Quantification of MDMA in seized tablets using benchtop ^{1}H NMR spectroscopy in the absence of internal standards

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Abstract

Recreational MDMA use is a worldwide problem. Tablet dosage varies, thus entailing a requirement for quantitative analysis. The quantification of MDMA in tablets using benchtop ^{1}H NMR spectroscopy *via* either linear regression ('manual' method) or partial least square regression ('automated' method) approaches are reported, without the need for an internal standard, and compared against contemporaneously obtained GC-MS data. Twenty samples were evaluated of which 15 were proven to contain MDMA, *via* qualitative NMR (hit score ≥0.97) and GC-MS (R_t = 5.6 min) analysis. Quantitative NMR analysis showed that the mean value of MDMA content was 42.6% w/w by the manual method and 45.9% w/w by the automated method. The mean value obtained from GC analysis was 44.0% w/w. A substantial proportion (n = 9) of the tablets tested possessed >190 mg of MDMA (range 133-223 mg, average of all techniques' calculations for each tablet). This value is higher than the reported average MDMA content of tablets by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), which was *ca.* 125 mg of MDMA per tablet in 2016.

Highlights

- Quantitative analysis of MDMA is achieved using a benchtop NMR spectrometer
- NMR-based quantification using linear or partial least square regression is described and the values obtained compared against contemporaneously obtained GC-MS data
- NMR-based quantification is achieved without an internal standard

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA, **1**, Fig. 1) is a synthetic entactogen, which shares a structural similarity to methamphetamine (**2**) and acts upon the central nervous system (CNS) producing mood enhancement, increased energy and other empathetic effects by increasing the intra-synaptic concentrations of the key neurotransmitters serotonin, dopamine and norephinephrine [1-5]. MDMA was first synthesised by Merck in 1912 as a potential appetite suppressant. Over the next century, the psychedelic properties of MDMA were explored[6], culminating in recent clinical trials demonstrating initial safety and efficacy for its treatment of post-traumatic stress disorder (PTSD), with potential for expansion to depression and anxiety disorders [7].

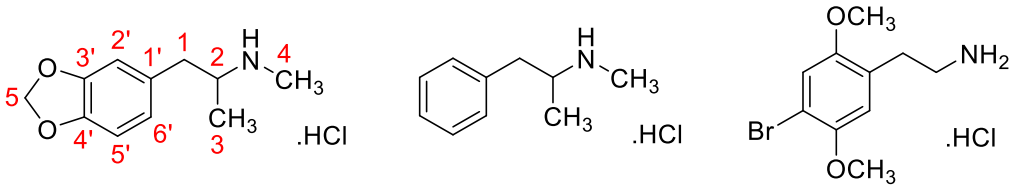

Fig. 1. Structures of 3,4-methylenedioxymethamphetamine (MDMA, **1**), methamphetamine (**2**) and 2,5-dimethoxy-4-bromophenethylamine (2C-B, **3**) hydrochloride.

During the 1970s and 80s, MDMA surfaced on the recreational drugs market. The widespread abuse and potential long-term health effects of MDMA led many countries to prohibit its possession, supply and manufacture. Currently in the UK, MDMA (or "ecstasy") is controlled as a Class A, Schedule 1 substance due to its illicit use as a recreational drug and its implication in a number of highly publicized fatalities [8-14]. The psychoactive substance is extremely prevalent [15-17] and encountered normally in tablet form, with each batch stamped with a particular motif e.g. the "Mitsubishi", "Punisher" or "Superman" logos, smiley faces or lettering. A number of UK-based studies have looked at the MDMA content within these formulations [18-21]. The first UK study by Cole *et al.*, carried out in 2002, employed high performance liquid chromatography (HPLC) to analyse a number of "White Dove" tablets (n = 82) obtained from the North West of England (1991 – 2001) and determined that MDMA content within these samples ranged from 73 – 89 mg with a mean content of 78.8 mg [21]. Subsequently Wood *et al.* also used HPLC to quantify the amount of MDMA present within surrendered ecstasy pills (n = 101) obtained from nightclubs in both London and Swansea in 2006. The researchers reported that the mean level (58.7 mg) in the samples was significantly lower than seen by Cole *et al.*, with a MDMA content ranging from 20 – 131 mg per tablet [19]. In 2019, liquid chromatography-tandem mass spectrometry (LC-MS-MS) was used by Couchman *et al.* to quantify the MDMA content within tablets (n = 412) collected from venues across the UK over a seventeen year period (2001 – 2018). The study demonstrated that the median amount of MDMA present was 74 mg with a range of 3 – 255 mg per tablet [18]. More recently, Blagbrough *et al.* have disclosed the ultra-high performance liquid chromatography (UHPLC) and high field nuclear magnetic resonance (NMR) analysis of ecstasy pills (n = 26) and the mean content within these products was 119.7 mg MDMA (range: 72.9 – 166.5 mg per tablet) [20]. Though there have been a number of reports of "super pills" (270 – 340 mg) circulating in the UK market, these quantitative studies support that the UK trend is in line with

data reported by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), which indicated an average MDMA content of tablets in 2016 as being *ca.* 125 mg [22].

Recreational MDMA/ecstasy use is a worldwide problem, due to its frequent use within nightclubs and music festivals. Recently, it was reported that MDMA was the most prevalent drug (40%) in a qualitative study that analysed 432 samples [15]. Cocaine (20%) and ketamine (17%) were the next most common. Another report from a UK festival onsite drug checking service reported that the most common drug identified was MDMA (57%) in either crystal / powder form or as pills of 230 tested samples [17]. 13.5% and 10% of samples were ketamine and cocaine respectively. Similarly, a report detailing the UK's first community-based drug safety testing in two city centres identified MDMA again as being the most prevalent drug (43.3%) out of the 171 samples surveyed [16]. Cocaine and ketamine were the next prevalent; each substance accounted for 12.9% of the substances sampled.

A number of groups have reported the quantification of MDMA using HPLC with ultra-violet (UV) detection [19, 21, 23-25], quadrupole electrospray time-of-flight (ESI-QTOF) UHPLC [20], gas chromatography-flame ionisation detection (GC-FID) [26], gas chromatography mass spectrometry (GC-MS) [27, 28], LC-MS (or LC-MS-MS) [18, 29-31] and electrochemical detection [23]. Chromatographic methods are considered the gold standard in academic and commercial forensic laboratories for the quantitative analysis of MDMA, but they suffer from some disadvantages (e.g. cost, technical complexity and portability) [32] and are not suitable for rapid and routine on-site testing. Nuclear magnetic resonance (NMR) spectroscopy, despite being a powerful analytical technique, has been underutilized for the detection and quantification of drugs within samples. The reason for low uptake of the technique in forensic settings is the perceived complexity of the measurement and the inherent cost of a superconducting magnet and the associated technical costs. However, a small number of studies that focus on the use of NMR spectroscopy to achieve high sample throughput coupled with quantification have been reported [20, 26, 33]. Hays disclosed a rapid, reproducible and versatile high field ^{1}H NMR method (pulse: 90°; scans: 8; pulse delay: 45 s) for determining both the purity and molecular weight of the illicit drugs (including MDMA) and adulterants in deuterium oxide using maleic acid (5 mg) as the internal standard [33]. Subsequently de Oliveira *et al.* utilised a 600 MHz ^{1}H NMR instrument to determine the content of MDMA in seized tablets (n = 38, 39 – 152 mg per tablet), also using maleic acid as the internal standard [26]. The ^{1}H NMR method (pulse: 90°; scans: 16; pulse delay: 25 s) was cross-validated by

GC-FID and shown to be more efficient and versatile for the detection and quantification of the target analyte, in terms of accuracy (< 5% relative error) and precision (<2% relative standard deviation) when compared to the GC-FID approach. More recently, Blagbrough *et al.* have reported the quantification of MDMA in seized tablets (n = 26), and in the presence of other psychoactive substances (*i.e.* methylone, eutylone and 3-trifluoromethylpiperazine), using a ^{1}H NMR method (pulse: 90°; scans: 16; pulse delay: 50 s) also acquired on a 600 MHz instrument and using maleic acid as the internal standard [20].

In this paper, the quantification of MDMA using 60 MHz benchtop ^{1}H NMR spectroscopy is reported using two different approaches. Benchtop NMR was utilised for this study due to the low running costs and ease of operation [34], which might be attractive to legal entities and healthcare providers interested in the screening of suspected MDMA tablets. The first approach employed is based on simple linear regression onto peak integrals, which we will call the 'manual' method. The second approach uses partial least squares regression of the MDMA content onto a wider spectral range; we will call this the 'automated' approach. Notably, quantification is performed in the absence of an internal standard and the results from the ^{1}H NMR analysis are compared against contemporaneously obtained GC-MS data.

2. Experimental

2.1 Instrumentation and materials

All reagents were of commercial quality (Sigma-Aldrich, Gillingham, UK or Fluorochem Limited, Hadfield, UK) and used without further purification. Solvents (Fisher Scientific, Loughborough, UK) were dried, where necessary, using standard procedures [35]. Racemic 3,4-methylenedioxymethamphetamine hydrochloride (MDMA, **1**) was prepared in-house using an adaptation of the method reported by Buchanan *et al.* and obtained as colourless crystals after recrystallization from isopropanol [36]. High-field ^{1}H NMR (10 mg/600 μL in d_6-DMSO) and ^{13}C NMR spectra (20 mg/600 μL in d_6-DMSO) were acquired on a JEOL JMN-ECS-400 (JEOL, Tokyo, Japan) NMR spectrometer operating at a proton resonance frequency of 400 MHz and referenced to the residual solvent peak (d_6-DMSO: ^{1}H-NMR δ = 2.50 ppm, ^{13}C-NMR δ = 39.52 ppm [37] respectively) and filtered prior to analysis. The purity of (**1**) was calculated using the relative concentration NMR determination method described by Pauli *et al.* [38]. Optical rotation values $[\alpha]_D^{22}$ (10^{-1} deg cm^2/g) were measured on a Bellingham & Stanley ADP-220 polarimeter (Bellingham & Stanley, Tunbridge Wells, UK) (MDMA.HCl,

$[\alpha]_D^{22}$ = 0 deg cm^2/g). Infrared spectra were obtained in the range 4000 – 400 cm^{-1} using a Thermo Scientific Nicolet iS10ATR-FTIR instrument (Thermo Scientific, Rochester, USA). High-resolution mass spectrometry (HRMS) data was obtained on an Agilent 6540 LC-QToF spectrometer in positive electrospray ionization mode. Melting points (uncorrected) were acquired on a Stuart SMP10 digital melting point apparatus (MDMA.HCl, Mpt. = 208-209°C). The twenty suspected MDMA tablets (M1 – M20) were provided to MANchester DRug Analysis and Knowledge Exchange (MANDRAKE), between 24th August 2018 – 29th March 2019, by Greater Manchester Police, in accordance with Manchester Metropolitan University's Home Office license requirements and agreed procedures.

2.2 Gas chromatography-mass spectrometry (GC-MS)

GC-MS analysis was performed using an Agilent 7890B GC and a MS5977B mass selective detector (Agilent Technologies, Wokingham, UK). The mass spectrometer was operated in the electron ionisation mode at 70 eV. Separation was achieved with a capillary column (HP5 MS, 30 m Å~ 0.25 mm i.d. 0.25 μm) with helium as the carrier gas at a constant flow rate of 1.2 mL/min. The oven temperature programme started at 50°C, increased at 30 °C/min and was held at 290 °C for 2 minutes. A 2 μL aliquot of the samples (qualitative analysis, calibration standards and test solutions) were injected with a split ratio of 50:1. The injector and the GC interface temperatures were both maintained at 280°C and 290°C respectively. The MS source and quadrupole temperatures were set at 230°C and 150°C, respectively. Mass spectra were obtained in full scan mode (50 – 550 amu) [qualitative analysis] and using selective ion monitoring mode (SIM) [quantitative analysis], using three specific fragment ions for MDMA (t_R = 5.6 min , *m/z* = 135.1, 77.0 and 58.0) and eicosane (t_R = 7.2 min, *m/z* = 71.0, 57.0 and 43.0) respectively [39]. *Calibration standards:* 10 mg of MDMA.HCl was weighed accurately into a 100.0 mL clear glass class A volumetric flask and diluted to volume with methanol to give a solution containing MDMA at 100 μg/mL. This solution was then further diluted with methanol and eicosane (100 μg/mL in methanol) added (in each case) to give calibration standards containing 5.0 μg/mL, 10.0 μg/mL, 15.0 μg/mL, 20.0 μg/mL and 25.0 μg/mL of each analyte and the internal standard at 10.0 μg/mL The five standards were injected six times. *Test samples:* In triplicate, 10 mg of the homogenised sample was weighed accurately into a 100.0 mL clear glass class A volumetric flask and diluted to volume with methanol to give a sample solution at 100 μg/mL. This solution was further diluted with methanol and eicosane (100 μg/mL in ethanol) added to give a test sample containing 10.0 μg/mL of the sample with

the internal standard at 10.0 μg/mL. Each seized sample was analysed in this manner twice to give two mean % w/w of MDMA present (termed run 1 and run 2).

2.3 Nuclear magnetic resonance (NMR) spectroscopy

Low-field ^{1}H NMR spectra were acquired on a bench-top Pulsar® (Oxford Instruments, Abingdon, UK) NMR spectrometer operating at a proton resonance frequency of 59.7 MHz. The temperature of the probe was calculated to be 308.5 K by measuring the separation (in Hz, Δδ) between the CH_2 and OH signals of neat ethylene glycol and using the equation T [K] = 466.5 - 102.00 Δδ [40]. *Qualitative analysis:* 5 – 10 mg of the powdered tablet was combined with d_6-DMSO (600 μL). The solution was filtered using a Whatman® 0.45 μm PVDF (polyvinylidene difluoride) syringe filter (Sigma-Aldrich, Gillingham, UK) directly into an NMR tube. After the sample had been inserted into the Pulsar® spectrometer, an automated procedure began whereby the instrument would lock on to the deuterated signature of the residual solvent peak (d_6-DMSO, δ = 2.50 ppm) before acquiring 16 scans. Following acquisition, the data were processed in MNova (Mestrelab Research, Santiago de Compostela, Spain) using an automated script file. The processed FID was then analysed using a pattern recognition algorithm, developed in-house using Matlab (The Mathworks Inc, Cambridge, UK). The algorithm employs a minimum distance classifier. The multivariate distance between the sample spectrum and each of the reference spectra is calculated. The sample is identified as the nearest reference compound, provided the 'match score' (equal to one minus the distance) exceeds an (empirically determined) threshold; if it does not, then the outcome is considered to be tentative, unreliable or unknown [15]. *Quantitative analysis:* 50 mg of the powdered tablet was combined with d_6-DMSO (1 mL). The solution was filtered using a Whatman® 0.45 μm PVDF (polyvinylidene difluoride) syringe filter (Sigma-Aldrich, Gillingham, UK) directly into an NMR tube. The individual ^{1}H NMR spectra of the test samples were acquired using four scans and a pulse delay of 30 s. The spectra were processed using a 1 Hz exponential in the t_1 direction and phased accordingly. Integrals of the aromatic (H2', H5' and H6'), 3,4-methylenedioxy (H5) and methyl (H3) groups were then obtained which had been referenced against an MDMA standard (100 mg/mL in d_6-DMSO). Each test sample was analysed five times. Spectral acquisition of each MDMA sample took ca. 2.5 mins.

3. Results and Discussion

The synthesis of a reference standard of racemic 3,4-methylenedioxymethamphetamine hydrochloride (**1**) was achieved using a modification of the previously reported method (Route

2) by Buchanan *et al.* from piperonylmethyl ketone (PMK) in 68% overall yield after recrystallization from isopropanol [36]. The hydrochloride salt was determined to be soluble (10 mg mL^{-1}) in methanol and dimethylsulfoxide and its purity confirmed to be >99.5% (by NMR). To ensure the authenticity of the material used in this study, the synthesised sample was fully structurally characterised and the spectral data (^{1}H NMR, ^{13}C NMR, FT-IR and GC-MS) with assignments for the synthesized reference material provided in the Supplementary Information (Fig. S1 – S4) for comparison. Analysis of the low field ^{1}H NMR spectrum (Fig. 2) of the reference standard highlighted three resonances that could be readily integrated, and thus would be easily quantifiable. These were the aromatic C-H signal (three coincident signals, H2', H5' and H6'), the 3,4-methylenedioxy protons (H5) and the methyl protons (H3) of the di-substituted propyl chain at 6.89-6.72, 6.01 and 1.11 ppm respectively. These three signals were the focus of subsequent analysis.

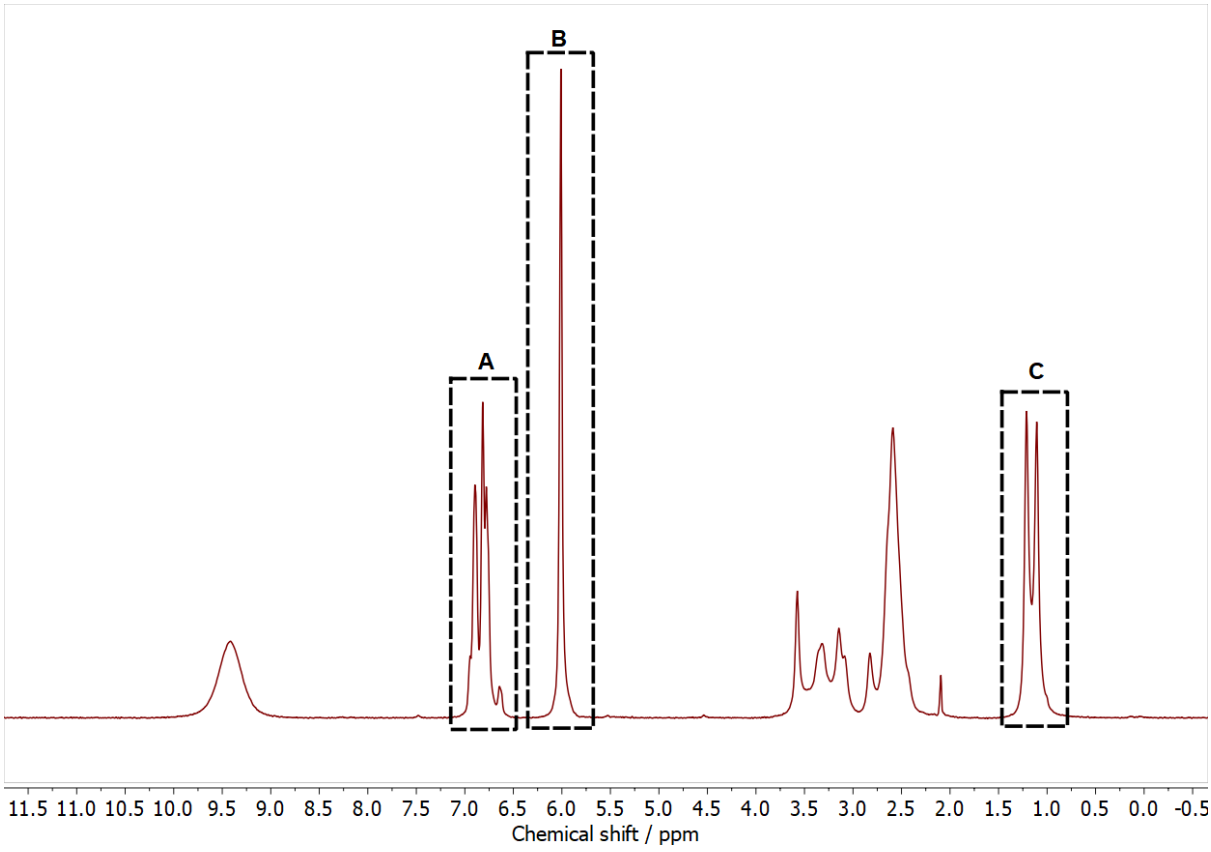

Fig. 2. ^{1}H NMR spectrum (60 MHz) of 3,4-methylenedioxymethamphetamine (**1**, 300 mg/600 µL in d_6-DMSO). The three signals of interest have been highlighted (A = aromatic protons [H2', H5' and H6'], B = 3,4-methylenedioxy protons [H5], C = methyl protons [H3])

3.1 Development of the quantitative methodology ('manual NMR method')

In order to quantitate the amount of MDMA present in a sample, the sample must fully relax between scans to ensure that representative integrals are obtained. Thus, to develop the quantification methodology, a series of samples of MDMA in d_6-DMSO in the range 5-300 mg mL^{-1} were prepared and these were analysed by ^1H NMR spectroscopy to obtain T_1 values for the three resonances of interest. The MDMA range was selected to encompass the range reported by the Drug Information and Monitoring Service (DIMS) as being regularly encountered (10 mg to >140 mg) [41] and also included so called "super pills" which contain ≥270 mg of MDMA per tablet [22].

The T_1s for the three signals ranged from 1.8 to 0.4 s over the concentration range investigated. The aromatic nuclei had the longest T_1 followed by the methylene protons and, finally, the methyl protons. A plot of inverse T_1 against concentration of MDMA is shown in Figure 3. As a sample relaxes in 5-6 T_1, the maximum relaxation delay needed to be *ca.* 12 seconds. However, to ensure complete relaxation, a relaxation delay of 30 s was used for quantification experiments, therefore facilitating accurate integration of the three peaks identified. Procedures published by Blagbrough *et al.* [20], Hays *et al.* [33] and de Oliveira *et al.* [26] utilised relaxation times of 50, 45 and 25 s respectively to quantify MDMA by ^1H NMR spectroscopy.

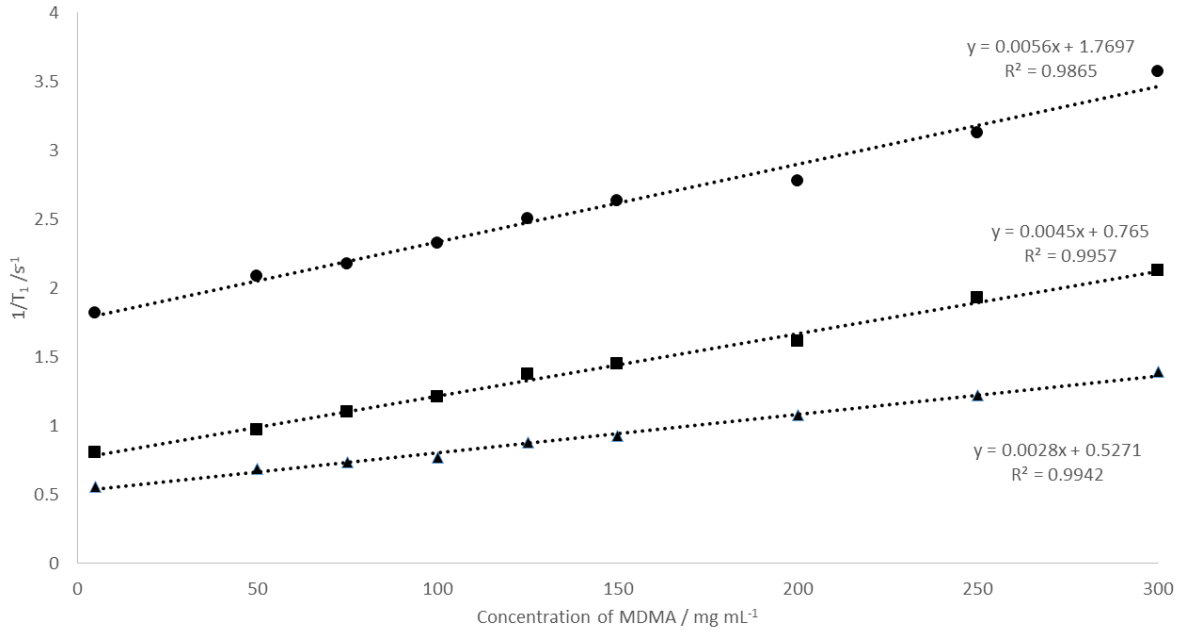

Fig. 3. Graph showing the inverse T_1 value for the aromatic (H2', H5' and H6', triangles), 3,4-methylenedioxy (H5, squares) and methyl (H3, circles) protons of MDMA at varying concentrations in d_6-DMSO

Each of the prepared MDMA samples was then analysed and the three signals of interest integrated using the same integral range for each spectrum to ensure consistency. Figure 4 shows the straight-line plot that was obtained for the average normalised signals of the samples utilised. Individual plots for the aromatic (H2', H5' and H6'), methylenedioxy (H5) and the methyl (H3) signals are included in the SI. The linearity of the plot was expected due to the signal intensity being proportional to the amount of ^{1}H nuclei in the sample.

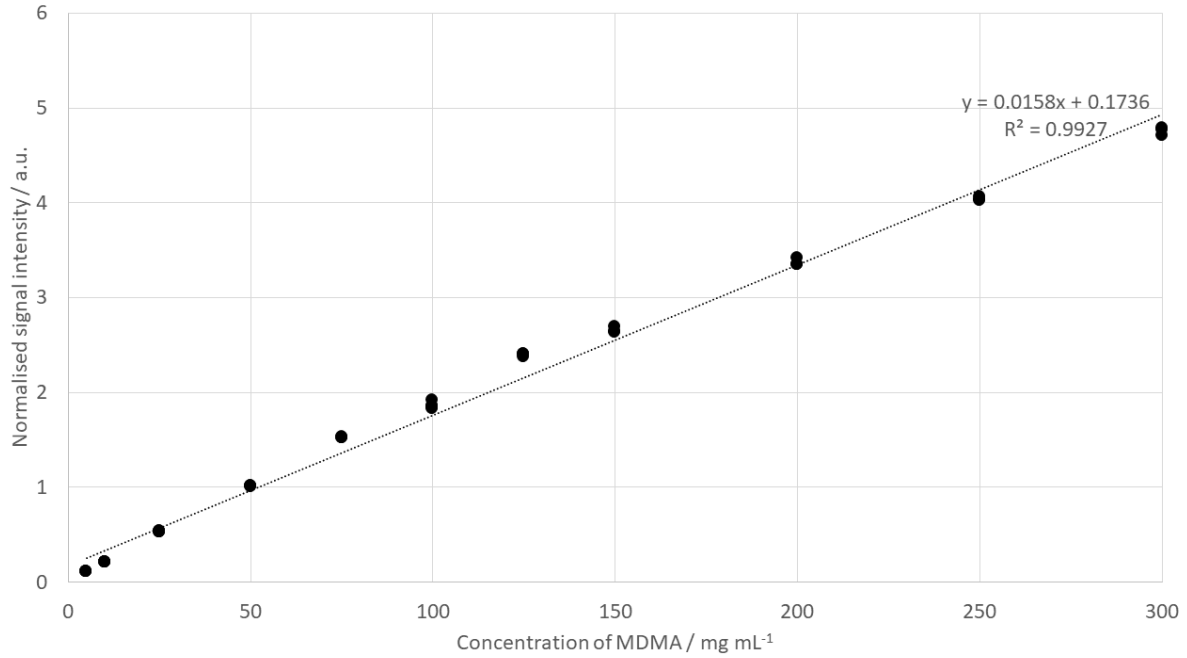

Fig. 4. Averaged normalised signal intensities for different concentrations of MDMA. The samples were normalised to the signal intensities for the 50 mg mL^{-1} samples. The ^{1}H NMR spectrum of each sample was collected five times and the average of the normalised signal intensity for each acquisition plotted as an individual point on the graph.

The limit of detection (LOD) was calculated for the three calibration plots produced using the standard deviation of y-intercepts of the regression line according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines [42]. The LODs ranged from 5.5 mg mL^{-1} for the aromatic nuclei (H2', H5' and H6') to 6.6 mg mL^{-1} for the methylenedioxy (H5) nuclei. Similarly, the limit of quantification (LOQ) was calculated and values ranged from 16.5 mg mL^{-1} for the aromatic nuclei to 21.9 mg mL^{-1} for the methylenedioxy nuclei. Table 1 summarises the findings. Using MDMA standards from 5 mg mL^{-1} to 150 mg mL^{-1} only reduced the LOD and LOQ to mean values of 3.3 and 10.1 mg mL^{-1} respectively. Furthermore, the mean signal-to-noise ratio of the three signal areas of interest is 36 for the 5 mg mL^{-1} reference sample. The ICH also states that signal-to-noise

ratios can be used to determine LOD and LOQ, with typical values being 3:1 and 10:1 respectively. Employing this approach would imply that the LOD and LOQ are both <5 mg mL^{-1}. Given that tablets typically contain 125 mg MDMA [22], the methodology employed is sufficient to quantify the material present. It should be noted that the LOQ values reported for MDMA quantification using high-field NMR instrumentation (600 MHz), was 0.67% of the molar ratio of MDMA relative to the internal standard (maleic acid), which equates to ca. 0.13 mg [26]. Again, the authors noted that the values are far below the lowest MDMA.HCl concentration determined in real samples. The difference in LOQ values could be due to a number of factors, the most pertinent being the difference in field strength (60 MHz compared to 600 MHz) and the number of scans used, both of which have a direct relationship on the signal-to-noise ratio.

Table 1. ^{1}H NMR validation results for the aromatic, 3,4-methylenedioxy and methyl proton environments

	Aromatic protons (H2', H5' and H6')	3,4-Methylenedioxy protons (H5)	Methyl protons (H3)
Slope	0.0165	0.0155	0.0155
Intercept	0.1486	0.1963	0.1760
Standard error of the intercept	0.0273	0.0340	0.0310
LOD / mg mL^{-1}	5.5	7.2	6.6
LOQ / mg mL^{-1}	16.5	21.9	19.9

3.2 Quantification of MDMA in a simulated mixture

To test the quantification method, a simulated mixture consisting of 780 mg MDMA hydrochloride and 2010 mg $CaCO_3$ was prepared and homogenised. $CaCO_3$ is a frequently used pharmaceutical excipient [43]. Aliquots (465 mg) of this powder were analysed using ^{1}H NMR spectroscopy. The ^{1}H NMR spectrum obtained was integrated and normalised relative to a reference sample of MDMA (50 mg mL^{-1}). Five separate samples were analysed. The mean values for the aromatic (H2', H5' and H6') signal, methylene (H5) signal and methyl (H3) signal were 133.0±1.8 mg, 123.3±2.2 mg and 132.2±2.1 mg respectively. Using all these integrated signals gave an overall mean of 129.5±2.0 mg. Each aliquot should contain 130 mg, so the values determined experimentally compare well with the expected value. The approach highlights the importance of using more than one signal in order to determine the amount of MDMA present in the sample; the lower average value of the methylene signal demonstrates this.

3.3 Automating the quantitative analysis ('automated NMR method')

In contrast to the 'manual' method, which treats each of the peaks of interest separately, the 'automated' method uses the spectral regions A, B and C as indicated in Fig. 2 simultaneously, in a multivariate approach. Internally cross-validated partial least squares regression (PLSR) was applied to these regions of the MDMA calibration series spectra. A 5-factor model was found optimal, with an estimated error in prediction of 4.5 mg mL^{-1}. This model was subsequently applied to the spectra of the seized samples to obtain predicted MDMA concentrations, discussed below.

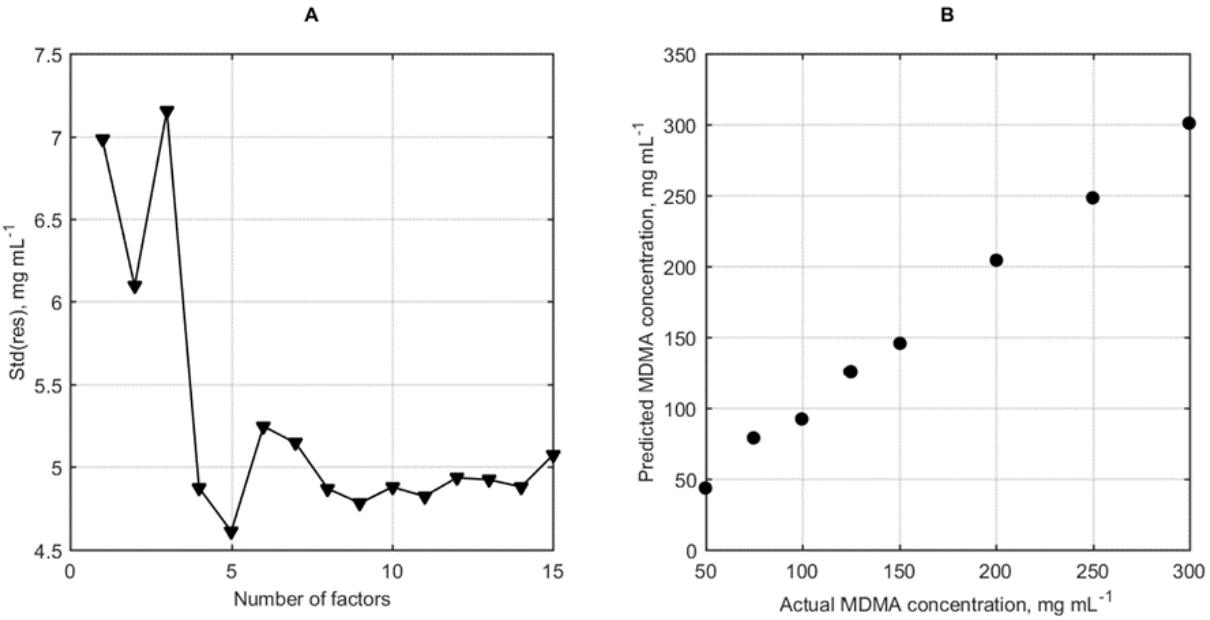

Fig. 5: A: Standard deviation of prediction residuals as a function of PLS model dimensions; and B: Cross-validated 5-factor PLS model of MDMA concentrations (actual against predicted)

3.4 Qualitative analysis of the seized tablets

20 tablets (MD1-MD20) were seized by Greater Manchester Police and provided to Manchester Metropolitan University through the MANchester DRug Analysis and Knowledge Exchange (MANDRAKE) partnership. All the tablets were suspected to contain MDMA. The drugs ranged in appearance as described in table 2. The seized tablets all possessed similar weights, with the exception of the green embossed "Twitter" tablets (n = 5). These had a mean weight of 0.20±0.03 g; for the remaining tablets (n = 15) the mean weight was 0.44±0.026 g. Initial analysis of the tablets was performed using qualitative NMR and GC-MS approaches to identify the active pharmaceutical ingredient (API) in each tablet.

The qualitative NMR data was acquired on a benchtop NMR spectrometer (60 MHz). Acquisition, analysis, processing and elucidation of the API present in the sample was automated, which has been described in full elsewhere.[15] Briefly, an algorithm compares the spectral data to a reference library of over 300 ^{1}H NMR spectra, ranking matches by a Pearson's correlation-based score. A threshold was set empirically at 0.838: match scores greater than this are indicative of a correct match, whereas identifications with lower scores are considered unreliable. This approach confirmed that tablets MD1-MD8 and MD14-MD20 all contained MDMA as the sole API, as the hit-scores were consistently 0.97 or above. Notably, grouping of the hit-scores values, as well as appearance, indicated the presence of six distinct types of tablet (four groups of tablets and two single tablets). MD19 and MD20 were the two single tablets, which were blue "Punisher" (hit score = 0.970) and blue "Superman" tablets (hit score = 0.989) respectively. MD1-MD8 were all red and white "iPhone" tablets, and these all yielded a hit-score of 0.986±0.001. MD14-MD16 and MD17-MD18 were pink "Heineken" and beige pharaoh tablets respectively, and these two groups had hit scores of 0.989±0 and 0.987±0. The final group, five green "Twitter" tablets, MD9-MD13, were identified by the pattern matching algorithm as containing the hallucinogenic phenethylamine, 2,5-dimethoxy-4-bromophenethylamine (2C-B, **3**), as the main API. These tablets were not investigated further, as the focus of the present study is the development of an automated quantitative NMR method for determining the MDMA content of a suspect tablet. Having ascertained that MD1-MD8 and MD14-MD20 all contained MDMA, the next step was the quantification of this API.

3.5 Quantification of MDMA in seized samples

The quantification of the seized samples was achieved using the manual and automated NMR methods. As both approaches are NMR-based, GC-MS data was also contemporaneously collected for comparison purposes. For the manual NMR approach, a reference sample consisting of 100 mg of MDMA was acquired, and the spectra of the samples were normalised to the reference spectrum in order for the calibration plot to be utilised.

MD1-MD8 all had the same physical appearance (red and white "iPhone" logo) and from the GC analysis, all of these tablets were determined to be ca. 50% w/w MDMA (range 209-219 mg) (Table 2). The amounts of MDMA in the tablets were in the range 200-241 mg as determined by the manual NMR method, and 212-244 mg by automated NMR. In general, the values from the NMR methods were in good agreement with those from the GC-MS analysis,

although a few samples gave somewhat higher values for the MDMA present in the tablet. Notably, this occurred for MD1, MD2 and MD6. For example, analysis of MD6 produced values for the MDMA content that were ca. 5% higher by the NMR methods (240.67±1.2 mg by manual, 244.03 mg by automated) than that obtained from the GC-MS analysis (218.86±5.0 mg). This sample showed the biggest difference between the NMR and GC-MS results. We note here a recent study that quantified MDMA in a tablet using UHPLC and NMR using an internal standard indicated differences between methods of ca. 8 mg for some of the tablets surveyed (ca. 1-1.5% differences in MDMA content w/w) [20]. From consideration of the various analyses of MD1-MD8, it is likely that these tablets were all produced from the same batch process. Furthermore, the crushed weight of these tablets were very similar; the average weight was 0.424±0.008 g.

Analysis of MD14-MD16, the pink "Heineken" tablets, again showed very good agreement between the NMR analyses, both automated and manual, and the GC-MS analysis. Similar to MD1-MD8, it is likely that MD14-MD16 were all produced from the same batch process. This is reflected in near identical qualitative hit scores, similar tablet weights (0.438±0.010 g) and similar MDMA contents (36-39% w/w by the NMR methods). GC-MS analysis indicated that the tablets contained 171-176 mg of MDMA, whereas the range determined by manual NMR was 159-163 mg, and by automated NMR, 165-173 mg.

MD17 and MD18 were two beige pharaoh tablets. These two samples were found to have the lowest % w/w MDMA from analysis by GC-MS (ca. 29% w/w). Interestingly, the manual NMR method resulted in substantial differences in the amount of MDMA present (5 and 9% w/w, equating to 26 and 48 mg difference of MDMA in the tablet compared to GC-MS analysis). Conversely, the automated approach gave very good agreement with the GC-MS analysis (ca. 29% w/w, 7 or 10 mg difference between automated and GC-MS analysis), especially when the estimated error of 4.5 mg mL^{-1} is accounted for.

The amount of MDMA in MD19 as determined by NMR and GC-MS differed substantially. Upon inspection of the ^{1}H NMR spectrum, an additional signal was observed as a downfield shoulder (centred at 1.23 ppm) on the methyl (H3) peak. Consequently, the integration value obtained for this signal is the combination of the methyl signal of MDMA and the additional signal, which is attributed to binder / filler material that was not removed by the filtration process performed. Thus, the reported amount of MDMA in the sample is greater than is

actually the case. The integral values for the aromatic and methylene signals indicate that the amount of MDMA present is 24.5±0.5 mg and 23.4±0.5 mg respectively, which equates to 45.98±1.9% of MDMA in the original 52 mg tablet. This compares well with the GC-MS data that reports 44-46% w/w. In contrast, the methyl (H3) signal returns that the sample contains 28.9±0.3 mg, equivalent to ca. 57% w/w MDMA content in the original tablet. The automated NMR outcome is similarly adversely affected; this is due to the multivariate approach treating the three spectral regions simultaneously rather than individually. This contrasts to the manual approach that treats each spectral region separately prior to reporting the mean of these regions. Evidently, the presence of this additional signal complicates the quantitation of MDMA in the sample by both the manual or automated approaches, and hence additional scrutiny of the data was required.

The additional signal in the ^{1}H NMR spectrum of MD19 is believed to be due to stearate being present in the sample. Previously, samples that have been prepared and analysed in the same way as described herein were shown to possess this signal [15]. Confirmation was obtained by doping a sample with magnesium stearate, which led to a peak at the same chemical shift. Thus, care should be taken when quantifying, as additional signals present in the integral regions adversely affect the calculation of the amount of MDMA present. The results for MD19 exemplify that a single integral region should not be used for quantitation by NMR. Notably, the qualitative GC-MS analysis returned MDMA only as being present in the sample. It did not detect the presence of stearate in the sample as it would need to be derivatized, such as through the use of silylating agents [44], in order to be detected.

Finally, analysis of MD20 by the automated NMR method indicated that the tablet contained 38.6% w/w MMDA (159 mg in the tablet). This compares well with the GC-MS analysis, which reported that the tablet contained 157 mg MDMA. However, the manual NMR analysis of MD20 was similar to MD17 and MD18, in that the value compared to GC-MS was 5% lower. The results for these three samples (MD17, MD18 and MD20) highlight the importance of a combined approach in terms of quantifying the amount of MDMA per tablet; a singular methodology should not be relied upon.

Table 2. Qualitative and quantitative data for the samples analysed that contained MDMA following NMR and GC-MS analysis.

Sample code	Description of tablet	Weight of tablet / g	Mean amount of MDMA in tablet determined by GC / mg ± SD	% w/w of MDMA in tablet determined by GC ± RSD		Hit score determined by qualitative NMR analysis	Amount utilised for NMR analysis / g	Mean amount of MDMA in tablet determined by manual NMR / mg ± SD	% w/w of MDMA in tablet determined by NMR (manual method) ± RSD	Mean amount of MDMA in tablet determined by automated NMR / mg	% w/w of MDMA in tablet determined by NMR (automated method)
				Run 1	Run 2						
MD1	Red and white iPhone	0.4417	218.61±2.2	49.56±1.0	49.43±0.8	0.984	0.0500	222.35±1.8	50.34±0.8	241.65	54.71
MD2	Red and white iPhone	0.4331	217.55±2.4	50.29±1.1	50.18±2.8	0.986	0.0495	219.51±1.3	49.54±0.6	236.53	53.58
MD3	Red and white iPhone	0.4261	212.96±1.0	49.92±0.5	50.04±1.8	0.985	0.0539	214.57±1.4	50.36±0.6	217.61	51.07
MD4	Red and white iPhone	0.4189	210.73±0.71	49.81±0.3	50.80±0.6	0.986	0.0522	211.54±1.1	50.50±0.5	221.18	52.8
MD5	Red and white iPhone	0.4202	208.92±2.0	49.94±1.0	49.50±0.7	0.986	0.0479	199.57±0.9	47.49±0.5	212.16	50.49
MD6	Red and white iPhone	0.4329	218.86±5.0	51.43±2.3	49.68±0.8	0.985	0.0493	240.67±1.2	55.59±0.5	244.03	56.37

MD7	Red and white iPhone	0.435	218.42±2.0	50.33±0.9	50.10±0.1	0.987	0.0492	211.75±1.1	48.68±0.5	223.16	51.3
MD8	Red and white iPhone	0.421	208.9±3.8	49.61±1.8	49.63±1.4	0.987	0.0504	209.04±1.2	49.65±0.6	217.36	51.63
MD14	Pink Heineken	0.45	175.61±4.4	38.68±2.5	39.19±1.6	0.989	0.0511	160.00±1.9	35.56±1.2	173.16	38.48
MD15	Pink Heineken	0.4334	170.76±2.4	39.82±1.4	38.99±2.1	0.989	0.0506	163.00±5.06	38.89±3.1	171.36	39.86
MD16	Pink Heineken	0.4299	171.05±2.2	40.21±1.3	39.37±2.6	0.989	0.0519	159.31±3.6	37.06±2.3	165.25	38.44
MD17	Beige pharaoh	0.4837	137.56±2.4	28.68±1.8	28.20±2.7	0.987	0.0512	111.30±4.6	23.00±4.1	145.50	30.08
MD18	Beige pharaoh	0.5072	153.45±1.4	30.49±0.9	29.99±0.6	0.987	0.0498	105.04±1.9	20.71±1.8	143.08	28.21
MD19	Blue punisher	0.4749	214.23±7.6	44.22±3.6	46.00±1.5	0.970	0.0520	233.57±3.9	49.18±1.7	251.74	53.01
MD20	Blue superman	0.4142	156.94±6.0	38.64±3.8	37.14±0.6	0.989	0.0496	135.74±2.9	32.77±2.1	159.55	38.58

4. Conclusion

Twenty seized tablets were analysed, of which 15 were found to contain MDMA via qualitative NMR (hit scores ≥0.97) and GC-MS (R_t = 5.6 min) analysis. If MD19 is excluded due to the presence of stearate in the analysed sample, which was not removed by the filtration process employed, then the qualitative NMR hit score for all samples improves to ≥0.984.

T_1 data of MDMA were acquired for samples in the range 5-300 mg mL^{-1} to assess the time-period required for complete relaxation. The aromatic (H2', H5' and H6'), 3,4-methylenedioxy (H5) and methyl (H3) were selected for analysis due to their isolated nature in the ^{1}H NMR spectrum. For the concentrations measured, T_1 values ranged from 1.8 to 0.4 s. To ensure complete relaxation, a relaxation delay of 30 s was used. For the quantitative analysis by NMR, spectral acquisition of each MDMA sample took ca. 2.5 mins to obtain four transients. We can conclude that benchtop NMR offers a rapid method for accurate identification of the API. Furthermore, due to minimal sample preparation, this approach could be utilised by legal entities and healthcare providers for rapid screening of suspected MDMA tablets.

The quantification of MDMA using benchtop ^{1}H NMR spectroscopy was assessed using two different approaches. The first was based on simple linear regression onto peak integrals ('manual' method) whereas the second utilised partial least squares regression of the MDMA content onto a wider spectral range ('automated'). NMR-based quantification was performed in the absence of an internal standard to reduce sample preparation. The results from the ^{1}H NMR analysis were compared against contemporaneously obtained GC-MS data. The NMR quantitative analysis of MD1-MD8 and MD14-MD20 gave mean values for the MDMA content across all tablets of 42.6% w/w by the manual method and 45.9% w/w by the automated method. The mean value obtained from GC analysis was 44.0% w/w. Notably, this means that a substantial proportion of the tablets tested contained >190 mg of MDMA (range 133-223 mg, average of all techniques' calculations for each tablet). A dose of 190 mg MDMA is sufficient to produce a physiological effect in a human (>1 mg kg^{-1} of bodyweight required to induce elevated temperature and cardiovascular effects [45], for example).

The MDMA content of the tablets surveyed herein is in line with reports that document an ever-increasing dosage. Wood *et al.* reported that the MDMA content of 101 tablets obtained from amnesty bins cited in UK night clubs in 2006 was 58.7±22.9 mg per tablet, with a range of 20 mg to 131 mg per tablet [19]. 96% of tablets contained less than 100 mg MDMA per

tablet. Subsequent reports have highlighted an increase in the content of MDMA in tablets. In 2015, DIMS reported that 53% of tablets surveyed contained over 140 mg of MDMA. This contrasts with only 3% in 2009 and, furthermore, only 42% of tablets surveyed in this year contained more than 70 mg MDMA per tablet [46]. In 2018, DIMS reported that 72% of tablets surrendered contained >150 mg [41]. 95% of tablets contained more than 100 mg. The evaluation of 412 tablets collected over the period 2001-2018 in the UK provided evidence that recent samples (2018) contained a median content of MDMA of 105 mg for the first time [18].

The MDMA-containing tablets tested in the present work confirm this continuing trend of tablets containing ever-greater amounts of MDMA. It should be noted that, although the amounts of MDMA found were not those of "super pills" (270-340 mg of MDMA per tablet) [22], each tablet contains 2.1-3.63 the amount needed to induce a physiological response in an average human [47] (62 kg).

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6. Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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