

**Taste evaluation of a novel midazolam tablet for pediatric patients: *in vitro* drug dissolution, *in vivo* animal taste aversion and clinical taste perception profiles**

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## Abstract

Harmonized methodologies are urgently required for the taste evaluation of novel pediatric medicines. This study utilized *in vitro*, *in vivo* and clinical data to evaluate the palatability of a novel midazolam chocolate tablet. *In vitro* dissolution experiments showed the crushed tablet to release within 5 min 1.68 mg of midazolam into simulated saliva. This translated to a drug level of 0.84 mg/ml in the oral cavity, which would be higher than the midazolam bitterness detection threshold concentration of 0.03 mg/ml determined in a rat ‘brief access taste aversion’ (BATA) model. The visual analogue scale scores of patients aged 4 – 16 years prescribed with midazolam pre-surgery showed a clear preference for the midazolam chocolate tablets ( $3.35 \pm 1.04$ ,  $n = 20$ ) compared to the control midazolam solution ( $1.47 \pm 0.62$ ,  $n = 17$ ). The clinical data was in agreement with the *in vivo* rodent data in showing the novel chocolate tablet matrix to be effective at taste-masking the bitter midazolam.

Keywords: midazolam, taste, pediatric formulations

Abbreviation: brief access taste aversion (BATA)

## 1.0 Introduction

In pediatric patients, the unpleasant taste of a medicine is one of the commonest causes of treatment barrier (1), and the importance of building palatability into pediatric medicines is now recognized by the pharmaceutical industry (2) and the regulatory authorities (3, 4). However, there is no specific regulatory framework for palatability assessment, which has resulted in a lack of harmonization of taste evaluation methodologies applied in the development of pediatric medicines (5, 6).

The simplest taste evaluation methods for peroral pediatric medicines is to determine the *in vitro* drug release profile of the medicines in simulated physiological fluids, the rationale being that only the freely soluble drug molecules can interact with taste receptors. While cheap and readily accessible, this method is better suited to solid medicines, not solution formulations, and provides a measure of taste only when human perception of the tastant is known to some extent (e.g. as a taste threshold concentration). Currently, there are no regulatory-defined parameters to simulate drug release in the oral cavity from medicines. Published studies that have utilized the *in vitro* drug dissolution method to evaluate medicine palatability have employed a range of dissolution medium (water (7) to pH 6.8 phosphate buffer (8, 9)), receptor fluid volume (10 ml (10) to 900 ml (8)), and dissolution time (2 min (8, 9) to 5 min (7)).

Electronic taste evaluation may be a better alternative (11) as it can profile the taste characteristic of the drug molecule via an array of sensors (12). Compared to *in vivo* models, electronic sensors also provide a more objective and consistent taste evaluation without incurring the ethical and safety concerns associated with using animal and human participants. Electronic tongues are, however, limited by their application to assess only aqueous solutions, and the sensor sensitivity can be affected by formulation pH and excipients (13).

An *in vivo* model that exploits the natural defense mechanism in most animals to avert bitter-tasting substances has also been developed for the palatability testing of medicines (14). Mildly water-deprived rats are presented with the liquid tastant, and an IC<sub>50</sub> dose, defined as the tastant concentration that causes a 50% drop in licking frequency compared to water, is determined. This model is advocated for novel lead compounds whose toxicology has not been adequately elucidated to safeguard taste evaluation in humans. Products for evaluation by this method have to be liquid formulations of appropriate viscosity, and if they are suspensions, to have particles below a threshold diameter to enable the liquid formulations to flow freely through the sipping tube for the animals to lick. It is difficult to establish the accuracy of taste evaluation in animal models because the *in situ* drug concentration in the oral cavity cannot be measured accurately. Even if this concentration is measurable, it may still not correlate with ‘palatability’ and ‘acceptable taste’ as these perceptions are dependent also on other factors, e.g. the age and taste receptor expression of the animal model used.

The most direct measurement of palatability of a product is to conduct taste evaluation in humans, ideally in the specified pediatric age groups to account for age-related sensitivities towards bitter and sweet tastes (15, 16). Pediatric taste studies are, however, associated with significant methodological and ethical barriers around participant recruitment. Parents and clinicians may resist a taste trial where a healthy child may accidentally ingest a pharmacologically active agent, especially a novel yet-to-be-approved potent agent. On the other hand, parents and clinicians struggling to achieve treatment compliance in a child who requires the intervention of an unpalatable drug may be more inclined to support a clinical trial to evaluate a potentially better tasting product of the drug. In such cases, however, the application of a cross-over study design with placebo or comparator tastant could be unethical. Children may also be less able than adults to describe taste, and taste trials involving children

may have to rely on scales of assessment that are yet to be validated for taste evaluation, e.g. the 5-point facial hedonic scale and visual analogue scales (6).

Midazolam is a bitter drug that has caused considerable grief in pediatric hospitals. It is widely prescribed as a pre-medication aimed at calming young patients scheduled for surgery and dental procedures. Taste masking of midazolam has been challenging, with both the commercial and extemporaneously compounded oral midazolam syrups known anecdotally for high rejection rates due to poor taste. Rejection of the medicine presents particular difficulties in children who are very anxious or uncooperative in the preoperative setting, e.g. autistic children. In the event of treatment failure, the uncooperative children may have to be physically restrained for the induction of anesthesia, posing, particularly in older children, significant safety risks for the patients, accompanying parent/guardian and attending medical staff.

Our laboratory has developed a novel chewable chocolate-based tablet containing 5 mg of midazolam HCl for use in children. These are small tablets measuring 10 x 5 x 5 mm with 3 score lines to facilitate dose division (Figure 1A). The aim of this study was to provide a first-in-kind comprehensive evaluation of the palatability of a pediatric dosage form using a range of methodologies and the midazolam chocolate tablet as a model formulation. We applied the *in vitro* dissolution test, the rat brief-access taste aversion (BATA) model and a clinical trial involving patients aged 4 – 16 years of age to the taste evaluation of the chewable midazolam chocolate tablet. The electronic tongue was excluded because it would involve conducting a dissolution experiment for the midazolam chocolate tablets and analyzing filtered aliquots of the dissolution medium in the E-tongue. The filtered aliquots would not provide an accurate measure of the effectiveness of the chocolate matrix in masking the taste of the released drug. In this regard, the E-tongue would not offer significant advantages to the dissolution experiments in evaluating the taste of the formulation. A correlation of the *in vitro* dissolution

profile, animal taste aversion profile and pediatric taste scores for the chewable tablet, together with issues associated with each of the methodologies, is discussed in the present study.

## **2.0 Materials and Methods**

Midazolam hydrochloride (HCl) (BP grade, Cambrex Profarmaco Milano, Italy) was used as received. All other chemicals were of analytical grade. Deionized water (PSI Water Filters, Launceston, TAS, Australia) was used throughout.

### *2.1 Preparation and storage stability of chocolate-based chewable tablets*

Chewable tablets each containing 5 mg of midazolam hydrochloride and measuring 10x5x5 mm were prepared by a melt moulding technique using generally regarded as safe excipients that included dark chocolate (Nestle Australia Ltd, Rhodes, NSW, Australia), hydrogenated castor oil, xanthum gum, polyethylene glycol 1450, and steviol glycosides. All ingredients, except for the dark chocolate, were of pharmaceutical grades (BP or USP) and obtained from registered pharmaceutical suppliers in Australia.

### *2.2 In vitro drug dissolution profiles*

*In vitro* drug dissolution was conducted in triplicates using a paddle rotating speed of 50 rpm (Varian VK 7010 Dissolution Apparatus, Agilent Technologies, Mulgrave, Victoria, Australia). Tablets weighed into separate porcelain crucibles were ‘masticated’ by crushing with a glass rod into small fragments (Figure 1B), and placed into 300 ml of simulated saliva (8 mg/ml sodium chloride, 0.19 mg/ml potassium phosphate monobasic, 2.38 mg/ml sodium phosphate dibasic, pH 6.8) (17) at 37 °C. One-ml aliquots were sampled at 0, 5, 15, 30, 45 and

60 min, and simultaneously replaced with 1 ml of blank dissolution medium. The aliquots were filtered (Fitropur S 0.2 µm filter, Sarstedt Australia Pty. Ltd, Adelaide, SA, Australia), diluted 1:1 v/v with blank dissolution medium, and analyzed by a validated high performance liquid chromatographic (HPLC) assay. Parallel experiments were conducted ~~under sink conditions~~ using 500 ml of 0.1M HCl as dissolution medium. Withdrawn aliquot samples were filtered, mixed 1:1 v/v with methanol and analyzed by HPLC. A third dissolution profile was obtained of intact tablets in 500 ml of 0.1M HCl. The cumulative amounts of drug released, expressed as a percent of initial drug load in the tablet, were plotted against sampling times to obtain the *in vitro* dissolution profiles.

Midazolam HCl was quantified by reversed-phase HPLC (Agilent 1260 Infinity binary pump HPLC, Agilent Technologies Australia, Mulgrave, NSW, Australia) with a C18 column (3.5 µm, 100 x 3.0 mm) (Sunfire, Waters Australia Pty Ltd, Rydalmere, NSW, Australia). Gradient elution at flow rate of 0.45 mL/min was employed. The mobile phase of 25% v/v acetonitrile (ACN) and 75% v/v potassium phosphate buffer (20 mM phosphate, pH 3.8) was graded to 100% ACN in 6 min, then held isocratic for the next 6 min. Midazolam was detected at 237 nm. Linearity of calibration graphs ( $R^2 > 0.99$ ) was demonstrated over the concentration ranges of 0.04 to 25 µg/ml and 0.5 to 20 µg/ml for the standard solutions of midazolam HCl in 0.1 M HCl and simulated saliva (pH 6.8), respectively.

### 2.3 *In vivo* rat taste aversion profile

Ten male Sprague Dawley rats (average weight  $719 \pm 126$  g) previously trained for the rat BATA experiments (18) were used for two independent tasting evaluations. Animal procedures were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986 (Project License PPL 70/7668).

Prior to experiments, the rats were mildly deprived of water for 22 hours but had access to food *ad libitum*. Taste evaluation was performed in consecutive 40-min sessions using one rat at a time. The rat was placed into a Lickometer Davis Rig MS-160 (DiLog Instruments, Tallahassee, Florida, USA) apparatus that held 16 sample tubes on a moving rack controlled by the Davis Collect Data software. Each tube was presented to the rat via a sipper tube for 8 s, in randomized order specified by the software. Over the 40-minute session, each sample was presented to the rat 4 times, with a 2-s deionized water rinse given between samples. The number of licks received for a tube was transmitted and recorded electronically onto a database.

The midazolam chocolate tablet was assessed against 5 controls - water, vehicle (water adjusted to pH 5 with HCl), 1 mg/mL solution of midazolam HCl in the vehicle, 2.5 mg/mL oral midazolam HCl syrup (a proprietary formulation manufactured by the WA Hospital Central Pharmaceutical Manufacturing Facility, Princess Margaret Hospital, Perth, Australia) and chocolate placebo tablets. The placebo and midazolam-loaded chocolate tablets were triturated in the vehicle (2 mL per tablet, mimicking a realistic volume of saliva) using a mortar and pestle to allow passage through the sipper tubes without blockage. Calibration of midazolam taste threshold was also performed with standard solutions of midazolam HCl dissolved in the vehicle at 6 concentrations (range of 0.003 to 1 mg/mL).

#### *2.4 Clinical taste evaluation in pediatric patients*

Taste evaluation was conducted in a prospective, open-label, single center, randomized, single-treatment trial involving children aged 4 – 16 years undergoing elective surgery and who had been prescribed midazolam for pre-medication by an anesthetist independent of the



study team at the Princess Margaret Hospital. The study was approved by the hospital Human Research Ethics Committee (HREC 2014102EP; anzctr.org.au: ACTRN12615000225516).

All participants were recruited on the day of their surgery. Written informed parental consent and, where appropriate, child assent were sought prior to inclusion into the study protocol. Each child was randomized by computer generated block randomization to receive by mouth either the chocolate-based midazolam tablet or a 5 mg/ml midazolam IV solution, which was the preferred mode of midazolam administration at the hospital. Participants in the chocolate tablet arm were instructed to chew the tablet before swallowing. Midazolam was dosed at 0.5 mg/kg to a maximum of 20 mg, but the treating anesthetists were free to change the dose according to the clinical need. Children in the study received doses of 6.25 to 20 mg, equivalent to 1¼ to 4 midazolam chocolate tablets, or 1.25 to 4 ml of midazolam solution.

After the child had taken the allocated midazolam sample, a trained research nurse would record on a five-point facial hedonic scale (Figure 2) whether the child liked the taste of the medication or not. Non-invasive pharmacodynamic data (time to onset of sedation) was also collected for each patient to determine the clinical effect of midazolam. The child patient was asked to record how much he/she liked the sample by putting a mark on a separate five-point facial hedonic scale, and whether he/she would be happy to take the sample again if unwell. The attending parent/guardian was also asked to give a score on a third five-point hedonic scale on how he/she perceived the child's response to the taste of the assigned medication.

## 2.5 Statistical analysis

Rat aversion data were analyzed using the *R* statistical software (<http://www.R-project.org>) after removal of data sets with  $\leq 1$  lick (19), and the comparison of means was performed using 2-tailed unpaired t-test. Non-parametric Mann-Whitney test and 2-tailed

unpaired t-test were applied on the clinical taste evaluation data. With the effect size  $d=1.0$  and  $\alpha=0.05$ , this trial has 80% power to detect a difference with 18 children in each group (G\*Power 3.1). The gender distribution and the participant's willingness to take the second dose were analyzed using 2-tailed Chi-square test. All statistical analyses were performed with PRISM 6 for Mac (GraphPad software). A  $P$  value  $<0.05$  was considered statistically significant.

### 3.0 Results

HPLC analysis of the tablets immediately after manufacture showed a midazolam hydrochloride content of  $5.12 \pm 0.16$  mg/tablet ( $n = 3$ ). The tablets were stable for at least 18 months when stored at ambient temperature. Residual midazolam content at 12 and 18 months of storage were  $96.84 \pm 4.18\%$  and  $91.00 \pm 0.01\%$  ( $n = 3$ ), respectively.

There was complete release ( $99.52 \pm 3.22\%$ ,  $n = 3$ ) of the midazolam HCl load from the masticated 5 mg tablets after 15 min in the 0.1 M HCl medium (Figure 3). The absence of extraneous peaks, and the complete release of intact drug suggests a lack of interaction between midazolam and the chocolate tablet matrix. At the zero time point, the drug already available from these tablets amounted to  $0.62 \pm 0.70$  mg, equivalent to  $14.26 \pm 16.18\%$  of the drug load in the tablets. Our data suggest that the pre-crushing of tablet led to faster drug release in the first 5 min compared with the intact tablet, the cumulative drug released differing by 0.62 mg at  $t=0$  and 1.79 mg at  $t=5$  min. If the tablets had not been crushed prior to exposure to the dissolution medium, the mean cumulative drug released at  $t=0$  and  $t=15$  min were  $1.25 \pm 0.47\%$  and  $62.78 \pm 4.87\%$ , respectively. The full drug load ( $\sim 100\%$ ) was released from the intact tablets at about 35 min (Figure 3). Changing the dissolution medium for the crushed tablets from 0.1M HCl to simulated saliva (pH 6.8) significantly inhibited the rate of drug

released. The cumulative drug released reached a plateau value of about 45% (6.46  $\mu\text{g/mL}$ ) at 10 min, and this level was maintained over the duration of the experiment (Figure 3).

Rat aversion to the taste of midazolam was indicated by a significant reduction in the number of licks when a sample was presented to the rat. Standard solutions containing up to 0.03 mg/mL midazolam HCl received comparable licking frequencies compared to the blank vehicle (water adjusted to pH 5 with HCl to facilitate dissolution of midazolam HCl) (Figure 4A). This suggests that the rats were unable to effectively detect the bitter drug presented at up to these concentrations. There was a significant drop in licking frequency when the drug concentration was increased to 0.1 mg/mL, and near abandonment of licking when the drug concentration was increased further to 1.0 mg/mL. Dose-response curve for the midazolam HCl standard solutions over the two test days suggests a mean  $\text{IC}_{50}$  value of  $0.30 \pm 0.29$  mg/mL (Figure 4A). There was no significant difference in the number of licks received between the vehicle and water.

Figure 4B shows that, compared to the standard 1 mg/mL midazolam HCl solution, the liquid midazolam chocolate sample prepared by triturating the 5 mg midazolam chocolate tablet in 2 mL of water was significantly better received by the rats. This is despite the chocolate sample having a 2.5 fold higher midazolam HCl content than the standard solution. The mean licking frequencies for the midazolam chocolate sample and placebo chocolate sample were comparable, suggesting that the rats were unable to detect the bitter drug when it was presented in the novel chocolate tablet matrix formulated by our laboratory. In contrast, the proprietary oral syrup prepared by the local hospital, which also contained 2.5 mg/mL midazolam HCl, was less well received.

Preliminary analysis of the clinical study was conducted based on data collected from 38 children aged between 4 to 16 years (Table 1). The data was not stratified for patient age due

to the small sample size. There were no significant differences in the demographic characteristics of participants in the midazolam chocolate tablet group and the control arm, which received the IV midazolam solution orally.

Non-parametric Mann-Whitney test and 2-tailed unpaired t-test were applied on the clinical taste scores and both tests yielded the same outcome. There was a clear preference for the novel midazolam chocolate tablets ( $n = 20$ ) compared to the IV midazolam solution administered orally ( $n = 17$ ; one child in this group was too drowsy to give a score). The largest mean score difference between the chocolate and comparator samples was provided by the child participants (Figure 5). When asked whether they would take the medicine again, 75% of participants ( $n=15$ ) in the midazolam chocolate tablet arm replied in the affirmative while only 35.3% of participants ( $n=6$ ) in the midazolam solution arm would take the dose again ( $P=0.015$ ). Time to onset of sedation (clinical effect of midazolam) was  $11.30 \pm 5.03$  min for the midazolam solution group and  $12.40 \pm 6.39$  min for the chocolate group ( $P > 0.05$ ).

#### **4.0 Discussion**

Of the 3 taste evaluation studies conducted for the novel midazolam chocolate tablets, the simplest and cheapest to perform were the *in vitro* dissolution experiments. There were no requirements for ethics approval and animal/patient recruitment, and consistent data were readily obtained within days. However, the lack of consensual dissolution methodology and simulated salivary fluid to employ (20) has significant implications on a drug with pH-dependent aqueous solubility. Midazolam is a weak base ( $pK_a$  6.04) (21). It is soluble in acidified water ( $>2.5$  mg/ml), but the solubility decreased to  $< 0.1$  mg/ml at  $pH > 5$ . In our study, the volumes of dissolution media employed were significantly higher than the volume of saliva in the oral cavity. This is to ensure the dissolution experiments could be optimally

conducted with the chocolate tablets in the USP dissolution apparatus. Despite the high volume of dissolution media used, drug saturation was apparent within 10 min of introducing the midazolam chocolate tablet into the simulated saliva (pH 6.8). This could underestimate drug release in the oral cavity *in vivo*. Conversely, the relative ease with which midazolam HCl dissolved in 500 ml of 0.1 M HCl would overestimate the oral drug level. The typical saliva volume in human is no more than 2 ml (22), but this low volume was difficult to apply in dissolution experiments for the chocolate-based tablets as it rendered the samples too thick to be filtered for HPLC analysis. The chewable midazolam chocolate tablets are not likely to remain in the mouth of patients for longer than 5 min, so the extent of drug released from the tablet into the oral cavity can be predictable from the drug concentrations in the 0 and 5 min aliquot samples. In this study, the sampling time for the dissolution experiments was extended to 60 min to also provide us with an estimate of the peroral drug bioavailability *in vivo*. There is no validated method to simulate tablet mastication for *in vitro* drug dissolution studies. In the absence of a defined method, we have improvised a reproducible, if rather crude, method for the chewable chocolate tablets. However, the dissolution data could not be directly correlated to taste aversion without prior knowledge of the taste detection and tolerance thresholds for midazolam in the pediatric patients.

Taste evaluation using the rodent BATA model allowed the test product to be simultaneously evaluated against a range of controls, and provided the *in vivo* IC<sub>50</sub> and taste thresholds for midazolam HCl. On the basis that taste aversion was reflected by a significant reduction in the licking frequency, the detection threshold for the bitter taste of midazolam HCl would be about 30 µg/ml in the rodent model.

Challenges associated with the rodent model included the requirement for animal ethics approval. Rats had to be purchased and trained and, while the trained rats could be re-used over several months, there were on-going agistment costs. Another disadvantage was the

inability to administer the solid tablet to the rats with the present lickometer. Instead, the tablet had to be rendered into a liquid, and the concentration of midazolam presented to the rats' oral cavity (2.5 mg/ml) could be higher than if the rats were administered with the chewable tablets. Animal behavior was also not always predictable. Despite the controlled experimental environment of dim lighting and minimal noise, some rats did become distracted and stop licking the sample presented. A waiting time to first lick of 20 seconds was imposed, and where a sample attracted 0 or 1 lick, whether or not this was due to the rats being distracted or the sample having an unpalatable taste, the data had to be discounted as outliers (possible false negatives) for the final data analysis. To enhance the reliability of data, 10 rats were used and every tastant presented 4 times, resulting in an experiment that took 7h to complete, discounting sample preparation time. There was no scope for automation, unlike the dissolution experiment, although replicate experiments could still be completed within days. An excellent analysis of the difficulties and precision associated with the BATA method has earlier been reported by our colleagues (18).

Of the 3 studies conducted, the most direct taste data was obtained from the clinical trial, which also provided an analysis of pharmacodynamic effects in the target pediatric patients. The facial hedonic scales provided by the children suggested that the chocolate formulation was able to significantly mask the bitter taste of midazolam, as was the finding that 75% of the participants in the test group were willing to take the chocolate tablets again. The facial hedonic scores also suggest that the children were more willing to differentiate between the tastes of the midazolam chocolate tablet and solution compared to their caregivers.

The clinical study was, however, the most expensive to conduct, and it took the longest time. Preclinical *in vitro* dissolution and animal taste data were required by the hospital ethics committee, and participation was restricted to patients prescribed with midazolam for pre-surgery sedation. This then exposed the study to the unique dynamics between sick children

and their caregivers. For example, a child attempting to exert some control over their condition might refuse a medication or rate it poorly; we had children giving a low score for the midazolam chocolate tablet who had then asked whether they could have a second tablet. We also had a small number of very young children who had insisted on scores based on preferred facial expression on the hedonic scale rather than the taste of the medication. These factors underscore the urgency of validating scoring tools, including the 5-point hedonic scale used in this study, for the taste assessment of pediatric medicines.

A cross-over study design that could have provided more robust comparative taste data was also not possible when the participants required only 1 dose of midazolam prior to surgery. In addition, the midazolam doses, which were prescribed by attending anesthesiologists independent of the study, did not always conform to the recommended study dose of 0.5 mg/kg. However, even if the recommended dose had been uniformly prescribed, the participants, whose body weights ranged from 15.6 to 72.4 kg, would still not receive the same number of tablet or volume of solution. This discrepancy might affect the taste perception scores, but there are no industry guidelines on how to normalize the taste scores of participants receiving different doses of a medicine.

The clinical taste data for the midazolam chocolate tablets correlated well with the rodent BATA data in showing the chocolate matrix was effective at masking the bitter taste of midazolam. The tablets were presented to the rats in a liquid form at a drug concentration of 2.5 mg/ml. Despite the concentration being 83 fold higher than the detection threshold of 0.03 mg/ml, the rats were unable to differentiate the taste of the midazolam chocolate liquid from the placebo chocolate liquid. In the clinical trial, the participants were instructed to chew the tablets prior to swallowing. The *in vitro* dissolution data for a crushed tablet showed 1.68 mg and 3.03 mg of midazolam HCl was released at 5 min into the simulated saliva (~~non-sink condition~~) and 0.1M HCl (~~sink condition~~) media, respectively. Taking the saliva volume to be

2 ml, the chewing of one midazolam chocolate tablet might therefore produce drug levels of 0.84 – 1.52 mg/ml in the oral cavity. Higher cumulative concentrations are expected for the trial participants as they had received 1¼ to 4 tablets. Despite this, the pediatric participants had given relatively favorable taste scores for the midazolam chocolate tablets.

Corresponding data for the midazolam solution was less correlative. This solution was a commercial IV midazolam injection without any taste masking agents. Its composition was similar to that of the midazolam standard solutions used in the rodent experiments. However, while the rats were clearly averse to sipping midazolam solutions stronger than 0.03 mg/ml, a significant number of the children were able to take the full dose (1.25 to 4 ml) of the 5 mg/ml midazolam solution. The administration of the solution via an oral syringe by the child participant or attending parent under the supervision of the research nurse might have made the solution more tolerable. However, it could also reflect a higher threshold tolerance for bitter taste in human than in rat, and/or importantly, the willingness of sick children to take medicines as a matter of routine regardless of the taste of the medicine.

The novel midazolam chocolate tablet was developed following feedback from clinicians about the high rejection rates amongst paediatric patients administered with the commercial and extemporaneously prepared peroral midazolam syrups. The bitter taste of midazolam hydrochloride is notoriously difficult to mask in liquid products; a recent study involving 45 paediatric patients aged 1-6 years old recorded a mean score of 3 (range 2-3 for ‘Really Bad’ to ‘Bad’) on a 7-point hedonic scale for a commercial midazolam syrup (23).

The collective *in vivo* taste data of this study suggest that the novel chocolate-based matrix formulation has the potential to provide an acceptable peroral midazolam product for paediatric (and dysphagic) patients. Chocolate is an important taste masking ingredient, but the matrix also employs other ingredients to enhance its taste masking capacity, and to provide storage stability at ambient temperature. The generic applicability of this chewable matrix to



formulate acceptable products of other bitter drugs is currently being investigated in our laboratory.

## **5.0 Conclusion**

Harmonized methodologies are urgently required for the taste evaluation and development of novel pediatric medicines. *In vitro* dissolution data can be rapidly and routinely generated to evaluate the effectiveness of taste masked medicinal products provided the *in vivo* taste threshold and tolerance concentrations of the drug are known. The latter may be determined using rodent taste aversion studies, which can also provide preliminary taste evaluation of the products *in vivo*. Ultimately, clinical studies involving the target pediatric patient population are required to validate whether the product has been effective in masking the aversive taste of a drug. For the midazolam chocolate tablets, the *in vivo* and clinical data concur in indicating that the novel chocolate tablet matrix was effective at taste-masking the unpalatable midazolam.

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## **Conflict of Interest/Disclosure**

The authors report no potential conflicts of interest.

### **Author Contributions**

L.C.C., B.S.vU-ST., S.S., C.T. and L.Y.L. wrote the manuscript. L.C.C., M.N., B.S.vU-ST., S.S., C.T. and L.Y.L. designed research. L.C.C., M.N., E.T., B.S.vU-ST., A.H.A.M-A., J.S. and L.Y.L. performed research. L.C.C., M.N., E.T., B.S.vU-ST, C.T., A.H.A.M-A., J.S. and L.Y.L. analyzed the data.

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## Figure Legends

- Figure 1** The midazolam chocolate tablet (A) Intact tablet; (B) Crushed tablet for *in vitro* dissolution studies.
- Figure 2** The five-point facial hedonic scale used in the clinical taste evaluation study.
- Figure 3** *In vitro* drug release profile from the midazolam chocolate tablet into simulated dissolution media at 37°C; (a) ▲ - whole tablet in 500 ml of 0.1 M HCl; (b) ■ - masticated tablet in 500 ml of 0.1 M HCl; and (c) ● - masticated tablet in 300 ml of simulated saliva, pH 6.8. Data represent mean ± SD,  $n = 3$ .
- Figure 4** Response of Sprague Dawley rats ( $n = 10$ , data represent the sum of 2 independent experiments conducted over 2 days, mean with 95% confidence interval) to the taste of different samples (A) midazolam standard solutions prepared by dissolving midazolam HCl in the vehicle, \*  $P < 0.0001$  compared to blank vehicle; and (B) test samples comprising deionized water; vehicle; dark chocolate placebo (500 mg dissolved in 2 mL of water); 2.5 mg/mL midazolam oral syrup; and 5 mg midazolam HCl chocolate tablet dissolved in 2 mL of water to give a chocolate liquid containing 2.5 mg/mL midazolam. Statistical significance is indicated by \*  $P < 0.05$ .
- Figure 5** Box and whisker plots (10<sup>th</sup> -90<sup>th</sup> percentile) of visual analogue scale data for the taste evaluation of the midazolam chocolate tablets (□) administered orally. The comparator was a commercial IV midazolam solution (■) administered orally at comparable doses, which was the current practice at the Princess Margaret Hospital for Children. Statistical significance is indicated by \* $P < 0.05$ , \*\* $P < 0.001$ , or \*\*\* $P < 0.0001$ .



Figure 1

A



B



Figure 2

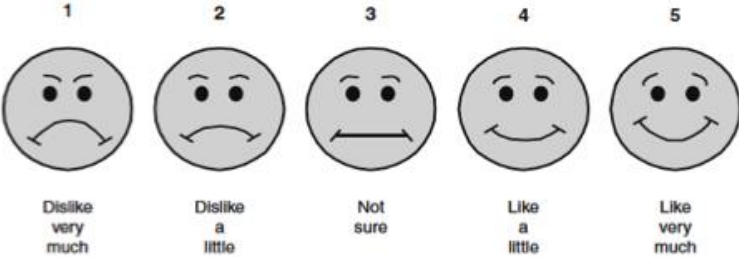


Figure 3

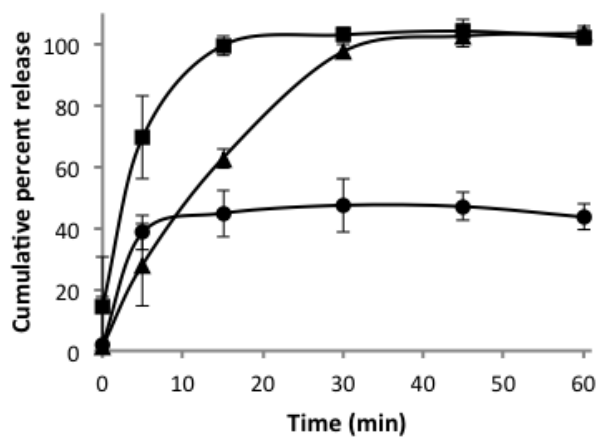


Figure 4

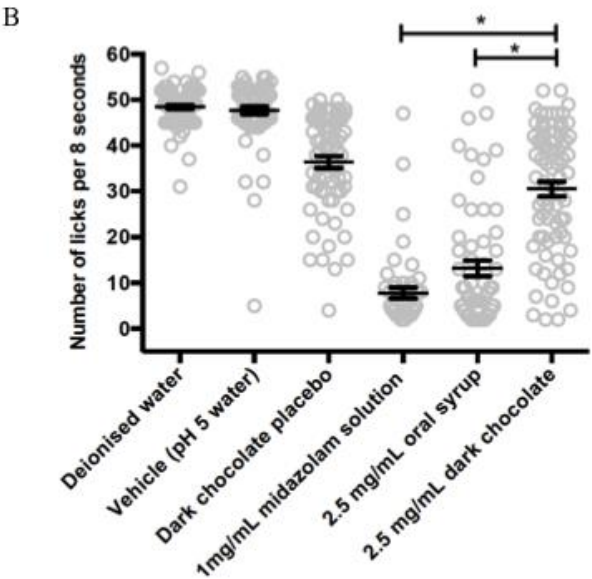
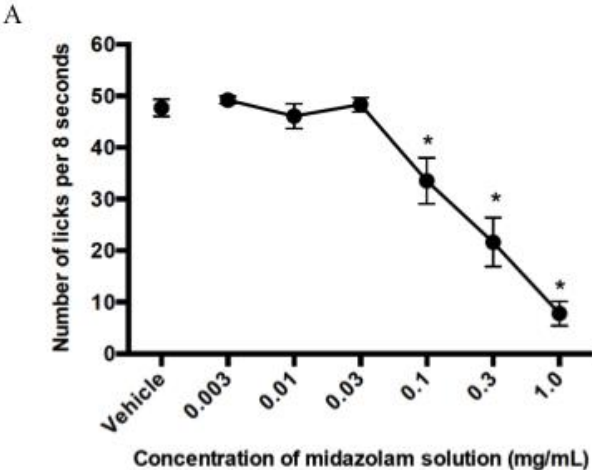


Figure 5

