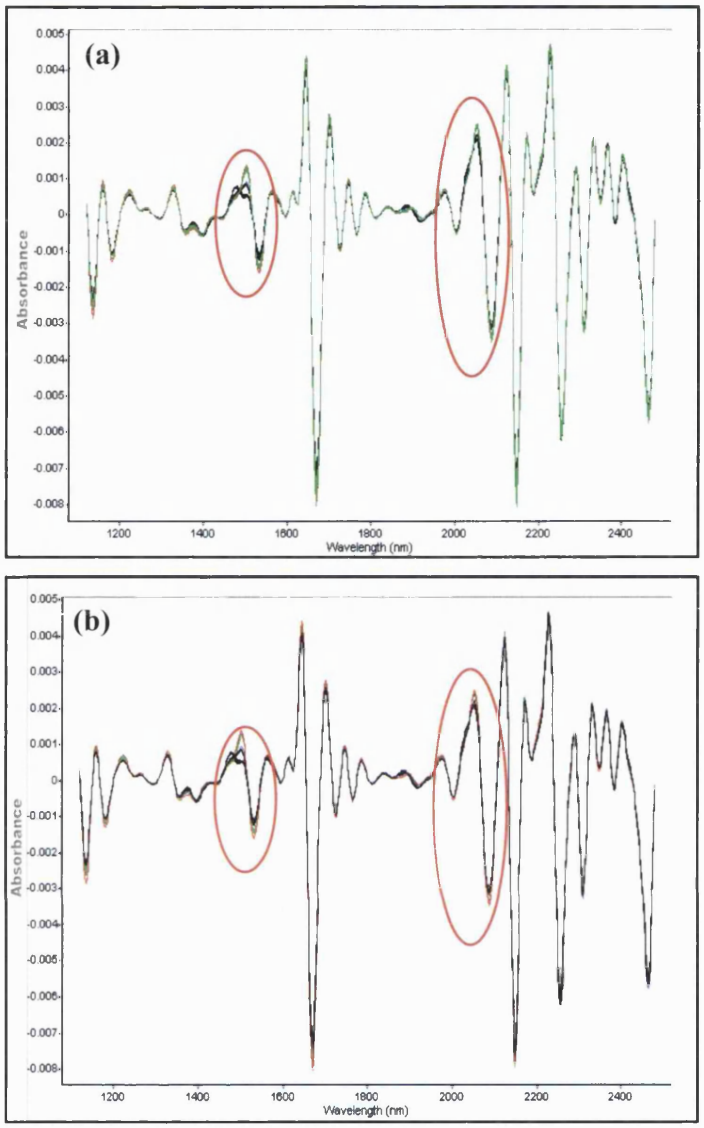

Figure 56: 2nd derivative spectra of individual containers for deliveries 2 (___), 4 (___) 5 & 6 (___) overlaid with (a) acceptable reference library (___) and (b) deliveries 1 & 3 (___)

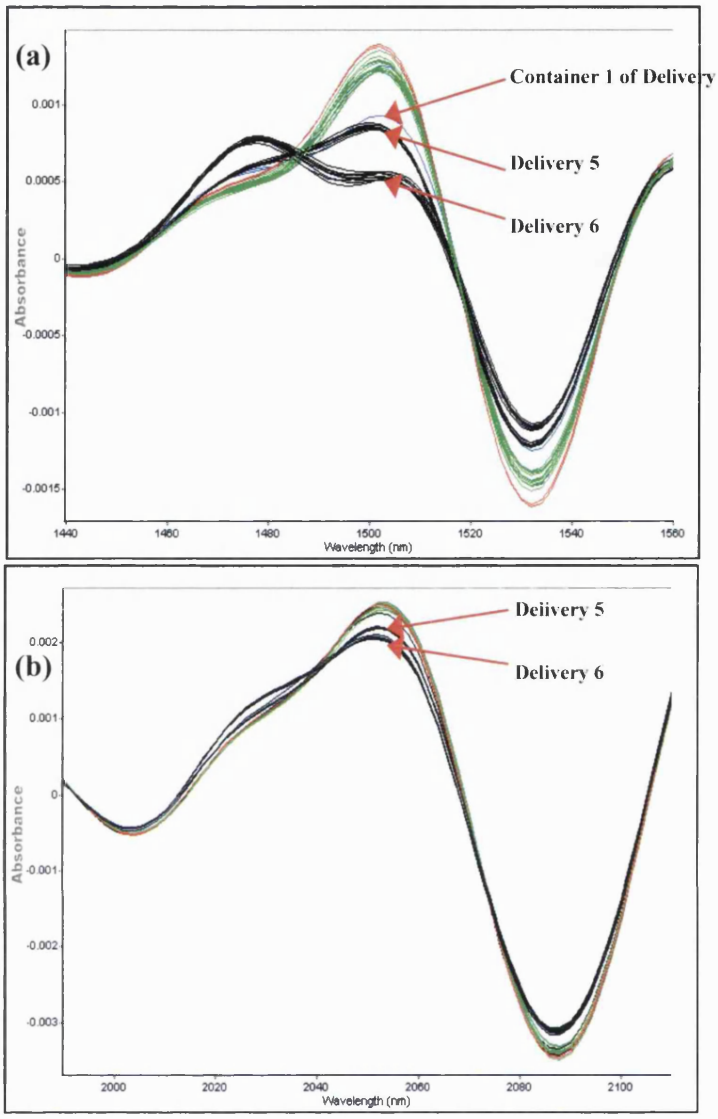

Figure 57: Zoomed 2nd derivative spectra of deliveries 2 (___), 4 (___) 5 & 6 (___) overlaid with acceptable reference library & deliveries 1 & 3 (___) at (a) 1440–1560 nm and (b) 1990–2110 nm

To investigate the source of the non-conformance in TNSD-RR for deliveries two, four and six, Figure 57(a) was examined as this image shows the region used for the TNSD-RR conformance model.

Deliveries five and six and container one of delivery two all showed the characteristic spectral deviation of material with high amine based residual content. The deviation aligns with that seen in deliveries with confirmed high amine based residual solvent content (see Figure 43).The deviation is more significant in delivery six than in both

delivery five and container one of delivery two, indicating that delivery six would be expected to have higher levels of amine based residual solvent. Note that container two of delivery two aligns with the acceptable reference library and conforming deliveries and as such is not seen as out of conformance.

Delivery four is not showing the spectral deviation expected for high amine based residual solvent, rather it indicates a deviation to the acceptable reference library in the opposite direction compared to deliveries with confirmed high amine based residual content. This may indicate delivery four is lower in amine based residual solvent content than the materials in the acceptable reference library.

Figure 57(a) also provides insight into the non-conformance seen in the MLR-DA conformance method for deliveries two, five and six as the absorbance at 1502 nm is the key x-variable in the MLR-DA regression equation. The marked reduction in the absorbance of deliveries five and six at this wavelength leads to the low MLR-DA prediction for these deliveries and subsequently the qualification of these deliveries as out of conformance.

It is interesting to note that both containers of delivery two are found to not conform despite container two of delivery two aligning with the acceptable reference library and other conforming deliveries in this region. The two wavelengths used in the MLR-DA model were scrutinised more closely as shown in Figure 58. Figure 58(a) demonstrates that container two of delivery two aligns well with conforming deliveries one and three at 1502 nm unlike container one which has markedly lower absorbance. However, the situation is reversed at 1614 nm with container one aligning to deliveries one and three while container two has higher absorbance. The inclusion of the absorbance at 1614 nm as an x-variable in the MLR-DA regression equation leads to the MLR-DA predicted result falling just beyond the control limits set from historically acceptable material.

It is also worth noting that the higher absorption for delivery four at both wavelengths leads to reducing the difference in the prediction results for delivery four and this delivery is not identified as a non-conforming delivery as it was with TNSD-RR. The outcomes for delivery two and four highlights the importance of careful selection of the additional 'stabilising' x-variables included in MLR based analysis.

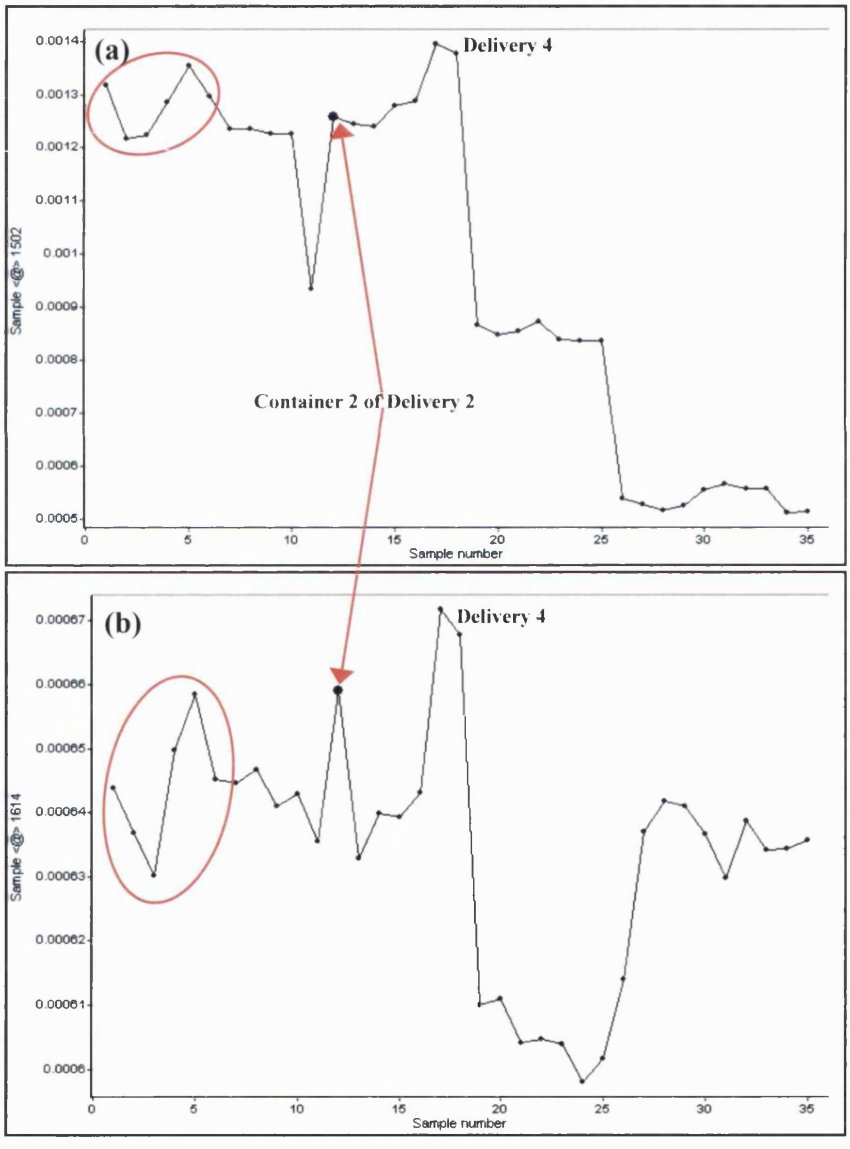

Figure 58: 2nd derivative absorbance of acceptable reference library (circled) and six deliveries at (a) 1502 nm and (b) 1614 nm

To investigate the PLS-DA non-conformance for deliveries four, five, six and container one of delivery two, the wavelengths used in the PLS-DA model (identified in Figure 46) were scrutinised more closely. The key wavelengths utilised in the 1st LV, 1502 nm and 2056 nm, are shown in Figure 57. As discussed with regards to TNSD-RR, Figure 57(a) clearly shows separation of the deliveries and containers with non-conformance compared to the acceptable reference library and the conforming deliveries. This is mirrored also in Figure 57(b) with deliveries five and six separating distinctly at 2056 nm.

The key wavelengths utilised in the 2nd LV, 1670 nm, 2148 nm and 2256 nm, are shown in Figure 59. These wavelengths show grouping of non-conforming and conforming deliveries. Note that in Figure 59(a), delivery four is absorbing more strongly than the conforming deliveries one, three and the acceptable reference library, while deliveries two, five and six have lower absorbance. As discussed with regards the TNSD-RR results, the non-conformance in delivery four is not the same as in deliveries five and six. It is also worth noting that delivery four was not identified as out of conformance with individual samples however it is flagged with the tighter control limits for the overall delivery control chart.

The key wavelengths utilised in the 3rd LV, 1494 nm and 1518 nm are also included in the region shown in Figure 57(a). As mentioned during model development, these wavelengths are not signifying peak absorptions; rather the variation at these wavelengths is related to changes to inflection points and slopes due to the surrounding peaks (the largest of which is the key wavelength in the 1st LV).

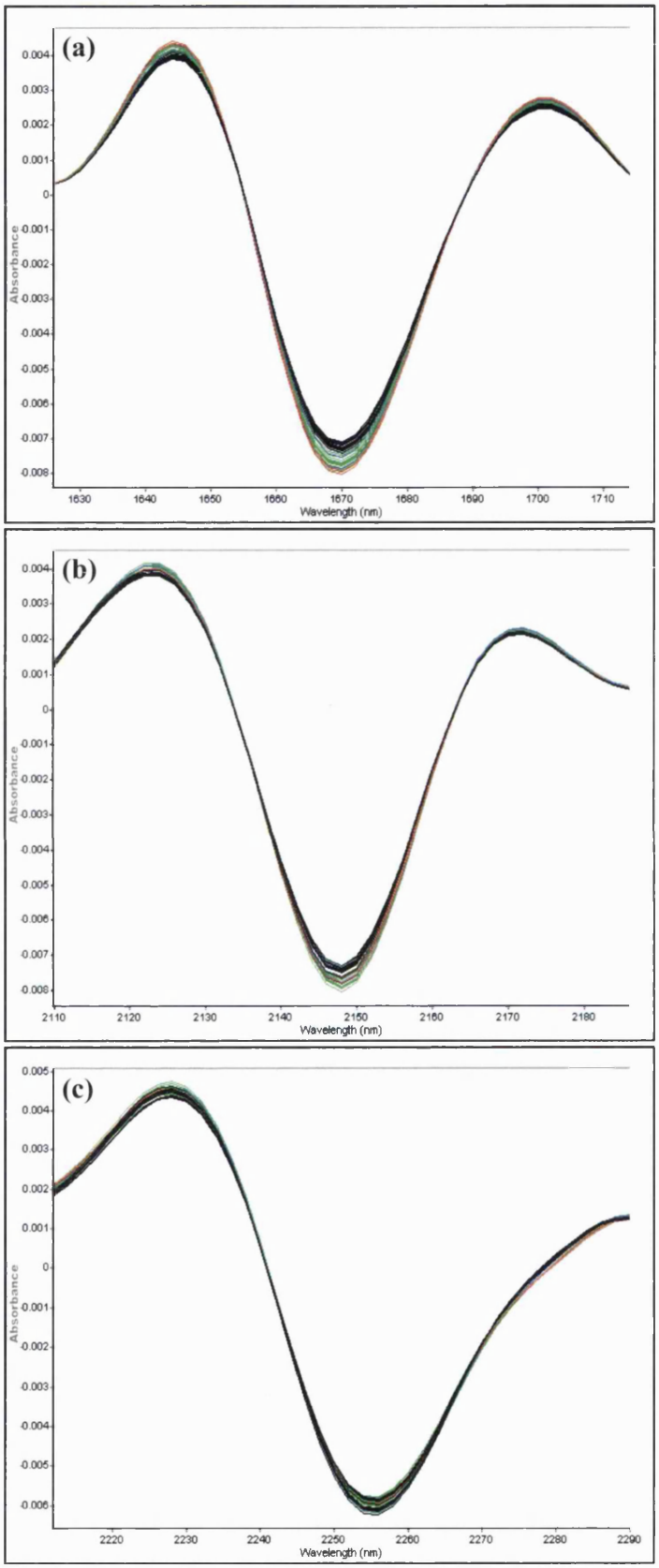

Figure 59: 2nd derivative spectra of deliveries 2 (___), 4 (___) 5 & 6 (___) overlaid with the acceptable reference library, deliveries 1 & 3 (___) at (a) 1670 nm , (b) 2148 nm and (c) 2256 nm

4.3.3 Material attribute quality conformance method discussion

4.3.3.1 Impact of non-conformance for the product and process

The results of the material attribute quality conformance for deliveries two, four, five and six alerted the Quality and Production departments that these deliveries had amine based residual solvent levels deviating from that found in material with acceptable levels. This assisted in scheduling decisions as the production team was forewarned that processing would likely be slowed for the deliveries with suspected high amine based residual solvent. Due to the ongoing investigation initiated by the first delivery with non-conformance of the global quality material conformance, all deliveries received full confirmation testing according to the registered specification and results aligned with the results on the CoA, passing all specified acceptance criteria.

As mentioned in 4.2.3.1, container two of delivery two was found to have become caked into one solid mass during storage and this container was rejected and not utilised in production. Close observation of the use of container one of delivery two in production showed significant impact to processing with the product blend sticking considerably to the tablet press, extending processing time two-fold and impacting the product quality through poor clarity on the tablet embossing. Delivery five showed similar delays in production, while delivery six was found to have an extreme impact on processing almost halting manufacturing with hourly stoppages to remove sticking material from punches. It was also found that the impact to processing was most severe for the smaller 5 mg dosage strength tablet compared to the 10 mg tablet. This was hypothesised to be a function of the tablet surface area and the different compaction force used during tabletting to meet the different hardness specification for the two dosage strengths (11-14 kg for 5 mg tablets compared to 28-30 kg for 10 mg tablets).

Delivery four, which showed an inverse variation to the other non-conforming deliveries, processed well in production. The identification of the non-conformance for this delivery assisted in understanding the relationship of the variation in the spectra and impact on processing. Through the non-conforming deliveries, it was demonstrated that the more extreme the non-conformance of the material attribute quality conformance the larger the impact in processing.

Data from the material attribute quality conformance method was utilised to assist with scheduling production of Norvasc® through either mixing material from deliveries predicted to impact processing with deliveries predicted to process well or allocating the deliveries with severe non-conformance to the 10 mg dosage strength. In doing so, material could be utilised with reduced impact on processing and product embossing appearance.

The approach also assisted a global investigation into the issue which identified impact to processing at several other Pfizer drug product manufacturing facilities. The severity of the impact appeared to vary related to tablet press model, compaction settings, tooling surface finish and quality and percent of magnesium stearate (lubricant) in the formulation. The production facility for which this research was performed, utilised the data to negotiate lot selection at the API manufacturing site so that, where possible, material predicted to impact the production facility would be redirected to other global sites with less severe impact while resolving the issue and implementing mitigation procedures.

4.3.3.2 Conformance method selection for continued implementation

Review of all three conformance methods within this research provided insight into the capabilities of the three approaches to suit practical implementation. Section 4.3.2.4 demonstrated that all three approaches were able to correctly differentiate non-conforming deliveries five, six and container one of delivery two which impacted processing.

TNSD-RR also identified the individual containers and overall delivery quality for delivery four to be out of conformance. For the purpose of the conformance method (to identify deliveries with unacceptable amine based residual solvent) this can be seen as a false negative. As TNSD-RR is based on the absolute variation to the average spectra of the acceptable reference library, the control chart does not give any indication of directionality. Absolute spectral differences are typically used for 2^{nd} derivative spectra as a single positive peak in raw spectra will have a central negative peak with two positive shoulder peaks in the 2^{nd} derivative spectra (see Figure 7). Thus, without the use of absolute SDs, deviations in the shoulder peaks will negate deviations in the central peak. The lack of directionality in the conformance control chart is therefore a limitation to the TNSD-RR approach. Both MLR-DA and PLS-DA conformance methods incorporate directionality of the deviation. This can be seen by the fact that the output for delivery four is at the opposite side of the centre line in the MLR-DA and PLS-DA control charts in Figure 53 and Figure 54. It is worth noting that delivery four is found as out of conformance for the PLS-DA SPC chart for overall delivery quality. Again, this could be considered a false negative given that delivery four processed well. However, the directionality of the control chart coupled with the fact that the individual containers were acceptable according to the individual container SPC chart lends useful information as to this delivery's level of amine based residual solvent content.

MLR-DA also identified container two of delivery two to be out of conformance. Whether this is a false negative is challenging as this container was not utilised in production. However, review of the data indicates that the cause of the variation was due to a relative change in the stabilising wavelength used in the MLR-DA calculation. This is an interesting outcome and it may be worth further scrutiny given the fact that this container formed a solid mass on storage while other containers and deliveries did not. There is therefore something uniquely different about this container which probably should have been flagged. However, this material attribute conformance method was established to analyse the acceptability of the amine based residual solvent content. For this reason, the identification of this container to be out of conformance by MLR-DA can be seen as a false negative. This demonstrates the criticality in identifying the most appropriate stabilising / supporting wavelengths when applying MLR analysis. It would be possible to redevelop the MLR model using different secondary stabilising wavelengths. However, given the high number of possible wavelengths, PLS-DA provides a more practical approach through the use of LVs to weight wavelengths appropriately in the regression equation.

Based on the lack of dimensionality in the TNSD-RR and the criticality of secondary wavelength selection in MLR-DA, this work indicates that PLS-DA is the optimal approach to use for continued implementation.

Further enhancements could be made through including more than two classes in the model (e.g. to categorise deliveries as acceptable, moderately or severely deviating) once further data was gathered and aligned with feedback on processability from production. Additionally, control limits could be established based on historical data for deliveries with extreme non-conformance to indicate deliveries that should be rejected (with agreement with API Supplier). However, the Pfizer facility was satisfied with the two class DA approach with control charts based on historically acceptable material.

Though the SPC charts in this research were performed in Minitab, the Pfizer facility chose to implement the conformance method and SPC charts with Excel due to operator familiarity and accessibility.

As was discussed in Section 4.2.3.2, vendor software does not often provide the capability to implement NIRS conformance methods. In general trending of results is not available in vendor software for at-line NIR instruments and SPC charts often need to be developed external to vendor software. Additionally, not all vendor software provide flexibility in the quality metrics available for normalised SD (as mentioned in Section 4.2.3.2, normalised SD algorithms may be missing entirely or report only the maximum value at any wavelength in the region used). The majority of vendor software includes MLR analysis and all include PLS regression.

The DeLight software utilised at the site allowed simple implementation of the methods in the facility. The DeLight software has the capability to reproduce the individual container I-chart including control limits to allow the operator to monitor the results in real time. As mentioned in 4.2.3.2, DeLight does not have the capability to reproduce the overall delivery SPC chart. As such, following data acquisition, data were exported to Excel for SPC trending and review by Quality Assurance personnel.

Figure 60 shows the user interface that operators were able to use to monitor the material attribute quality conformance in real time during material receipt testing. In this case, the user interface incorporated output for both the global and material attribute quality conformance methods in one combined simple user interface for warehouse personnel.

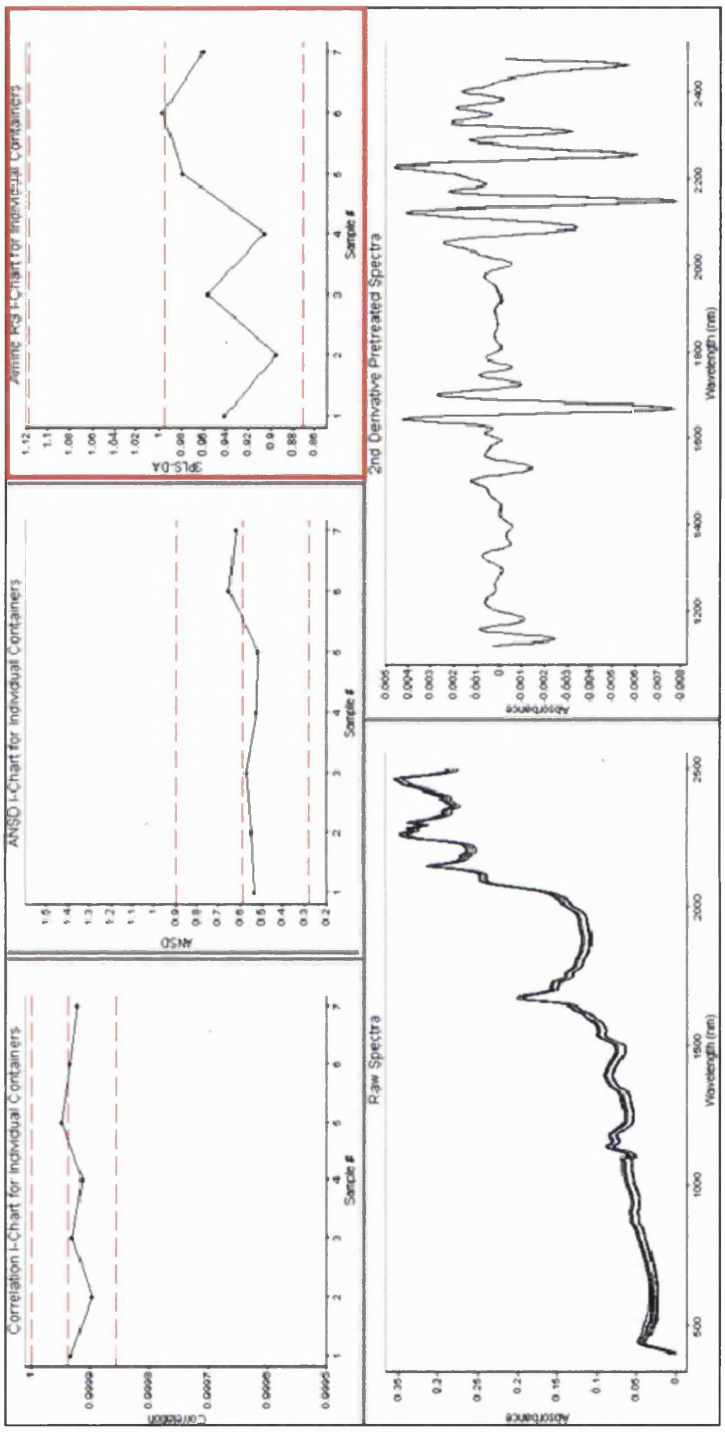

Figure 60: Operator interface for developed within DeLight Software with material attribute quality conformance individual container control chart marked in red

4.3.4 Summary - criticality of research

This research investigated the use of continuous data output from three qualification algorithms (TNSD-RR, MLR-DA and PLS-DA) and SPC charting to create a material attribute quality conformance approach for monitoring undesirable amine based residual solvent content in amlodipine besylate. Typically, qualification algorithm output is used simply as a categorical classification technique (pass / fail) and the extended use of the data with SPC has not been previously reported.

Three material attribute quality conformance methods were developed and applied in parallel to real deliveries of amlodipine besylate at a Pfizer facility and the value of the work was demonstrated in the ability to identify non-conforming deliveries as described in 4.3.3.1. The benefits and limitations to the three different methods were compared and commentary was provided as to the availability of vendor software to easily implement such approaches.

Material attribute quality conformance methods were demonstrated to enable rapid identification of material variation with the potential to greatly impact the pharmaceutical manufacturing process and / or product quality. The approach facilitated processability prediction and appropriate scheduling of material use in manufacturing and also provided the means to negotiate preferred manufacturers lot selection based on established causality. The work also continued to provide valuable information into the global effort to establish the root cause of material variation impacting production of a key Pfizer product.

4.4 Review of research outcomes

The research described in Chapter 4 successfully addressed the identified need for the development of novel applications of NIRS for at-line analysis of materials aligned with PAT. The innovative approach of NIR Material Conformance enables powerful process understanding of the material variation and its impact on product quality and process performance. Rather than the traditional application of NIR for material identification or the use of the NIR data for pass / fail categorisation of material qualification, the developed NIR Material Conformance approach utilises the continuous nature of chemometrics qualification algorithms coupled with SPC to monitor material rapidly at receipt and assess whether the delivery conforms to statistical expectations compared to both the validated static model and continuously evolving historical deliveries. Deviations from SPC can be identified prior to impact to product quality and process performance. Behaviours of the material in the formulation and in future process steps can be predicted based on enhanced causal knowledge.

The approach goes beyond the black box approach to qualification model development. To gain real process information requires a skilled practitioner to delve deeper into the analysis of spectral deviations and the chemometrics behind the algorithms available in software.

To harness the breadth of possible applications and the diverse use of chemometrics for such Material Conformance methods, vendor software will need to evolve to enable greater functionality in both chemometrics algorithms as well as statistics. Pfizer continues to work with strategic vendors to attempt to influence the direction of software updates. However, as discussed in Section 3.5, this has not been overly successful to date.

The research met the desired objectives of developing novel ways to apply at-line NIR for material analysis, aligning with the PAT philosophy described in Chapter 1 and addressing the gaps in material analysis identified in Chapter 2. The work was well received by the Pfizer facility from warehouse operators and Quality personnel through to management and provided an unprecedented level of insight into the material characteristics of an important raw material.

CHAPTER 5 APPLICATION OF AT-LINE NIRS FOR PAT

APPLICATIONS IN SOLID DOSAGE PHARMACEUTICAL PRODUCTION – TABLET ANALYSIS

5.1 Introduction

As discussed in Section 2.5, historical application of NIRS for intact tablet analysis has concentrated on the API potency prediction followed by content uniformity determination to decrease the cycle time through the laboratories, exploiting the advantages of NIRS analysis being fast and non-destructive. These methods are not typically applied to gain process understanding and thus not aligned with the PAT philosophy. Applying NIRS at-line for process monitoring rather than as product release testing in the laboratory provides a mechanism for gaining process understanding and inherent quality improvements.

The proposed conformance methodology builds on the material attribute quality conformance approach discussed in Section 4.3. The focus of NIR tablet conformance methodology is to assess whether the quality attribute of interest (typically the API content) fits within a previously established acceptable and/or 'normal' population and combines qualitative or semi-quantitative chemometrics with SPC techniques.

Though different algorithms have historically been used to assess the conformance of a sample spectrum to the predetermined acceptable set of spectra (particularly applied for raw material quality assessment), any algorithm result, spectral processing output or straight absorbance value can be utilised in a conformance method.

A benefit of conformance methodology over traditional quantitative methods, is that reference chemistry can be restricted to samples used to validate the method, greatly reducing the large extent of reference chemistry which is typically conducted when

developing quantitative NIRS methods. This leads to rapid development of the conformance methods.

Section 5.2, describes the investigation of qualitative NIRS tablet conformance methods to provide a method to assess the API content of tablets and determine the quality of the product by demonstrating that the product is consistent to the predetermined threshold of acceptability (obtained from a historical set of acceptable batches). Once established, this method provides a mechanism to monitor the quality of the tabletting process by trending the result for a large number of tablets throughout manufacturing, providing the opportunity to establish normal process signatures for the tabletting process.

Section 5.3 extends the NIR tablet conformance methodology with semi-quantitative regression based chemometric algorithms. Rather than utilising reference values, this semi-quantitative approach extends DA by the use of nominal values in the regression methods explored. The semi-quantitative output is then applied using SPC techniques to monitor the tabletting process. Once more, the combination of SPC with the conformance model data provides a window of insight into the manufacturing process.

Although the explored conformance methodology is not targeted for regulatory filing, aspects of analytical method validation for such qualitative and semi-quantitative methods are explored in Section 5.4 to address concerns on how to validate the underlying analytical methods which will provide a mechanism for quality assurance. Fit for purpose validation of the methods is discussed, aligning with ICH Guidelines on Analytical Method validation.

5.2 Qualitative tablet component quality conformance

The development and application of a Qualitative Tablet Component Quality Conformance method is described in this section demonstrating the value of this research. Continuing from the product focus in Chapter 4, Norvasc® 5 mg tablets was selected as the target product as it was the highest volume and highest value product at the manufacturing facility in which the work was undertaken. The component of interest was the active component of Norvasc® 5 mg tablets, the amlodipine base.

5.2.1 Materials and methods

5.2.1.1 Design of analysis

A minimum of five batches were required to establish the normal range of spectra for Norvasc® 5 mg tablets and comprise the historical dataset used to develop the conformance model and establish SPC charts. To ensure a suitable degree of natural process and material variability in the tablets was represented in the historical data set, ten batches were obtained covering several years and compressed on all tablet presses utilised for the product at the manufacturing facility. A subset of these batches was then chosen to be included in the historical dataset equally representing each of the tablet presses utilised, with the remaining batches available to assist in optimising and verifying the suitability of the method.

To represent typical within batch variability, 10 tablets for each batch were utilised for the historical dataset. Various qualitative algorithms were explored to select the most appropriate modelling approach for the qualitative tablet component quality conformance method. As production processes are highly controlled, the natural range of concentrations of the API in production tablets is very narrow and the likelihood of sampling tablets from the tails of the distribution to extend the range of the method is

very low. As such, extended range tablets were manufactured at pilot scale. Though ideally extended range tablets would be made based on DoE, exploring variation of all components in the matrix, given financial and time constraints, a simple spiking with API (amlodipine besylate) and dilution with diluent (microcrystalline cellulose PH 102) approach was selected to develop the extended range using a production blend of the Norvasc® formulation. Tablets were then compressed to mimic the same physical traits of the Norvasc® production tablets. The extended range tablets were used to assist in optimising and verifying the suitability of the method.

The developed qualitative models were then applied to nine commercial tablet batches with appropriate frequent time based sampling to allow the development of process based SPC charts. Six batches, manufactured on the three tablet presses the facility uses to manufacture Norvasc®, were used to establish normal process behaviour and the remaining three commercial batches (one from each tablet press model) were utilised in assessing the suitability of the complete Qualitative Tablet Component Quality Conformance Method.

5.2.1.2 Reagents and samples

Non-film coated Norvasc® tablets were manufactured at development (pilot batch) and production scales (over a four year period). Norvasc® 5 mg production tablets nominally have 5 mg amlodipine base content per 200 mg tablet (i.e. 2.5% weight basis). Norvasc® are formulated with the API amlodipine besylate rather than the active component, amlodipine base. For ease of discussion, amlodipine base is termed the "amlodipine active" and amlodipine besylate the "amlodipine API" for the remainder of Chapter 5.

Development scale tablets were produced by dry blending the amlodipine API with the other tablet components (microcrystalline cellulose PH102, dibasic calcium phosphate, sodium starch glycollate and magnesium stearate) and compressing into 200 mg tablets. The amlodipine API and microcrystalline cellulose PH 102 (nominally 62.0% weight basis) were varied in the different development batches while keeping the remaining components constant to form a suitable range of amlodipine active content, expressed as 75%, 85%, 115% and 125% of label claim.

For each development batch, blends were pressed into white, octagonal shape tablets (average thickness 3.43 mm, and average mass 200 mg), with a bisect line on one face. The development tablets were devoid of embossment and markings.

HPLC grade methanol and purified water (both Riedel-de Haën, Seelze, Germany) and potassium dihydrogen orthophosphate (Fisher Chemicals, Loughborough, Leicestershire, UK) were used for the HPLC mobile phase and diluent solvents.

5.2.1.3 NIR apparatus and software

NIR transmission spectra were measured using a FOSS NIRSystems 6500 Series II spectrophotometer (FOSS NIRSystems Inc., Silver Spring, MD, USA) configured with an InTact™ tablet transmittance analyser (NR-1650) with an Indium-Gallium-Arsenide (InGaAs) detector. The spectrophotometer was controlled by DeLight software, version 2.3b and D2NIRS software, version 1.2a (DSquared Development, La-grande, OR, USA).

NIR spectra were measured for individual tablets over the wavelength range 600-1900 nm at 2 nm intervals. Each recorded spectrum was the average of 32 individual scans (a total of 35 seconds scan time per tablet) and recorded with respect to an air reference. The ten tablets in each of the ten production batches as well as ten

tablets from each of the four 75% to 125% development batches were scanned in a typical laboratory temperature / humidity environment of 20° to 25° C / 60% relative humidity using custom made tablet holders to minimise light leakage and assure reproducible sample positioning and presentation. Tablets were placed in the tablet holders with the same orientation to ensure the bisect line (and embossing for production tablets) contribution to scatter was consistent to minimize spectral variation. A total of 140 spectra were saved for threshold and method validation. NIR spectra for individual tablets sampled throughout commercial production for the nine batches studied were acquired using the same procedure.

Individual tablet components were evaluated utilising a 1 mm deep top loading transmission cell (glass 20-C 1 mm cell (Starna Pty Ltd, Australia)) for presentation for transmission NIR analysis.

Data analysis and model development were achieved using DeLight software, version 2.3b and DMentia 1.1b software (DSquared Development, La-grande, OR, USA), Minitab version 16.1 (MinitabTM, Inc.) and Microsoft® Excel, version 9 (Microsoft® corporation). Minitab® 16 version.1.16 (Minitab Inc, State College, PA, USA) was utilised for statistical evaluation and SPC chart development.

5.2.1.4 HPLC apparatus, software and methods

Reference chemistry measurements were made using a Thermo Separation Products and Varian integrated HPLC systems (Aligent Technologies, Palo Alto, CA, USA) with UV detection. The columns used for this reversed-phase method were Supelcosil LC-18-DB with a 50 mm length × 4.6 mm internal diameter stainless steel columns (Supelco) with 5 μm particle size packing. The operating temperature was ambient (20° to 25 °C).

The amlodipine active content of individual tablets was measured by isocratic reversed-phase HPLC with UV detection at 237 nm. The mobile phase was purified methanol: 0.03 M phosphate buffer (600:400, *v/v*), and the flow rate was 1.5 mL/ min. The phosphate buffer was made by dissolving 4.08 g of potassium dihydrogen phosphate in distilled water in a 1 L volumetric flask which was then made up to volume.

Sample solutions were prepared by dissolving an individual tablet in 200 mL of mobile phase, and then diluting 25 mL of this stock solution to make a 50 mL working sample solution. Duplicate 20 µL injections were made for each sample and the peak areas measured. To calibrate the system, standard solutions of the amlodipine active were prepared by dissolving 35 mg of amlodipine API reference standard in 200 mL mobile phase and diluting by the same factor as used for the tablet samples. Duplicate injections (20 µL) were made for each standard and the measured peak areas used to construct a peak area vs. mass of amlodipine active calibration curve.

5.2.2 Qualitative tablet component quality conformance method development

5.2.2.1 Spectra pre-treatment

As described in Section 4.2.2.1, the gentlest mathematical spectral pre-treatment should be applied to remove noise and reduce specular reflection without masking or hiding spectral features that may be useful in the analysis. The spectra from the ten production and four extended range tablet batches were utilised for optimising mathematical pre-treatments and derivative transforms. Table 14 describes the optimised pre-treatment with the associated desired effect. Figure 61 shows the success of the mathematical pre-treatment to remove noise and normalise baseline shifts due to pathlength effects of within and between batch tablet thickness variations and blend density scattering effects. The success of the pre-treatment can be seen in Figure 61.

Table 14: Optimisation of data pre-processing treatments for tablet analysis

Extract 800–1360 nm	Remove regions of high noise and non-linearity of the detector response as well as region below 800 nm impacted by product colour in the visible region
Five point smoothing	Provide continuous curve through the 2 nm data interval
SNV normalisation	Remove baseline, path length & multiplicative scatter effects

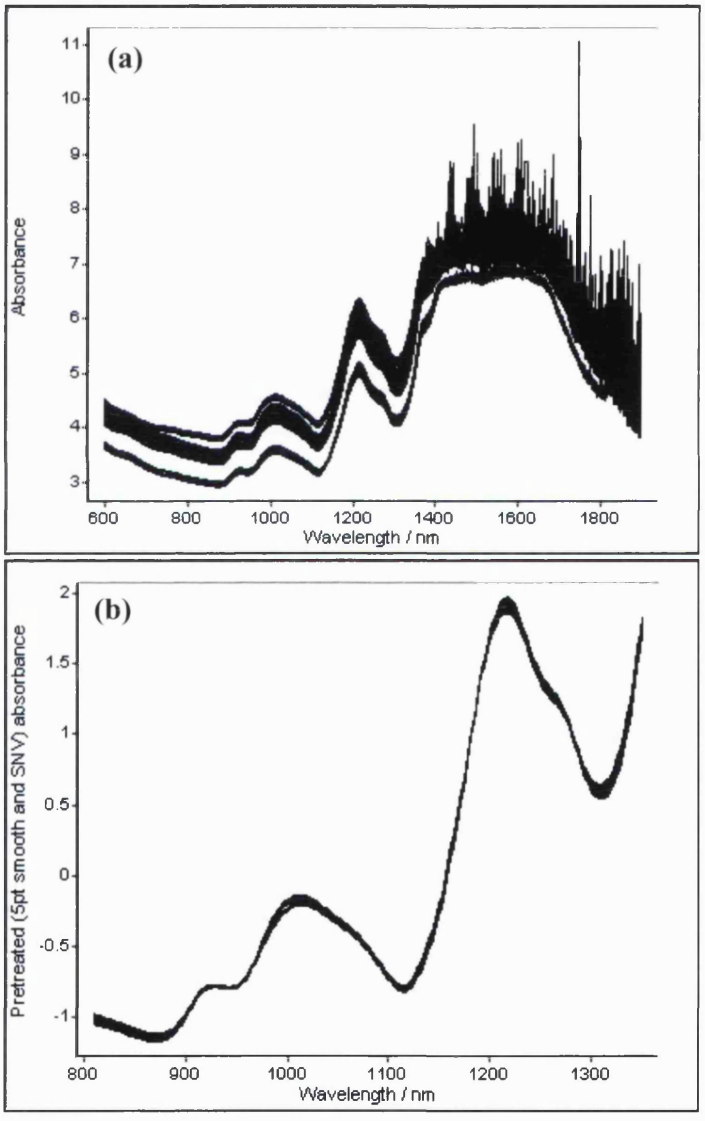

Figure 61: (a) Raw NIR spectra of complete data set across full wavelength range, (b) pretreated spectra NIR with reduced wavelength range, five point smoothing and SNV normalisation

Derivatives are commonly applied to further remove slope effects and enhance spectral peak features. For complex matrices such as tablet formulations, it is important to apply appropriate derivative treatment to extract useful spectral features from the overlapping absorption bands of both the API and other components in the formulation without loss of information. This is vital in Norvasc® tablets as the percentage of amlodipine active in the formulation is low (2.5% on weight basis).

The typical derivative transform used for tablet analysis recommended by FOSS is 2nd derivative. The resulting spectra from application of 1st and 2nd derivative transforms with five point derivative gap are shown in Figure 62.

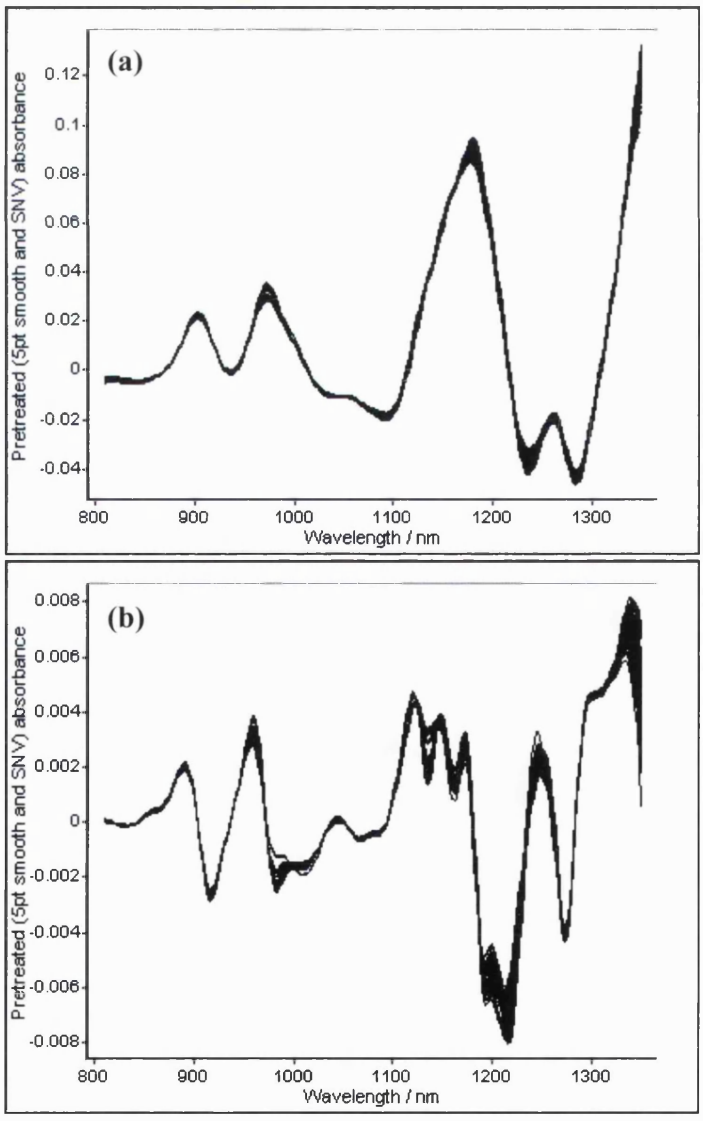

Figure 62: (a) 1st derivative and (b) 2nd derivative data with five point derivative gap

To determine the optimum parameters suitable for the Norvasc® tablet data, impact of the treatments for a region of the spectra specific to the amlodipine API was reviewed. To identify the region related to the amlodipine API, the ten commercial and the four development batches (75% to 125% label claim) were utilised to evaluate the correlation coefficient between the nominal amlodipine active content and NIR absorbance at each wavelength for both first and second derivative maths treatment using the lowest gap for both 1st and 2nd derivative transforms. The correlation plots in Figure 63 demonstrate regions at 1100–1140 nm and 1120–1150 nm have high stable correlation.

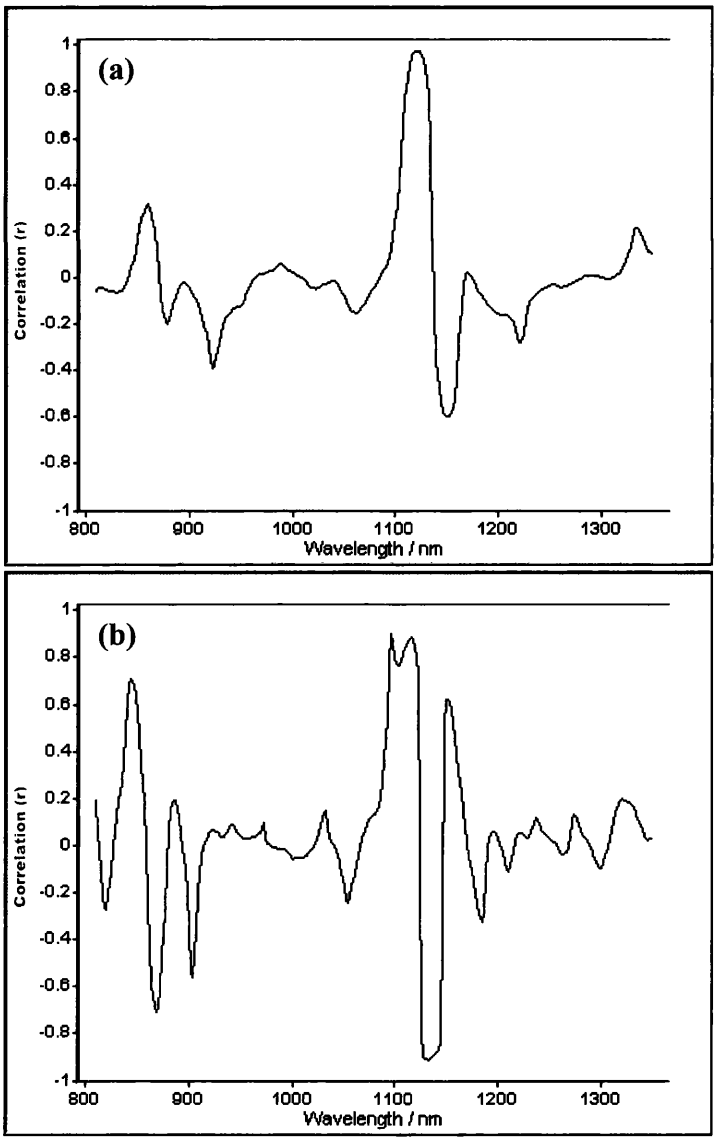

Figure 63: Correlation to nominal amlodipine active content of (a) 1st derivative and (b) 2nd derivative data with five point derivative gap

The spectrum of each individual formulation component was overlaid to examine the region identified in the correlation plots in Figure 63 to ensure that the correlation seen is due to amlodipine active absorption and not dilution or other effects. Figure 64 shows the spectra of all components with a five pt derivative data interval.

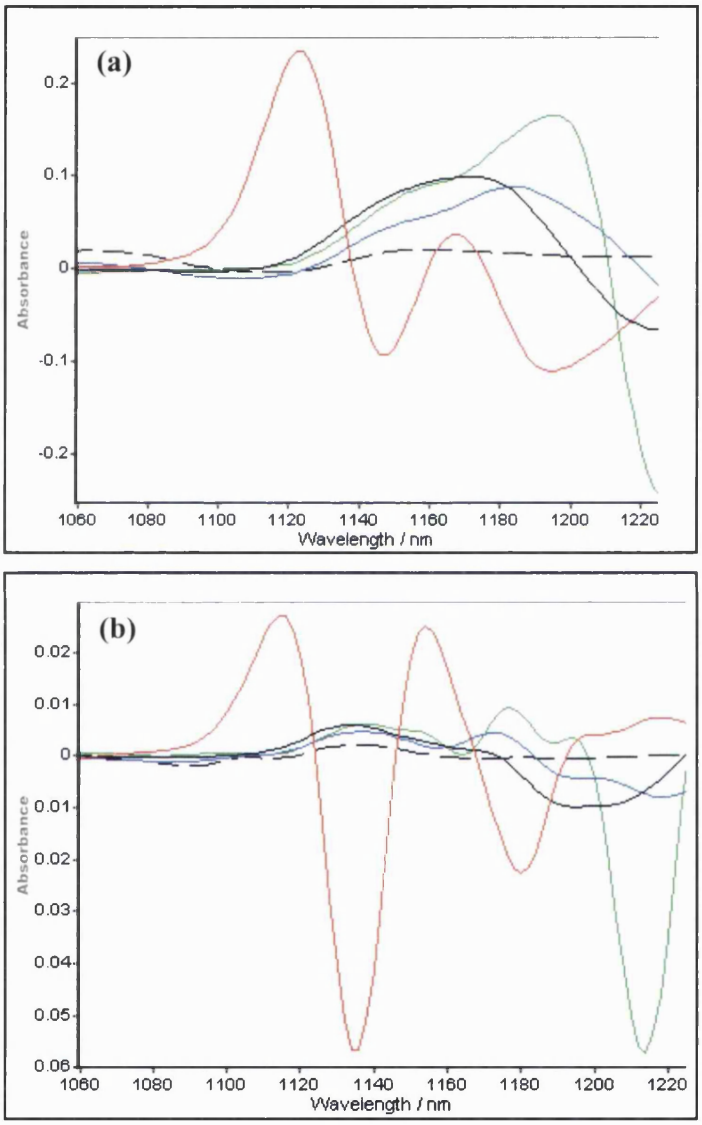

Figure 64: Overlay of (a) 1st derivative and (b) 2nd derivative spectra of Norvasc® tablet components; amlodipine besylate (___), sodium starch glycollate (___),calcium phosphate (_ _ _), microcrystalline cellulose (___), and magnesium stearate (___)

Visualisation of component spectra identified the peaks at 1122 and 1136 nm as the most promising wavelengths for specific measurement of the amlodipine active for 1st and 2nd derivative mathematical treatments respectively.

The NIR absorbance at these wavelengths correspond to second overtone N-H and aromatic C-H vibrations (Figure 2) enabling good specificity in the measurement of amlodipine active as the remaining components of the tablet matrix do not contain aromatic and N-H functional groups. Note that both the amlodipine active (amlodipine base) and the besylate ion contain aromatic functional groups.

First and second derivatives with varying data intervals were visually and statistically evaluated using the ten commercial and the four 75% to 125% development batches to assess the correlation of NIR absorbance with the nominal amlodipine active content at the regions identified in Figure 64. Table 15 shows the correlation at the centre of the stable correlation region and the associated range of derivative spectral absorption for different derivative gap intervals. Note that the centre of the stable region shifted as indicated for the 1st derivative spectra as the data interval gap increased.

Table 15: Optimisation of derivative data transforms for tablet analysis

	First derivative		Second derivative	
Data gap Interval	**Correlation (wavelength)**	**Absorbance range (and values)**	**Correlation (wavelength)**	**Absorbance range (and values)**
5	0.9735 (1122 nm)	0.007624 (0.01017-0.01779)	-0.9038 (1136 nm)	0.002048 (0.001371-0.00342)
6	0.9661 (1122 nm)	0.007016 (0.01039-0.01741)	-0.9034 (1136 nm)	0.001869 (0.001643-0.003513)
7	0.9535 (1120 nm)	0.006413 (0.007090-0.01350)	-0.9034 (1136 nm)	0.001687 (0.001905-0.003591)
8	0.9391 (1120 nm)	0.005841 (0.007496-0.01334)	-0.9041 (1136 nm)	0.001508 (0.002141-0.003649)
9	0.9156 (1118 nm)	0.005359 (0.004768-0.01013)	-0.9058 (1136 nm)	0.001339 (0.002344-0.003682)
10	0.8845 (1116 nm)	0.004922 (0.002449-0.007372)	-0.9086 (1136 nm)	0.001183 (0.002508-0.003691)

It was found that 1st derivative data transform resulted in improved correlation of absorbance to nominal amlodipine active content and a five point derivative gap enhanced absorbance sensitivity (higher absorbance and wider absorbance range).

5.2.2.2 Model development

PCA of the optimised pre-treated spectra of the ten historical production batches was used to assist in the selection of six production batches that represented the range of acceptable variation in excipient and amlodipine active variation in commercial production (which covered four years of manufacture). Six PCs were required to describe 90% of the spectral variance.

While the first two PCs explained 75% of the variance, it should be noted that the spectral variance explained is primarily physical scattering effects (the separation of scores into two groups in Figure 65(a) relates to production batches manufactured on the two tablet press brands, Korsch and Fette. Note that two Fette and one Korsch tablet presses were utilised at the manufacturing facility to compress Norvasc®.

The production batches at the extremes of the PCA scatter plots (representing four years of production and all tablet presses) were chosen for the historical dataset, representing the natural physical variation as well as variation due to the chemical components of the formulation. The remaining four batches were utilised for validation (covering three years of production batches and all tablet presses).

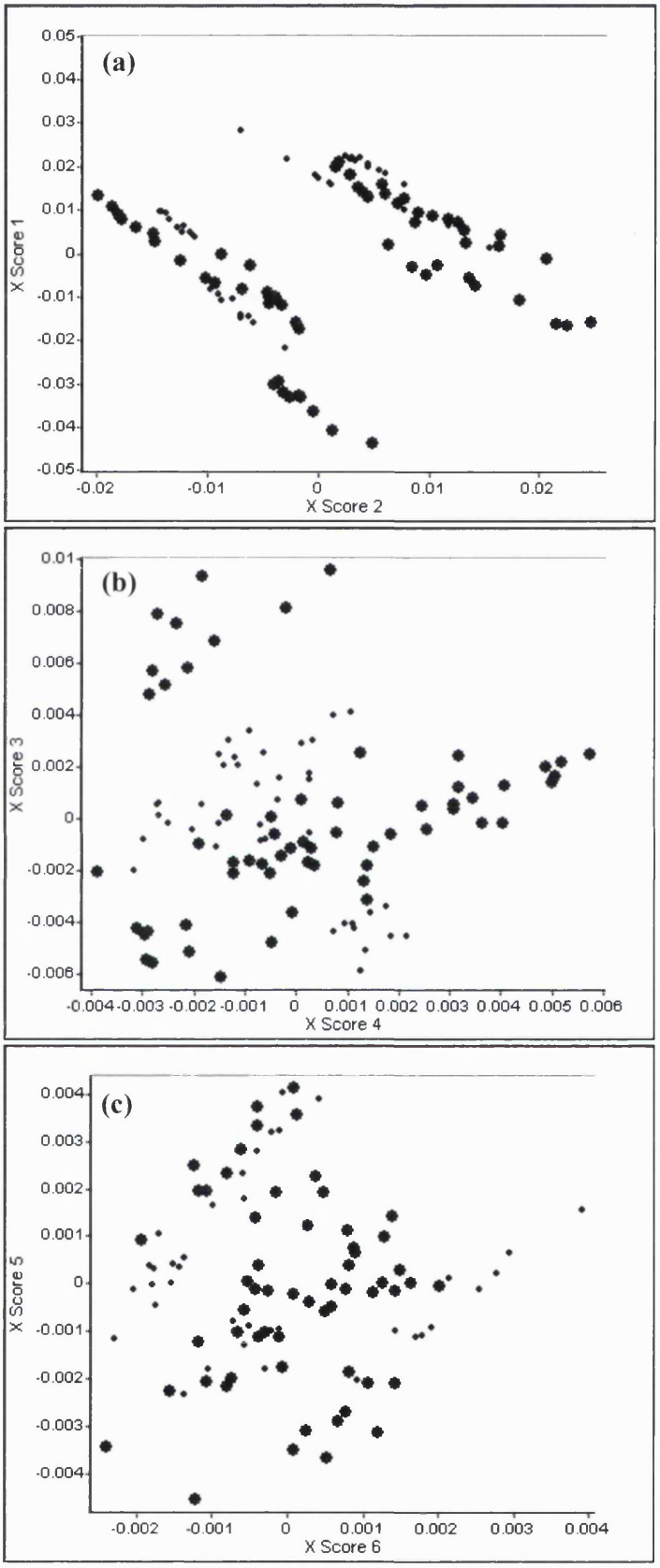

Figure 65: PCA scores scatter plot for the ten production batches for (a) PCs 1 & 2, (b) PCs 3 & 4 and (c) PCs 5 & 6 with selected historical dataset set in bold

Options for simple non-regression qualification chemometric techniques were reviewed that would be specific for the tablet attribute of interest, the amlodipine active content. The simplest technique is the use of single wavelength absorbance values. Absorption at 1122nm was previously shown to be appropriately specific to amlodipine API (see Figure 63(a) and Figure 64(a)) and was selected as one of the qualitative metrics for further investigation for the conformance method.

Correlation and normalised SD algorithms over a reduced range were considered. As it was desired that the conformance method would indicate direction of deviation when not in conformance, average normalised SD over reduced range with sign maintained (absolute value not taken) was selected for further investigation. For ease of discussion this is termed ANSD-RR hereafter.

Given the heavy influence of physical effects in the PCA, it was determined that PCA-MD would not provide specificity for amlodipine active content without training the PCA with the extended range batches. Such training would equate to a regression or discriminant analysis approach and is explored and discussed in Section 5.3.

Normalised SD models were developed in DMentia software using the default threshold of 3. The model was then applied in DeLight software to predict normalised SD results for the historical dataset. ANSD-RR values were then calculated from the normalised SD value across the wavelength range 1110–1132 nm (the region with correlation over 0.75 in the correlation plot shown in Figure 63(a)). The absorbance value at 1122 nm for the pre-treated 1st derivative spectra were also obtained from DeLight software (termed 1122 nm Absorbance for ease of further discussion).

5.2.2.3 Establishment of the conformance SPC charts

Once the conformance chemometrics have been established and models created, SPC charts must be established that will monitor results within and between batches over time. This ensures that, although the metric is calculated against a static model (in the case of the ANSD-RR method), batches can be compared to historically acceptable deliveries over time and trends towards non-conformance can be identified prior to failure.

For tablet analysis, assessment on a unit dose level is required (at the individual tablet basis) as well as overall batch quality. Additionally, within batch trend analysis is required when NIR analysis is performed throughout production at the process monitoring level.

To establish the individual tablet SPC chart, the 1122 nm Absorbance and ANSD-RR data for the six batches in the historical dataset (tabulated in Appendix 3 on page 297) were exported into Minitab software to establish normality to allow the development of Shewhart[137] SPC charts. The Graphical Summary function in Minitab was used to represent the data and assess normality for each conformance metric (Appendix 4 on page 299). No evidence of non-normality was observed (p>0.05) at the 95% confidence level. Normality p-value for each set of data is shown in Table 16.

Figure 66 shows the Minitab I-chart for the historical dataset. The mean and standard deviations applied in establishing the control charts are shown in Table 16. Control limits (___) were set at three standard deviations from the mean (___) to represent the "Voice of the Process" (VOP).

Table 16: Parameters used to generate the SPC charts for the qualitative tablet component conformance methods

	1122 nm Absorbance	ANSD-RR
***p*-value**	0.279	0.053
Mean (Absorbance)	0.01411	-0.00002
Standard Deviation (Absorbance)	0.0004070	0.8422

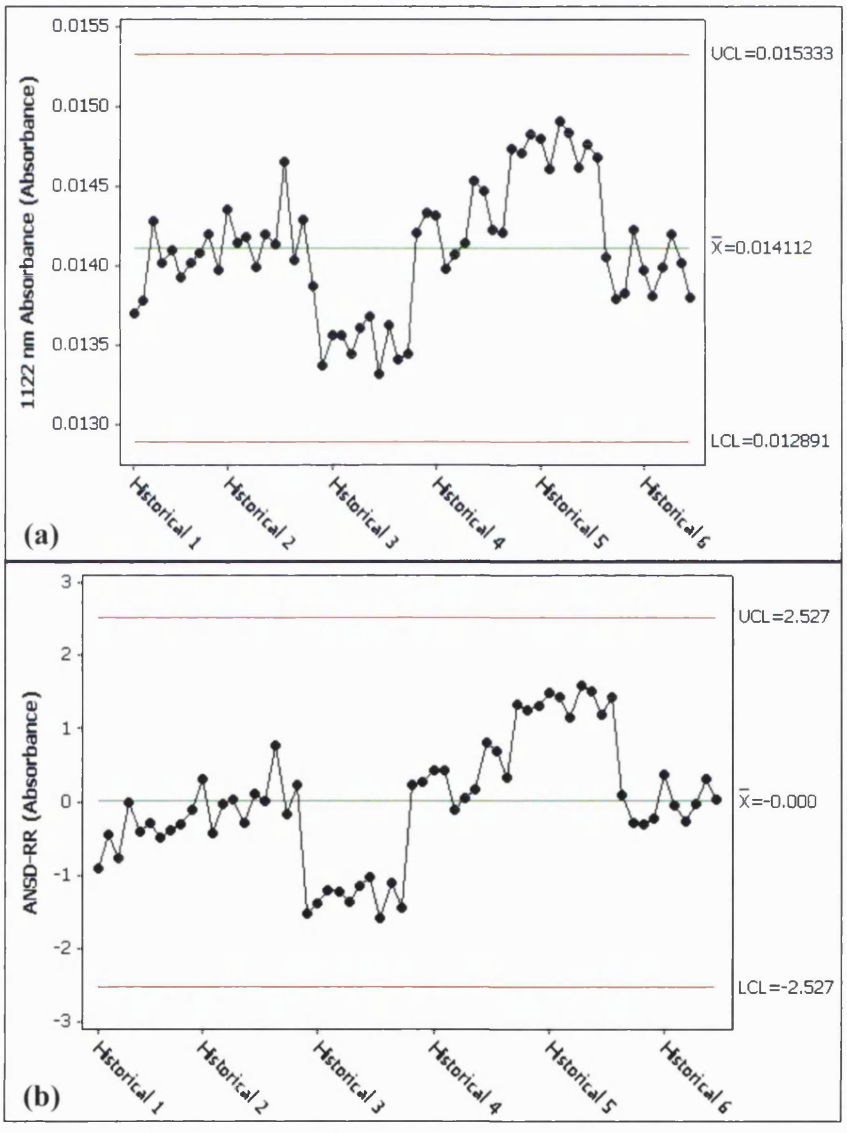

Figure 66: I-chart for the historical dataset for (a) 1122 nm Absorbance & (b) ANSD-RR

To demonstrate that the determined SPC chart with VOP limits are appropriate, the SPC chart was applied to new tablets from four commercial production batches and the development tablets intentionally manufactured outside of normal amlodipine active content.

The SPC chart for 1122 nm Absorbance data shown in Figure 67(a) demonstrates that the thresholds are appropriate to monitor when individual values fall outside the established normal process (marked in red with "1") and also when trends occur such as runs of tablets on either side of the mean (marked in red with "2").

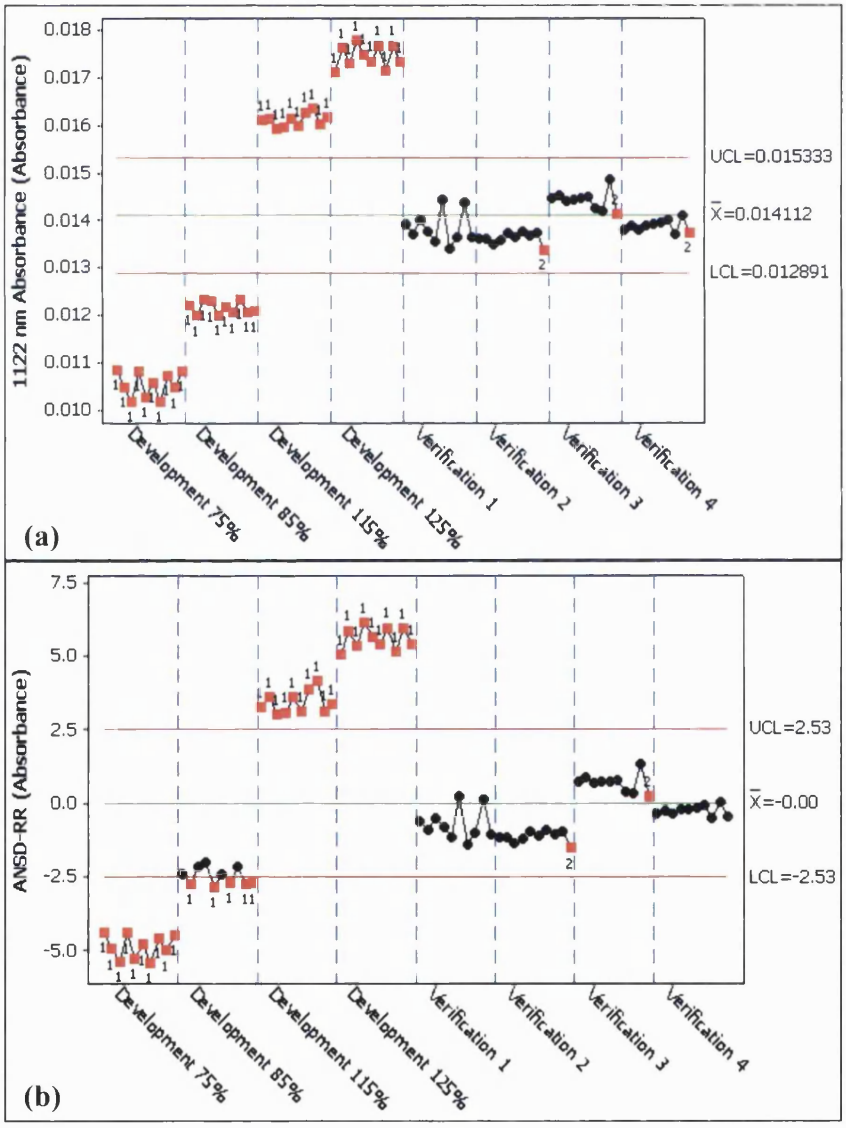

Figure 67: I-chart for the verification data showing verification batches for (a) 1122 nm Absorbance and (b) ANSD-RR

The control chart for ANSD-RR shown in Figure 67(b) shows that the VOP limits indicate some of the tablets that are at the limits of acceptable amlodipine active content (85% label claim) are within the expected normal population of the process. This indicates a lack of sensitivity (analyte response) of the method (potentially from averaging in response of other components absorbing near to the amlodipine API). However, it also may indicate that the development tablets were not at the nominal amlodipine active content.

It is important to establish thresholds that also effectively flag when the amlodipine active content of tablets fall outside the acceptable "Voice of the Customer" (VOC) range rather than just the VOP, i.e. when the amlodipine active content falls outside an acceptable level rather than just outside the established normal process variation. For a product developed under QbD, the VOP may be considered the normal operating range while the VOC may be considered the limits of the defined design space.

Large discussions have been held within the pharmaceutical industry regarding application of the Pharmacopeial content uniformity concept to large sample sizes.[119] However, looking at what is an acceptable content for each individual tablet that reaches the market, we can apply a conservative threshold for the VOC of an absorbance that is equivalent to a amlodipine active content of 15% from target label claim.

The VOC limits can be set by the use of tablets with known amlodipine active content and correlating the algorithm output to amlodipine active content. One of the key benefits of the conformance methodology is the reduced number of samples required to validate the approach and hence the reduced reference chemistry required. Two tablets from each of the verification batches were used to establish the VOC limits by performing a simple linear regression on the algorithm output and HPLC assay results (in mg per tablet) acquired through reference chemistry.

The data used to establish the VOC limits are shown in Table 17 and indicate that the tablets in the 85% development batch would be expected to just be beyond 'acceptable' tablets (assaying at an average of 83% label claim, while the 115% development batch would be expected to fall just within the 'acceptable' tablet range (assaying at an average of 114% label claim). Note that the 75% Development batch was assayed at 67% of label claim (while the other development batches were assayed closer to the targeted percentage of label claim). This indicates an error likely occurred in calculating or dispensing the excipient used to dilute the Norvasc® blend during the manufacture of the 75% development batch.

Table 17: Reference chemistry results used to establish VOC limits

	HPLC Assay (mg / tablet)	% Label Claim	1122 nm Absorbance	ANSD-RR (Absorbance)
75% Development 1	3.42	68.4%	0.01084	-4.401
75% Development 2	3.35	67.0%	0.01049	-4.954
85% Development 1	4.20	84.0%	0.01221	-2.390
85% Development 2	4.09	81.8%	0.01199	-2.757
115% Development 1	5.74	114.8%	0.01611	3.243
115% Development 2	5.68	113.6%	0.01592	2.993
125% Development 1	5.94	118.8%	0.01711	5.051
125% Development 2	6.03	120.5%	0.01763	5.859
Production 1-1	5.04	100.7%	0.01392	-0.6385
Production 1-2	4.89	97.8%	0.01370	-0.9206
Production 2-1	4.91	98.1%	0.01362	-1.154
Production 2-2	4.80	96.0%	0.01360	-1.177
Production 3-1	4.800	96.0%	0.01446	0.7235
Production 3-2	4.96	99.1%	0.01453	0.8534
Production 4-1	4.89	97.7%	0.01380	-0.3828
Production 4-2	4.95	98.9%	0.01390	-0.2465

The regression and resulting equations are shown in Figure 68. The VOC limits can be established from the equation for 4.25 mg / tablet (85% of label claim) and 5.75 mg / tablet (115% of label claim). The calculated VOC limits for the two metrics are shown in Table 18.

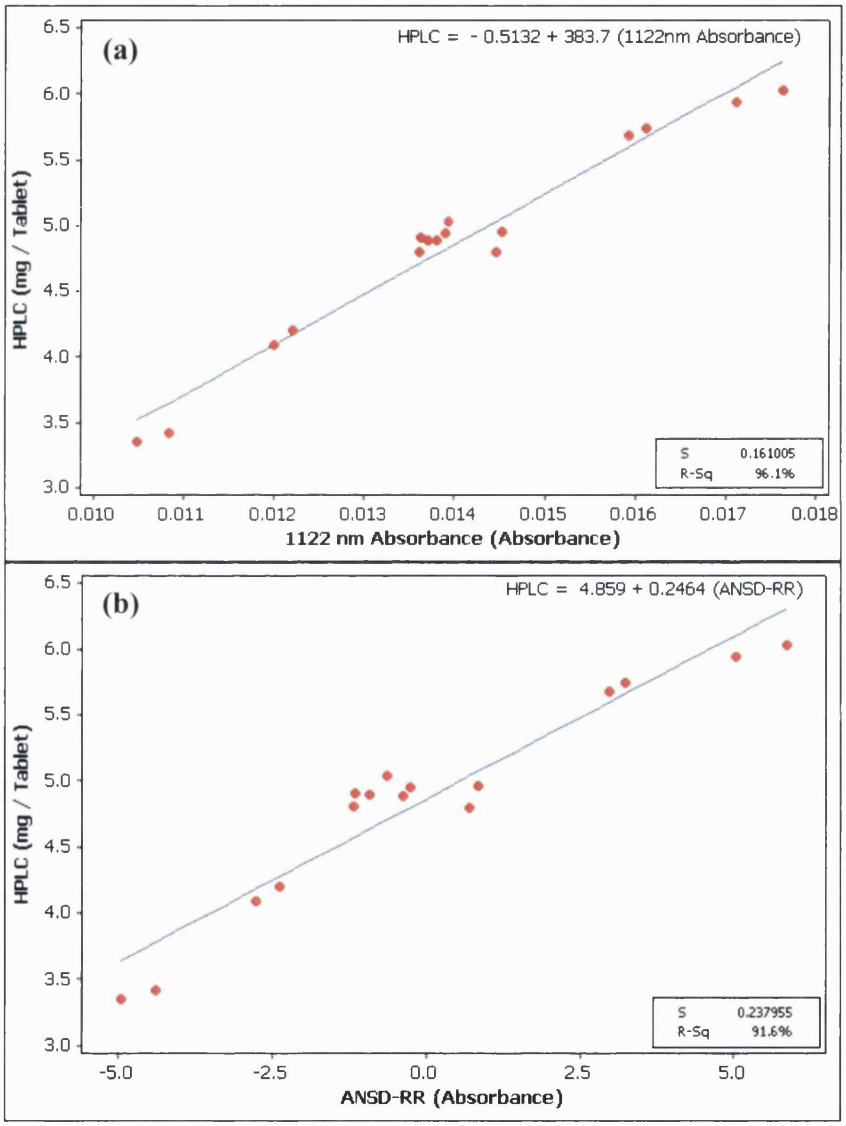

Figure 68: Regression fitted line and equation for sixteen representative tablets from the verification batches for (a) 1122 nm Absorbance and (b) ANSD-RR

Table 18: Calculated VOC limits for 1122 nm Absorbance and ANSD-RR metrics

	1122 nm Absorbance	ANSD-RR
85% Label Claim	0.01241	-2.472
115 Label Claim	0.01632	1.668

Figure 69 shows the control chart of the verification batches with both the VOP limits (___) and the VOC Limits (_ _ _). Note that the VOC limits are offset from the VOP limits as the VOC limits are centred about the target assay content (label claim) and not based on the process mean (___).

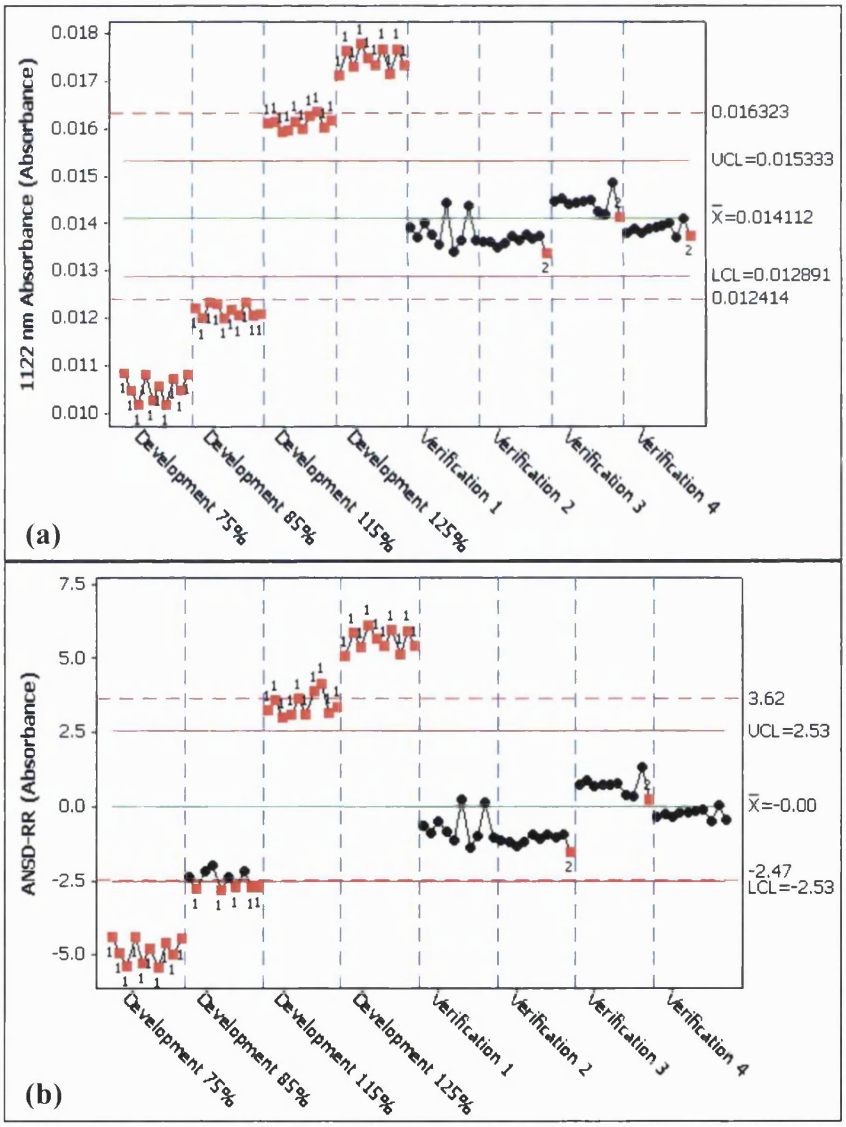

Figure 69: I-chart for the verification data showing verification batches with VOC and VOP limits for (a) 1122 nm Absorbance and (b) ANSD-RR

Applying the equations determined in Figure 68 to the process mean values used to establish the VOP limits found that the process mean was equivalent to a HPLC amlodipine active content results of 4.902 mg and 4.859 mg per tablet for the 1122 nm Absorbance and ANSD-RR methods respectively. The average of the HPLC results for the verification production tablets (shown in Table 17) confirmed the offset to label claim with the average assay 4.903 mg per tablet.

It is also of note that the suspected sensitivity issue of the ANSD-RR method was confirmed through the addition of the VOC limits. The VOC limits fall within the VOP limits at low amlodipine active content showing that the method is not capable of accurately discriminating tablets at the limits desired. This may be a factor of non-linear response in the ANSD-RR metric (r^2 of 0.916 as shown in Figure 68b).

The verification results of the 1122 nm Absorbance method show that the derived SPC charts for the individual tablets (reflecting both the VOP and VOC) is appropriate to be applied during the tabletting process to monitor individual tablets as they are manufactured. The SPC chart correctly identified that the 115% development batch tablets were at or just within VOC limits while identifying the 85% development batch tablets were at or outside of the VOC limits. The 1122 nm Absorbance control chart also showed sensitivity with separation of the tighter VOP limits from the VOC limits.

The developed SPC charts would provide sufficient insight into the content uniformity of processes when applied throughout tabletting operations by identifying individual tablets beyond 85 – 115 % label claim, thus allowing manufacturing to count the number of tablets beyond the acceptable range, arrange for confirmatory testing and determine batch compliance to content uniformity as described in Sandell's[119] approach for content uniformity for large n.

The established individual SPC chart addresses the important question of whether each individual tablet is within the normal process limits and also whether the tablets analysed will meet quality requirements (average amlodipine active content and content uniformity). However, there are key process questions that the individual tablet SPC chart alone does not address, namely whether:

1. any abnormal process trends exist for a given batch compared to normal batch trends,
2. any abnormal within batch process trends exist for a given batch,
3. the overall variation within the batch is typical.

To enable monitoring of tablets throughout the process to address these questions, the number of tablets to be tested at each sampling point and the frequency of sampling must be established. The number of samples to be taken at each time point should be kept to a minimum to prevent burden on operators, while being sufficient to give a representative set of samples to derive the time point sample mean to represent the amlodipine active content in the product at that point of tabletting. Five tablets were considered appropriate to yield an accurate estimate of the sample point mean. Increasing this number will only give more information about the consistency of the individual tablet punches on the tabletting press turret (typically composed of 20 to 40 punch / die sets).

The frequency of sampling must be set to ensure adequate description of the process trends and should be considered proportional to the frequency of any known process events (e.g. tablet press operational adjustments). With modern automatic tablet presses it is suggested that half hourly to hourly sampling would be sufficient. The batch size and resulting tabletting process run time should also be considered to ensure that a minimum of 20 sample positions are achieved. A minimum of 20 sampling positions

will provide opportunities to identify runs and cyclic patterns in the data. Consideration should also be taken as to whether different frequency should be applied at tablet press stoppages, shift changes or bulk blend feed changes (e.g. changing holding bins, or refilling rate of tablet press feed hopper).

The tablet press compression speed and batch size for Norvasc® 5 mg tablets at the facility at which this work was conducted led to the tabletting operation requiring 35-40 hours. Product was collected in pails with one pail equating to approximately 30 minutes of tabletting. It was determined that five tablets would be taken at the completion of each pail at approximately 30 minute intervals to ensure that each sample point represents equal portions of the tablet product (by tablet volume manufactured). This testing frequency required less than three minutes of analysis time for each sampling point.

To address whether any abnormal process trends exist for a given batch compared to typical batch trends, individual tablets analysed throughout tabletting for six process batches were reviewed. The data were represented in a time series plot to establish whether any characteristic process trends exist for the Norvasc® tabletting process. Figure 70 shows the trend of the 1122 nm Absorbance result for six commercial batches manufactured on three models of tablet presses with five tablets sampled every half hour.

No characteristic process signature occurs batch to batch. Batch four and five (manufactured on two different tablet press brands) show an increase in absorbance over the length of the process run then a decline near the end of the batch while batch six shows a decrease in absorbance throughout the batch. As there is no typical systematic process signature, process signature trajectory modelling across batches would not be beneficial.

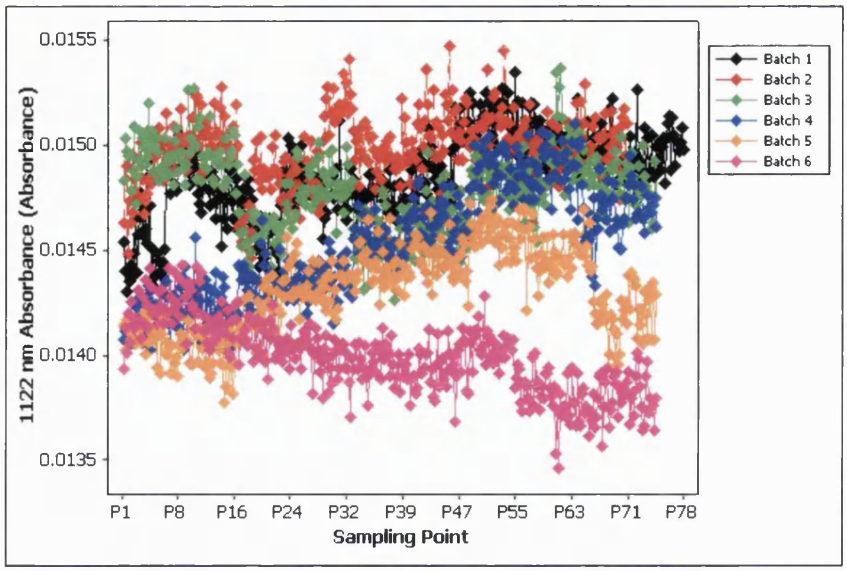

Figure 70: Time series chart of six commercial batches showing lack of common process signature across the tabletting operation

To address the second process monitoring question, SPC charts to assess within batch trends for a time ordered subset of the process were required. These control charts would identify deviations from the process and allow monitoring of changes to the process trends within a batch. Assessment of the six process batches (with five tablets sampled at half hourly intervals), determined that the I-chart and Moving Range (MR) chart for Sample Means and the Run chart were appropriate control charts to assess within batch process trends.

The I-chart for Sample Mean identifies any process deviation using within-batch control limits set at three standard deviations from the batch mean value. The MR chart for Sample Mean identifies any sudden shift from one time point to the next time point beyond what is normally observed for that batch. The Run chart complements the I-chart by objectively identifying the presence of trends, patterns or deviations which cannot be explained by random variation.

It should be noted that the standard X-bar chart could not be used as the process limits are based on the within sample point variation (representing the variation in tablet punch/ dies in the tablet press turret), therefore the I-chart for Sample Means is used to ensure that the process control limits are based on between sample point and across the process variation.

Figure 71 and Figure 72 show an example of the I-MR and Run chart respectively for the first of the six batches used to develop the SPC strategy. The I-MR chart (Figure 71) shows that several sampling points are beyond the expected population (beyond three standard deviations of the batch mean) and also that there are four sudden shifts between sampling points. The Run chart (Figure 72) also indicates that there is a significant degree of clustering of points on one side of the mean (p-value for clustering <0.05). These plots provide insight into the tabletting operation. This information may be able to be aligned with tablet press operation parameters, powder blend charging and also operator shift patterns to enable further improvements to process capability.

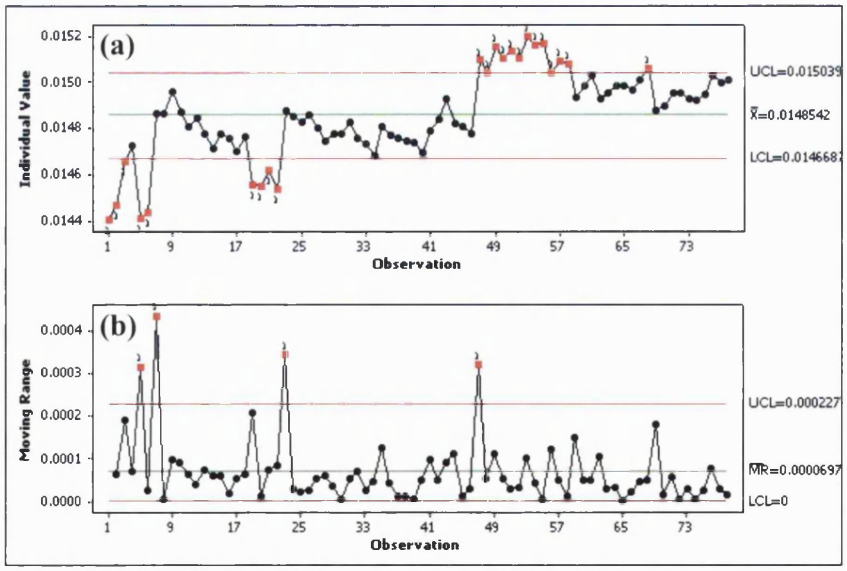

Figure 71: I-MR chart of 1122 nm Absorbance for the 1st historical batch showing within batch trends with (a) I-chart for sample mean (Absorbance) and (b) MR chart for sample mean (Absorbance)

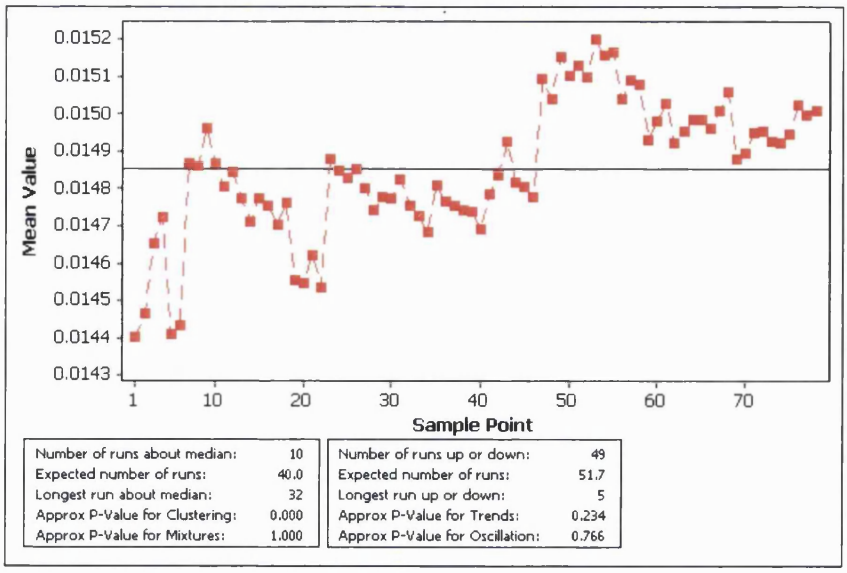

Figure 72: Run chart of 1122 nm Absorbance (Absorbance) for the 1st historical batch showing within batch trends

To address whether the overall variation within a batch is typical, a mechanism to monitor both the overall spread and the variation pattern of results in a large sample set was required. Six real process data sets were used to investigate various methods for monitoring the spread and variation pattern aspects of the process.

The fact that the sample size differs between batches when samples are taken on a time basis complicates the establishment of appropriate measures. Sampling by product volume (per product pail) was an attempt to limit this effect however fill height per pail was subjective (manual switch of pail by operator).

Standard statistical theory postulates that the probability of sampling at the extremes of the sample population increases as sample size increases. However, this is the situation when randomly sampling from a population, where each tablet has equal probability of being selected for testing. This situation does not occur with time-based process monitoring as tablets are targeted specifically for analysis based on the manufacturing process time. In time based sampling, samples at the extremes of the sample population will be captured in greater numbers only if the frequency of sampling increases at the

time points of increased process instability or extremes of process trends. The process is likely to be most unstable at the start of manufacturing and at the very end of the tabletting process where powder flow to the powder press can become disrupted from normal mass flow characteristics. Sampling is no longer random, therefore conventional probability calculations no longer prevail.

An assumption can be made that the sampling frequency has been set appropriately to capture the breadth of the process and that the subset of tablets taken from the process is truly representative of the process. Here we must assume that the operation speed of the tablet press does not change throughout the manufacturing process so that each time point represents an equal portion of the process throughout manufacturing. In this instance the range calculated across all samples in the subset will be a reflection of the range of the process, regardless of the sample size (given a fixed batch size) and thus the range can be used to indicate the spread of the batch population.

The six commercial process batches were used to calculate the typical range for the product. The range of the 1122 nm Absorbance was calculated and the Graphical Summary function in Minitab used to represent the data and assess normality (Appendix 4 on page 299). No evidence of non-normality was observed (p>0.05) at the 95% confidence level. The normality p-value is shown in Table 19. Note that output is an estimate only as the number of data points is small, reducing the power of the normality tests.[135]

Figure 73 shows the range Minitab I-chart of 1122 nm Absorbance for the six commercial Norvasc® batches. The mean and standard deviations applied in establishing the chart are shown in Table 19. The control limits (___) were set at three standard deviations from the mean (___) value.

Table 19: Parameters used to generate the batch range SPC chart for the qualitative tablet component quality conformance method

***p*-value**	0.553
Mean (Absorbance)	0.001032
Standard Deviation (Absorbance)	0.000058

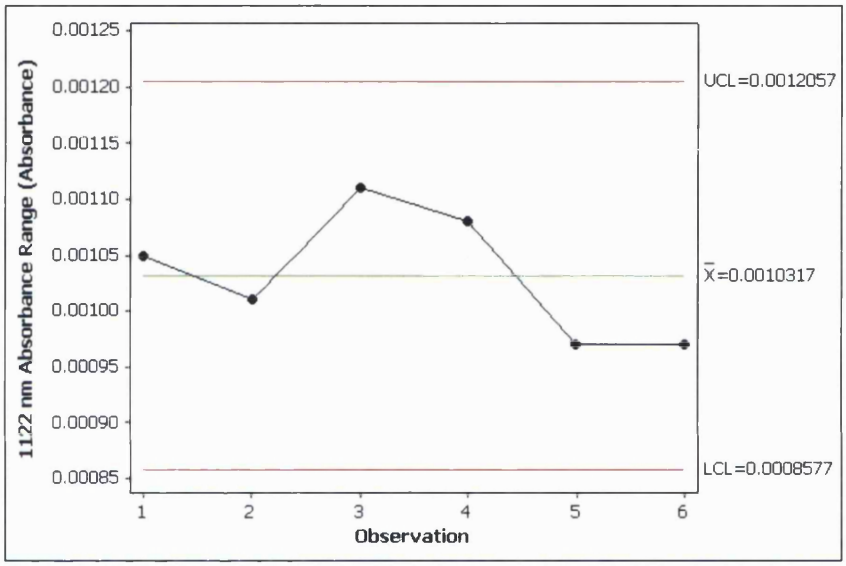

Figure 73: I-chart of the range of 1122 nm Absorbance for the six commercial Batches

The determination of the variability within the range is more complicated as the calculation typically used, standard deviation, is in itself 'n' dependent and does not just rely on the sampling plan capturing an unbiased representative view of the process in successive batches. The presence of process trends also affects the use of a standard deviation calculation as the standard deviation calculation assumes the population is randomly distributed about the mean. It may then be of more interest to assess the distribution pattern of the data, independent of the distribution orientation to the mean.

Distribution histograms are typically performed with the y-axis as the frequency of the occurrence of results within contiguous equal sized divisions (or bins) of the range of values. The use of frequency percent (frequency over the sample size) rather than just the frequency ensures that the analysis is independent of sample size. The convention on selecting the number of bins to use for developing a distribution histogram is to use the square root of the number of samples. However, to be able to directly compare the distributions histograms batch to batch, the same number of bins is required. For the Norvasc® tablet process, the mean sample size was 370 tablets. This is likely a good estimate of future sample size given the tablet press speed of operation and batch volume. Therefore 19 bins were chosen for the analysis (close to the square root of 370). Figure 74 depicts the frequency distribution histograms for the six commercial batches of tablets with the percent frequency occurrence across 19 bins.

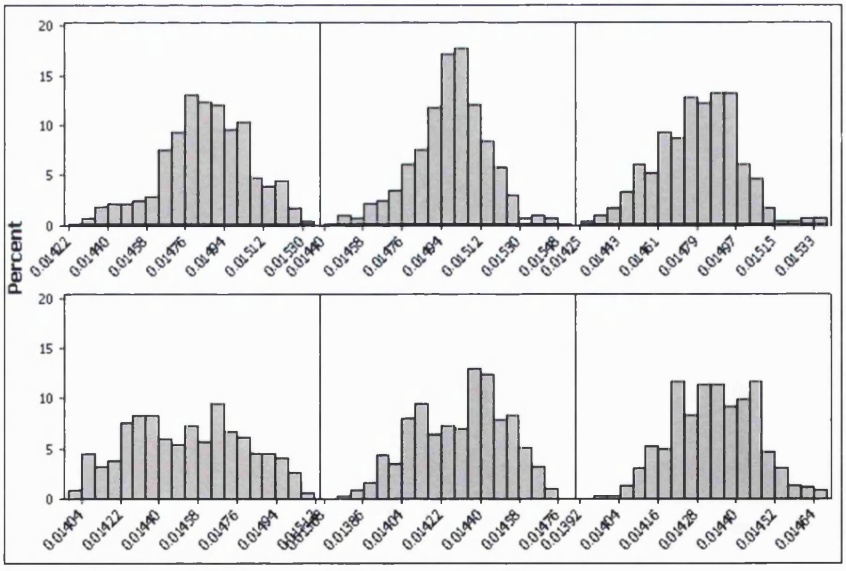

Figure 74: Distribution histograms for 1122 nm Absorbance for the six commercial batches

The shape of the distribution histogram (standardised across batches by the use of percent frequency and constant binning) can be used to establish the boundary of acceptable distribution where the limits are three standard deviations from the mean

distribution curve (calculated from the six historical batches). This control mechanism is analogous to the monitoring control methods available and routinely utilised for on-line particle size monitoring PAT analyzers. However the application of such an approach is novel to NIRS methods.

Figure 75 demonstrates the resulting distribution profile SPC chart with VOP limits (___) set at three standard deviations from the mean frequency percent in each bin for the six commercial batches used to establish the SPC strategy. A boundary limit of 0.0 was utilised for the lower limit at any bin that exceeded the allowed range of percent values.

Note that although Minitab allows for frequency percent distribution histograms with manual binning, Minitab does not have the capability to develop the distribution profile SPC chart (without extensive macros which are not ideal within a GMP environment due to software validation requirements). The distribution profile SPC chart was therefore developed using Excel.

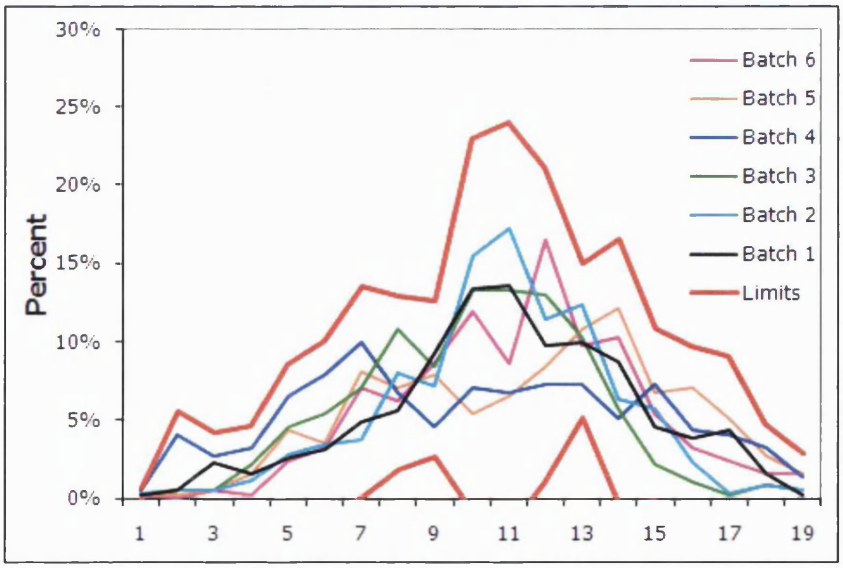

Figure 75: Overlay of distribution profiles for six commercial batches with the derived VOP limits (___)

The application of I-MR for Sample Mean, Run charts, Range I-charts and distribution profile SPC charts all provide significantly greater process understanding than simply counting tablets that fall beyond 85 – 115 % Label Claim; the current best practise for applying content uniformity assessment to large sample sizes.[119] This is an example of clear separation of process based analytical analysis verses regulatory directed quality compliance driven analysis. It is this author's position that such conformance based approaches to process data are more scientifically sound and provides much greater assurance of the quality of product produced by a well understood and controlled process.

5.2.2.4 Implementation of the developed method concepts in manufacturing

The developed qualitative tablet component quality conformance method was applied to three additional commercial batches independent of the batches used to establish the conformance method to assess the suitability of the approach for implementation.

The application of the individual tablet SPC chart to the new commercial process batches is shown in the three graphs in Figure 76 where five tablets were sampled at half hourly intervals throughout the tabletting process. Figure 76 demonstrates that no individual tablet falls outside the VOP or VOC limits. This demonstrates that the process has generated tablets that conform to previous product history and that tablets will meet the desired quality. Note that batches two and three are shifted towards lower absorbance compared to batch one.

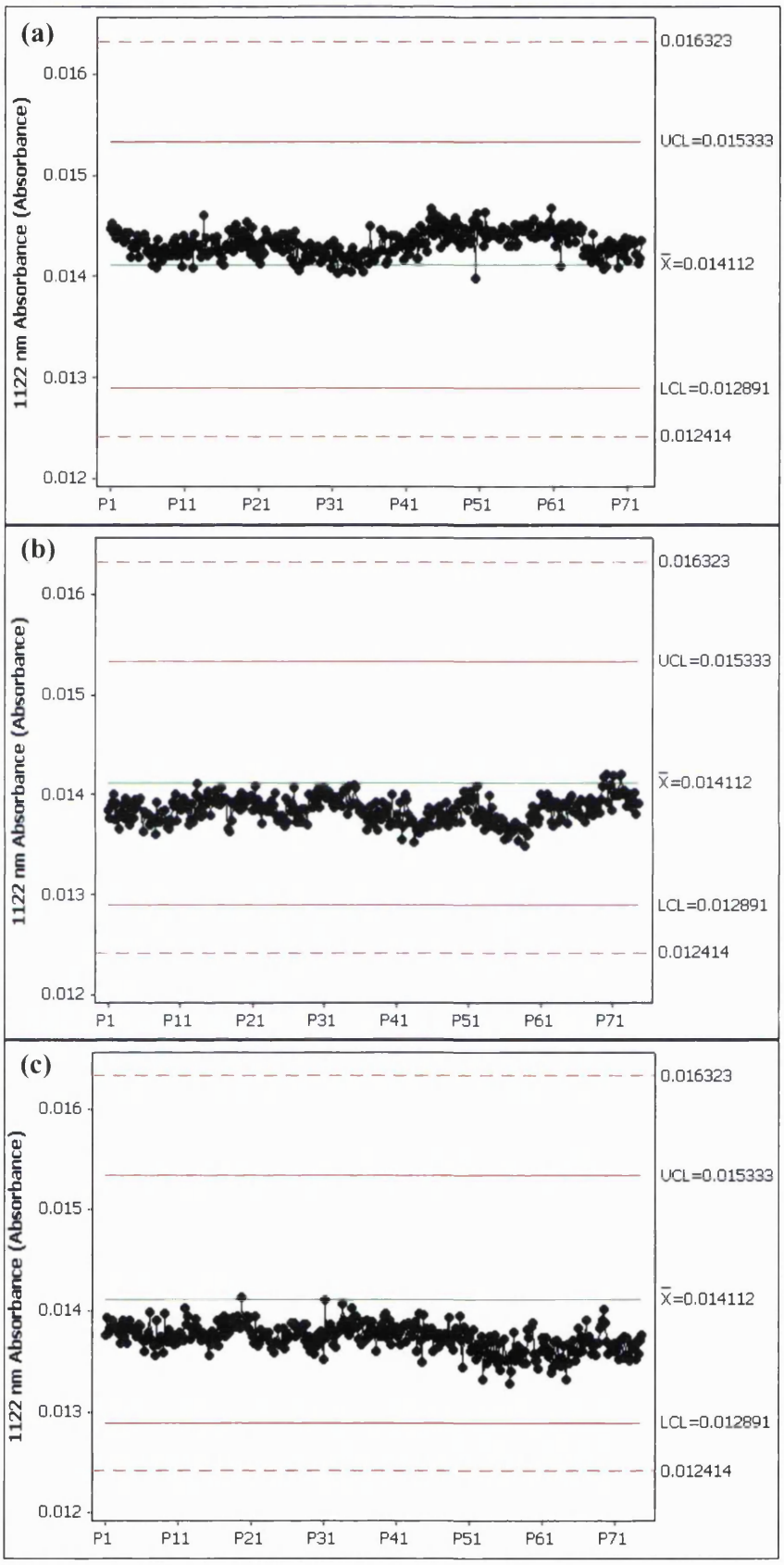

Figure 76: Individual tablet SPC chart of 1122 nm Absorbance for three production batches; (a) batch 1 to (c) batch 3 with VOP (___) and VOC (_ _ _) limits & product mean (___)

The application of the within batch control charts for these three process batches are shown in Figure 77 and Figure 78. The I-MR for sample mean SPC chart for the three batches (Figure 77) indicates that within the three batches there are excursions beyond the within batch process limits (flagged in red). The MR portion of the chart also has identified that there are occasional sudden changes between time points that are higher in magnitude than expected for batch one and batch three. This may align with tablet press adjustments, refill of blend in press feed hoppers or operator shift patterns and is worth further investigation to identify whether any of these factors can be improved to positively affect the process capability. This was not conducted at the time of scanning and was a missed opportunity for process knowledge.

The Run charts for the three batches shown in Figure 78 indicate that all three batches have a higher proportion of clusters of points on one side of the mean than would be statistically expected. As the *p*-value is less than 0.05 at 95% confidence, the distribution of points in clusters is seen as likely due to non-random effects. This indicates that there is likely to be a non-random cause of variation in the data yielding the process signature for the process. The first batch also has a higher proportion of runs of increasing or decreasing data than would be expected ($p<0.05$ for trends). The presence of runs suggests the presence of underlying process trends and visually there does appear to be a general cyclic pattern across the batch mean throughout the batch. This information could provide insight into the tablet press operation through the day and across different operator shift patterns. Further batches should be monitored with these trend charts to establish any root causes for the within batch process signature (clusters, point to point steps and runs) and whether there are opportunities for process improvement.

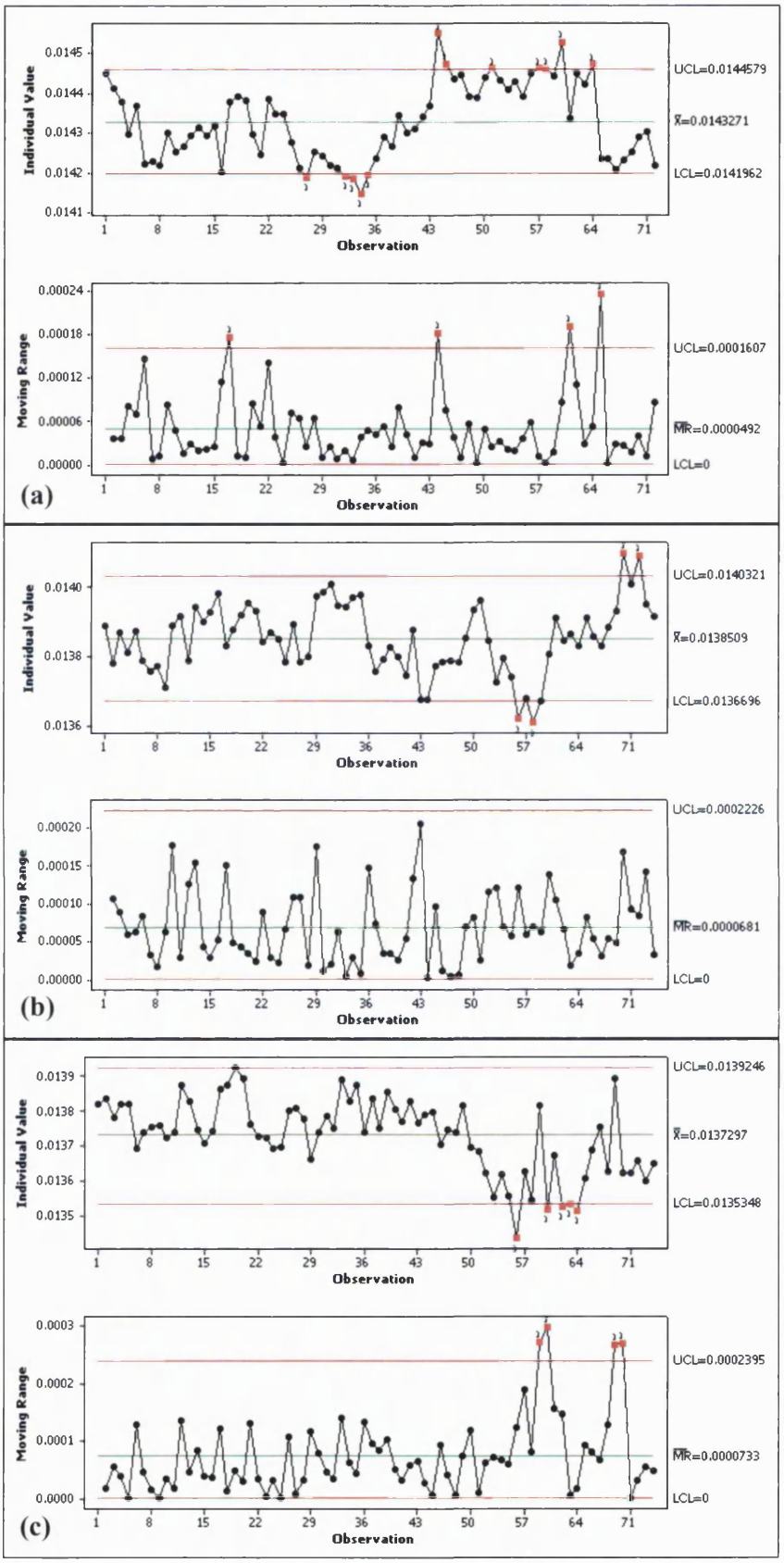

Figure 77: I-MR charts of 1122 nm Absorbance (Absorbance) for three production batches; (a) batch 1 to (c) batch 3 with within batch process limits (___) and batch mean (___)

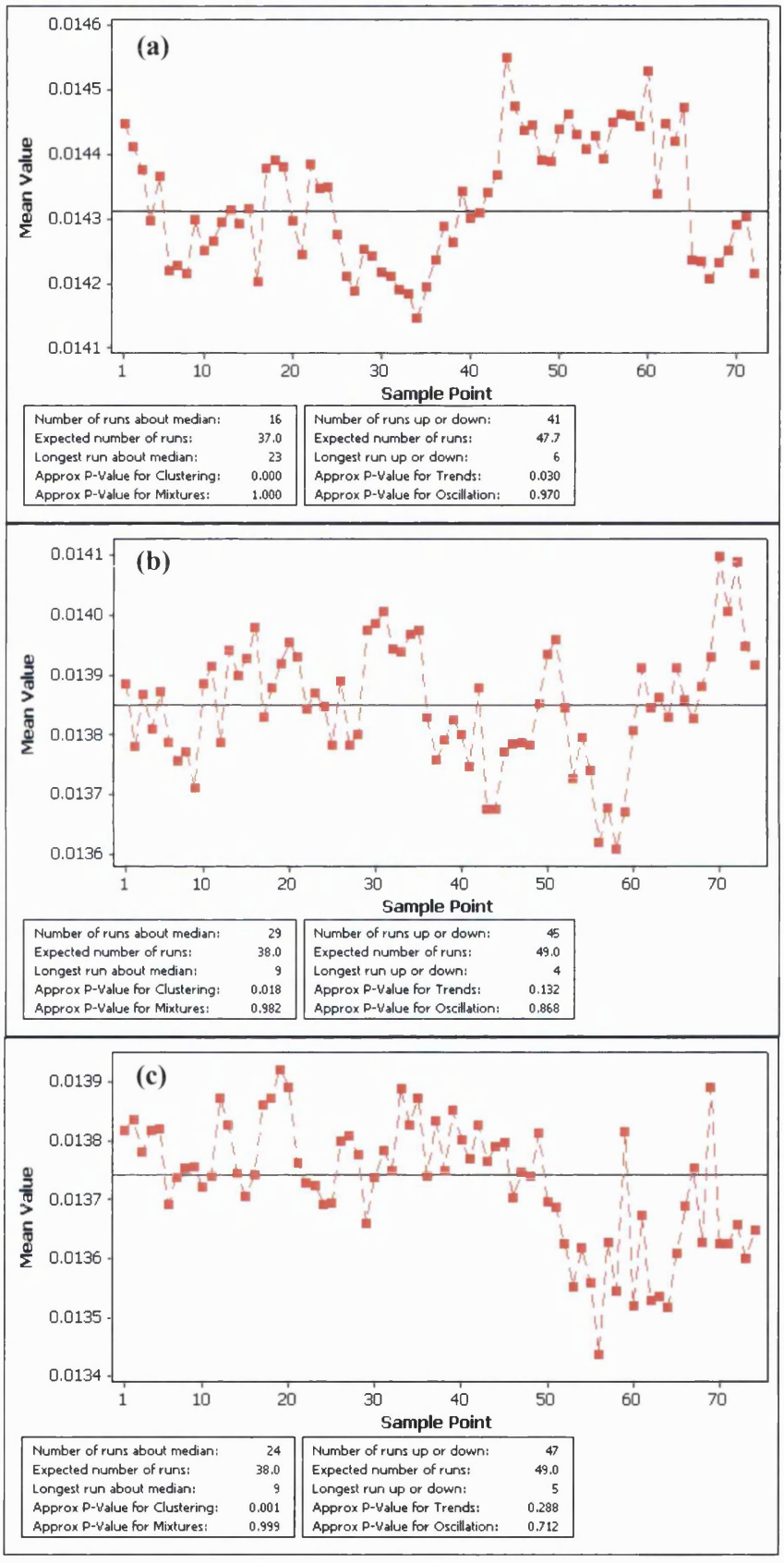

Figure 78: Within batch Run chart of 1122 nm Absorbance (Absorbance) for three production batches; (a) batch 1 to (c) batch 3

Figure 79 shows the Range I-chart for the three additional batches of the product using the established threshold from the six production batches. Figure 79 demonstrates that the three batches have unexpectedly narrow range compared to the six batches used to establish the conformance Range chart limits. This may indicate the production process was maintained more tightly and the tablets are more uniform or that the uniformity of the blend under compression was improved. The breach of the lower control limit in the SPC chart would warrant further investigation to determine why the range of the data is narrowed in these batches and to determine how to influence the variability of tablet product in future deliveries. This is an opportunity for learning about the process and gaining process insight and process understanding. Once the reason for these two batches to have reduced variability is understood, the historical SPC chart could be updated to include these batches in the limit calculations.

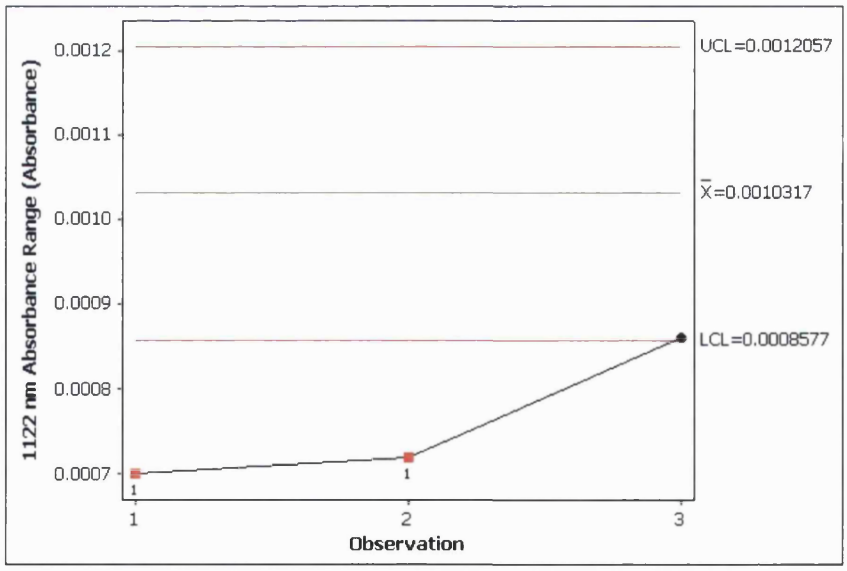

Figure 79: I-chart of Range of 1122 nm Absorbance (Absorbance) for three production batches

Figure 80 demonstrates that the distribution profile of the amlodipine active content for the three additional batches is within the acceptable distribution limits and that the batches each conform to expectation. Note that the extremes of the distribution (the 'tails') are reduced in percent frequency despite the profiles being wholly with the established normal profile for Norvasc® 5 mg tablets. This directly led to the out of trend range output seen in Figure 79.

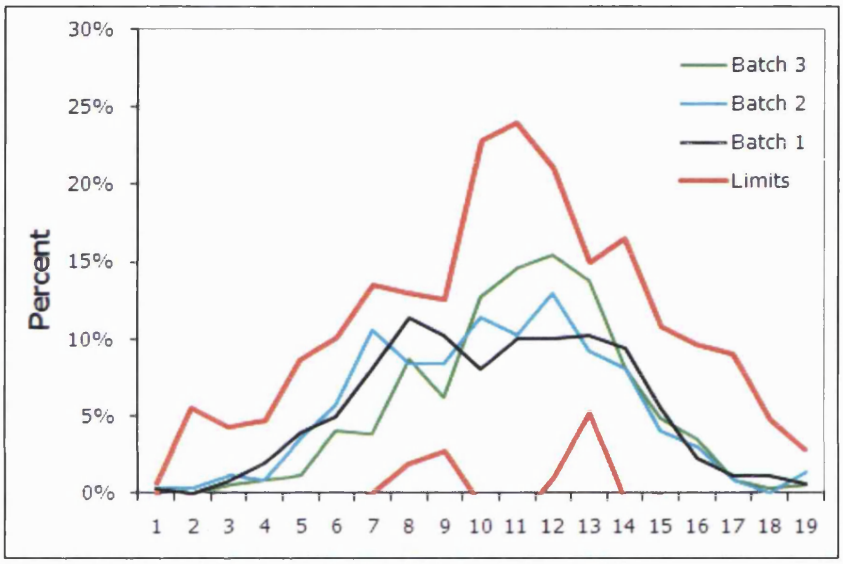

Figure 80: Distribution profile of 1122nm Absorbance (Absorbance) for three production batches with established distribution profile SPC limits (___)

5.2.3 Qualitative Tablet Component Quality Conformance Method Discussion

The qualitative conformance methods investigated, and the resulting univariate qualitative tablet component quality conformance method for Norvasc® 5 mg tablets, were extremely rapid to develop with very limited reference chemistry (only 16 tablets). This compares sharply with the traditional quantitative NIRS methods, which typically require extensive development time and considerable reference analysis (in the order of 100–200 tablets). The qualitative approach, in its simplicity, requires less experienced NIRS analysts to develop compared to traditional regression based approaches. Competency in NIRS is still required to be able to select the qualitative metric that is

most suited given the matrix and component of interest. Any of the diverse chemometric techniques discussed in literature that are applied to classification of materials may be utilised to develop a conformance approach. This research has shown that qualitative metrics that provide continuous data with their signs are preferred so that the direction of any identified deviation can be quickly discerned by the operator. Within this work ANSD-RR was found to lack sensitivity despite appearing to be specific to the amlodipine active content. Regression based approaches may be necessary if a qualitative approach with sufficient specificity, linearity and sensitivity in response for the attribute of interest cannot be achieved.

The developed conformance method includes the use of various SPC charts to monitor individual tablets and within batch variation as well as conformance batch to batch. Consideration of the ease of use within manufacturing operations (appropriate complexity and easy to use operator interface) must be balanced with providing sufficient information on the quality of the product and process capability.

During the development of this approach it was noted that vendor software typically lacks the capability to provide flexibility to implement qualitative methods for purposes other than identification and most also lack the ability to perform process relevant statistics and develop and display SPC charts in real time. Some software has in-line process modules in the software which can handle continuous data collection as the process runs. However, these still generally apply the traditional identification and quantitative algorithms in a continually updating mode. Many do not chart the results, but rather tabulate the prediction results. Those that do graph results have limited user customisation and / or require costly server data storage. There is also a general lack in consideration to establishing conformance limits related to product history rather than the static model. Current NIRS vendor software does not provide the means to perform distribution profile analysis. If a manufacturing site has the software utilised for on-line

particle size analysis, data could be exported and analysed in this software, otherwise the data must be exported to Excel to perform this analysis manually. Ideally automated capability would be available in NIRS vendor software. As part of this research, Pfizer has worked with some NIRS software vendors to develop the ability to provide SPC charts with minimal success. There continues to be a lack of flexibility to develop and implement a range of SPC charts such as has been outlined in this research.

The DeLight software has the capability to reproduce the individual tablet I-chart including VOP and VOC limits to allow the operator to monitor the results in real time. It is possible to reproduce the I-chart and MR-chart for Sample Means within DeLight, however, the control limits must be manually overlaid on the data by the operator at the end of the batch as it is only once the batch is complete that the within batch process control limits can be determined and overlaid on the plots. This can be performed with appropriate steps outlined in a SOP. It is not possible to reproduce the Run chart or distribution profile SPC chart within DeLight. Data must be exported to create these conformance plots in Excel and Minitab and within the manufacturing environment this would likely be performed by Quality Assurance personnel and would be valuable information to include in batch documentation. Figure 81 shows the user interface that operators can use to monitor the tablet conformance method in real time during tabletting operations.

The developed approach provides a simple conformance SPC chart view for operators to monitor during manufacturing within NIR software, while also providing considerable process information about conformance within and between batches through the use of additional SPC metrics at batch completion in external software (Excel and Minitab).

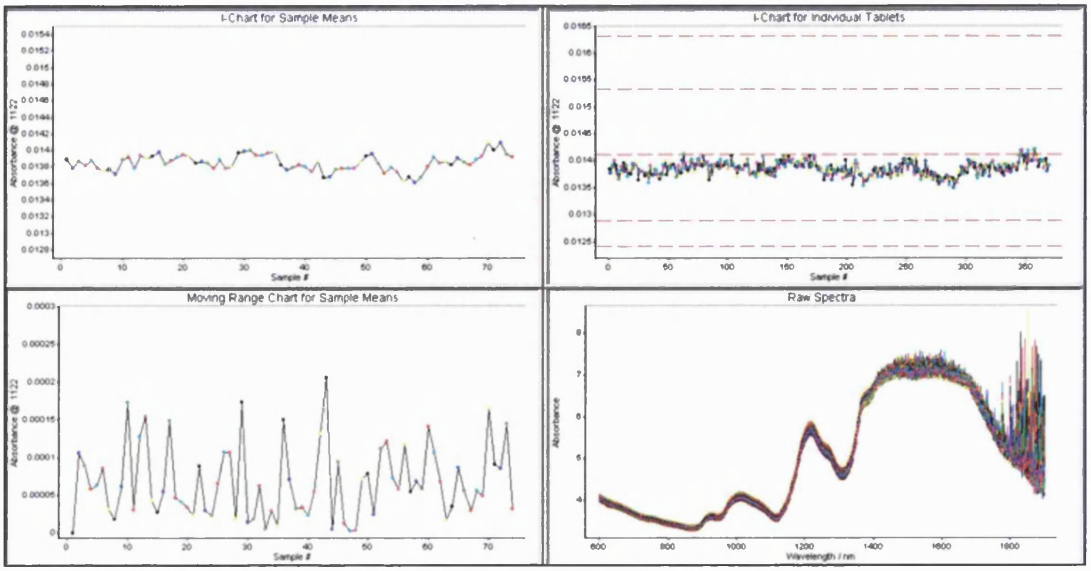

Figure 81: Operator interface for the developed qualitative tablet component quality conformance SPC charts within DeLight software

The application of the qualitative conformance to commercial batches with frequent sampling through manufacture demonstrated significant insight into the tabletting operation. This is demonstrated through the application of the method to commercial tablets monitored throughout tabletting discussed in Section 5.2.2.4. The individual tablet I-chart showed that each batch produced tablets that were within the historically established product SPC limits (both at the VOP and VOC level). The I-MR and Run charts, however showed each batch contained within batch variation that was seen as significant at the 95% confidence level. It is possible that these events were within the normal random behaviour of the process and within the 5% of the population expected to fall beyond the limits. Further work may be valuable in understanding how events identified in the I-MR chart and Run charts relate to tabletting press operation activities. This was not undertaken at the time that the qualitative methods were applied to the commercial batches. The three batches studied indicated the tablets were within an acceptable distribution that fell within the established expected distribution (within the SPC control limits for the product) however the distribution profile indicates a narrower

distribution with smaller tails resulting in lower than expected range in the conformance method metric (range I-chart showed abnormally low range for two assessed batches).

It is important to understand the value of the information in terms of process knowledge and how the new understanding relates to quality perspectives. Questions such as those listed below may need to be considered;

- When does an out-of-conformance occurrence become an actionable manufacturing deviation?
- What data or reports should be part of the batch record?
- Does deviation to historical norm necessitate an investigation

Hesitation to adopt such applications may result if the output is reviewed within the traditional quality assurance mindset.

5.2.4 Summary - Criticality of research

This research investigated the use of data from two qualitative techniques (simple univariate output and ANSD-RR) coupled with SPC charting to create a qualitative tablet component quality conformance approach. Typically a qualification algorithm output is used simply as a categorical classification technique and the extended use of the data coupled with SPC for tablet process monitoring has not been previously reported.

A conformance method based on univariate output was developed and applied to real commercial batches of Norvasc® 5 mg tablets at a Pfizer facility and the value of the work was demonstrated in the ability to interrogate batch to batch and within batch trends in conformance as discussed in Section 5.2.2.4. The benefits and challenges to the implementation for the conformance method were discussed including a

commentary on the availability of vendor software to easily implement such approaches.

Qualitative tablet component quality conformance methods can be applied for any tablet component, not just the API. Success for the active component for Norvasc® 5 mg tablets, which is at a low percentage by weight of the formulation, indicates that this approach would be applicable for any component for any product, provided a qualitative method can be developed with sufficient specificity, sensitivity and the appropriate linearity of response. The requirement for the qualitative approach to provide specificity, sensitivity and appropriate linearity was demonstrated with the ANSD-RR approach lacking sensitivity and hence not meeting the needs of the application to monitor tabletting at the process and VOC levels.

The application of the qualitative tablet component quality conformance method was demonstrated to provide an opportunity to gain greater insight in to the tabletting process and enable real time identification of deviation of process and product from the normal operation. This approach allows rapid remedial action to prevent any occurrence that would have the potential to greatly impact the pharmaceutical manufacturing process and/ or product quality. This work is clearly aligned with the philosophy of PAT through providing an in depth understanding of tabletting operations, providing opportunity for process optimisation and improvement while also assuring quality.

5.3 Semi-quantitative tablet component quality conformance

Although research in Section 5.2 has demonstrated the ability to establish a qualitative tablet component quality conformance method, the success requires the qualitative method to have sufficient specificity, selectivity and linearity as described in Section 5.2.3. Where development of such methods is challenging, a semi-quantitative approach using regression based chemometric techniques may be more appropriate. The development and application of a semi-quantitative tablet component quality conformance method using regression based chemometrics is described in this section demonstrating the value of this research.

Norvasc® 5 mg tablets were selected as the target product as it was the highest volume and highest value product at the manufacturing facility that the work was undertaken. The use of the same research subject as was studied in Section 5.2 allowed direct comparison of the qualitative and semi-quantitative conformance approaches with no further analytical testing or NIRS analysis.

5.3.1 Materials and Methods

5.3.1.1 Design of analysis

The historical dataset established for the qualitative tablet component conformance method research in Section 5.2.1.1 was utilised to develop the semi-quantitative conformance method and establish SPC charts. The verification batches established in Section 5.2.1.1 (including four commercial batches and the four development scale extended range batches) were used to assist in optimising and verifying the suitability of the method. Various regression algorithms were explored to select the most appropriate modelling approach for the semi-quantitative tablet component quality conformance method.

The use of the same data sets for the semi-quantitative approach as with the qualitative approach enabled direct comparison of the fit of both approaches for the purpose of monitoring the tablet operation without the need to take into account differences in the underlying sample sets.

The developed semi-quantitative tablet component quality conformance models were then applied to the same nine commercial tablet batches outlined in Section 5.2.1.1 to establish normal process behaviour (six batches) and assess the suitability of the complete semi-quantitative tablet component quality conformance method (three batches).

5.3.1.2 Reagents and samples

Non-film coated Norvasc® 5 mg tablets at production scales (over a four year period) and development tablets at pilot batch scale were manufactured as described in Section 5.2.1.2.

HPLC grade methanol and purified water (both Riedel-de Haën, Seelze, Germany) and potassium dihydrogen orthophosphate (Fisher Chemicals, Loughborough, Leicestershire, UK) were used for the HPLC mobile phase and diluent solvents.

5.3.1.3 NIR apparatus and software

NIR spectra for method development and verification (140 spectra in total) and for tablet operation monitoring (nine commercial batches) were measured as described in Section 5.2.1.3.

Individual tablet components were evaluated during specificity assessment utilising a 1 mm deep top loading transmission cell (glass 20-C 1 mm cell (Starna Pty Ltd, Australia)) for presentation for transmission NIR analysis.

Data analysis and calculation of the thresholds was achieved using DeLight software, version 2.3b and Dementia 1.1b software (DSquared Development, La-grande, OR, USA), Minitab version 16.1 (Minitab, Inc.) and Microsoft® Excel, version 9 (Microsoft® Corporation).

5.3.1.4 HPLC apparatus, software and methods

Reference chemistry measurements were made as described in Section 5.2.1.3.

5.3.2 Qualitative Tablet Component Quality Conformance Method Development

5.3.2.1 Spectra pre-treatment

The mathematical pre-treatment (800–1360 nm extract, SNV and five point smoothing) and derivative transform (1st derivative with five point gap) optimised in 5.2.2.1 was applied to all spectra for the semi-quantitative tablet component quality conformance method.

5.3.2.2 Model development

As univariate qualification methods were previously reviewed in Section 5.2, multivariate regression techniques were explored. The simplest multivariate regression technique, MLR (refer to Section 0), and the more traditionally applied PLS regression (refer to 0), were chosen for further analysis.

Rather than full regression techniques, a hybrid of DA (refer to Section 3.3.3) using nominated values at five levels was chosen to reduce the volume of reference analysis and create a semi-quantitative approach. The reference analysis results (in mg per tablet) described in 5.2.2.3 on 16 tablets from the verification batches (results tabulated in Table 17) were used to nominate values for each tablet in the historical batches used to develop the regression model. Originally the intention was to apply the theoretical amlodipine active content, 3.75, 4.25, 5.75 and 6.25 mg per tablet , for each of the ten tablets in the 75 %, 85 %, 115 % and 125 % development batches. However following the assay of the 16 tablets during the development of the qualitative tablet component quality conformance method development (Section 5.2.2.3), it was apparent that assigning the theoretical value was not appropriate due to the hypothesis error in formulating the 75% development batch. Therefore, each tablet in the historical dataset used to develop the calibration curve was nominated the amlodipine content based on the result obtained from the HPLC analysis of the two representative tablets from the verification data at each concentration level. The nominated amlodipine active content for the ten tablets in the 75 %, 85 %, 115 % and 125 % development batches were 3.35, 4.15, 5.70 and 6.00 mg per tablet respectively. All production samples were nominated amlodipine active content values of 4.90 mg per tablet based on the two representative tablets assayed for each of the four verification batches. This result was lower than the expected 5.00 mg per tablet (label claim) indicating either a slight offset in process target (possibly tied to a purity assumption in dispensing the amlodipine API in the formulation) and or systematic bias in the HPLC reference method. Note that the discrepancy between expected and HPLC determined amlodipine active content is within acceptable validation limits for a HPLC method.

For the purpose of discussion MLR-DA with nominated values is termed MLR-NV and PLS-DA with nominated values is termed PLS-NV hereafter.

The MLR-NV model (with two terms) was developed in DMentia software utilising the historical dataset (composed of six production batches) and the first five development spectra of each content level (for ease of discussion this data set is termed Regression Dataset). Initially, the DMentia software was allowed to automatically pick the optimum wavelengths for the two terms in the regression. The wavelength 1122 nm was selected as the primary variable while 1282 nm was selected for the secondary variable.

Figure 82 shows the correlation plot for the first term in the MLR-NV regression indicating that 1122 nm is the optimum wavelength for the primary wavelength as the maximum occurs at a stable correlation. The primary wavelength is the same as that identified in Section 5.2.2.1 as highly correlated to the amlodipine besylate content and the wavelength utilised in the development of the qualitative tablet component quality conformance method.

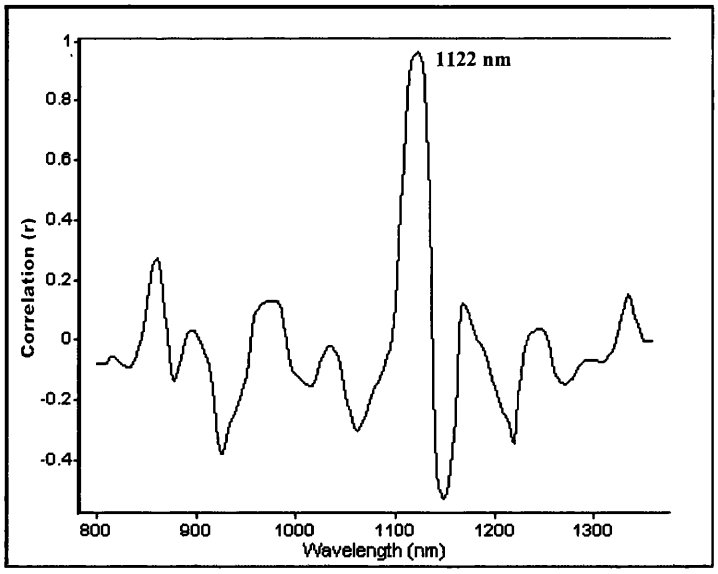

Figure 82: Correlation plot for the 1st MLR-NV wavelength term

The correlation plot for the 2nd term (Figure 83) shows the correlation at each wavelength to the residual content (predicted content from 1st term subtracted from the nominated content). This shows that spectra are strongly correlated to the residual content between 1172-1194 nm and 1266-1294 nm with the maximum at 1282 nm.

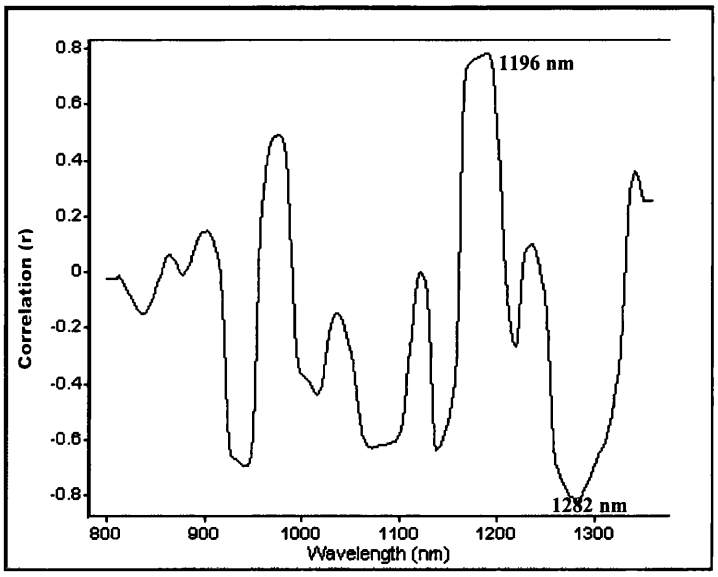

Figure 83: Correlation plot for the 2nd MLR-NV wavelength term

Figure 84 shows the 1st derivative spectra focused on these wavelength regions to illustrate the suitability of the wavelength choices. There is clear discrimination between the content levels at 1122 nm indicating this wavelength is ideal for the primary MLR-NV wavelength term, while there is no differentiation by content at 1196 nm or 1282 nm. As 1284 nm is a peak maximum, this wavelength would provide a more stable wavelength selection as the secondary term rather than 1196 nm and 1282 nm. Including the spectral information at this wavelength will stabilise the prediction and correct for any underlying systematic noise in the spectra and as such 1284 nm was selected as the secondary stabilising MLR-NV term.

Note that the correlation at 1284 nm in the 1st term correlation plot is very low confirming that this wavelength is not collinear with 1122 nm.

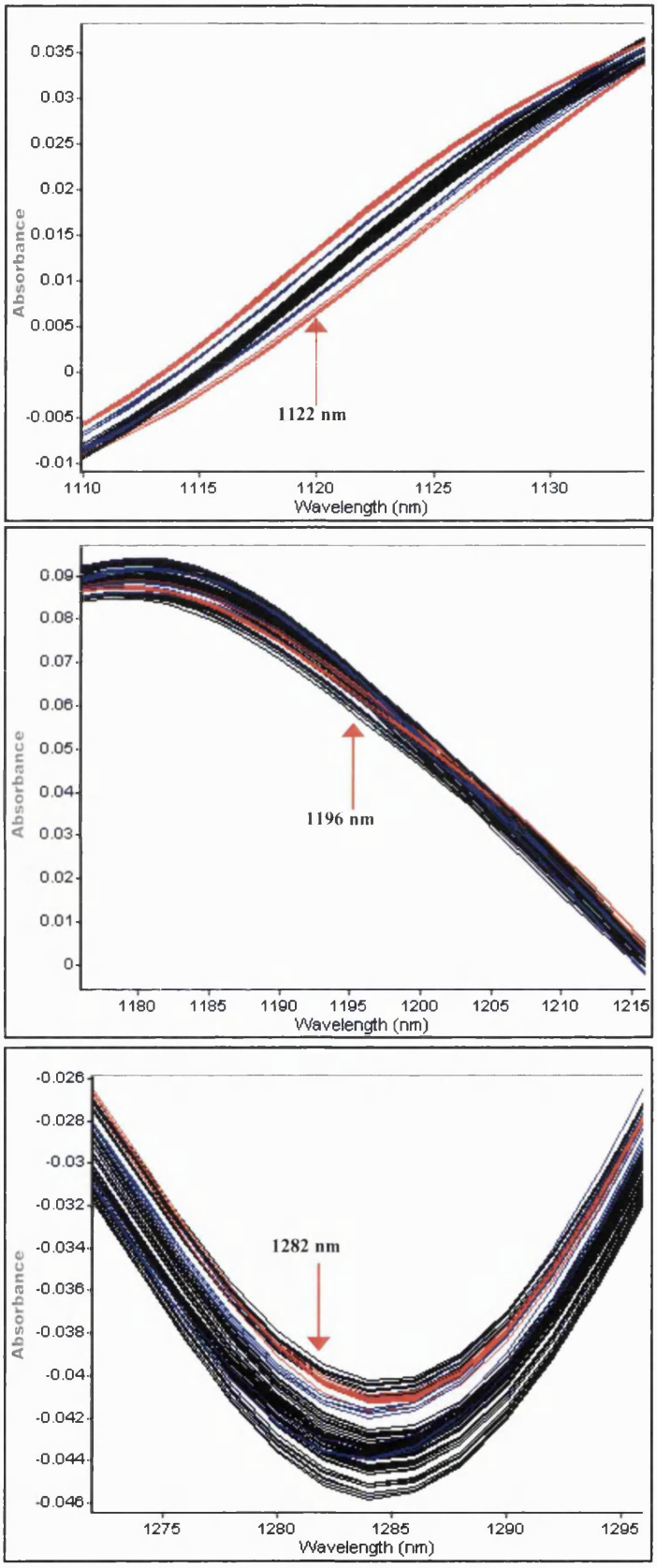

Figure 84: Zoomed 1st derivative spectra marked with MLR-NV wavelengths for the Regression Dataset with; production batches (---), 85 % & 115% development batches (---) and 75 % & 125% development batches (---)

A third term was reviewed however the third term had little impact on the MLR-NV model statistics and wavelength selection was not clear (correlation appeared low with no prominent features thus indicating that a third term will include random noise in the regression). As such a two term model was chosen for further study.

The MLR-NV results for the Regression Dataset used to create the model are tabulated in Appendix 5 on page 301. The MLR-NV model statistics are show in Table 20 and demonstrates the model is very capable of relating the correlation of tablet content (r^2>0.95) with low error (SEE of 0.08634 represents a 1.727% error in estimating content at label claim).

Table 20: Model statistics for the MLR-NV model

r2	0.9764
SEE (mg / tablet)	0.08634
slope	0.9470
Intercept (mg / tablet)	0.2584

To assess the capability of the MLR-NV model to predict satisfactorily for samples independent of those used in the model, deliveries external to the Regression Dataset were predicted with the models in DeLight and the prediction statistics reviewed. Four production batches (ten tablets for each batch) and the remaining development tablets (five tablets for each batch) were used to verify the model. The predicted results are tabulated in Appendix 6 on page 303. The SEP for the MLR-NV predictions was calculated according to Equation 17 and found to be 0.1024, yielding a SEP: SEE ratio of 1.19. This close agreement in errors indicates that the MLR-NV model is very capable when predicting new deliveries. Figure 85 depicts the residuals of the predictions and indicates a degree of non-random scatter about zero.

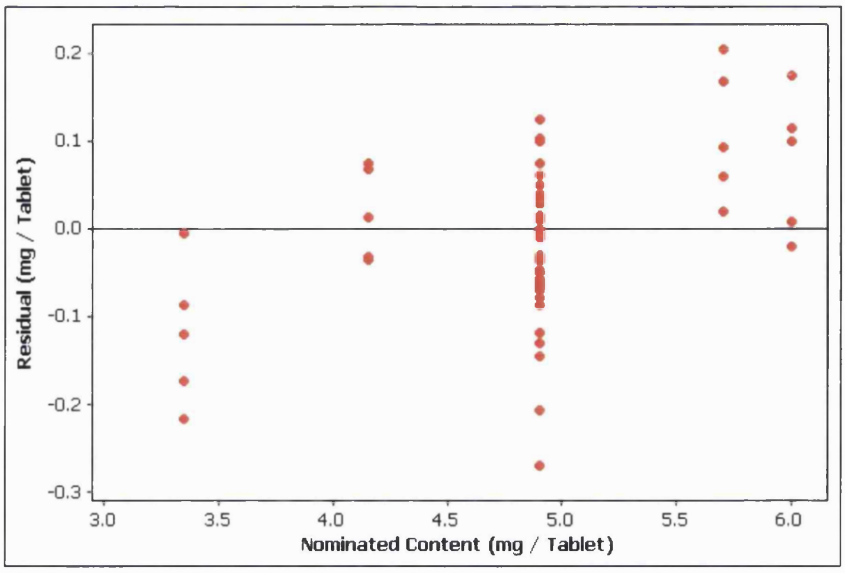

Figure 85: MLR-NV residuals plot for prediction of verification tablets

Despite the MLR-NV yielding appropriate prediction error, the non-random pattern of the residuals indicates that there is a need to include further variables in the MLR to explain all correlations in the data to the amlodipine active content. Including additional wavelengths (increasing the terms in the MLR-NV analysis) is one option, however the initial assessment showed little clarity in selection of the 3rd term and investigation of PLS based models was preferred.

PLS-NV models were developed over the 810–1340 nm range in DMentia software utilising the Regression Dataset. The range was reduced from 800–1360 nm due to spreading of the data at the extremes of the range as an effect of the SNV correction which had a strong influence on the PLS regression. Given the complexity of the formulation (five components) and tablet physical matrix, an initial model was developed with eight LVs to explore the variables and their relationship to the amlodipine active content.

Review of the scores plots in Figure 86 and Figure 87, show that the data separates relative to the amlodipine active content predominantly along the first and second LV axes.

Differences between batches and between commercial and development batches (independent of amlodipine active content) appear to be explained in the 2nd through 5th LVs. Note also that the magnitude of the scores for fifth to eighth LVs in Figure 87 are an order of magnitude lower than the earlier variables.

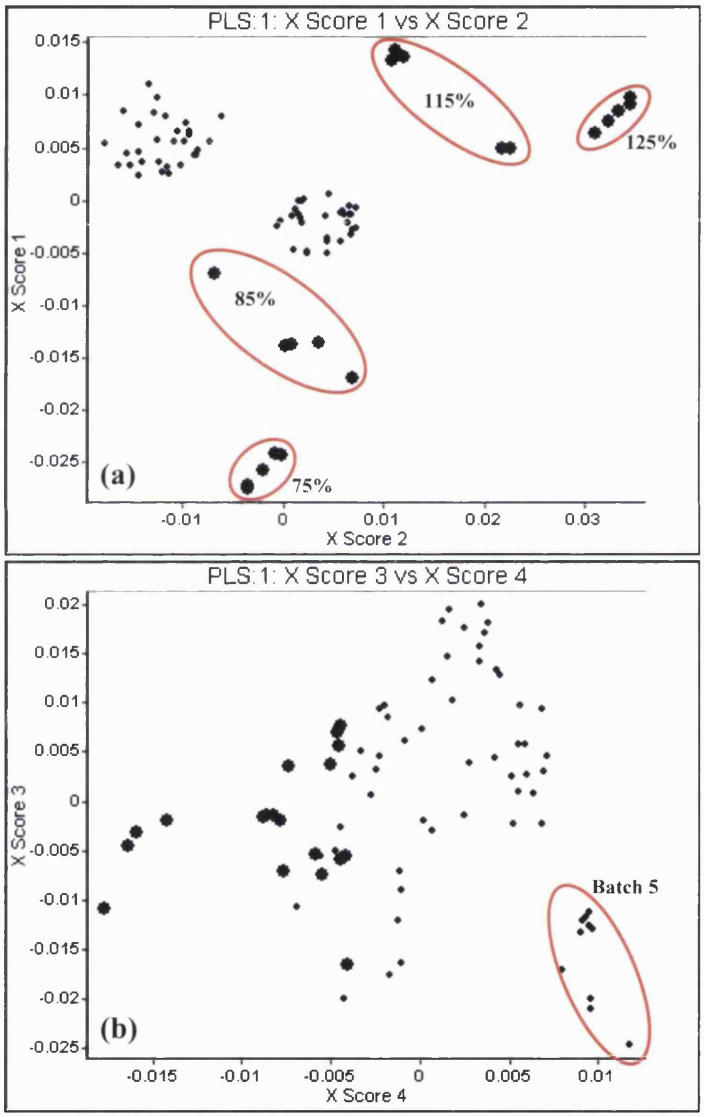

Figure 86: LV scores plots for the regression data set (a) LVs 1 vs. 2 and (b) LVs 3 vs. 4 with samples from the development batches in bold

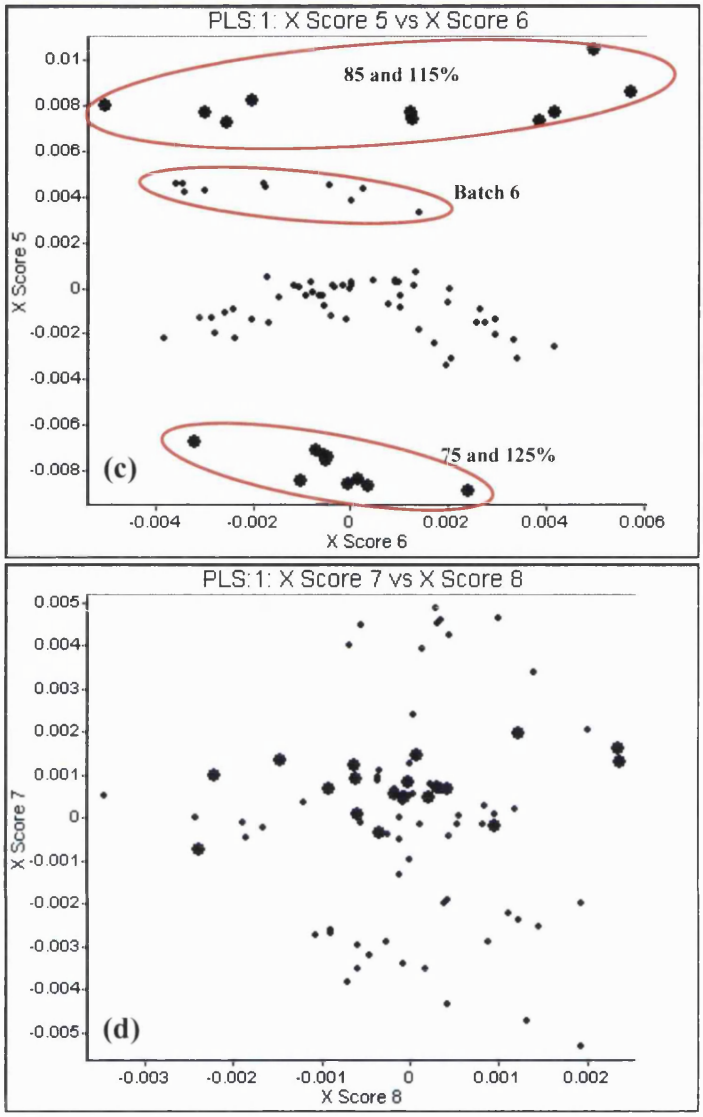

Figure 87: LV scores plots for the regression data set (a) LVs 5 vs. 6 and (b) LVs 7 vs. 8 with samples from the development batches in bold

Examination of the weights (red lines) of the LV spectral loadings (black lines) in Figure 88(a) shows that the first LV includes the wavelength (1122 nm) that has previously been identified to be highly correlated to the amlodipine active content. The wavelengths of variation included in the next two LVs (refer to Figure 88(b)-(c)) relate to O-H functional group absorbance (at ~970 nm) commonly seen in free moisture (refer to Figure 2) and C-H functional group absorbance (1180–1240 nm) commonly seen in aliphatic compounds (refer to Figure 2) indicating contribution from excipients such as microcrystalline cellulose, magnesium stearate and sodium starch glycollate.

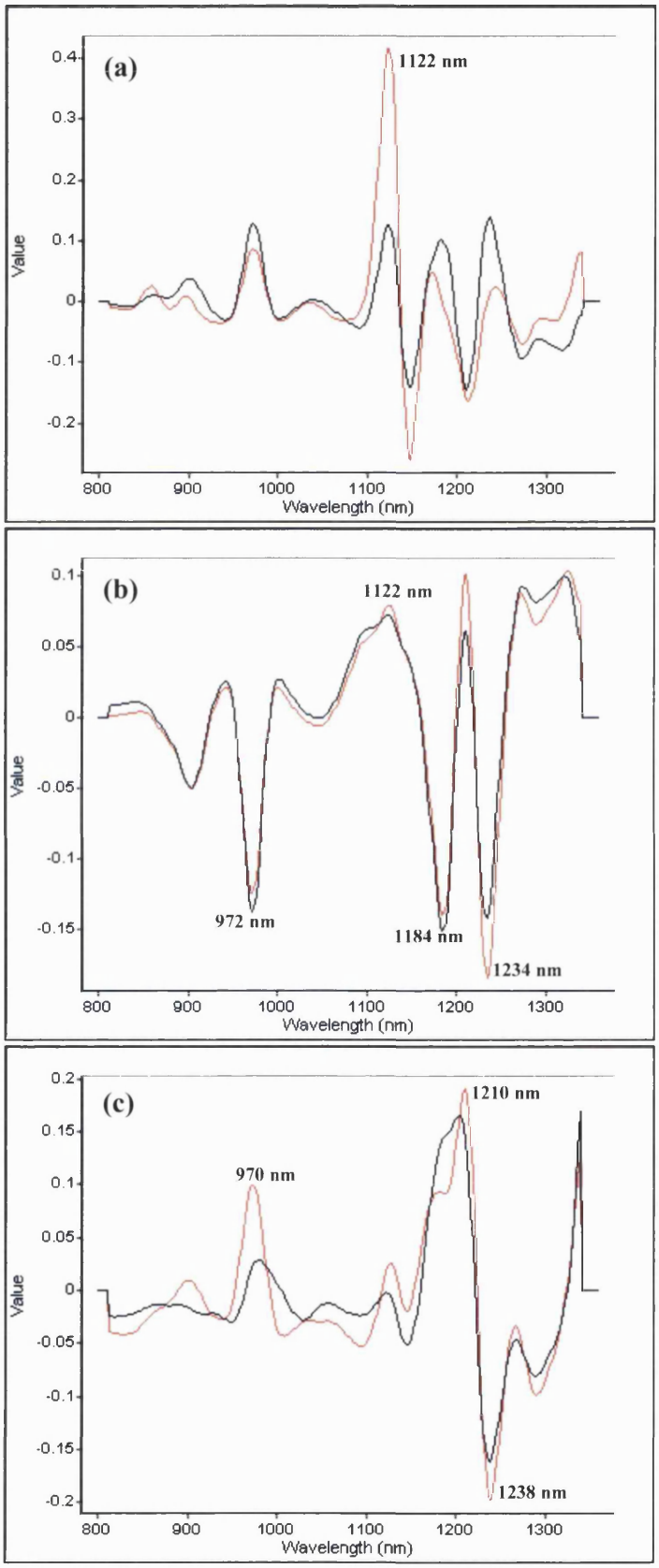

Figure 88: Weighted loading for the PLS-NV model of regression dataset (a) LV 1 to (c) LV 3 with loadings in black and weights in red

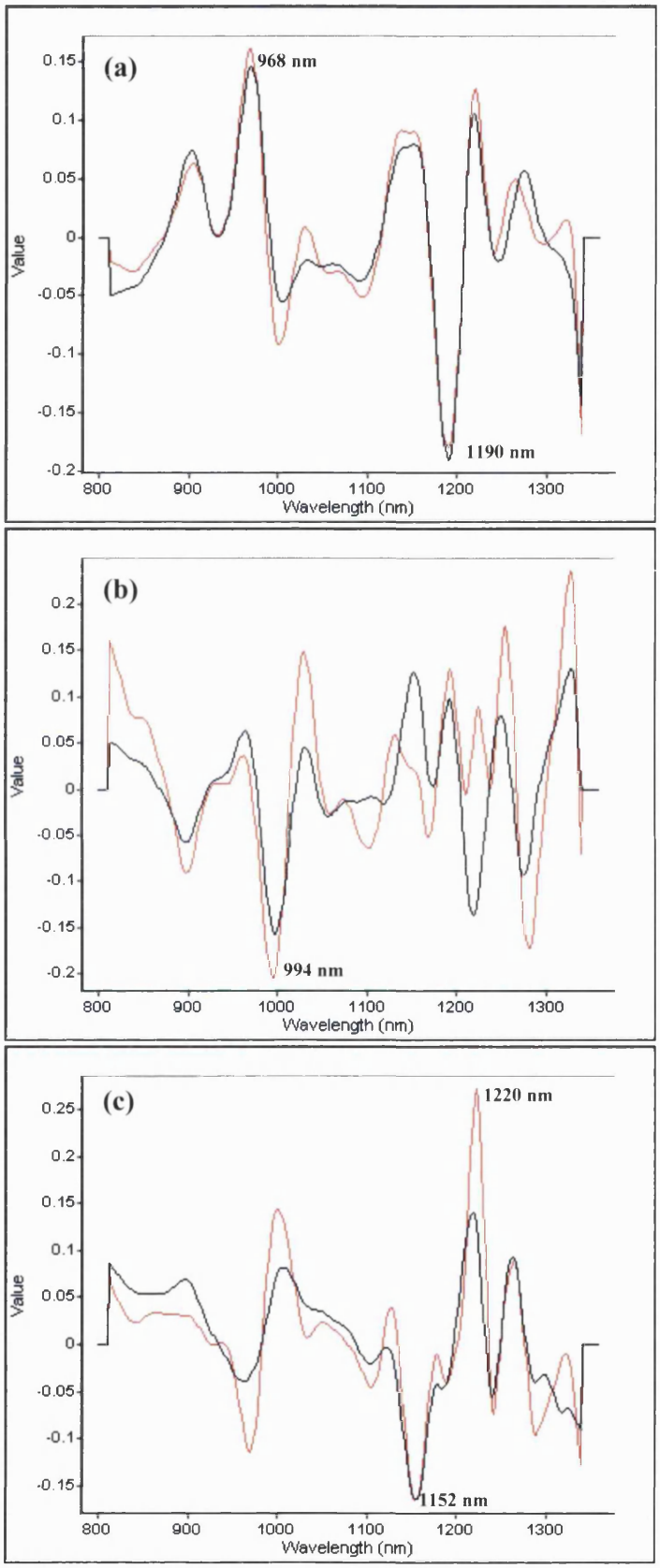

Figure 89: Weighted loading for the PLS-NV model of regression dataset (a) LV 4 to (c) LV 6 with loadings in black and weights in red

The wavelengths of variation included in the fourth LVs (refer to Figure 89(a)) also relate to O-H functional group absorbance (at ~970 nm) and contributions from excipients. The variation explained in fifth LV is less obvious (Figure 89(b)), relating to the differences between batch six and the other production batches and also differences seen in the spike and dilute development batches compared to the production batches. This is likely due to physical effects and tablet press as no distinct regions of the wavelength are identified as assignable to functional group absorption of particular formulation components and many wavelengths are affected (which would be expected to be the case with physical effects affecting the entire spectrum). The sixth LV (Figure 89(c) once more shows contribution from the region likely indicative of a C-H functional group absorbance and therefore is likely to be modelling residual batch to batch or individual tablet to tablet matrix variations.

PRESS was calculated for the eight LVs with leave-one-out cross validation to assist in selecting the optimum number of variables to use in the PLS-NV model. As expected, Figure 90 shows a continued improvement in the SEE as an increasing number of LVs are included in the model. This relates to the model being better able to describe the Regression Dataset spectra as more spectral detail is included. The SECV shows a marked improvement as the second LV is included and then decreasing error in parallel to the SEE with the closest agreement occurring at the fourth LV.

Observation of the variation removed in the x-axis (spectral variance) as each LV is included in the model shows a steep increase with the inclusion of the first five variables (reaching 81.8% variance explained) then a slower increase thereafter. The majority of the variation in the y-axis (amlodipine active content variance) is explained in the first and second LVs (explaining 77.5% of the variance) with a gradual increase in the variation explained for subsequent variables.

Review of the scores, the loadings and the PRESS output indicates that five LVs may be optimal and balances an improved error without over fitting and including noise and unrelated variation which may lead to lack of robustness of the model over time.

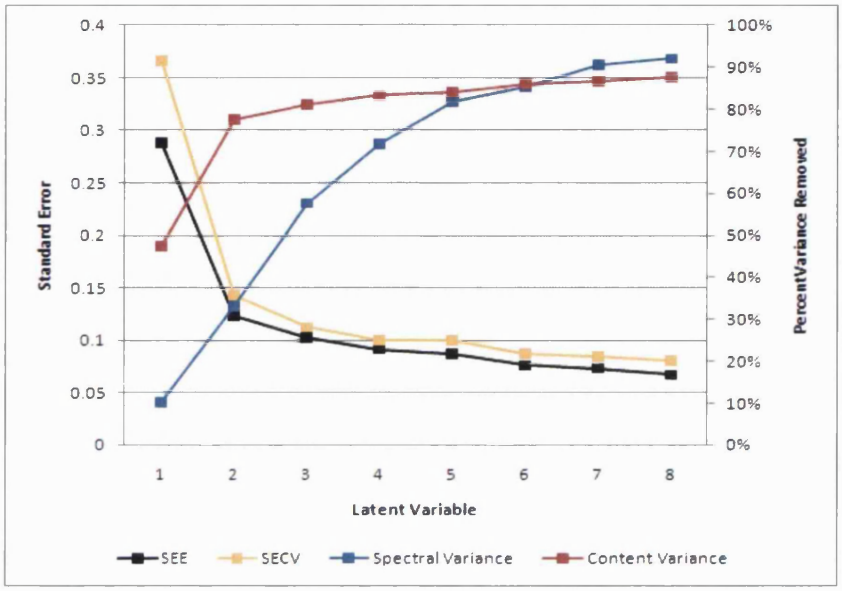

Figure 90: SE and variation for the PLS-NV models developed with varying number of LVs

The PLS-NV results for the Regression Dataset used to create the model are tabulated in Appendix 5 on page 301. The PLS-NV model statistics for the model is shown in Table 21 and indicates that the PLS-NV model with five LVs is capable of relating the correlation of amlodipine active content (r^2>0.95) with low error (SEE of 0.08805 represents a 1.761% error in estimating content of label claim). Also note the model compares well with the MLR-NV model statistics described in Table 20 with similar correlation and error.

Table 21: Model statistics for the PLS-NV model with five LV

r2	0.9745
SEE (mg / tablet)	0.08805
slope	0.9745
Intercept (mg / tablet)	0.01243

To assess the capability of the PLS-NV model to perform satisfactorily for samples independent of those used in the model, the verification data set utilised to assess the MLR-NV model was applied to assess the PLS-NV model developed on five LVs. The predicted results are tabulated in Appendix 6 on page 303.

The SEP for the PLS-NV model was calculated according to Equation 17 and found to be 0.09220 yielding a SEP:SEE ratio of 1.05. This close agreement in the errors indicates that the PLS-NV model is very capable when predicting new deliveries.

Figure 91 depicts the residuals of the prediction and indicates a slight degree of non-random scatter about zero. The slight linear variation in the data will result in low potency tablets being predicted slightly lower in expected content while over potency tablets would be predicted slightly over expected content. This is equivalent to erring on the side of caution. Note that the PLS-NV model shows more homoscedastic behaviour than was seen in the MLR-NV model (refer to Figure 85).

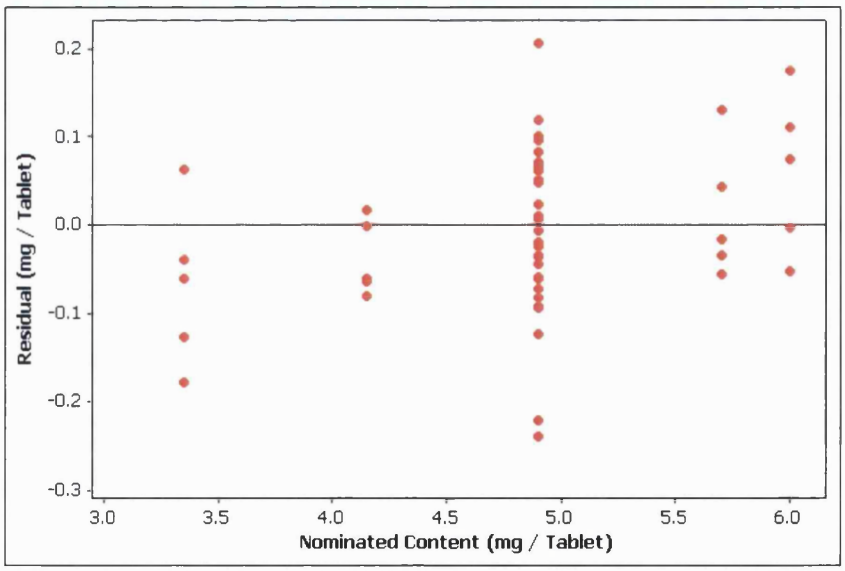

Figure 91: PLS-NV residuals plot for prediction of verification tablets modelled with five LVs

Following review of the MLR-NV and the PLS-NV models, the five LV PLS-NV model was selected for continued development of the semi-quantitative tablet component quality conformance method.

5.3.2.3 Establishment of the conformance control charts

As was discussed in 5.2.2.3, once the conformance chemometrics have been established and models created, SPC charts must be established that will monitor results within and between batches over time.

To establish the individual tablet conformance control chart, the predicted amlodipine active content from the PLS-NV model with five LVs for the six historical dataset (tabulated in Appendix 6 on page 303) were exported into Minitab software to establish normality to allow the development of Shewhart[137] control charts. The Graphical Summary function in Minitab was used to represent the data and assess normality (Appendix 7 on page 305). No evidence of non-normality was observed (p>0.05) at the 95% confidence level. The normality p-value is shown in Table 22.

Note that one tablet result was identified as a potential outlier. This individual tablet was assayed by HPLC and confirmed that the amlodipine active content was higher (HPLC assay of 5.20 mg / tablet) than would be expected from the data within the rest of the historical data set. As HPLC confirmed the result, it was determined that it was valid to include the tablet result in the historical dataset for the development of the SPC charts.

Table 22: Parameters used to generate the SPC charts for the semi-quantitative tablet component quality conformance method

***p*-value**	0.061
Mean (mg / tablet)	4.903
Standard Deviation (mg / tablet)	0.0875

Figure 92 shows the I-chart for the historical dataset. The mean and standard deviations applied in establishing the control charts are shown in Table 22. Control limits (___) indicating the VOP were set at three standard deviations from the historical dataset mean (___) value. The confirmed higher amlodipine content sample is marked in red with a "1" notation as the sample is beyond three standard deviations from the mean.

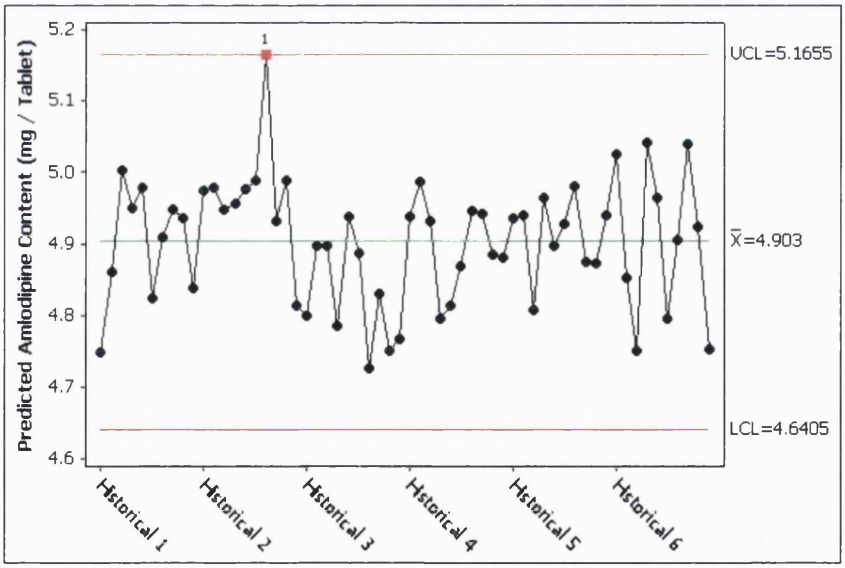

Figure 92: I-chart for the historical dataset for the PLS-NV model

As the units of measure for the output from the PLS-NV predictions are in mg per tablet, VOC limits can be applied directly. Aligned with the VOC limits applied in the qualitative tablet component quality conformance method, limits were set at ±15 % of label claim (4.25 and 5.75 mg per tablet).

To demonstrate that the determined VOP and VOC limits are appropriate, tablets from four further commercial production batches and the remaining five extended range development tablets were assessed with the developed SPC chart. Figure 93 demonstrates that the thresholds are appropriate to monitor when individual values fall outside the established normal process as all development batch samples are marked in red with the"1" denoting the samples exceed the VOP limits.

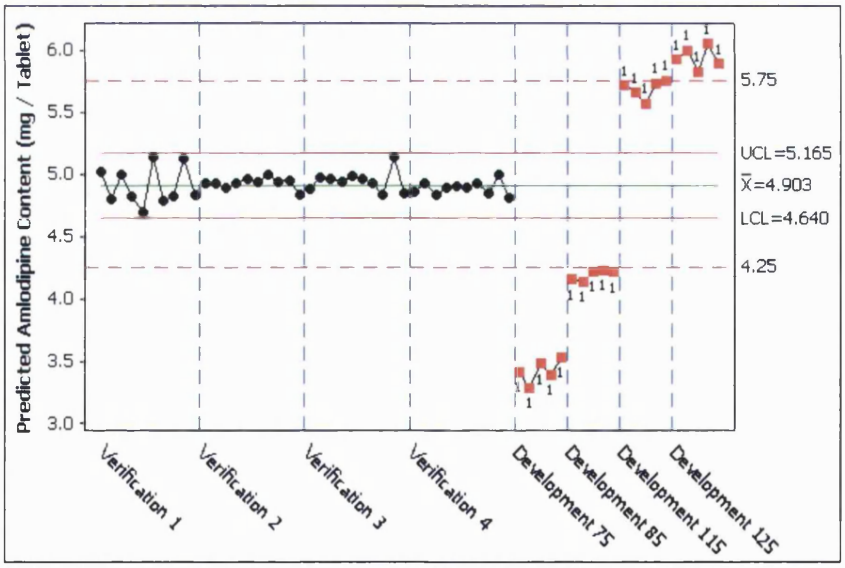

Figure 93: I-chart for the verification data showing verification batches with VOC (_ _ _) and VOP (___) limits

The tablets assayed by HPLC and used to nominate amlodipine active content values (shown in Table 17) indicate that the tablets in the 85 % development batch would be expected to just be beyond 'acceptable' tablets (assaying at an average of 4.15 mg per tablet), while the 115 % development tablets would be expected to fall just within the

'acceptable' tablet range (assaying at an average of 5.71 mg per tablet). The SPC chart shown in Figure 93 aligns directly with these expectations demonstrating the suitability of the VOP and VOC limits.

The verification results of the PLS-NV method show that the derived SPC chart for the individual tablets (reflecting both the VOP and the VOC) is appropriate to be applied during the tabletting process to monitor individual tablets as they are manufactured. The SPC chart correctly identified that the 115% development samples were at or just within VOC limits while identifying the 85% development were at or outside of the VOC limits.

The developed SPC charts would provide sufficient insight into the content uniformity of processes when applied throughout tabletting operations by identifying individual tablets beyond 85–115 % label claim allowing manufacturing to count the number of tablets beyond the acceptable range, arrange for confirmatory testing and determine batch compliance to content uniformity as described in Sandell's[119] approach for content uniformity for large n.

The established individual control chart addresses the important question of whether each individual tablet is within the normal process parameters for that product and also whether the tablets analysed will meet quality requirements (average amlodipine active content and content uniformity). To assess the key process questions identified in 5.2.2.3 (within batch trends and overall batch variation conformance), control charts aligned with that developed for the qualitative tablet content conformance method (described in 5.2.2.3) were developed. The batches of Norvasc® 5 mg tablets utilised in the development of the process monitoring SPC chart strategy in 5.2.2.3 were again utilised for the semi-quantitative tablet component quality conformance method to allow direct comparison of the two approaches.

For each of the nine batches (representing the use of three tablet presses), five tablets were taken at half hourly intervals throughout tabletting operations. This frequency was set based as per discussion in Section 5.2.2.3 on page 208.

Although no typical systematic process signature was seen in the qualitative tablet component conformance method (refer to Figure 70), the data output from prediction with the PLS-NV model were reviewed for the same six commercial batches. Once more, Figure 94 shows no characteristic batch to batch process trends / trajectories existed for the Norvasc® tabletting process. As there was no typical systematic process signature, process signature trajectory modelling across batches was not pursued.

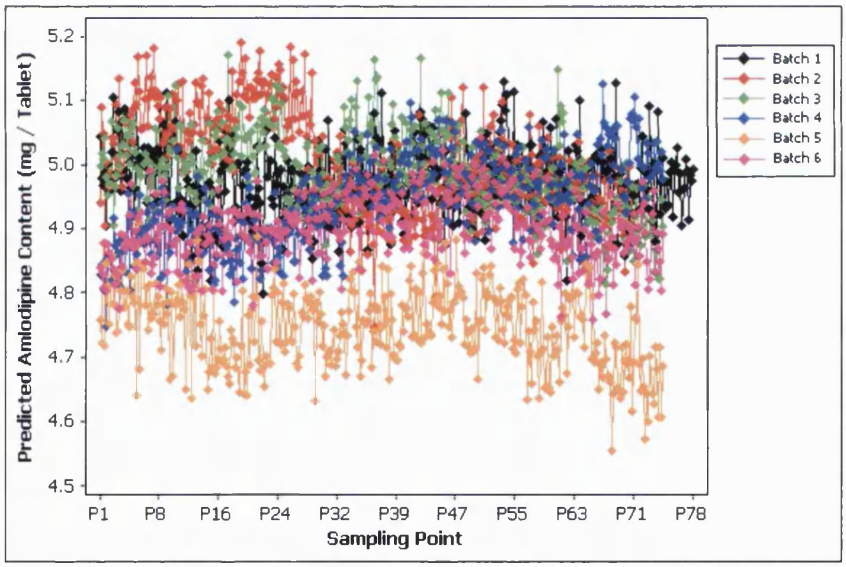

Figure 94: Time Series chart of six commercial batches showing lack of common process signature across the tabletting operation

I-MR chart for Sample Means and Run charts were developed as control charts to assess within batch process trends. Figure 95 and Figure 96 show an example of the I-MR and Run charts for the first of the six historical batches used to develop the control chart strategy.

The I-MR chart (Figure 96) shows that several sampling points are beyond the expected population (beyond three standard deviations of the batch mean marked with a "1") and also that there is a series of data points on one side of the mean (marked with a "2"). No sudden shifts between sampling points were identified in the MR chart.

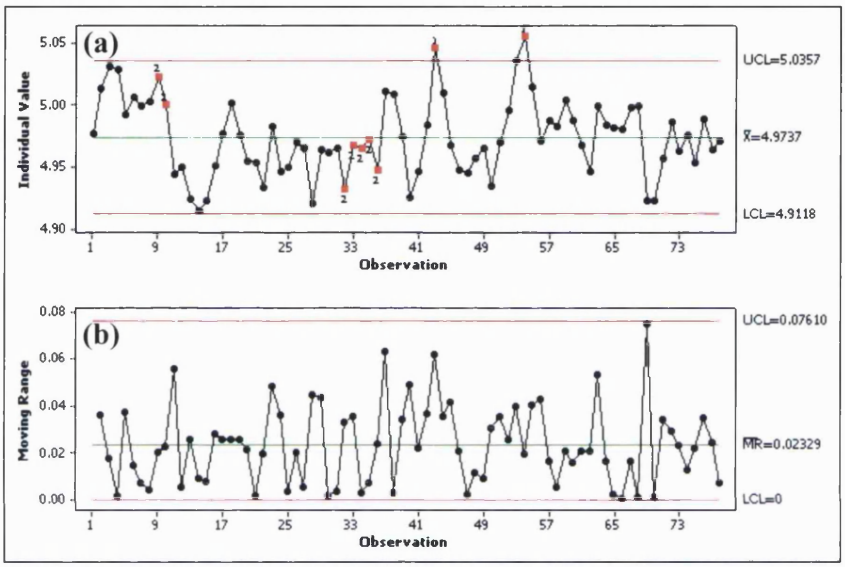

Figure 95: I-MR chart of predicted amlodipine active content (mg / tablet) for the 1st historical batch showing within batch trends with (a) I-chart for sample mean and (b) MR chart for sample mean

The Run chart (Figure 96) indicates that there is a significant degree of clustering of points on one side of the mean (p-value for clustering <0.05) and also a degree of trending up and down (p-value for trends < 0.05). Data were not found to alternate up or down or to have any significant oscillating / cycling pattern (p-value for mixtures and oscillations > 0.05).

As was noted in the qualitative tablet component quality conformance method, these plots provide insight into the tabletting operation. The information may be able to be aligned with tablet press operation parameters, powder blend charging and operator shift patterns to enable further improvements to process capability.

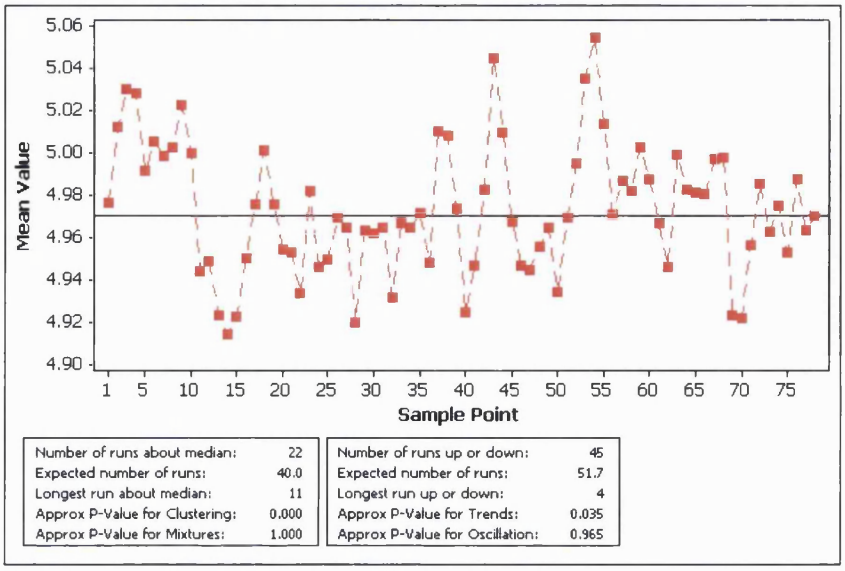

Figure 96: Run chart of predicted Amlodipine active content (mg / tablet) for the 1st historical batch showing within batch trends

An I-chart for ranges was calculated across all samples in the six commercial process batches to establish a SPC chart to monitor the spread of amlodipine active content across the batch. Once more an assumption is made that the systematic sampling by time provides a means to capture the approximate spread of the batch population. As this sampling strategy was applied for all batches, the assumption is that the SPC chart developed with six historical commercial batches will approximate future manufactured batches.

The range of the predicted amlodipine active content (from the PLS-NV model) was calculated and the Graphical Summary function in Minitab used to represent the data and assess normality (Appendix 7 on page 305). No evidence of non-normality was observed (p>0.05) at the 95% confidence level. The normality p-value is shown in Table 23. Note that output is an estimate only as the number of data points is small, reducing the power of the normality tests.[135]

Figure 97 shows the Minitab I-chart for ranges for the historical dataset. The mean and standard deviations applied in establishing the control charts are shown in Table 23. The control limits (___) were set at three standard deviations from the mean (___).

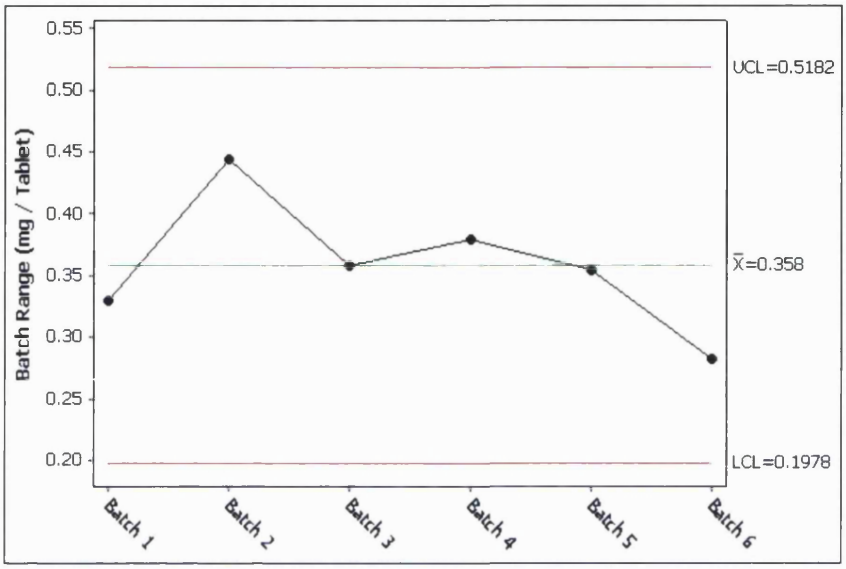

Figure 97: I-chart of the range of PLS-NV predicted amlodipine active content for the six commercial batches

Table 23: Parameters used to generate the range SPC chart for the semi-quantitative tablet component quality conformance method

***p*-value**	0.690
Mean (mg / tablet)	0.3580
Standard Deviation (mg / tablet)	0.0534

Distribution profiles were derived from frequency distribution histograms of the PLS-NV model predictions as discussed in Section 5.2.2.3. To enable direct comparison batch to batch and the development of an appropriate control mechanism, the distribution histograms were standardised through the use of percent frequencies rather than absolute frequencies and also by the use of 19 data bins (as discussed in Section

5.2.2.3). Figure 98 depicts the frequency distribution histograms for the six commercial batches of tablets with the percent frequency occurrence across 19 bins.

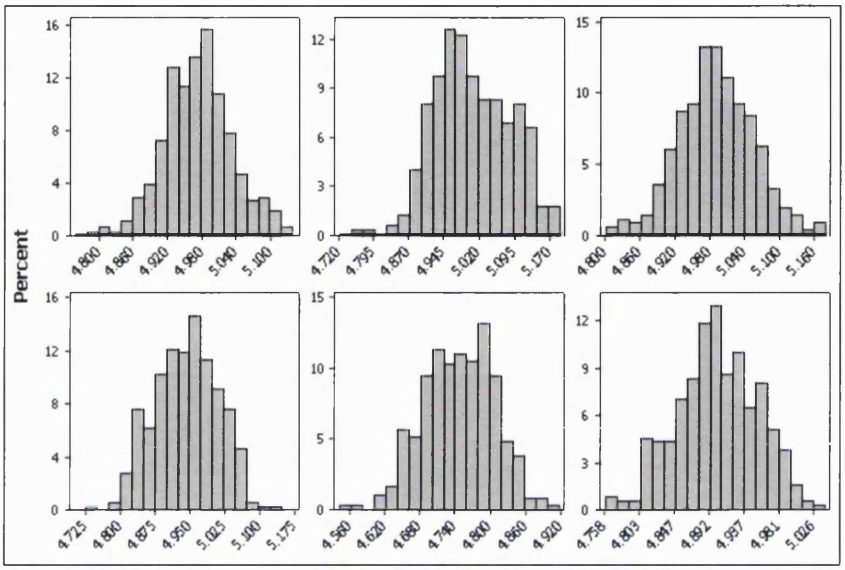

Figure 98: Distribution histogram of the PLS-NV predicted amlodipine active content for the six commercial batches

The curves of the distribution histograms (standardised across batches by the use of percent frequencies and constant binning) were used to establish the boundary of acceptable distribution (control limit was set as three standard deviations from the mean of the established typical distribution). Figure 99 shows the control chart for the distribution profiles developed from the six commercial batches.

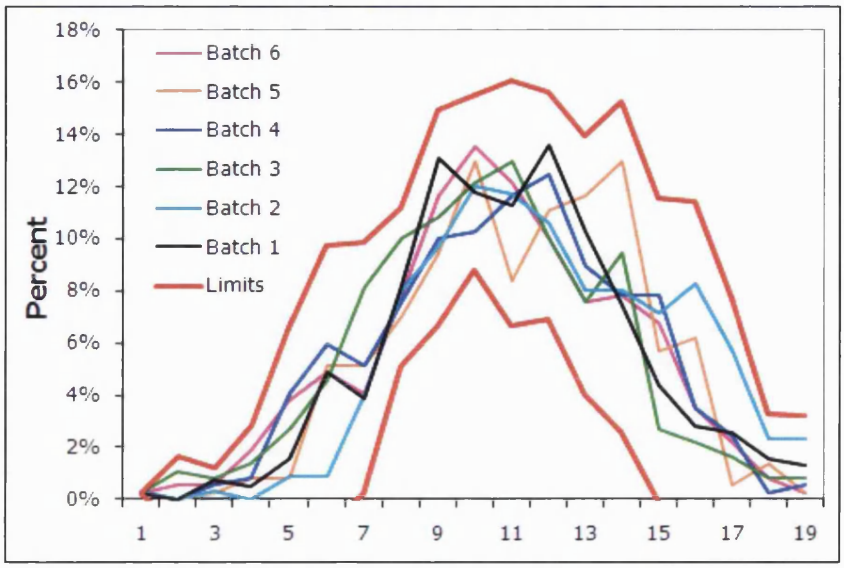

Figure 99: Overlay of distribution profiles for six commercial batches with derived control limits (___)

The developed semi-quantitative tablet component quality conformance method provides significantly greater assurance of the quality of the product through providing a mechanism to demonstrate the product is manufactured using a well understood and controlled process.

5.3.2.4 Implementation of the developed method concepts in manufacturing

The application of the individual tablet control chart to commercial process data (five tablets sampled at half hourly intervals throughout the tabletting process) is shown in Figure 100.

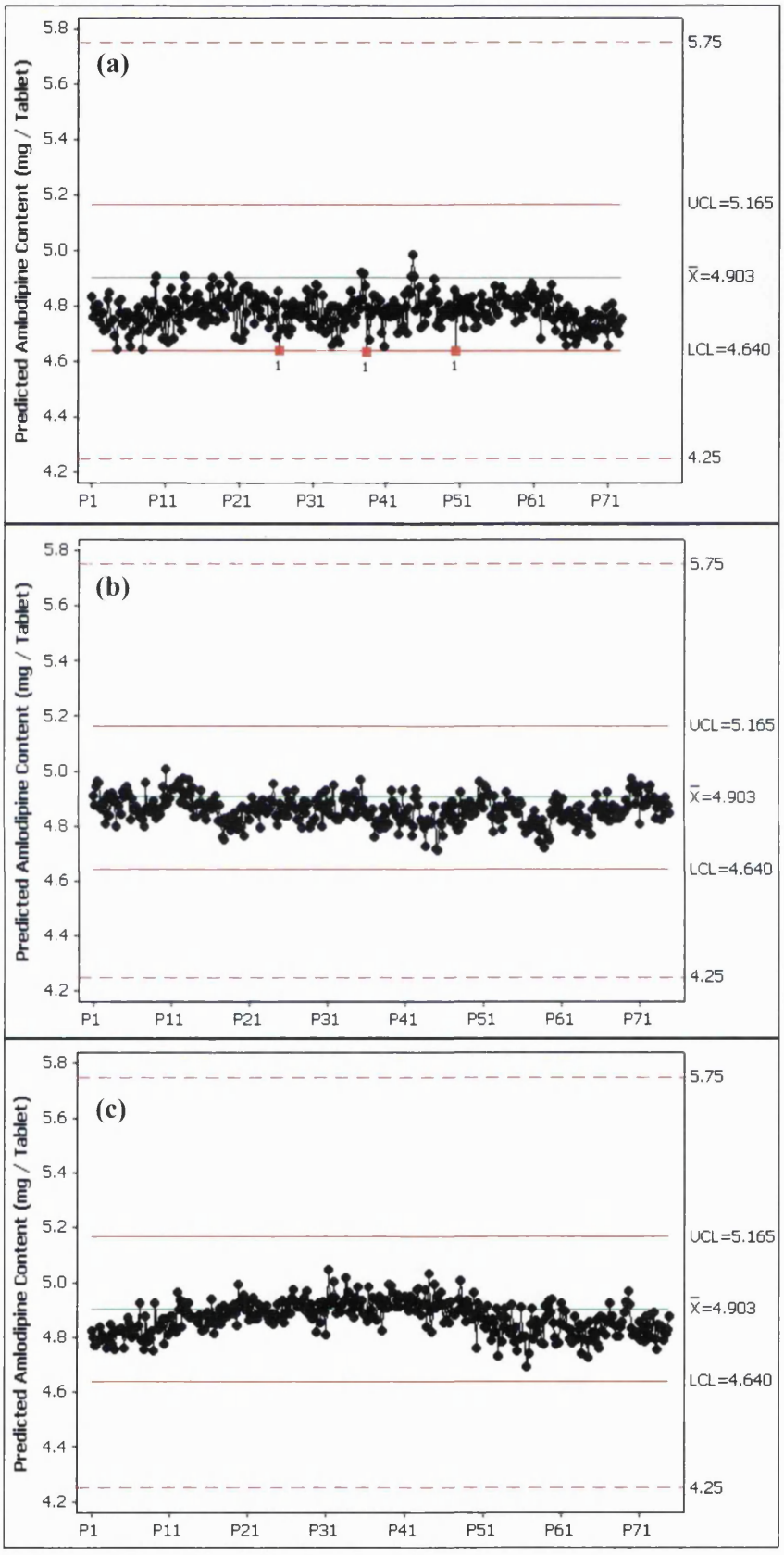

Figure 100: Individual tablet SPC chart of predicted amlodipine active content (mg / tablet) for three production batches; (a) batch 1 to (c) batch 3 with VOP (___) and VOC (_ _ _) limits and product mean (___)

Figure 100 demonstrates that batch two and three are centred about the VOP mean with no tablets beyond the VOP limits. Batch one is shifted to lower amlodipine active content with three individual tablets at or just outside the VOP limits. It may have been of interesting to rescan the out of trend tablets alongside a control to verify that the analysis was representative with no operator errors. This was not conducted at the time of scanning. No individual tablets fell near the VOC limits demonstrating tight processing control and that tablets will meet the desired quality expectations. The shift in batch one mean from the established product mean is of interest and indicates an opportunity for process understanding and improvement.

The application of the within batch control charts for the three process batches used previously are shown in Figure 101 and Figure 102. The I-MR control charts for the three batches shown in Figure 101 indicate that within the three batches there are excursions beyond the within batch process limits (flagged in red and marked with a "1"). Batch one and three also have occurrences of nine consecutive sample points on one side of the line (flagged in red and marked with a "2" in the chart). The MR chart also has identified that there are occasional sudden changes between time points that are higher in magnitude than expected for batch one and three. This may align with tablet press adjustments, refill of blend in press feed hoppers or operator shift patterns and is worth further investigation to identify whether any of these factors can be improved to positively affect the process capability. As mentioned in Section 5.2.2.4, this was not conducted at the time of scanning and was a missed opportunity for process knowledge.

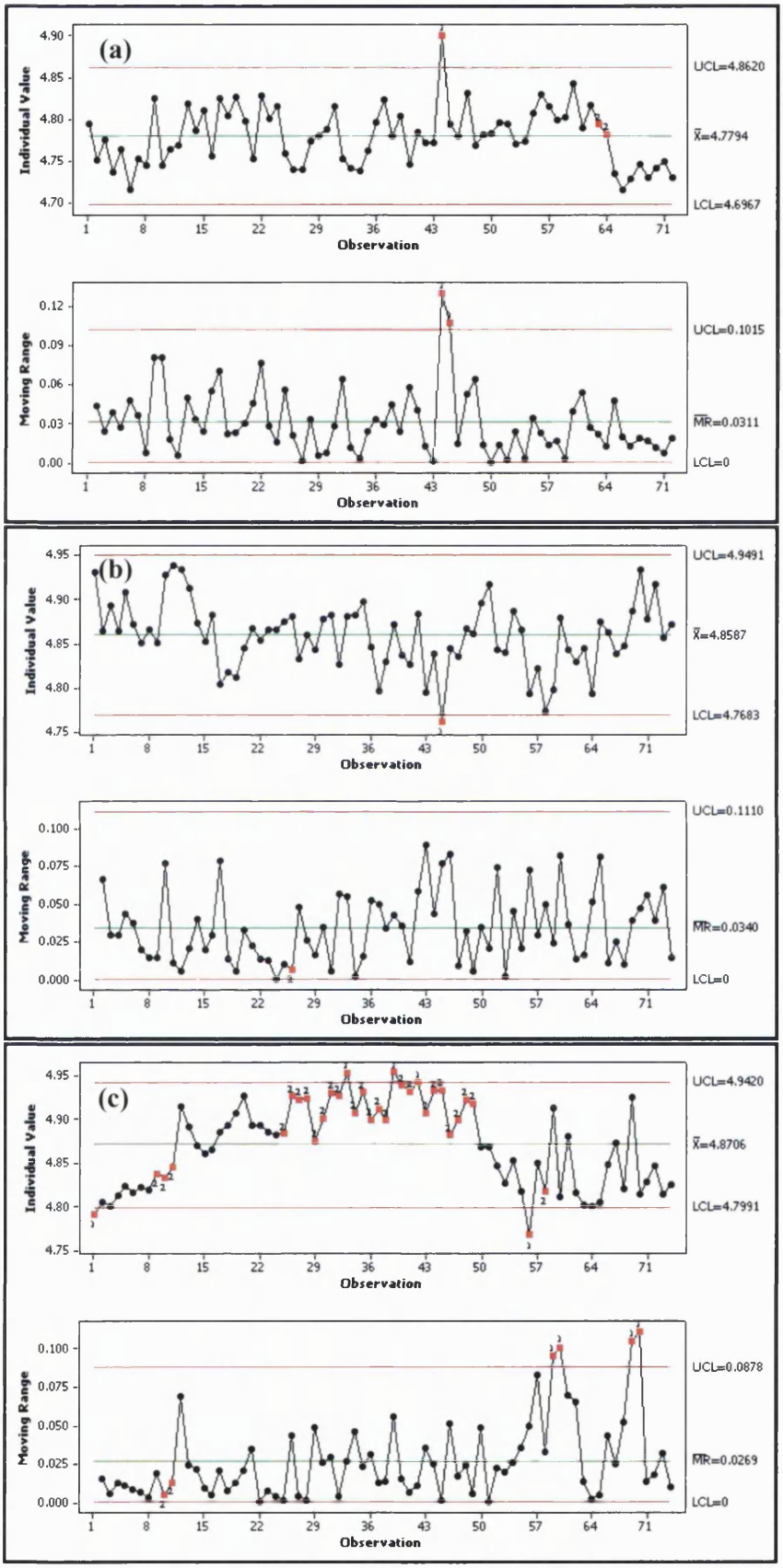

Figure 101: I-MR chart of predicted amlodipine active content (mg / tablet) for three production batches; (a) batch 1 to (c) batch 3 with within batch process limits (___) and batch mean (___)

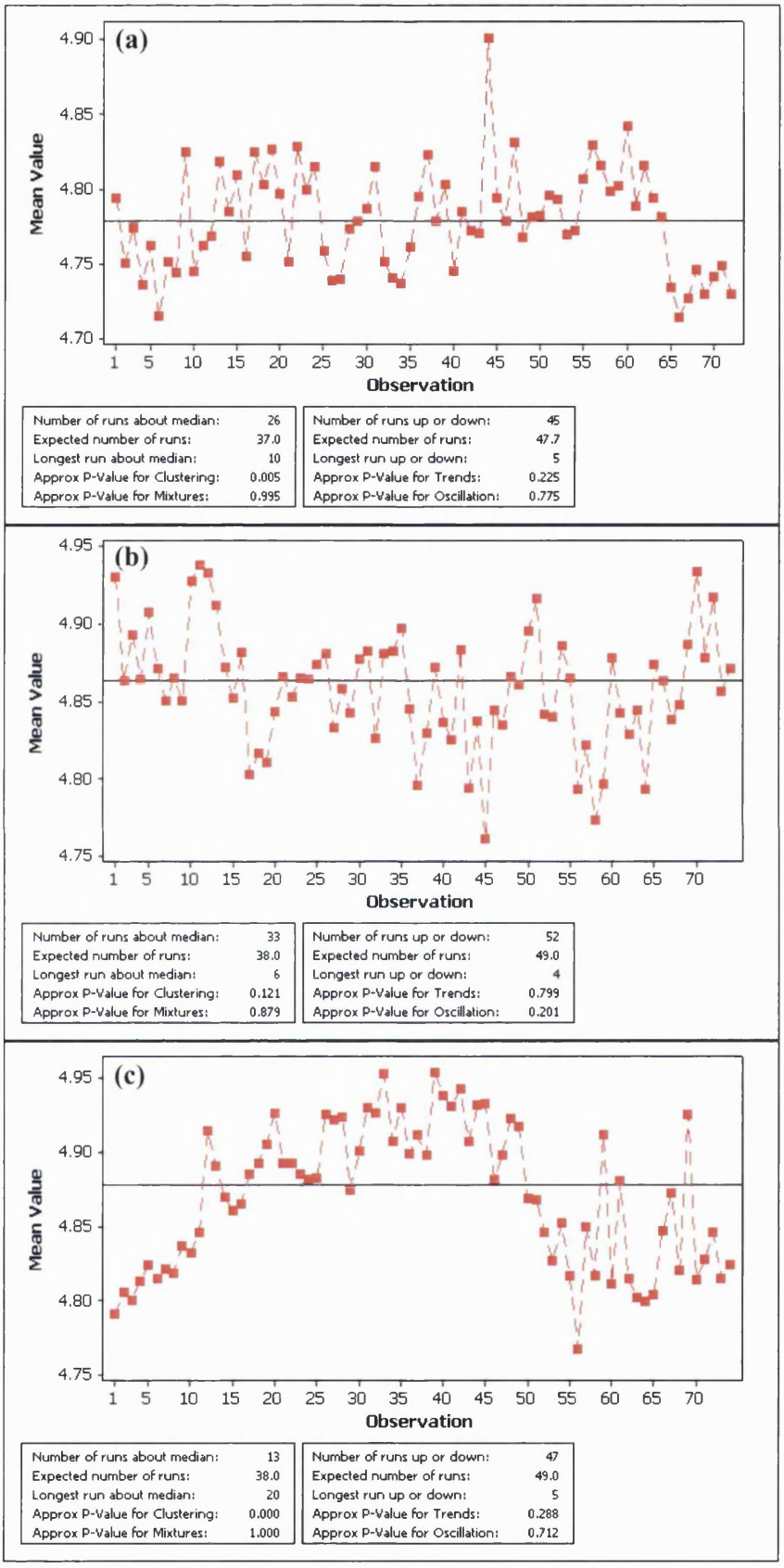

Figure 102: Within batch Run chart of predicted amlodipine active content (mg / tablet) for three production batches; (a) batch 1 to (c) batch 3

The Run charts for the three batches shown in Figure 102 indicate that Batches one and three have a higher proportion of clusters of points on one side of the mean than would be statistically expected (as (p<0.05) at 95% confidence, the distribution of points in clusters is seen as likely due to non-random effects. No batch is seen to have a significant number of consecutive runs, oscillations/cycles or alternating values (mixtures).

The information regarding within batch process trending could provide insight into the tablet press operation through the day and across different shift patterns or the impact of charging blend into the blender. Further batches should be monitored with these trend charts to establish any root causes for the within batch process signature (clusters, point to point steps) and whether there are opportunities for process improvement.

Figure 103 shows the Range I-chart for the three additional batches of the product using the established threshold from the six commercial batches. The range of all three batches conforms to the established control limits indicating that the range of the amlodipine active content is typical for Norvasc® 5 mg tablet batches.

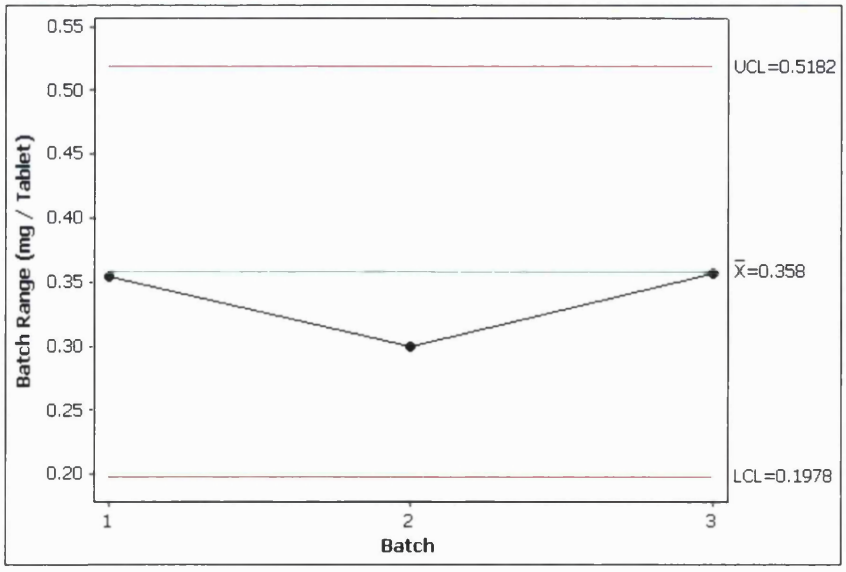

Figure 103: I-chart of range for predicted amlodipine active content (mg / tablet) for three production batches

Figure 104 shows the derived distribution profile SPC chart for the three verification production batches. The distribution profile SPC chart indicates that batch one is shifted to lower amlodipine active content; breaching the control limits at lower amlodipine active content bins. Additionally batch two has a higher percentage of tablets at the centre of the distribution and a narrower curve indicating production was controlled more tightly about the process mean. Batch three had a distribution profile well aligned with the established typical process.

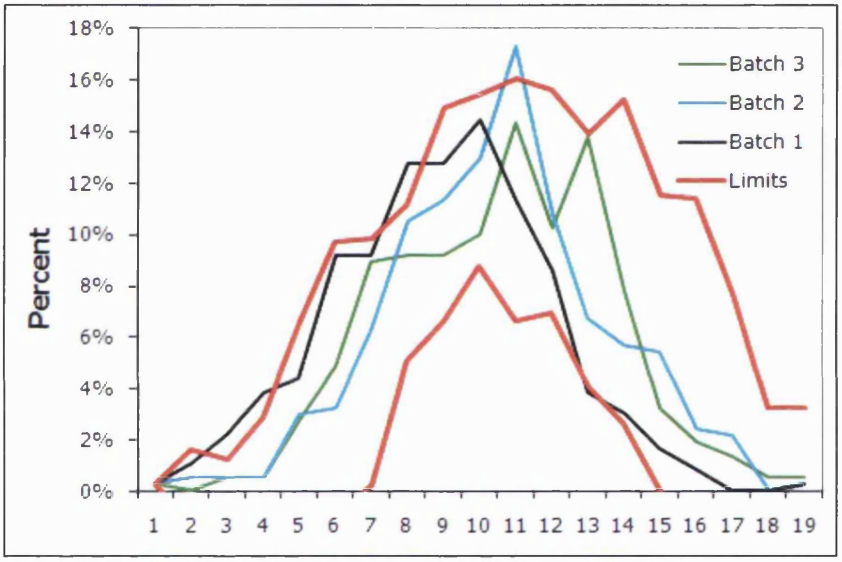

Figure 104: Distribution profile of predicted amlodipine active content of three production batches with established distribution profile SPC limits (___)

5.3.3 Semi-quantitative tablet component quality conformance method discussion

As with the qualitative tablet component quality conformance method discussed in Section 5.2.3, the semi-quantitative tablet component quality conformance method for Norvasc® 5 mg tablets was extremely rapid to develop with approximately 10% of the reference chemistry compared to that typically required for traditional quantitative NIR methods. The semi-quantitative approach requires similar chemometric expertise as is required for development of traditional fully quantitative methods. The key difference in approaches is the use of nominated reference values rather than individual reference

values for each tablet used in the regression calibration and verification sets and the coupling of the output of the model to historical process oriented SPC trending.

The use of theoretical content would require no reference chemistry. However, the developer would have to be supremely confident that the extended range tablets developed were at the target content, relying on the ability to formulate the extended range tablets. The use of nominated values can lead to increased prediction errors due to averaging the content for any given set of samples. Therefore, knowingly choosing to add another source of uncertainty by using the theoretical amlodipine active content with no reference chemistry was not considered ideal. As such, it was determined that the use of reference chemistry of a minimum number of tablets to enable the assignment of nominated content is an appropriate balance of introducing error into the regression while providing rapid development and implementation.

While any regression model could be utilised in the development of a semi-quantitative tablet component quality conformance approach, this work assessed two common multivariate regression techniques, MLR and PLS. It was found that for this complex direct compression low dose product PLS, with nominated amlodipine active content, was more capable at correlating the amlodipine active content within the tablet form, overcoming matrix absorption and physical effects.

The vendor software limitations identified in Section 5.2.3 are similar, except that regression chemometrics are typically standard functionality in most vendor software. The main limitation is with the inability of the vendor software to incorporate SPC functionality requiring the export of data and use of external software for SPC trending. Within GMP production environments, the routine export of data is typically avoided as is the use of macros requiring software validation. Additionally, the use of multiple software programs requires additional training and operator capability. Until industry as

a whole demands functionality such as that applied in this research, vendors will likely be reluctant to incorporate such features.

As discussed in Section 5.2.2.4 the DeLight software has the capability to reproduce the individual tablet I-chart including VOP and VOC limits to allow the operator to monitor the results in real time. The I-MR chart for sample means can also be replicated within DeLight without the control limits. The Run chart and distribution profile SPC chart cannot be replicated within DeLight and data must be exported to create these conformance plots in external software. Figure 105 shows the operator user interface for monitoring the tablet conformance method in real time during tabletting.

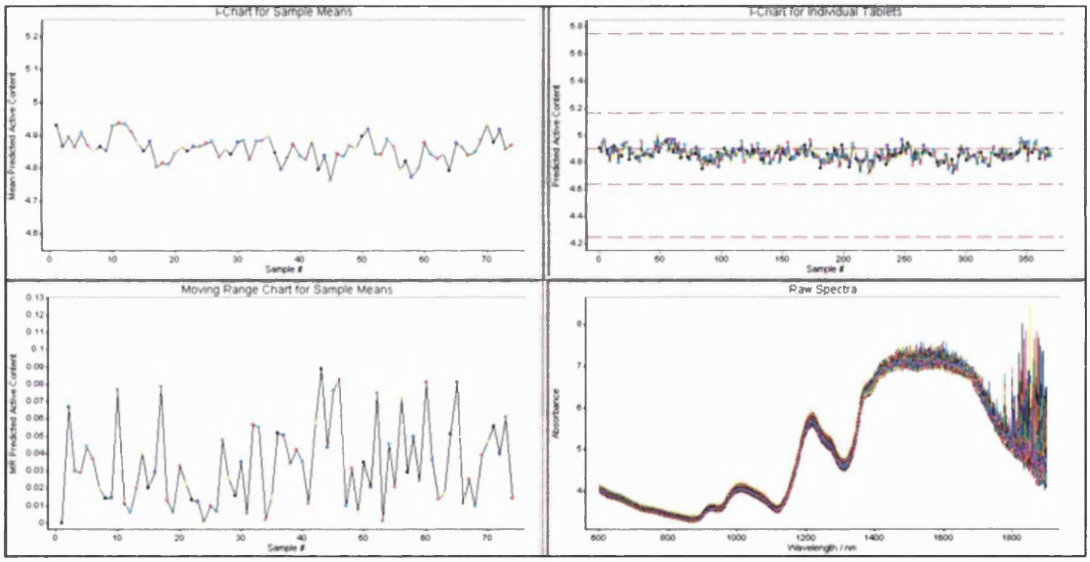

Figure 105: Operator interface for developed semi-quantitative tablet component quality conformance SPC charts within DeLight software

The developed semi-quantitative tablet content quality conformance method was shown to provide simple easy to use real time SPC charts to identify individual tablets deviating from expected behaviour as well as valuable process information regarding within batch trending as well as the range and distribution profile of Norvasc® 5 mg tablets at batch completion.

This is demonstrated through the application of the method to commercial tablets monitored throughout tabletting discussed in Section 5.3.2.4. The individual tablet I-chart showed that batch one produced three tablets that were at the VOP limit established for Norvasc® 5mg tablets based on the established historical control limits. As all tablets were within the VOC limits and also as the entire batch was shifted to lower predicted amlodipine active content, these tablets were not analysed by reference chemistry. However this would be an option if quality assurance were particularly concerned at the predicted result of any given tablet. Performing reference chemistry could assist in investigating any unexpected deviation (as part of established site SOPs).

The I-MR and Run charts showed batch one and two had minimal within batch variation while batch three had more numerous out of conformance events that were seen as significant at the 95% confidence level. It is possible that these events were within the normal random behaviour of the process and within the 5% of the population expected to fall beyond the limits. Further work may be valuable in understanding how events identified in the I-MR chart and Run charts relate to tabletting press operation activities and why batch three was seen to contain significantly different process signature that the other two batches. Such data was not collected at the time that the semi-quantitative method was applied to the commercial batch data. Note that these within batch SPC charts could not be generated in real time and as such, within batch variations were only identified at batch completion and opportunity to relate observations to real time process operation events was missed.

The I-chart for range demonstrated that the range of amlodipine active content for the entire batch was consistent with historical expectation. The distribution profile SPC chart indicates a narrower distribution with smaller tails resulting in lower than expected range in the conformance method metric for batch two. This batch could be reviewed more closely to determine why this batch has shown greater process capability

(tighter distribution with less trends and clustering) and can be considered a 'gold standard' batch to replicate in future manufacturing.

5.3.4 Summary - Criticality of Research

This research investigated the use of data from two semi-quantitative techniques (MLR and PLS with nominated reference values) coupled with SPC charting to create a Semi-quantitative Tablet Component Quality Conformance approach. There has been limited application of 'reduced reference' quantitative method development through the use of gravimetrically developed and nominal or theoretically assigned reference values[110] and the use of discriminant analysis applied with regression analysis of tablets.[103] However, the use of the data coupled with SPC for analysis of large number of tablets through process monitoring has not been previously reported.

Two semi-quantitative conformance methods were developed and compared for suitability for the application and PLS-NV found to be the most applicable. A conformance method based on PLS-NV model coupled with SPC was developed and applied to real commercial batches of Norvasc® 5 mg tablets at a Pfizer facility and the value of the work demonstrated in the ability to interrogate batch to batch and within batch trends in conformance as discussed in Section 5.3.2.4. The benefits and challenges of implementing the conformance method were discussed including a commentary on the availability of vendor software to facilitate such approaches.

The semi-quantitative tablet component quality conformance method was demonstrated to provide an excellent means to gain greater insight into the tabletting process and can enable real time identification of deviation of process and product from the normal operation allowing rapid remedial action to prevent any occurrence that would have the

potential to greatly impact the pharmaceutical manufacturing process and / or product quality.

With an appropriate DoE and development batch programme, the semi-quantitative tablet quality conformance approach could be applicable to any key tablet component, not just the active component as studied in this research. Success for the active component for Norvasc® 5 mg tablets at low percentage of the formulation indicates that this approach would be applicable for any component for any product where an appropriate semi-quantitative model can be developed.

This work is clearly aligned with the philosophy of PAT through providing an in depth understanding of tabletting operations, providing opportunity for process optimisation and improvement while also assuring quality. The work was implemented into the Pfizer Canada manufacturing facility and is the basis of amlodipine active content alternate testing within the real time release strategy for Norvasc® 5 mg current being implemented at the Pfizer Sydney site.

5.4 Interpretation and appropriateness of ICH guidelines on analytical method validation to conformance methodology

The qualitative and semi-quantitative conformance approaches developed in Section 5.2 and Section 5.3 are built upon the initial ability to develop an appropriate model or metric for the tablet component of interest - the active component in individual tablets.

The methods were developed rapidly with minimum reference chemistry and the development of the control charts and implementation of these charts on real batches indicates successful methods.

However, to satisfy review by those used to reviewing traditional analytical methods, this section provides a summary of the validity of the method with reference to the principles outlined by the ICH Harmonised Tripartite Guideline - Q2 (R2): Validation of Analytical Procedures: Text and Methodology.[144]

The EMEA (now known as EMA) published a note for guidance on NIRS in 2003[143] which provided guidance on validation of NIRS analytical methods. For many years this was widely used in industry to direct analytical method validation activities, however this document did not cover the use of qualitative methods (other that as pass / fail classification) or semi-quantitative methods. The EMA issued an updated guidance in 2012[145] which removed the section providing detailed guidance on validation in lieu of the applicability of the ICH Q2 (R2) guidance following harmonisation activities. Throughout this period, the application of ICH Q2 (R2) approach to analytical method validation of PAT based methods has been inconsistent, as discussed by De Bleye,[146] and literature mainly describe validation of off-line and at-line quantitative NIRS methods[90, 96, 147, 148] and less frequently in-line in-process control analysis.[83]

Directly applying validation guidelines according to the ICH Q2 (R2) guidance is not appropriate, as semi-quantitative methods are not within the scope of this document. Many of the validation principles in the ICH guidance do stand, however, they should be considered with respect to the purpose of the conformance methods. The various traditional validation characteristics are discussed in this section in relation to the applicability to conformance methodology.

5.4.1 Specificity

The ICH guidance document defines specificity as:

> "*Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present*".[144]

NIR spectra consist of overlapping absorption bands, therefore there is difficulty in stating unequivocally that the absorbance at a specific measured wavelength is solely due to the API. To demonstrate method specificity, it is important to demonstrate that the model or metric used in the conformance method appropriately targets the attribute of interest and addresses the aspect of interference from other components in the matrix as well as interference from unrelated spectral effects (e.g. scatter effects).

The qualitative tablet component quality conformance method was developed with a univariate method at a single wavelength. Therefore, to demonstrate specificity, this single wavelength must be shown to be specific to the attribute of interest. Through examination of the matrix components, it has previously been shown that the wavelength 1122 nm was found to be related to API absorbance (Figure 61). Amlodipine besylate specificity within the product matrix was further demonstrated by overlaying development tablet spectra at 75%-125% label claim with normal production tablet cores (nominally at 2.5% w/w) (Figure 64).

Furthermore, throughout the development of the method tablets from a variety of production batches manufactured over a four year timeframe utilising components from different manufacturers lots were utilised to build in robustness to typical variations in the tablet matrix into the method. This provides added assurance that the SNV, 1st derivative absorbance at 1122 nm is specific for changes to the amlodipine active content.

The semi-quantitative tablet component quality conformance method was developed on a PLS-NV model. Examination of the LVs of the model in Figure 88 demonstrated that the primary variable in the model was the absorbance at 1122 nm and as described earlier, this wavelength demonstrates clear specificity to the amlodipine active content.

Additionally, the inclusion of a variety of production batches manufactured over an extended period and capturing natural variation in physical characteristics of the tablets, demonstrated the developed semi-quantitative method is specific to the amlodipine active in the presence of factors such as scattering phenomenon and that these factors do not interfere.

5.4.2 Linearity

The ICH guidance document defines linearity as:

> "*The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample*".[144]

The linearity of a NIRS conformance method can be directly assessed according to the ICH principles. The linearity of the qualitative tablet component quality conformance model was established earlier and visually demonstrated in Figure 68. The data used in the regression were tabulated in Table 17. The regression statistics are shown in Table 24. Note that the large slope coefficient relates to the different order of magnitudes in the axes (absorbance vs. mg / tablet).

Table 24: Linearity outcomes for the qualitative tablet component quality conformance model

	Intercept (Absorbance)	Slope
Coefficients	-0.5132	383.7
95% Confidence Interval	-1.135 – 0.1089	339.6 –427.7
Standard Error	0.2901	20.53
Correlation	0.9805	
R^2	0.9614	
Standard Error (Absorbance)	0.1610	

The linearity of the semi-quantitative tablet component quality conformance model was calculated by regression of the predicted amlodipine active content from the PLS-NV model against the paired HPLC reference values for the 16 tablets that were used to establish the nominated values. The regression statistics are shown in Table 25. The data used in the regression are tabulated in Table 26 and linearity is visually demonstrated in Figure 106.

Table 25: Linearity outcomes for the semi-quantitative tablet component quality conformance model

	Intercept (mg / tablet)	Slope
Coefficients	-0.1160	1.022
95% Confidence Interval	-0.3407 - 0.1086	0.9768 - 1.068
Standard Error	0.1047	0.02130
Correlation	0.9970	
R^2	0.9940	
Standard Error (mg / tablet)	0.06371	

Table 26: Reference chemistry and predicted amlodipine active content for the PLS-NV model

	HPLC Assay (mg / tablet)	PLS-NV Predicted Content (mg / tablet)
75% Development 1	3.42	3.49
75% Development 2	3.35	3.42
85% Development 1	4.20	4.14
85% Development 2	4.09	4.10
115% Development 1	5.74	5.78
115% Development 2	5.68	5.71
125% Development 1	5.94	5.81
125% Development 2	6.03	6.04
Production 1-1	5.04	5.02
Production 1-2	4.89	4.80
Production 2-1	4.91	4.92
Production 2-2	4.80	4.92
Production 3-1	4.80	4.88
Production 3-2	4.96	4.97
Production 4-1	4.89	4.85
Production 4-2	4.95	4.93

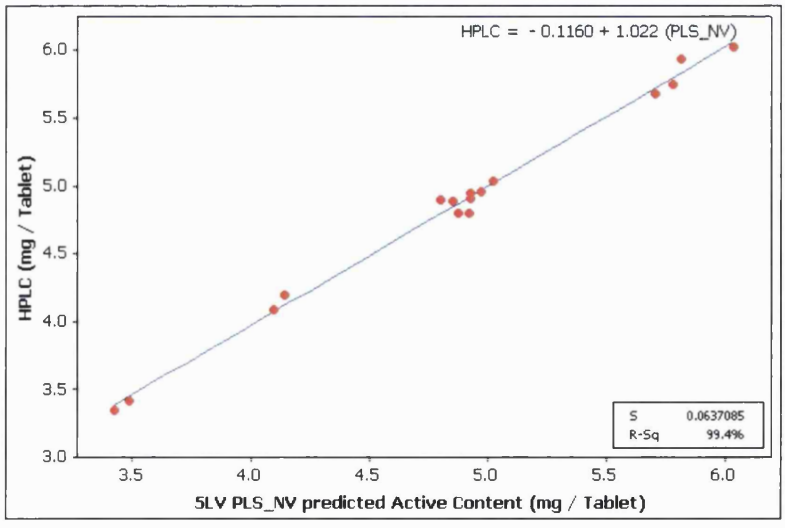

Figure 106: Regression fitted line and equation for sixteen representative tablets from the verification batches for the PLS-NV model with five LVs

The linearity assessment results demonstrate that both the qualitative and semi-quantitative NIR tablet component quality conformance methods are appropriately linear and fit-for-purpose.

5.4.3 Precision (repeatability and intermediate precision)

The ICH guidance document defines precision as:

> *"The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility".*[144]

The precision of a NIRS conformance method can be directly assessed according to the ICH principles. It is simpler to assess than in conventional circumstances due to the non-destructive nature of NIRS measurements. There is no issue regarding homogeneity of the samples used to perform the test, the sample is simply rescanned.

The repeatability (short term precision or measurement variability) of the methods was determined by the same operator scanning the same tablet six times. The data for the repeatability assessment is shown in Table 27. The standard deviations and relative standard deviations of the tablet component quality conformance model outputs for the replicate scans were extremely small as anticipated.

Table 27: Repeatability outcomes for the tablet component quality conformance models

Replicate	1122 nm Absorbance (Absorbance)	PLS-NV Prediction (mg / tablet)
1	0.013909	4.9375
2	0.013910	4.9406
3	0.013911	4.9428
4	0.013910	4.9406
5	0.013907	4.9455
6	0.013908	4.9423
Mean	0.013909	4.9416
Mean ± 95% Confidence Interval	0.013909 ± 1.2×10-06	4.9416 ± 0.002145
Standard Deviation	1.5×10-06	0.0027
Relative Standard Deviation	0.01058%	0.05425%

The intermediate precision (mid term precision or within laboratory variability) was assessed by two different analysts measuring the same set of samples on separate days by NIRS. It can be seen from Table 28 that the Pooled relative SE (operator one and operator two pooled results) were very low (considerably less than 1.0%).

A test for equal variance was performed in Minitab and the intermediate precision data for both tablet component quality conformance models were found to be equivalent across the studied range at 95% confidence (p>0.05) as shown in Figure 107.

Table 28: Intermediate precision outcomes for the tablet component quality conformance models with analysis by different analysts on different days

Sample	1122 nm Absorbance (Absorbance)		PLS-NV Prediction (mg / tablet)	
	Day 1 Analyst 1	Day 2 Analyst 2	Day 1 Analyst 1	Day 2 Analyst 2
75% 1	0.009767	0.009681	3.1708	3.1517
75% 2	0.009891	0.009813	3.2071	3.1943
85% 1	0.01174	0.01163	4.0110	3.9867
85% 2	0.01170	0.01159	3.9321	3.9369
115% 1	0.01586	0.01581	5.5257	5.5212
115% 2	0.01582	0.01580	5.4972	5.4991
125% 1	0.01786	0.01780	6.1190	6.1112
125% 2	0.01791	0.01787	6.0729	6.0782
Production 1	0.01387	0.01395	4.9115	4.8989
Production 2	0.01388	0.01395	4.9726	4.9736
Production 3	0.01385	0.01383	4.8401	4.8328
Production 4	0.01390	0.01397	4.9860	4.9892
Production 5	0.01372	0.01378	4.9656	4.9530
Production 6	0.01396	0.01399	5.0588	5.0205
Production 7	0.01385	0.01393	5.0248	5.0048
Production 8	0.01392	0.01398	5.0297	5.0105
Pooled Mean	0.01384		4.8277	
SE	0.000071		0.01218	
Relative SE	0.513%		0.252%	

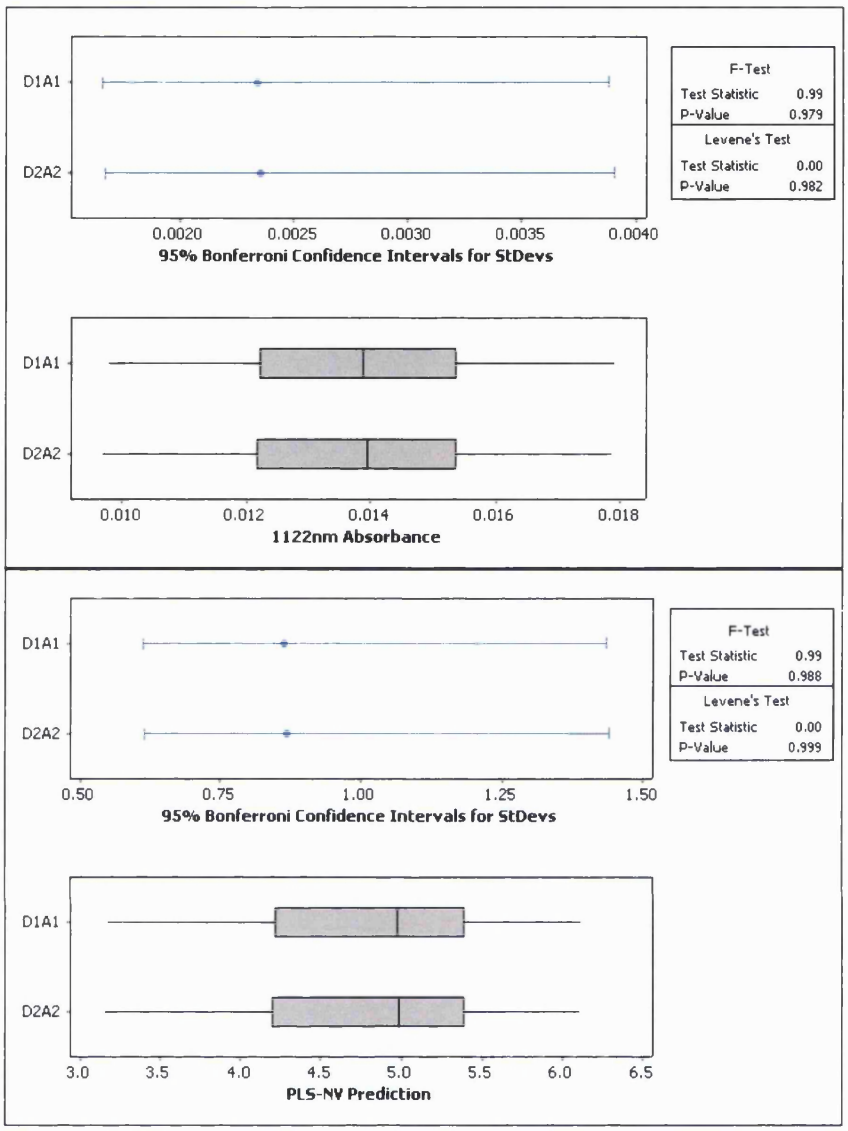

Figure 107: Minitab test for equal variances output for sixteen paired intermediate precision samples for (a) 1122 nm Absorbance and (b) PLS-NV model with five LVs

Reproducibility was not studied as the method was applicable only to the product manufactured at the site for which the research was conducted and on the instrument on which it was developed (only instrument at the site). There was no plan to transfer the method between instruments or laboratories. Any future transfer of the method would be assessed fully under a method transfer protocol (which would include reproducibility studies).

5.4.4 Accuracy

The ICH guidance document defines accuracy as:

> *"The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness".* [144]

However, it is further stated that *"accuracy may be inferred once precision, linearity and specificity have been established".* [144]

Accuracy cannot be assessed for the qualitative tablet component quality conformance method as the units of measure (Absorbance units), though correlated to the traditional method, are on a different scale and magnitude. Therefore accuracy of the qualitative tablet component quality conformance method is inferred from the established acceptable precision, linearity and specificity.

Accuracy can be assessed for the semi-quantitative tablet component quality conformance method as for other traditional quantitative NIRS methods, through the comparison of the predicted NIRS results to an established true (reference) values. The data used in the accuracy determination were those applied to assessing linearity, tabulated in Table 26. The accuracy statistics are shown in Table 29 demonstrating that the semi-quantitative tablet component quality conformance method is accurate with a relative SEP of less than 2% and very small bias. A paired T-test for means was also performed which demonstrated that there was no significant difference between the means of the two analysis methods at 95% confidence (p-value>0.05).

Table 29: Accuracy outcomes for the tablet component quality conformance models

	HPLC (mg / tablet)	PLS-NV Prediction (mg / tablet)
Mean	4.854	4.861
Pooled Mean	4.858	
SEP	0.0637	
Relative SEP	1.311%	
Bias	-0.00694	
t_{crit}	2.13	
t_{stat}	0.43398	
***p*-value**	0.67048	

5.4.5 Range

The ICH guidance document defines range as:

> *"The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity".*[144]

The range of a NIRS conformance method can be directly assessed according to the ICH principles. The qualitative tablet component quality conformance method was determined to have a range 0.01049 - 0.01763 Absorbance which is equivalent to 3.512 - 6.251 mg per tablet once the established equation correlating model response to reference data is applied (refer to Figure 68).

The semi-quantitative tablet component quality conformance method was determined to have a range 3.256 – 6.047 mg per tablet based on the predicted values of the Regression dataset used to develop the PLS-NV model.

5.4.6 Robustness

The ICH guidance document defines range as:

> *"The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage".*[144]

As samples were collected from a variety of production batches, manufactured over a four year timeframe from different component manufacturers lots and compressed on different tablet presses, the conformance methods inherently include seasonal changes (such as environmental aspects), wear and tear of the NIR spectrometer (including part servicing and lamp replacement), component material variations, formulation (weighing and blending) variation as well as processing (compression, thickness) variations. The intermediate precision result has demonstrated the conformance method is robust to changes in analyst and daily fluctuations. Thus, it is deemed that the method is inherently robust and appropriately fit for purpose.

5.5 Comparison of qualitative and semi-quantitative conformance methods for applicability in the manufacturing setting

Though conformance methods based on qualitative and semi-quantitative algorithms were both explored (Sections 5.2 and 5.3 respectively) and demonstrated to provide a mechanism for monitoring the Norvasc® 5mg tabletting process, a manufacturing facility would select one approach for a given application.

Both approaches provided an opportunity for increased understanding of within and between batch variability and provided opportunity for process improvements. There were slight differences in the trends seen for the same batches studied by both

approaches, most notably in the I-chart for sample means for batch three (Figure 77 compared to Figure 101). These differences are related to the impact of matrix and physical effects on the conformance algorithm. It is expected that the semi-quantitative method may be less impacted by these affects assuming sufficient representation of matrix and physical effects are built into and appropriately weighted in the regression model.

The semi-quantitative approach provided an advantage over the qualitative tablet component quality conformance method by providing control charts based on the natural unit of measurement of the analyte, in this case mg per tablet. However, this can also be a distraction within production with operators or quality personnel becoming fixated by the predicted numerical value (quality compliance mindset) rather than utilising the information given to gain further understanding of the process. This could lead to unnecessary, time consuming, deviation investigations of what may be the inherent process signature and variability characteristics of a given product. If this is a concern, it may be preferred to opt for a qualitative approach.

The main disadvantage of the semi-quantitative approach compared to the qualitative approach described in Section 5.2 is the higher complexity in the chemometrics and thus the need for greater NIRS analytical expertise to develop the method. However the advantages of rapid development and reduced reference chemistry are still achieved.

The manufacturing facility for which the research was undertaken chose to progress the semi-quantitative approach. The facility was then sold and exited the Pfizer manufacturing network in 2008 and outcomes of routine application of the approach since 2008 are not available.

The developed tablet component quality conformance method would be simple to apply in a non-routine setting for validation or process optimisation activities and would

provide opportunity to very quickly implement a means for real-time tablet monitoring to deliver process understanding to enable process enhancements and improvements. When supporting validation activities, historical SPC charts can be developed for an existing process and then product manufactured following the process improvement or approved material or process change can be compared to the established baseline. The conformance method development can be tailored to the specific validation activity (for example a conformance method can be developed to monitor a key excipient in the product before the introduction of an alternate supplier).

It is worth noting that within the research and development realm, insufficient batches are typically manufactured to develop a historical SPC approach in early product development. In such cases, the same qualitative or semi-quantitative approach with reduced reference chemistry can be implemented with the associated statistical charts without historical limits to aid in visualisation of batch distribution and within and between batch variability. Within batch I-MR, Run charts and distribution profiles could be very valuable when reviewing DoE's performed while developing the final dosage form process.

5.6 Review of research outcomes

The rapid development of NIRS Tablet Conformance analysis was successfully demonstrated in Chapter 5 through the application of the approach to the active tablet component, amlodipine base, in Norvasc® 5 mg tablet finished cores. This work successfully addressed the identified need for process oriented application of NIRS aligned with the PAT initiative to monitor tabletting production.

The novel approach of tablet component conformance analysis enabled extensive deep understanding of the within and between batch process behaviour while also assessing

the ability of individual tablets to meet the quality expectations of customers at a more significant sampling level with no impact to regulatory commitments.

Conformance models based on qualitative and semi-quantitative algorithms were explored in Sections 5.2 and 5.3 respectively. In both cases the reference chemistry required was reduced to only 16 tablets. The models were rapidly developed and validated as fit for purpose with respect to the expectations outlined in the ICH Harmonised Tripartite Guideline - Q2 (R1): Validation of Analytical Procedures: Text and Methodology[144] in Section 5.4. SPC charts were developed for qualitative and semi-quantitative model predictions based on commercial historical process batches.

The application of the developed conformance methodologies to commercial production batches demonstrated the success of the approach in gaining in-depth process knowledge of the tabletting process and would facilitate process enhancements leading to improved product quality for the consumer.

One of the greatest difficulties faced with implementing the new approaches to data analysis in real time within the process environment is the limitations in the software that runs the NIR spectrometer. This is an area which will need to be improved to enable industry to implement advanced PAT applications using NIR. However, this will require the companies that develop the software to accept that industry is moving in new ways and requires a desire in those companies to move with the industry to harness the full potential of NIRS for PAT applications.

The research met the desired objectives of developing novel ways to apply at-line NIR for material analysis, aligning with the PAT philosophy described in Chapter 1 and addressing the gaps in material analysis identified in Chapter 2.

CHAPTER 6 RESEARCH SUMMARY AND THESIS

CONCLUSION

PAT, though often considered a new initiative, has been actively pursued since the early 1990's, though the term PAT was cemented in pharmaceutical technology language following the FDA launch of the Guidance for Industry on PAT. During Chapter 1 the history of PAT was reviewed highlighting the different drivers of PAT and the perceived benefits from the regulatory, manufacturer and consumer view point. Different modes and different stages of application of PAT in pharmaceutical manufacturing were reviewed following a brief discussion on the constraints impacting implementation of PAT broadly in the industry. The future of PAT was considered in relation to the current economic and business climate within which the pharmaceutical industry currently operates.

Application of PAT measurement systems as fully in-line and on-line integrated systems are often thought to be the final desired state to allow unmanned analysis of large sample size to fully describe the process performance and product quality. However, integrated applications often have increased cost and challenges for implementation preventing widespread deployment. Similarly, applications that impact regulatory timelines for new products or have long lead time for regulatory approvals detract from the value of implementing PAT due to long periods before return on investment can be achieved. It was noted that pharmaceutical products are dominated by solid dosage forms and in particular by tablets. It was thus concluded that at-line PAT applications for process understanding and monitoring and control for solid oral dosage forms that avoid regulatory impact are an economically viable area for widespread deployment of PAT and worthy of focussed scientific research.

The application of NIRS to support PAT initiatives was explored in Chapter 2, where it was demonstrated that NIRS has a rich history of application within the pharmaceutical industry. The PAT initiative has focused the use of NIRS in the pharmaceutical industry as a process understanding and improvement tool rather than as a direct replacement to conventional QC testing as NIRS was often previously applied.

The deployment of NIRS within the PAT framework was reviewed for solid dosage form processes and two process areas of particular scientific need were identified. Despite significant use of NIRS in material testing, the traditional use of NIRS as a replacement identification method falls short of the aims of the PAT initiative. There was a strong need for NIRS to be applied to material characteristics that relate to product quality and process performance to develop the deep understanding of the causal relationships between input materials and processes and the resulting output products.

Additionally, it was determined that there was a gap in current practise and research in the application of NIRS to tablet component analysis beyond alternate assay and content uniformity measurement. Directly replacing traditional QC testing by NIRS does not progress PAT philosophy. Rather, the application of NIRS must delve into the analysis of the tablet component throughout the tabletting operation, relating the observed NIRS spectra to relevant process performance and product quality metrics. A novel approach was needed in applying NIRS for these two aspects of solid dosage form manufacturing aligned with the PAT philosophy.

To enable the development of novel PAT applications of NIRS, common mathematics and chemometrics treatments and analyses along with univariate and multivariate statistical process control concepts were reviewed in Chapter 3.

Novel applications of at-line NIRS were investigated and developed aligned with the PAT philosophy to establish an innovative system of analysis that combined chemometrics and spectral analysis with statistical process control.

Various chemometric algorithms were explored to enable rapid monitoring of global spectral quality as well as the quality of specific critical-to-process material attribute within a statistical process control framework which related material characteristics to historically demonstrated acceptable product quality and process performance. The developed material conformance methods were implemented within a commercial pharmaceutical facility in Pfizer and the success of the approach clearly demonstrated by the identification and investigation into non-conforming deliveries in Chapter 4. The conformance approach provided unforeseen opportunity to gain understanding of causal relationships between raw materials and an important Pfizer product. The valuable information gained through the application of raw material conformance methods enabled rapid identification of potential product or processing issues at material receipt and allowed schedule modification to prevent production facility impact, provided customer supply assurance and facilitated rapid root cause analysis. The statistical process control charts and NIRS user interface were well received by the warehouse operators despite challenges in available statistical and chemometrics features within vendor NIR software.

Novel approaches to within and between batch statistical process control for tablet quality conformance were also developed. Tablet component conformance methods were created using rapid to develop qualitative and semi-quantitative chemometrics algorithms that required minimal reference analysis. The chemometrics methods were then coupled with statistical process control analysis to develop a strategy of analysis that assessed the tablet component of interest within intact tablets sampled in large sample size throughout tabletting operations. The SPC charts that were developed

assessed the ability of individual tablets to meet historically established normal component behaviour for the product as well as conventional quality metrics. Additionally, within batch and between batch variability was analysed to fully assess the conformance of a batch of tablets to expected product quality and process performance. This work, described in Chapter 5, included the novel adaption of distribution profile control charts typically applied to particle size measurement. The tablet component conformance methods provided an opportunity for extensive process monitoring and unsurpassed in-depth process understanding. The approach was found to be approachable for plant operators through to quality assurance analysts.

The research described in Chapter 4 and Chapter 5 was shown to provide exceptional business value and advanced the critical science based understanding of the pharmaceutical formulation. The research was well aligned with the PAT initiative; clearly meeting the overall aims of the research in developing PAT aligned at-line applications of NIRS and the specific aims and objectives outlined in Section 2.6.

6.1 Future Work

The approach demonstrated in this thesis is broadly applicable with the potential to extend beyond the solid dosage form studied. Raw materials are applicable to all manufacturing processes and the physicochemical nature of NIRS analysis enables assessment of both chemical and physical material attributes in a single analysis.

The approach has since been rolled out as a standard methodology within Pfizer and has been applied most recently in manufacturing sites in Egypt and Venezuela.

Similarly, though the focus of this work was tablet component analysis and the aspect studied was the active component, the tablet component conformance methodology can be applied for excipients and also for any other process step in solid dosage form

manufacture or more broadly across manufacturing to other finished dosage forms. Further work is currently planned to extend the conformance approach to at-line conformance analysis of moisture for tray drying applications as well as incorporating the concepts in NIRS support of process validation.

There is also potential applicability of the conformance methodology for PAT techniques beyond NIRS. Any process analysis measurement would benefit from both the reduced reference approach as well as the broader use of statistical process control analysis to shed greater light on the process under study.

The work highlighted gaps in currently available chemometric and statistical process control capabilities within NIR instrument control software. A recent brainstorm by PAT experts within Pfizer identified this as one area of future focus to close the gaps and enable PAT support for advanced paradigms. NIRS vendors will need to be willing to add capability to meet the evolving needs of the pharmaceutical industry; otherwise pharmaceutical manufacturers will pursue other strategic partnerships with software and hardware vendors that are willing to work towards the future directions of PAT deployment.

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APPENDIX 1 – GLOBAL MATERIAL QUALITY CONFORMANCE

MINITAB GRAPHICAL SUMMARIES WITH NORMALITY ASSESSMENT

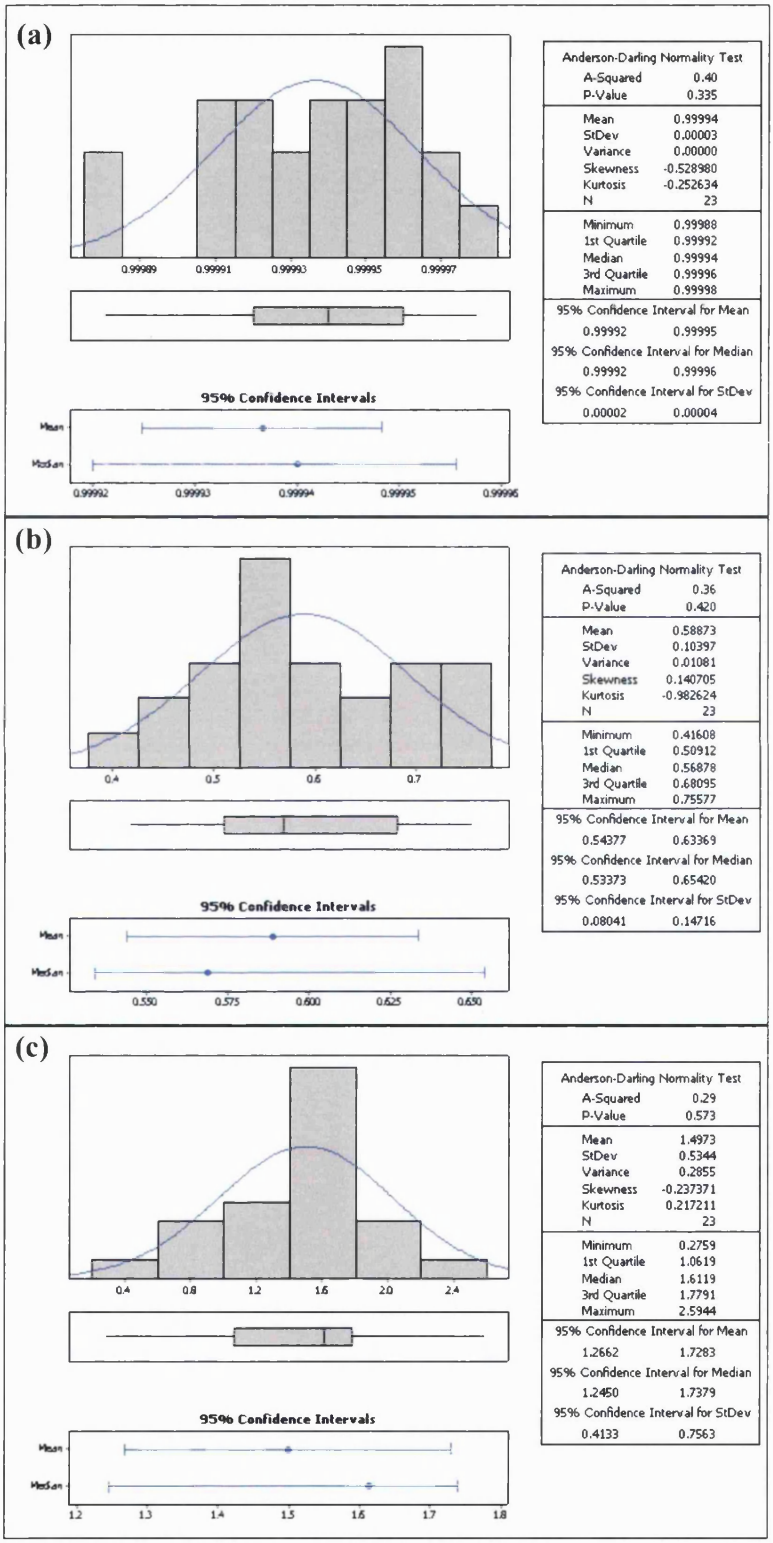

Figure 108: Minitab graphical summary with normality results for individual container conformance results for historical deliveries: (a) correlation, (b) ANSD and (c) PCA-MD

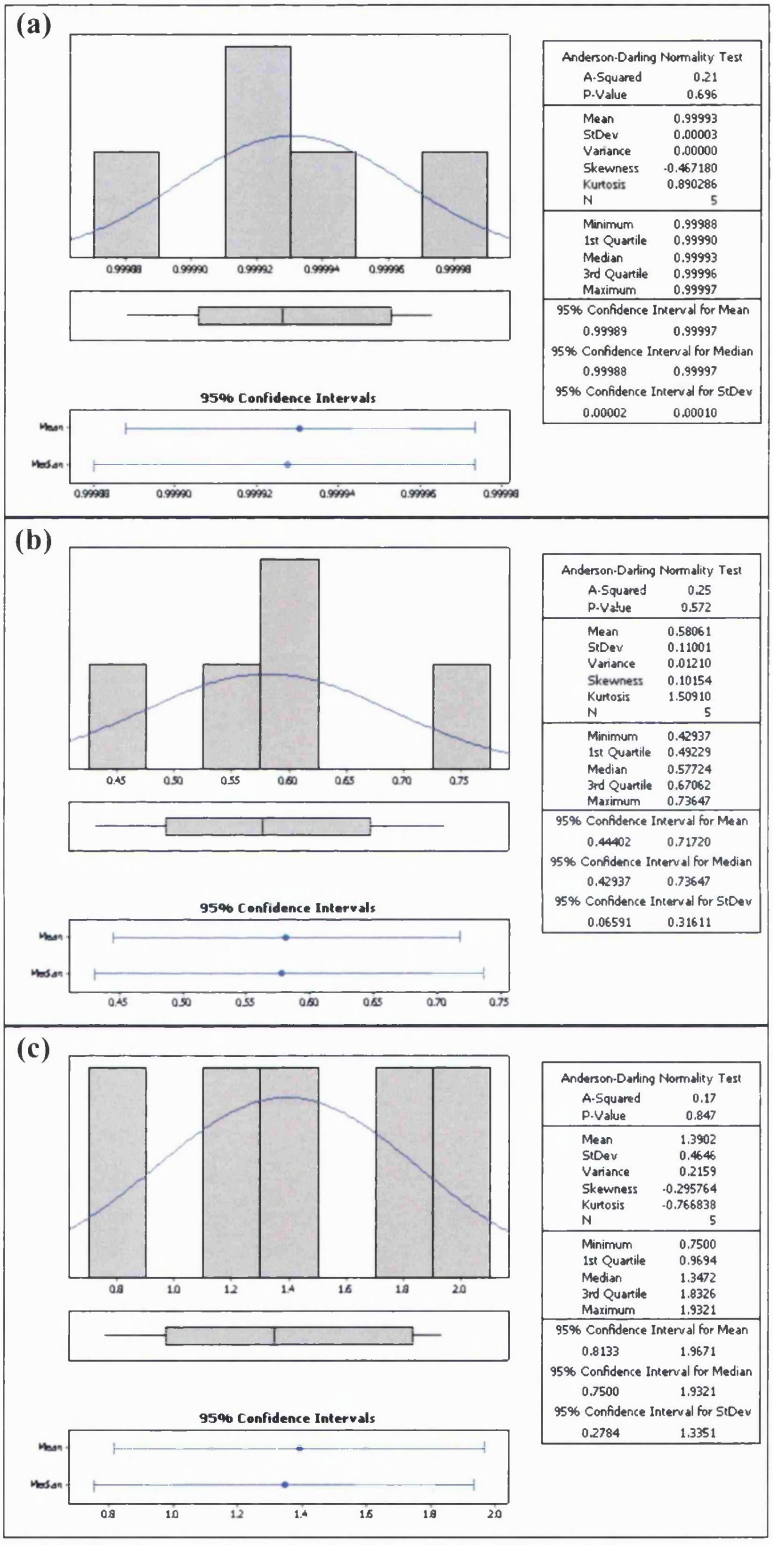

Figure 109: Minitab graphical summary with normality results for overall delivery conformance results for historical deliveries: (a) correlation, (b) ANSD and (c) PCA-MD

APPENDIX 2 – MATERIAL ATTRIBUTE QUALITY CONFORMANCE

MINITAB GRAPHICAL SUMMARIES WITH NORMALITY ASSESSMENT

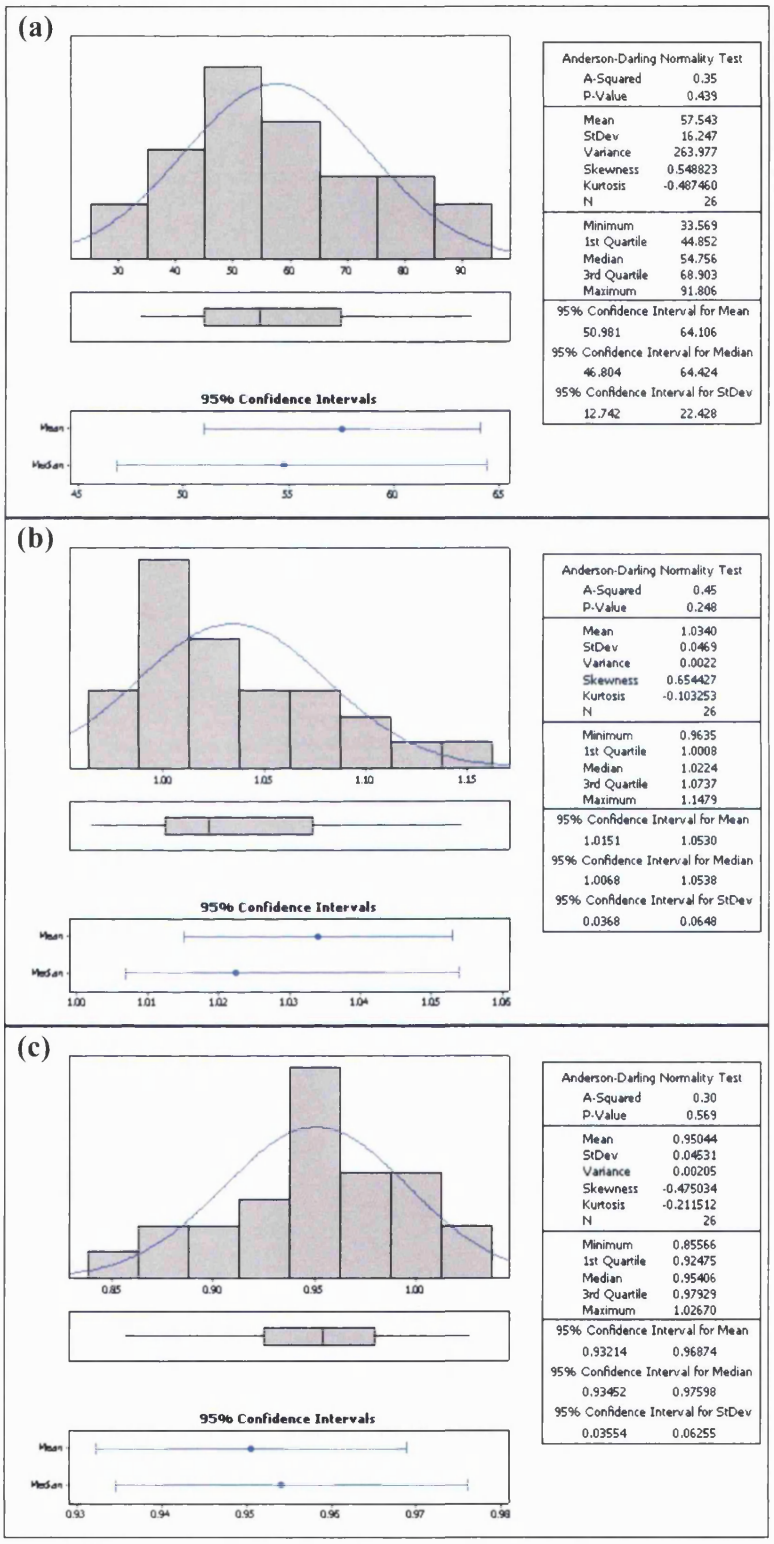

Figure 110: Minitab graphical summary with normality results for individual container conformance results for historical deliveries: (a) TNSD-RR, (b) MLR-DA and (c) PLS-DA

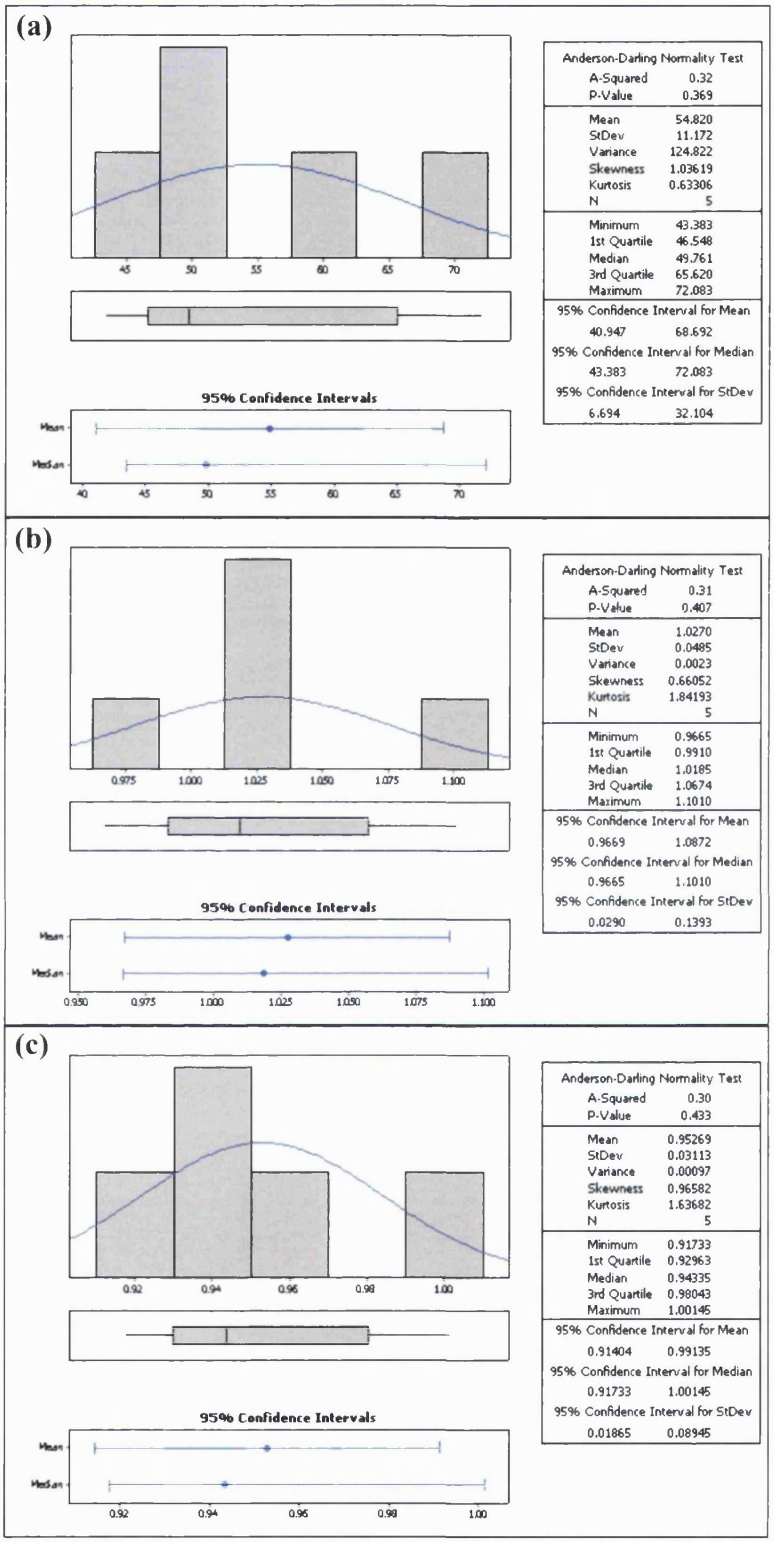

Figure 111: Minitab graphical summary with normality results for overall delivery conformance results for historical deliveries: (a) TNSD-RR, (b) MLR-DA and (c) PLS-DA

APPENDIX 3 – QUALITATIVE TABLET COMPONENT QUALITY CONFORMANCE PREDICTION RESULTS FOR THE HISTORICAL THRESHOLD BATCHES

Batch -Tablet	1122 nm Absorbance	ANSD-RR	Batch -Tablet	1122 nm Absorbance	ANSD-RR
1-1	0.0137	-0.8785	**3-1**	0.01337	-1.532
1-2	0.01378	-0.7909	**3-2**	0.01356	-1.24
1-3	0.01428	-0.2146	**3-3**	0.01356	-1.284
1-4	0.01402	-0.5173	**3-4**	0.01345	-1.358
1-5	0.0141	-0.4265	**3-5**	0.01361	-1.237
1-6	0.01393	-0.5338	**3-6**	0.01368	-1.119
1-7	0.01402	-0.5031	**3-7**	0.01332	-1.542
1-8	0.01408	-0.4412	**3-8**	0.01363	-1.186
1-9	0.0142	-0.274	**3-9**	0.01341	-1.422
1-10	0.01397	-0.5322	**3-10**	0.01345	-1.384
2-1	0.01436	0.1596	**4-1**	0.01421	0.2149
2-2	0.01415	-0.1038	**4-2**	0.01434	0.3871
2-3	0.01418	-0.06524	**4-3**	0.01432	0.3859
2-4	0.01399	-0.3254	**4-4**	0.01398	-0.002242
2-5	0.0142	0.04181	**4-5**	0.01407	0.135
2-6	0.01414	-0.03106	**4-6**	0.01415	0.2215
2-7	0.01466	0.4745	**4-7**	0.01454	0.7107
2-8	0.01404	-0.1887	**4-8**	0.01447	0.6531
2-9	0.01429	0.1281	**4-9**	0.01423	0.3836
2-10	0.01387	-0.3953	**4-10**	0.01421	0.3038

Batch -Tablet	1122 nm Absorbance	ANSD-RR	Batch -Tablet	1122 nm Absorbance	ANSD-RR
5-1	0.01474	1.297	**6-1**	0.01406	0.2609
5-2	0.01471	1.331	**6-2**	0.01379	-0.015
5-3	0.01483	1.457	**6-3**	0.01383	0.07393
5-4	0.0148	1.395	**6-4**	0.01423	0.4888
5-5	0.01461	1.209	**6-5**	0.01397	0.1486
5-6	0.01491	1.483	**6-6**	0.01381	-0.01242
5-7	0.01484	1.467	**6-7**	0.01399	0.1678
5-8	0.01462	1.268	**6-8**	0.0142	0.4114
5-9	0.01477	1.436	**6-9**	0.01402	0.2207
5-10	0.01468	1.276	**6-10**	0.0138	-0.03544

APPENDIX 4 – QUALITATIVE TABLET COMPONENT QUALITY CONFORMANCE MINITAB GRAPHICAL SUMMARIES WITH NORMALITY ASSESSMENT

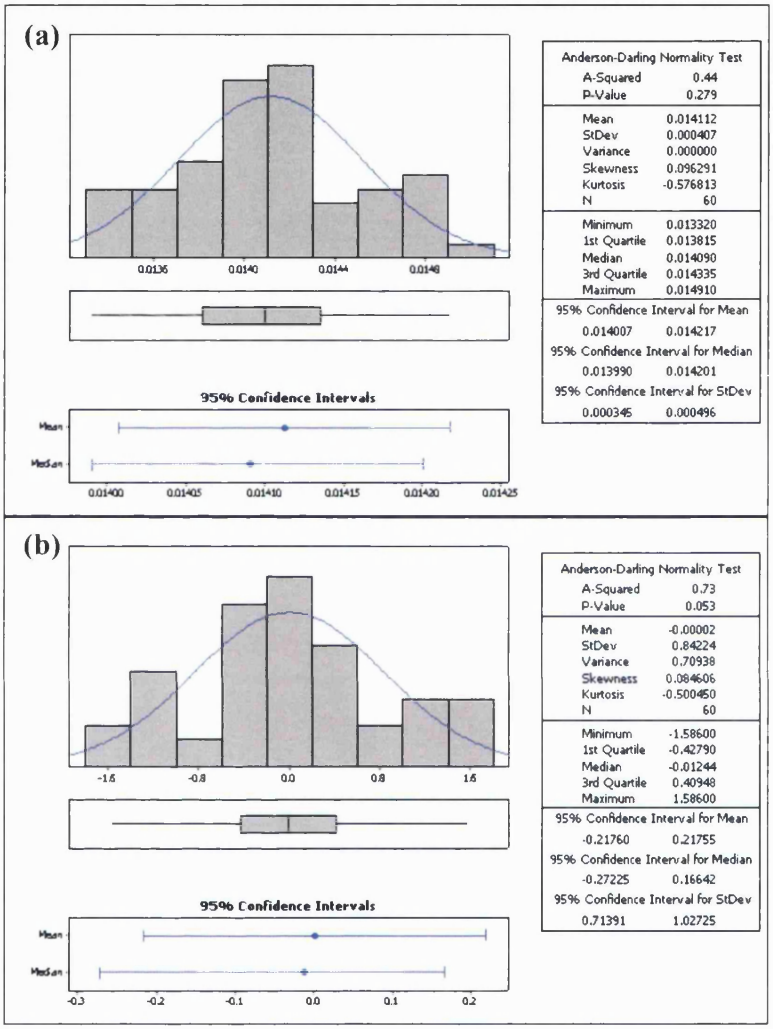

Figure 112: Minitab graphical summary with normality results for the individual tablet historical dataset for (a) 1122 nm Absorbance and (b) ANSD-RR

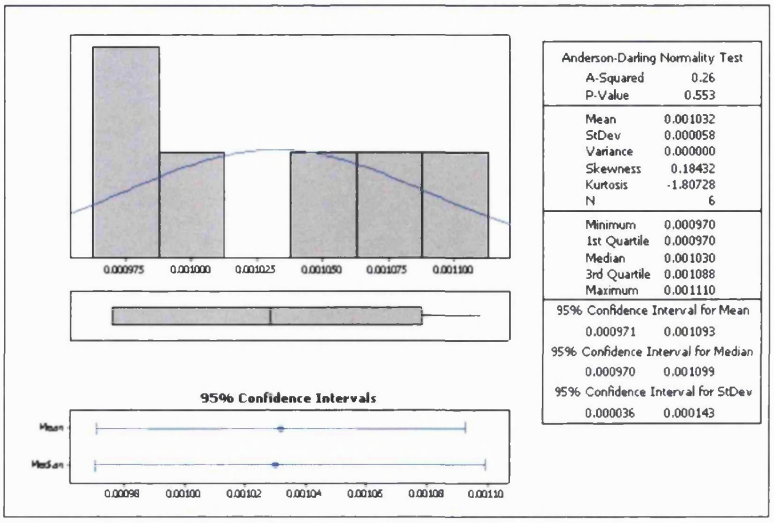

Figure 113: Minitab graphical summary with normality results for the range of 1122 nm Absorbance for the six commercial batches

APPENDIX 5 – SEMI-QUANTITATIVE TABLET COMPONENT QUALITY CONFORMANCE PREDICTION RESULTS FOR THE REGRESSION DATASET

Batch -Tablet	MLR-NV	PLS-NV	Batch -Tablet	MLR-NV	PLS-NV
1-1	4.827	4.748	**3-1**	4.84	4.8
1-2	4.892	4.86	**3-2**	4.884	4.896
1-3	5.046	5.003	**3-3**	4.895	4.896
1-4	4.979	4.95	**3-4**	4.824	4.784
1-5	5.008	4.978	**3-5**	4.917	4.937
1-6	4.879	4.824	**3-6**	4.926	4.886
1-7	4.95	4.909	**3-7**	4.778	4.726
1-8	4.986	4.947	**3-8**	4.873	4.83
1-9	4.991	4.936	**3-9**	4.797	4.751
1-10	4.898	4.837	**3-10**	4.824	4.767
2-1	4.994	4.974	**4-1**	4.947	4.937
2-2	4.968	4.978	**4-2**	4.981	4.986
2-3	4.959	4.947	**4-3**	4.95	4.932
2-4	4.948	4.957	**4-4**	4.837	4.795
2-5	4.96	4.977	**4-5**	4.853	4.813
2-6	4.953	4.989	**4-6**	4.9	4.869
2-7	5.155	5.166	**4-7**	4.996	4.946
2-8	4.907	4.932	**4-8**	4.969	4.941
2-9	4.969	4.989	**4-9**	4.899	4.884
2-10	4.823	4.813	**4-10**	4.903	4.881

Batch -Tablet	MLR-NV	PLS-NV	Batch -Tablet	MLR-NV	PLS-NV
5-1	4.946	4.935	**75%-1**	3.55	3.488
5-2	4.936	4.939	**75%-2**	3.457	3.423
5-3	4.913	4.807	**75%-3**	3.31	3.256
5-4	4.968	4.965	**75%-4**	3.539	3.494
5-5	4.904	4.897	**75%-5**	3.345	3.272
5-6	4.978	4.927	**85%-1**	4.091	4.143
5-7	4.978	4.98	**85%-2**	4.028	4.098
5-8	4.881	4.875	**85%-3**	4.114	4.213
5-9	4.91	4.872	**85%-4**	4.057	4.072
5-10	4.923	4.94	**85%-5**	4.14	4.22
6-1	4.913	5.025	**115%-1**	5.687	5.777
6-2	4.789	4.852	**115%-2**	5.496	5.574
6-3	4.756	4.751	**115%-3**	5.611	5.705
6-4	4.948	5.042	**115%-4**	5.617	5.737
6-5	4.873	4.964	**115%-5**	5.514	5.591
6-6	4.802	4.795	**125%-1**	5.808	5.814
6-7	4.86	4.905	**125%-2**	5.992	6.038
6-8	4.946	5.04	**125%-3**	5.881	5.906
6-9	4.886	4.923	**125%-4**	6.04	6.047
6-10	4.773	4.753	**125%-5**	5.957	5.978

APPENDIX 6 – SEMI-QUANTITATIVE TABLET COMPONENT QUALITY CONFORMANCE PREDICTION RESULTS FOR THE VERIFICATION DATASET

Batch -Tablet	MLR-NV	PLS-NV	Batch -Tablet	MLR-NV	PLS-NV
1-1	5.029	5.024	**3-1**	4.929	4.877
1-2	4.885	4.799	**3-2**	4.96	4.972
1-3	5.044	4.993	**3-3**	4.947	4.96
1-4	4.899	4.818	**3-4**	4.934	4.936
1-5	4.8	4.694	**3-5**	4.962	4.982
1-6	5.17	5.139	**3-6**	4.969	4.962
1-7	4.825	4.781	**3-7**	4.9	4.922
1-8	4.89	4.817	**3-8**	4.838	4.829
1-9	5.17	5.122	**3-9**	5.106	5.139
1-10	4.909	4.832	**3-10**	4.849	4.849
2-1	4.966	4.924	**4-1**	4.824	4.852
2-2	4.957	4.92	**4-2**	4.861	4.925
2-3	4.946	4.894	**4-3**	4.798	4.833
2-4	4.95	4.925	**4-4**	4.838	4.891
2-5	4.986	4.959	**4-5**	4.864	4.907
2-6	4.964	4.937	**4-6**	4.859	4.891
2-7	5.018	4.995	**4-7**	4.904	4.922
2-8	4.978	4.935	**4-8**	4.797	4.839
2-9	4.986	4.944	**4-9**	4.937	4.993
2-10	4.871	4.834	**4-10**	4.774	4.805

Batch -Tablet	MLR-NV	PLS-NV	Batch -Tablet	MLR-NV	PLS-NV
75%-1	3.469	3.411	**115%-1**	5.606	5.717
75%-2	3.355	3.287	**115%-2**	5.532	5.657
75%-3	3.523	3.477	**115%-3**	5.495	5.569
75%-4	3.437	3.39	**115%-4**	5.64	5.734
75%-5	3.567	3.529	**115%-5**	5.68	5.757
85%-1	4.075	4.152	**125%-1**	5.9	5.926
85%-2	4.081	4.133	**125%-2**	5.992	6.003
85%-3	4.137	4.214	**125%-3**	5.824	5.825
85%-4	4.184	4.231	**125%-4**	6.02	6.053
85%-5	4.181	4.211	**125%-5**	5.884	5.889

APPENDIX 7 – SEMI-QUANTITATIVE TABLET COMPONENT QUALITY CONFORMANCE MINITAB GRAPHICAL SUMMARIES WITH NORMALITY ASSESSMENT

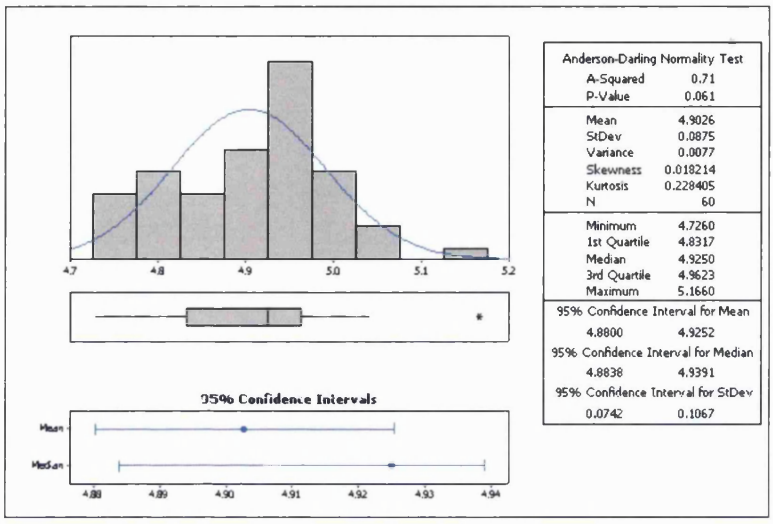

Figure 114: Minitab graphical summary with normality results for the PLS-NV predictions for the historical dataset

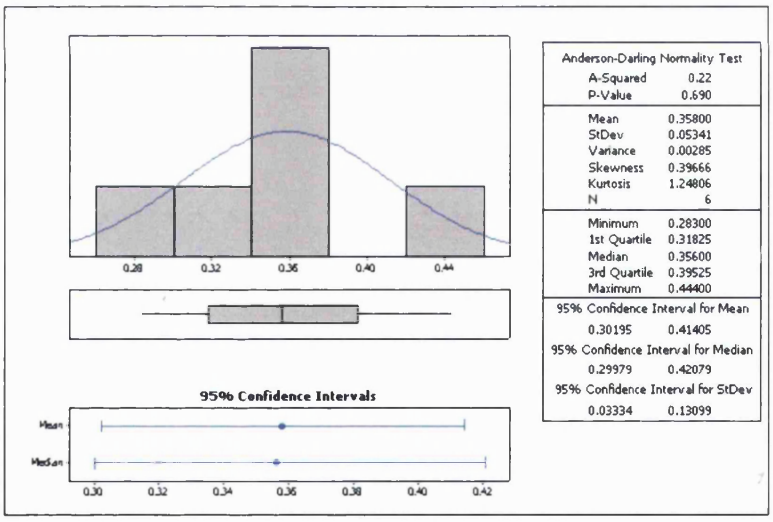

Figure 115: Minitab graphical summary with normality results for the range of PLS-NV predicted amlodipine active content for the six historical commercial batches