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RESEARCH ARTICLE

Variability in content and dissolution profiles of MDMA tablets collected in the UK between 2001 and 2018 – A potential risk to users?

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Abstract

3,4-Methylenedioxymethamphetamine (MDMA, Ecstasy) tablets are widely used recreationally, and not only vary in appearance, but also in MDMA content. Recently, the prevalence of high-content tablets is of concern to public health authorities. To compare UK data with other countries, we evaluated MDMA content of 412 tablets collected from the UK, 2001–2018, and investigated within-batch content variability for a sub-set of these samples. In addition, we investigated dissolution profiles of tablets using pharmaceutical industry-standard dissolution experiments on 247 tablets. All analyses were carried out using liquid chromatography–tandem mass spectrometry (LC–MS/MS). Our data supported other studies, in that recent samples (2016–2018) tend to have higher MDMA content compared to earlier years. In 2018, the median MDMA content exceeded 100 mg free-base for the first time. Dramatic within-batch content variability (up to 136 mg difference) was also demonstrated. Statistical evaluation of dissolution profiles at 15-minutes allowed tablets to be categorized as fast-, intermediate-, or slow-releasing, but no tablet characteristics correlated with dissolution classification. Hence, there would be no way of users knowing a priori whether a tablet is more likely to be fast or slow-releasing. Further, within-batch variation in dissolution rate was observed. Rapid assessment of MDMA content alone provides important data for harm reduction, but does not account for variability in (a) the remainder of tablets in a batch, or (b) MDMA dissolution profiles. Clinical manifestations of MDMA toxicity, especially for high-content, slow-releasing tablets, may be delayed or prolonged, and there is a significant risk of users re-dosing if absorption is delayed.

KEYWORDS

3,4-methylenedioxymetamfetamine, Ecstasy, MDMA, public health

1 | INTRODUCTION

3,4-Methylenedioxymethamphetamine (MDMA, Ecstasy) is a common recreational drug particularly popular amongst nightclub and music festival attendees because of the empathogenic/entactogenic nature of the MDMA "high".[1] Use is typically associated with younger populations. One study in the UK of 482 education centres found approximately one-fifth (18.9%) of the adolescents (16–21 years)

questioned had at some time used MDMA.[2] In England and Wales in 2017, there were 56 deaths attributed to MDMA toxicity.[3]

An additional consideration more recently is the availability of MDMA via the Darknet, purchased using cryptocurrencies, of which there are many.[4,5] There is some evidence that the perception amongst users is that drugs bought via the Darknet are (a) more likely to be "as advertised", ie, not containing an entirely different compound or not adulterated with other compounds and (b) of higher content.[6-8] Though limited and not necessarily representative of the global marketplace, evidence available from the Netherlands suggests that MDMA tablets bought online are of a statistically significant, but only slightly higher content, than those bought on the street (131 and 121 mg, respectively).[8] Current concerns for risks posed to recreational Ecstasy users focusses on the prevalence of high-content MDMA tablets. Tablets containing 200–300 mg MDMA have been reported, including some tablets which had clearly been produced for sale at specific events, bearing the event logo.[9]

That users may be actively seeking means to ensure the MDMA content of the drugs they are buying is some evidence of the problem which exists regarding variability in drug dosage. Indeed, a number of home-testing kits, typically using Marquis reagent or similar colour tests, are now commercially available.[10,11] Furthermore, variability in MDMA dosage (for tablets) and purity (for crystals) has been reported by a number of groups,[9,12-20] including a study previously carried out by this group for a sample of MDMA tablets collected in the UK in 2006,[21] which showed wide variability in the MDMA content of tablets in the UK [(range 20–131 mg MDMA hydrochloride per tablet (16.8–110 mg MDMA free-base)].

Dissolution testing is commonplace in the quality control and batch production of drugs in pharmaceutical laboratories, and for bioequivalence testing when developing new formulations or generic versions of existing drugs.[22,23] Standard protocols are available.[22] During drug and formulation development, dissolution data are used to determine pharmacokinetic parameters and guide decisions on dose frequency. Such data for illicit drugs are unsurprisingly sparse but are potentially of value for policymakers and for informing harm reduction strategies. Notwithstanding the known problem of MDMA content variability, two MDMA tablets with markedly different dissolution profiles could pose significantly different clinical risks to users.

In this study, we have analysed MDMA tablets collected in the UK using liquid chromatography with tandem mass spectrometry (LC–MS/MS). We have analysed representative samples from 2001 to 2018 to quantify MDMA content, including quantification for multiple tablets within a batch to assess within-batch content variability. The study aims to update our previous study with more recent MDMA tablets, examine these data longitudinally, and to see how the UK data compare to those collected in Spain and Portugal.[24] In addition, we have taken a sub-set of MDMA tablets and, for the first time to our knowledge, report data from dissolution studies to demonstrate variability in MDMA tablet dissolution.

2 | METHODS

2.1 | Chemicals and reagents

MDMA and MDMA-D_5 certified reference solutions (1 g/L and 100 mg/L in acetonitrile, respectively), MDMA hydrochloride reference standard, hydrochloric acid, and formic acid were all from Sigma Aldrich (Poole, UK). LC–MS grade methanol was from Rathburn (Walkerburn, Scotland, UK). Isopropanol was from VWR (Lutterworth, UK). Water was deionised in-house (> 18 megohm cm, Elix®, Watford, UK).

2.2 | MDMA tablets and crystals

Tablets analysed were those collected from amnesty bins, or seized during entry searches, from a number of large music festivals and nightclubs in the UK. All were initially identified as potentially containing MDMA by visual identification, based on size, shape, colour, and markings/logos, to tablets found to have contained MDMA previously (TICTAC visual drug identification and information system for solid dose drugs, version 23.1, TICTAC Communications Ltd., London, UK).[25] All TICTAC database entries were confirmed by gas chromatography–mass spectrometry (GC–MS) analysis (7890-5975C, Agilent Technologies, Santa Clara, CA USA,). All tablets were stored in sealed 7 mL glass sample containers (VWR, Lutterworth, UK) at room temperature (nominally 20–25°C). Only whole tablets were included in the analysis, ie, any damaged tablets, or those with obvious fragments missing, were excluded from the study.

2.3 | MDMA content measurement

Tablets were crushed and homogenised using a clean agate pestle and mortar. The crushed tablets were stored in glass sample containers at room temperature. For analysis, approximately 10 mg of tablet was weighed accurately (AE240, Mettler Toledo, Leicester, UK), and the weighed powder transferred to a 100 mL volumetric flask, which was made to volume with a solution of 50% (v/v) methanol in deionised water. After thorough mixing by inversion (30 minutes), 1 mL portions were diluted (1 + 9, v/v) with 50% (v/v) methanol in deionised water and were stored refrigerated (2–8°C) in 15 mL screw-cap tubes (Sarstedt, Leicester, UK) prior to analysis. MDMA calibrators (N = 6, 2 mL each calibration level) were prepared by dilution of MDMA stock solution (1 g/L) over the range 0.50–100 mg/L in 50% (v/v) methanol in deionised water.

For analysis, prepared samples and calibrators (50 μL) were diluted with MDMA-D5 solution (10 mg/L, 50 μL) in 1.5 mL micro-centrifuge tubes and 0.1% (v/v) formic acid in deionised water (900 μL). Portions of these mixtures (20 μL) were diluted further with 0.1% (v/v) formic acid in deionised water (380 μL) directly into 96-well plates with eluent A, 0.1% (v/v) formic acid in deionised water. Diluted samples (2 μL) were injected onto a reversed-phase (C18), tapered-bore column (2.7 μm, 25 x 2.0–0.5 mm i.d., WarpLCMS, Penn Valley, CA,

USA) and analysed using gradient elution (Table S1) on an Acquity™ LC instrument (Waters, Manchester, UK). Analytes were monitored using a TQD MS (Waters, Manchester, UK), operating in positive electrospray ionisation, multiple reaction monitoring (MRM) mode. Ion transitions (collision energy, V) were m/z 194.1 to 104.9 (24) and 163.0 (12), and 199.2 to 107.0 (26) and 165.0 (12) for MDMA and MDMA-D_5, respectively. The dwell time for each MRM transition was 25 ms. Ionisation source conditions were capillary voltage 0.5 kV, cone voltage 3 V, extractor voltage 3 V, source temperature 150°C, desolvation gas temperature 450°C, and desolvation gas flow rate 900 L/h. Data were analysed using MassLynx™ (version 4.1) and Microsoft Excel (2016 version).

For external quality control (EQC) to assess the method accuracy, portions of crushed MDMA tablets (*N* = 10) covering a range of MDMA content were sent to a second laboratory (LGC, Teddington, UK) for blinded, independent analysis. The LGC results (MDMA content, as free-base, per tablet) were compared against those obtained in-house. For internal quality control (IQC) and to assess between-assay precision, a selection of tablets (*N* = 3 of the 10 samples chosen for EQC) were chosen and were analysed with each of the batches.

For the tablets which were expected to contain additional compounds, extracted tablets were analysed using an LC–MS/MS method containing MRM transitions for more than 100 drugs and metabolites (two MRM transitions per analyte, 12-minute gradient elution), used in our laboratory for routine forensic toxicology investigations.

2.4 | Dissolution testing

A 7-vessel dissolution bath (Pharma Test DT70, Hainburg, Germany) was used. Dissolution solvent (900 mL per vessel) was 0.05 mol/L aqueous hydrochloric acid. Bath temperature was maintained at 37 ± 0.5°C, and the stirrer rate was 50 rpm. Samples (5 mL) were taken using a volumetric syringe via the sampling line and filter before addition of the tablet (t0), and subsequently after 5, 10, 15, 20, 30, 45, 60, 90, and 120 minutes. A final sample was drawn at 180 minutes (stirrer rate increased to 150 rpm for the final 60 minutes). After each sample was drawn, the solution was replaced with 5 mL blank dissolution solution to maintain the vessel volume. Samples drawn at each time point were collected into 15 mL screw-cap tubes and stored at 2–8°C prior to analysis.

To confirm the stability of MDMA in the dissolution solution for the 180-minute duration of the experiment, a solution of MDMA (0.1 mg/L, prepared in the dissolution solution) was incubated at 37°C and samples were drawn and analysed at each of the planned time points. This experiment also tested for any loss of analyte in the dissolution bath system (eg, via adsorption to the vessel or syringe filter).

As for the content measurement, MDMA calibrators (*N* = 6, 2 mL each calibration level) were prepared by dilution of certified MDMA reference solution (1 g/L), this time over the range 0.50–200 mg/L in 50% (v/v) methanol in deionised water. In addition, internal quality control (IQC) solutions were independently prepared from MDMA hydrochloride at 100, 10.0, and 1.00 mg/L (84.8, 8.48, and 0.85 mg/L MDMA free-base, respectively) in 50% (v/v) methanol in deionised water.

For analysis of the collected samples, portions of the sample (10 μL) were mixed with MDMA-D5 solution (20 μL, 10 mg/L) and diluted with 0.1% formic acid in deionised water (1.5 mL) directly into 96-well plates. Two injections were made from each well (one from each autosampler needle).

Analysis was carried out using a 1290 Infinity II™ LC system coupled with an Ultivo™ triple quadrupole mass-spectrometer (both Agilent Technologies, Santa Clara, CA, USA). Prepared samples (2 μL) were injected using a dual-needle autosampler onto a Raptor™ biphenyl column (5.0 x 3.0 mm, 2.7 μm, Thames Restek, High Wycombe, UK) fitted directly to the MS ionisation source to permit rapid gradient analysis.26 LC parameters are summarised in Table S1. Ionisation source conditions were sheath gas temperature 350°C, sheath gas flow-rate 9 L/min, nebulizer 55 psi, drying gas temperature 350°C, drying gas flow-rate 11 L/min, and capillary voltage 2.5 kV. Ion transitions (collision energy, V) were m/z 194.1 to 104.9 (17) and 163.0 (5), and 199.2 to 107.0 (21) and 165.0 (5) for MDMA and MDMA-D_5, respectively. The dwell time for each MRM transition was 10 ms, and the accelerator voltage was 9 V.

2.5 | Data processing

Unlike pharmaceutical dissolution testing, in which all tablets are expected to contain the same dose of active pharmaceutical ingredient, the MDMA content of each tablet was unknown prior to analysis. Dissolution profiles were plotted as (a) concentration (mg/L) versus time and (b) percent dissolved (relative to the 180-minute time point) versus time. Statistical comparisons were made using one-way analysis of variance (ANOVA) carried out using Minitab® (version 18) and illustrated using Microsoft Excel (2016 version). For all calculations, MDMA was measured as free-base rather than the hydrochloride salt.

3 | RESULTS

3.1 | MDMA content

Calibration was linear over the range 0.50–100 mg/L (typical $r^2 > 0.9984$). The within-batch imprecision (% CV) for the three IQC tablets (initial measured MDMA content 134, 71 and 34 mg free-base) was <13.3% for each of the five batches, and the between-batch imprecision was <11.8%. Comparison of the EQC sample results (*N* = 10 tablets, MDMA content range 9–134 mg by the in-house method) showed excellent agreement ($y = 1.0241x - 1.1456$, $r^2 = 0.9956$). EQC results demonstrating our method accuracy are summarised in Figure S1. The stability of MDMA in the dissolution solution at 37°C for the 180-minute duration of the experiment, was 98.0 ± 1.5%.

For the tablets used to investigate MDMA content (*N* = 412), the median MDMA content tended to decrease overall between 2001

and 2009, but then increased from 2010 onwards (Figure 1). It should be noted that the number of tablets available for this study from 2009, 2010, and 2012 was low: 4, 2, and 6, respectively. This was attributed to the low purity and availability of MDMA-containing ecstasy during these years, and the increase in availability of then-legal novel psychoactive substances. That said, there was a statistically significant difference ($p < 0.001$) in MDMA content for those tablets collected pre-2010, and those collected post-2010 (Figure 2).

The median (range) MDMA content per tablet, tablet mass (mg) and MDMA content expressed as a percentage of the tablet mass by year is summarised in Table 1. There was only a weak correlation between total tablet mass and MDMA content ($r^2 = 0.2919$, Figure S2). Overall, the median tablet mass increased over the period studied (Figure 1).

The within-batch MDMA content and tablet mass variability ($N = 45$ batches of tablets) are summarised in Figure S3, ordered by year. The median (mean, range) difference in tablet mass (heaviest minus lightest tablet) within a batch was 5 (14, 0–80) mg. The median (mean, range) content difference (highest minus lowest MDMA content) was 10 (19.5, 2.2–136) mg. The mean within-batch precision in MDMA content (% CV) was 16.4%. Twenty-two (49%) of the batches had a within-batch imprecision less than 10%.

Of the tablets tested which were expected to contain additional, non-MDMA, compounds, 22 (65%) were found to contain caffeine. Other compounds identified (number of tablets) were amphetamine (1), methamphetamine (1), 3,4-methylenedioxyethylamphetamine (MDEA) (3), cocaine (1), ketamine (3), methylephedrine (1), phentermine (1), 1-benzylpiperazine (BZP) (6), 1,4-dibenzylpiperazine (DBZP) (6), meta-chlorophenylpiperazine (mCPP) (3), diphenhydramine (2), and N,N-diallyl-5-methoxy-tryptamine (5-MeO-DALT) (1). From the visual identification made by TICTAC, two tablets were expected to contain methylone, in addition to MDMA, but were found to contain MDMA only. MDMA content of those with additional compounds was significantly lower than the MDMA-only tablets ($p < 0.001$).

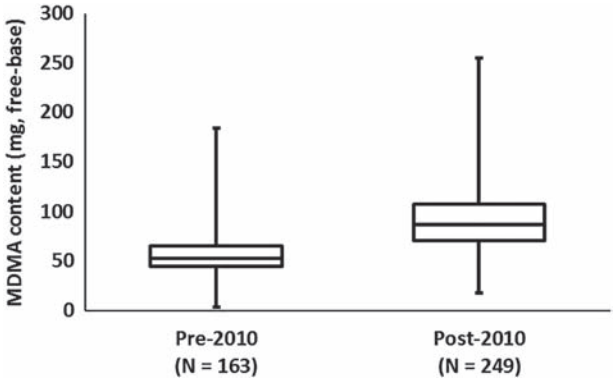

FIGURE 2 MDMA content of tablets collected pre-2010 and post-2010. Plotted are median, 25th and 75th percentiles (boxes) and minimum and maximum (whiskers) MDMA content for all tablets

3.2 | Dissolution

Calibration curves were fitted with a quadratic curve (weighted by the reciprocal of x^2, not forced through the origin) over the concentration range 0.1–200 mg/L (typical $r^2 > 0.9998$). The within-batch and between-batch ($N = 9$ batches) imprecisions for the three independently-prepared IQC solutions were all <13.7% and < 13.8%, respectively. Mean within-batch accuracy (% nominal concentration, $N = 30$ replicates of each IQC) was 92%–96%, and the mean between-batch accuracy (mean accuracy values for each IQC from 9 batches) was 104%–115%.

The MDMA tablets were expected to be immediate release formulations and the 180-minute time point was chosen pragmatically as the time when dissolution was complete. Inspection of the dissolution curves showed plateaus of relative concentration confirming this expectation. Using data from the 180-minute time point, accounting for the dissolution vessel volume of 900 mL, and assuming complete dissolution at the 180-minute time point, the MDMA content of each of the tablets included in the dissolution experiment was calculated.

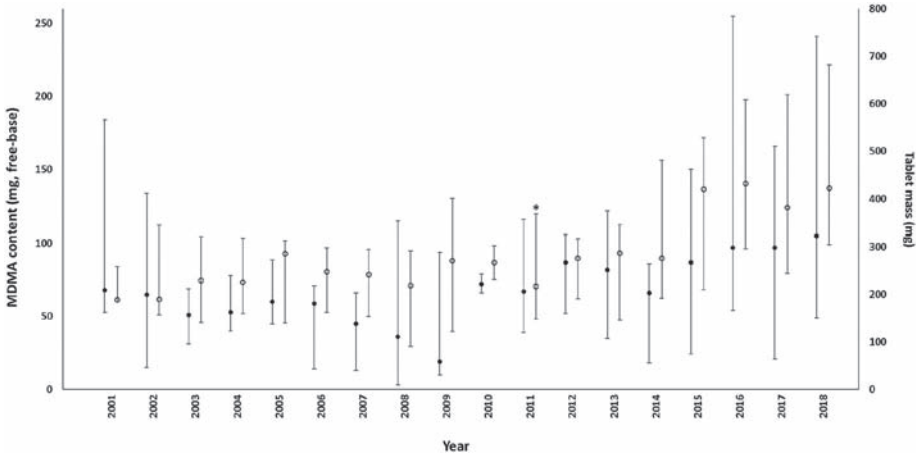

FIGURE 1 Median (range) MDMA content (mg, as free-base, filled circles) and median (range) tablet masses (mg, empty circles) by year. For the tablet mass, a single tablet (year 2011, 1045 mg) was excluded from these data (indicated by asterisk)

TABLE 1 Summary MDMA data (tablet mass, MDMA content, and 'purity'), 2001–2018 [Colour table can be viewed at wileyonlinelibrary.com]

Year	N	Median [range] Tablet Mass (mg)	Median [range] MDMA Content (mg, free-base)	Median [range] MDMA "Purity" (%)
2001	12	189 [185–259]	68 [53–184]	31 [27–72]
2002	22	190 [157–345]	65 [15–134]	35 [4–49]
2003	26	230 [141–322]	51 [31–69]	23 [14–34]
2004	26	226 [160–318]	53 [40–78]	23 [15–37]
2005	14	286 [140–313]	60 [45–89]	27 [15–37]
2006	11	248 [163–299]	59 [14–71]	21[7–44]
2007	28	242 [154–295]	45 [13–66]	21 [4–27]
2008	20	219 [91–292]	36 [3–115]	21 [1–39]
2009	4	271 [122–402]	19 [10–94]	13 [3–23]
2010	2	268 [232–303]	72 [66–79]	27 [26–28]
2011	22	217 [148–1045]	67 [39–116]	33 [11–39]
2012	6	276 [191–317]	87 [52–106]	35 [16–39]
2013	12	287 [146–346]	82 [35–122]	28 [14–38]
2014	26	276 [192–480]	66 [18–86]	23 [4–41]
2015	41	420 [210–528]	87 [24–150]	28 [10–34]
2016	46	432 [296–609]	97 [54–255]	23 [11–59]
2017	64	382 [244–619]	97 [21–166]	25 [5–40]
2018	30	422 [304–682]	105 [49–241]	26 [10–58]
TOTAL	**412**	**295 [93–1045]**	**71 [3–255]**	**25 [1–72]**

Data from 247 dissolution experiments were included in the analysis. The median (25th and 75th percentiles) data from each time point were plotted to produce dissolution profiles, using both MDMA concentration (mg/L) versus time, and percent dissolved versus time. Assuming complete dissolution at 180 minutes, the concentration at this time point gave another measure of MDMA content and provided further data for the variability in MDMA content of the tablets tested; median (range) MDMA content 70 mg (7–182 mg) free-base after accounting for dissolution vessel volume.

The distribution of the percent released was examined for each time point. Analysis of data at the 15-minute and 30-minute time points revealed a tri-modal distribution at 15 minutes, and a bi-modal distribution at 30 minutes. These were confirmed by analysis of variance ($p < 0.001$) which showed three distinct groups based on the percent dissolved at 15 minutes (Figure 3A); mean (95% CI) percent dissolved: fast-releasing 95.0 (93.4–96.6) %, intermediate-releasing 68.4 (66.5–70.3) %, and slow-releasing 39.4 (37.7–41.1) %, and two distinct groups at 30 minutes (Figure 3B): fast-releasing 95.7 (94.4–97.1) % and slow-releasing 57.3 (55.2–59.3) %. Using these distribution data, tablets were then classified for each of the two time points into (a) slow-releasing (≤ 54% dissolved), (b) intermediate-releasing (55%–81% dissolved), or (c) fast-releasing (≥ 81% dissolved) for the 15-minute time point, and as (a) slow-releasing (< 77% dissolved) or (b) fast-releasing (≥ 77% dissolved) for the 30-minute time point. Dissolution profiles produced for each tablet class using the median (25th and 75th percentiles) percent dissolved at each time point are shown in Figure 4. At the 15-minute time point, there were 95 (38%) fast-releasing tablets, 67 (27%) intermediate-releasing tablets, and 85 (35%) slow-releasing tablets. At the 30-minute time point, there were 172 (69%) fast-releasing tablets and 75 (31%) slow-releasing tablets. The highest content slow-releasing tablet (based on the 15-minute time point) contained 156 mg MDMA free-base (185 mg MDMA HCl). All MDMA crystal samples, including those wrapped in 'bombs', were classified as fast-releasing at both time points. There were no statistically significant differences in MDMA content based on dissolution classification at either 15-minute ($p = 0.527$) or 30-minute ($p = 0.067$) time points (Figure 5). Furthermore, dissolution classification was not always consistent within the batch of tablets. At the 30-minute time point, there was discrepant classification (that is, at least one tablet in the batch was classified differently to the remainder) in 10 (16%) of the batches tested (for example, Batch 89, $N = 5$ tablets, contained two fast-releasing and three slow-releasing tablets).

The proportion of fast-releasing, intermediate-releasing, and slow-releasing tablets by year is shown in Figure 6. Overall, the proportion of slow-releasing tablets decreased (64.3% of tablets 2001–2003 to 17.0% of tablets 2016–2018), and the proportion of fast-releasing tablets increased from 21.4% of tablets 2001–2003 to 52.3% of tablets 2016–2018 over the period studied, but there was still significant between-tablet variability in dissolution in each year group.

3.3 | Tablet characteristics – correlation with MDMA content and dissolution

With few exceptions (eg, a batch of heart-shaped tablets in 2005), round/circular tablets were predominant until 2010. Thereafter, more elaborate-shaped tablets became more common. Likewise, prior to 2010, the majority of tablets were white in colour, after which more colourful, and even multi-coloured tablets became more common (Figure 7). Statistically, white tablets and round tablets contained significantly less MDMA than the remainder of the tablets tested (both $p < 0.001$). There was no association (Chi squared test) between the white/non-white tablets and the dissolution classification at either 15-minutes ($p = 0.272$) or 30 minutes ($p = 0.729$), nor between the round/non-round tablets and dissolution classification at either time point ($p = 0.055$ and 0.246 at 15-minutes and 30-minutes, respectively).

4 | DISCUSSION

Previous studies have highlighted the risk of variability in MDMA content of Ecstasy tablets.[9,26] Furthermore, a number of studies have suggested that in recent years, the amount of MDMA in tablets is increasing.[9] Data from the UK presented from the MDMA content measurement portion of this study support these observations (Figure 8).[24] Our data from 2006 showed the highest content tablet

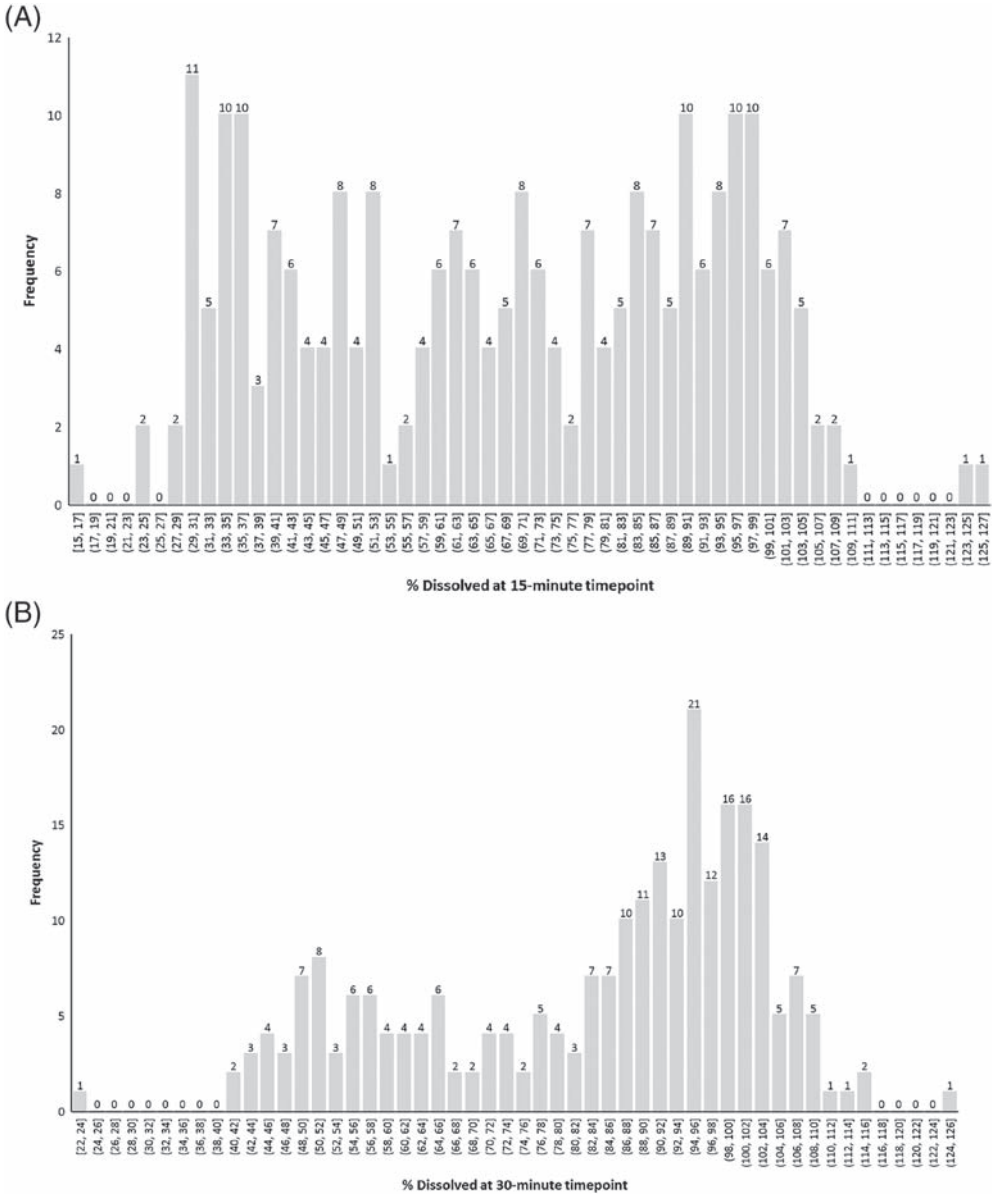

FIGURE 3 Histograms showing the % dissolved (relative to the 3-hour time point), at A, the 15-minute and B, the 30-minute time point for all tablets (N = 247)

contained 110 mg MDMA free-base (131 mg MDMA HCl).[21] However, in 2018, the median MDMA content exceeded 100 mg (equivalent to 118 mg MDMA hydrochloride) for the first time, and the highest content tablet contained 241 mg MDMA free-base (286 mg MDMA HCl), more than double the content of the highest of the 2006 tablets (Table 1). We observed a statistically significantly lower MDMA content for white and for round tablets, though this can be explained by the fact that these tablets tended to be from earlier years, when MDMA content was lower (Figure 1). That said, white, round tablets should not universally be considered "low-dose" – one such tablet from 2001 had an MDMA content of 184 mg free-base (219 mg MDMA HCl). Two tablets from a batch which were labelled with "199.9 mg" (presumably to reflect the apparent MDMA dose) were in fact found to contain 78.3 and 90.0 mg MDMA free-base (Figure 7).

The samples collected and used for this study provided the opportunity to investigate the variability in MDMA content within batches of tablets. Though for many of the batches tested the number of tablets was low (minimum two tablets per batch), the data revealed that the within-batch reproducibility of tablet mass was generally good. This is likely a reflection of the reproducibility of modern tablet-pressing equipment.[9] However, some batches showed large differences, even in the mass of tablets. One batch (Batch 14, N = 4) showed a difference of 80 mg between the heaviest and lightest tablet in the batch (MDMA content of the heaviest and lightest tablets in this batch 46 and 22 mg MDMA free-base, respectively).

With respect to the MDMA content of tablets within batches, there was greater variability than in the tablet mass (median difference in MDMA content 10 mg). Almost half the batches tested showed "good" tablet-to-tablet reproducibility (% CV < 10%). However, a

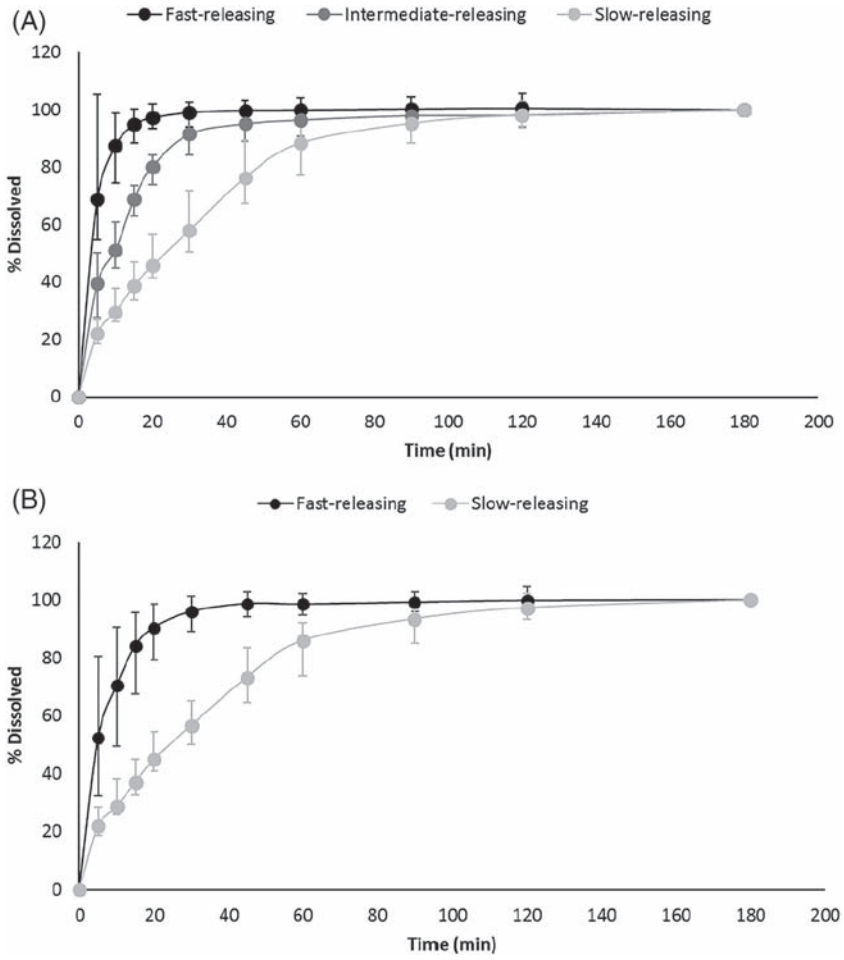

FIGURE 4 Dissolution profiles showing the median (25th and 75th percentile) % dissolved vs time, A, for tablets classified as fast-releasing, intermediate-releasing, and slow-releasing at the 15-minute time point, and B, for tablets classified as fast-releasing and slow-releasing at the 30-minute time point

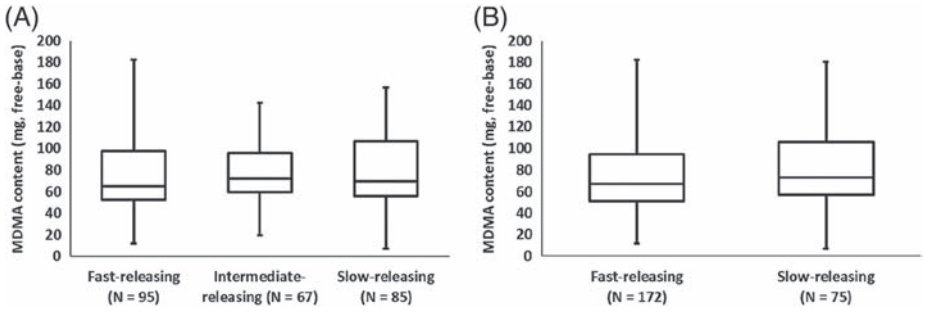

FIGURE 5 MDMA content of tablets based on dissolution classification at A, 15-minute and B, 30-minute time points. Plotted are median, 25th and 75th percentiles (boxes) and minimum and maximum (whiskers) MDMA content for all tablets

number of batches showed marked differences in MDMA content despite similar tablet mass. For example, in one batch (Batch 1, N = 4 tablets), despite all tablets being very similar in mass (range 256–259 mg), significant variability in content was observed (range 70–184 mg MDMA free-base). When producing tablets in the pharmaceutical industry, homogeneity of the bulk powder using high-shear mixing tools is essential to ensure content uniformity of the active pharmaceutical ingredient, and forms an integral part of release-testing protocols.[27] Variability in MDMA content within a batch of illicit tablets, when the tablet mass is consistent, most likely represents incomplete mixing of the MDMA and tablet excipients prior to pressing and of course demonstrates the inevitable lack of quality control for illicitly produced tablets. For MDMA users, our data suggest that analysis of a single tablet from a batch cannot guarantee that all others in the same batch are of the same content. For users tending to base their tablet ingestion (ie, the number of tablets) on previous experiences, this could prove dangerous. For instance, a user may be accustomed to taking 200 mg MDMA per night. If they are informed upon analysis that in a batch of tablets each tablet contains 50 mg MDMA, then they will plan to ingest four tablets from that batch. In reality, content variability of MDMA tablets may mean that, in total, they far exceed this dose, and hence increase their risk of toxicity.

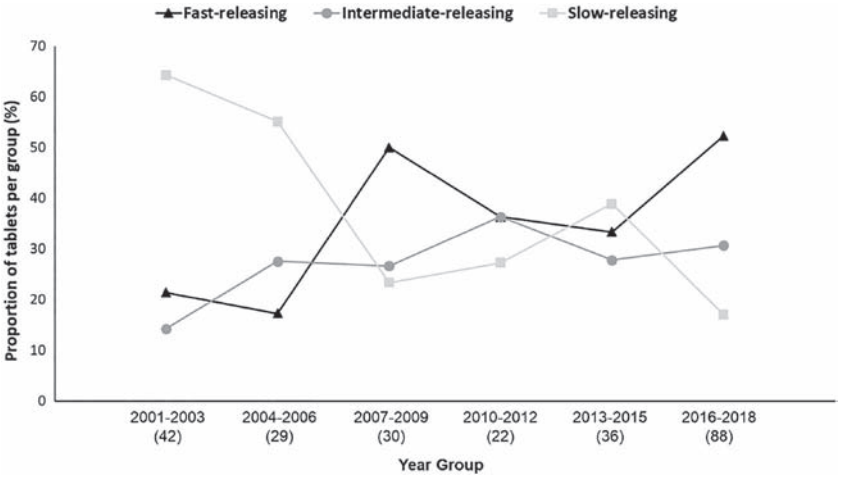

FIGURE 6 Proportion of tablets classified as fast-releasing, intermediate-releasing, and slow-releasing at the 15-minute time point by year. The number of tablets for each year group is shown on the x-axis

FIGURE 7 The evolution of ecstasy tablets. Example tablets from A, 2001 to 2003, B, 2010 and C, D, 2016–2018, showing the difference in tablet colour, shape, and design. Examples of two of the tablets shown in the centre of D (labelled "199.9 mg") were analysed for MDMA content and dissolution, and were found to contain 78.3 and 90.0 mg MDMA free-base. Both were classified as fast-releasing at the 30-minute time point, and intermediate-releasing at the 15-minute time point [Colour figure can be viewed at wileyonlinelibrary.com]

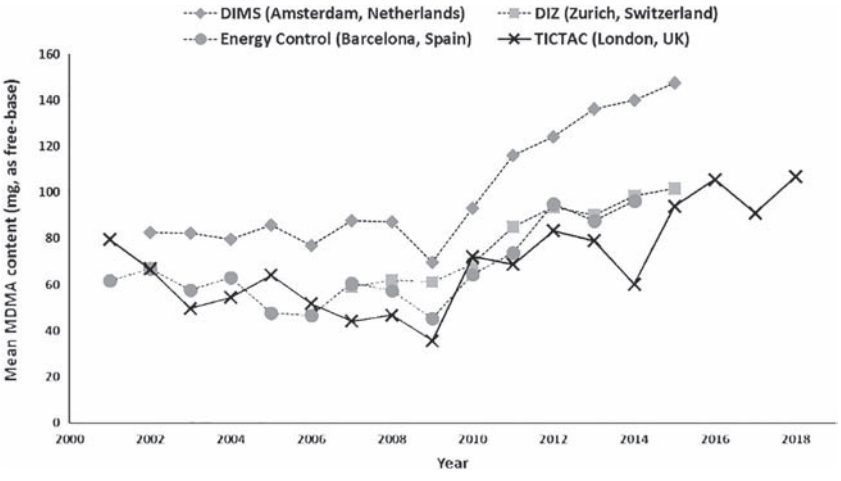

FIGURE 8 Mean MDMA content (mg, as free-base, *N* = 412 tablets) of tablets seized in four European countries between 2000 and 2018. Non-UK data from Szigeti et al.[24]

LC–MS/MS was chosen as the analytical method for these studies for a number of reasons. Primarily, the specificity of MS when compared to the more normal ultraviolet (UV) detection allowed for the analysis of tablets with unknown excipients, contaminants, or adulterants which may interfere in UV systems. In addition, LC–MS/MS allowed for the inclusion of isotopically-labelled MDMA as an internal standard for quantitation. Finally, for the dissolution experiments in particular, where the number of samples to be analysed was large, ultra-rapid, high-throughput analysis was permitted (36 seconds per injection).[28]

Measurement of MDMA content is useful for implementing harm-reduction strategies and providing information on trends for both MDMA users and healthcare professionals. Typically, as in the first part of this study, these data are presented as retrospective audits of substances collected or purchased over a number of years, carried out in laboratory settings by groups such as the Drug Information and Monitoring System (DIMS) in the Netherlands, which has been in existence since 1992[29,30] and the National Detection System of Drugs and Toxic Substances (SINTES) in France.[13] Some laboratory-based groups, for example DanceSafe in the United States and Canada, and most recently the Welsh Emerging Drugs and Identification of Novel Substances (WEDINOS) project in Wales and the UK publish their analytical findings online.[31-33] A review of the analytical techniques used in European testing laboratories was compiled as part of the Trans-European Drug Information (TEDI) project.[20,34]

Non-laboratory-based (in-field or mobile) testing of MDMA and other substances is also commonly carried out, for instance at music festivals, nightclubs, and in city-centres, with the aim of rapidly informing users of risk(s), for example due to circulation of high-content tablets, and in an effort to reduce harm. A number of organisations, including some already mentioned that also offer laboratory-based testing, carry out such testing services, for example Check It! (Austria), Check!n (Portugal), Energy Control (Spain), Safer Dance (Switzerland), and The Loop (UK).[30] Field-based drug identification tends to be limited to colourimetric reagent tests, spectroscopic methods, eg, infra-red (IR), Fourier transform IR (FTIR), and Raman spectroscopy, and occasionally thin layer chromatography (TLC). Whilst these methods are cheap, rapid, and easily carried out without the need for elaborate laboratory infrastructure, colourimetric reagent tests are highly subjective, and there are limitations of spectroscopic methods in both sensitivity and specificity (particularly for tablets and powders containing mixtures of compounds or for very low concentration adulterants).[20,35-37] Further, the results produced by spectroscopic methods are dependent on (a) up-to-date libraries to include the latest substances, and (b) complex in-built identification algorithms for the analysis of mixtures. For the latter, such libraries are now available, and are ever-growing to include new compounds, eg, TICTAC spectral libraries for novel psychoactive substances.[38] Mobile HPLC with ultraviolet and MS detection (single quadrupole) has also been used by the Check It! organisation in Austria, with throughput of approximately 40 samples per hour, using a modified laboratory van which can be deployed to music festivals. The LC–MS/MS method used for the dissolution profiling in this study provides additional evidence that, although significantly more expensive than colourimetric and spectroscopic methods, these specific and sensitive methods can in fact be carried out very rapidly (up to one 96-well plate per hour throughput using the method described). Through implementing novel, miniaturised instrumentation, such as the triple quadrupole MS used for the dissolution analyses in this study (Ultivo™), these techniques are also becoming more amenable to non-laboratory environments.

Most importantly, the data in the second part of this study showed significant differences in the dissolution profile of MDMA tablets. These data highlight an extremely important additional risk with regard to variability in MDMA tablets. There is little doubt that higher-content MDMA tablets pose a greater risk of toxicity to users than low-content tablets.[26,39,40] Content variability aside, risks for fast-releasing and slow-releasing tablets should also be considered, especially since our data suggest no definite way to know a priori which class a tablet is likely to be in (eg, based on colour or shape), and there were no statistically significant differences in MDMA content for fast-releasing, intermediate-releasing, or slow-releasing tablets. For high-content tablets, if the tablets are fast-releasing, toxicity may manifest rapidly (within 15 minutes or less) and hence will require rapid emergency treatment in cases of overdose. However, the highest risk could be posed by high-content, slow-releasing tablets. Peak plasma concentrations following oral administration of these tablets may be some hours after ingestion based on dissolution data from this study. For these tablets, users expecting a high 15–30 minutes after ingestion may re-dose before peak concentrations for the first tablet have been reached, exacerbating any toxicity.[41] Healthcare workers should be aware that high-content, slow-releasing tablets are in circulation, and that users presenting with MDMA toxicity may not have reached peak plasma concentrations if they have ingested (multiple) slow-release tablets.

These data also impose an inherent limitation for rapid MDMA analysis. Whilst identification and content measurements can be made quickly and in remote locations, to assess dissolution profiles takes a much longer time (180 minutes using the method described, plus analysis time thereafter), and requires additional specialist equipment in a laboratory setting.

Knowledge of the MDMA content alone does not fully assess the risk for a tablet, or batch of tablets. The dissolution studies were conducted in standard aqueous buffer at acidic pH and whilst the findings of this study illustrate the potential variability in release rates from the tablets analysed, it should be noted that the in vitro–in vivo correlation of these data to predict physiological outcomes has not been confirmed. Further work would be needed to obtain this evidence. At present, there is no correlation with other tablet characteristics or parameters to identify fast- and slow-releasing tablets. In this work, we did not carry out any further tablet characterisation experiments (eg, assessment of tablet hardness or friability) to correlate to dissolution, nor did we assess dissolution of any crushed tablets. Although the tablets were stored in dark, dry conditions and room temperature, we cannot be certain that storage did not have a detrimental effect on content. Finally, our data do not account for

the inherent risk of illicitly manufactured drugs from non-pharmaceutical excipients, for example heavy metals, which should not be overlooked.[42] The nature of these and other diluents (often sugars such as lactose) needs to be explored.

5 | CONCLUSIONS

Recreational MDMA use continues to be widespread. Recent concerns have focussed on the prevalence of high-content MDMA tablets in circulation, and our data support these observations.

However, in addition, we have demonstrated that variability in dissolution profiles of MDMA tablets must be considered. Rapid drug identification and quantitation are useful and transferable to non-laboratory settings, but in the light of these new data, do not and cannot fully assess the risk to users due to the presence of fast- and slow-releasing MDMA tablets on the illicit market.

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DECLARATIONS OF INTEREST

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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