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¹H quantitative NMR and UHPLC-MS analysis of seized MDMA/NPS mixtures and tablets from night-club venues†

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3,4-Methylenedioxymethamphetamine (MDMA) in the UK has increased in purity and the contents sold as MDMA (ecstasy, E) have increased in complexity. Night-club scenes remain overall the biggest venues where such powders or tablets are consumed. HPLC and GC are the gold standard analytical methods in forensic laboratories for the quantification of seized samples of illicit drugs. However, complex mixtures of such samples may be a limiting factor for chromatographic techniques. NMR is used for the structural elucidation of newly isolated natural products or synthesized compounds, but also the inherent ability of ¹H NMR to quantify compounds is a powerful tool that is employed for quantification and purity testing in the pharmaceutical industry. In this study, a ¹H quantitative NMR (q-NMR) method is developed for the quantification of seized samples from night-clubs. These samples are shown to contain mixtures of MDMA and other NPS, e.g. ethylone, methylone, trifluoromethylpiperazine (TFMPP), *N,N*-dimethyl-3,4-methylenedioxyamphetamine (MDDMA), 4-bromo-2,5-dimethoxyphenethylamine (2C-B), and 4-iodo-2,5-dimethoxyphenethylamine (2C-I). The method is applied to MDMA tablets seized from similar venues, and compared with UHPLC and UHPLC-MS, resulting in a good agreement across techniques. ¹H q-NMR provides a fast (15 min) and robust analytical method for the quantification of complex seized samples without resorting to tedious sample preparation or obtaining reference standards.

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Introduction

3,4-Methylenedioxymethamphetamine (MDMA, ecstasy, E) is considered to be by far the most popular of the phenethylamines in Europe, as demonstrated by the amount seized, 5.3 million tablets and 295 kg of powder in 2016.¹ This popularity is the result of many factors such as the high purity (higher than seized amphetamine and methamphetamine) and the low price of only 6–11 euros/tablet.¹ The health risks of high-potency products and the continued emergence of new substances, together with the changing patterns of drug use are among the issues highlighted in the European Drug Reports of 2016² and 2018,¹ and recently further reinforced in the June 2019 Report.³

Cathinones are the second most popular class by seizures among novel psychoactive substances (NPS) (after synthetic cannabinoid receptor agonists, SCRAs) and the leading NPS available in the powdered form.¹ They are based on the natural product *S*-cathinone, with many opportunities in the chemical structure for modifications with e.g. alkyl substituents resulting in a large number of derivatives, e.g. methylenedioxy substituents giving rise to analogues such as butylone, ethylone and

methylone.^{4,5} The pharmacology and dose of cathinones and MDMA are of importance, especially in the context of analytical and forensic toxicology. MDMA works primarily on serotonin receptors (5-HT-R), but also possesses dopamine release action.^{4,5} Mephedrone is a more potent inducer of locomotor activity in rodents than MDMA.⁴ Therefore, taking both types of drug can lead to serious consequences such as tachycardia, hypertension, hyperthermia and dehydration.^{6,7} The association of cathinones with each other and with other NPS has been described by Zuba and Byrska.⁸ Using LC, GC-MS and NMR, they showed that the most common cathinones detected were 3,4-methylenedioxypropylone (MDPV) and butylone, found with piperazines. Toxicological identification of post-mortem cases showed MDMA with methylone, and MDMA with ethylone.^{9,10}

NPS continue to increase in number, e.g., 101 NPS were discovered for the first time in 2014. They can be toxic, as the taken dose is not known, and occasionally they have fatal consequences, often extensively reported in the media. MDMA trafficking continues to grow in complexity and tablets are commonly sold containing different doses of MDMA, often mixed (cut) with other NPS. Therefore, identification and quantification of drugs of abuse is still a challenge. New detection strategies and reports towards providing point-of-care/in-the-field NPS sensors have been reviewed.¹¹

The simultaneous detection of substances present in drugs of abuse is increasingly important as some materials are known

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On the other hand, NMR requires no solvent preparation, no method development and no stationary phase (column) with which the compounds will interact.^{16,18} NMR allows a simultaneous structure elucidation and quantification of the targeted analyte in a relatively fast time compared to various different chromatographic techniques and NMR will detect organic

Recent research has led to the rapid identification of NPS, including MDMA, using a low-field (LF) (60 MHz) benchtop ^1H NMR spectrometer.²¹ Indeed, the screening, detection and quantification of illicit drug “spice” samples using LF NMR spectroscopy has been explored by Gilard and co-workers showing that the recent introduction of benchtop cryogen-free

LF NMR spectrometers (60 MHz) can provide useful insights, in the form of diagnostic signals, to chemical structure.²² However, the greater spectral band overlap compared to data acquired from a superconducting magnet NMR spectrometer did present a data analysis challenge.^{23,24}

In this study, the quantitative analysis of multicomponent MDMA with other NPS, such as cathinones, phenethylamines, and piperazines, will illustrate the complexity of the samples from UK night-club venues. Additionally, a cross-method confirmation of q-NMR using an IS method is used to assay different MDMA tablets seized from night-club venues in Bristol. Validation is by UHPLC and UHPLC-MS using MDMA-d₅ as an IS for the latter.

Experimental section

Chemicals and sample preparation

NMR grade solvents D₂O 99.9% and TMSP-2,2,3,3-d₄ 98.0% were purchased from Cambridge Isotope Laboratories (Goss Scientific, UK). Methanol and water for UHPLC and UHPLC-MS analysis were Lichrosolve Honeywell LC-MS grade. $\pm$ -MDMA 1.0 mg mL⁻¹ in methanol solution reference standard, $\pm$ -MDMA-d₅ 1.0 mg mL⁻¹ in methanol solution reference standard, maleic acid (MA) 99.94% and dimethyl sulphone (DMS) 99.96% are TraceCERT certified for quantitative NMR analysis, and acetanilide (99%) were purchased from Sigma-Aldrich (UK).

Drug samples were provided by the Drug Expert Action Team (DEAT), Avon and Somerset Constabulary, in sealed evidence bags containing numerous drug samples in different forms (capsules, crystals, powders, tablets and plant materials) in different packaging (small packs, magazine twists). Samples in small packs in the form of crystals and powders weighing between 50–250 mg were from different night-club venues in Bristol, while MDMA tablet weights ranged between 188–645 mg. For quantitative analysis, crystals and powders were weighed using a Sartorius analytical balance MC 5 followed by extraction with D₂O containing IS maleic acid (2.0 mg mL⁻¹) and 0.5% of TMSP-2,2,3,3-d₄ (1.0 mL) for NMR spectroscopic analysis.

For MDMA quantitative analysis, tablets of different sizes and shapes were photographed for documentation, followed by manual pulverization using a mortar and pestle. 10.0 mg of powder was weighed using a Sartorius analytical balance MC 5, transferring into a 7.0 mL glass screw neck specimen vial (Fisher Scientific), then extraction with D₂O (2.0 mL) containing maleic acid (1.0 mg mL⁻¹) as an NMR quantitative IS with sonication for 30 minutes, filtration through a Sartorius Minisart® 0.2 µm filter followed by taking a final volume of 1.0 mL for NMR spectroscopic analysis. For UHPLC analysis, the sample was diluted 100-fold into a MS vial. Samples were quantified using a 6-point calibration curve 1.5, 3.1, 6.3, 12.5, 25.0, and 50.0 µg mL⁻¹ prepared in UHPLC solvent. For UHPLC-ESI MS quantitative tablet analysis, the sample was extracted with LC-MS grade water and diluted 10 000-fold into a MS vial and spiked with MDMA-d₅ (500 ng mL⁻¹) as an IS. Samples were quantified using an 8-point calibration curve from 32.25, 62.50,

125.0, 250.0, 500.0, 1000, 2000, and 4000 ng mL⁻¹. Each concentration was spiked with IS MDMA-d₅ (10 µL of 25 µg mL⁻¹) to achieve a final IS concentration of 500 ng mL⁻¹. The response was calculated as the ratio of the area under the curve of the target compound to that of the IS.

Instrumentation

NMR spectroscopy. NMR spectra were recorded on a Bruker AVANCE III 500 MHz spectrometer, ¹H and ¹³C frequencies are 500.130 and 125.758 MHz respectively. The probe was a variable temperature BBFO+ with three channels, temperature was 25 °C. Chemical shifts were referenced to 0.00 ppm for TMSP-d₄ or the HDO residual solvent peak at δ 4.76 (HDO) and are reported in ppm. Coupling constants (*J*, line-separations, absolute values) are rounded to the nearest 0.5 Hz. Structural elucidation was achieved with 2D NMR Correlation Spectroscopy (COSY), Heteronuclear Single Quantum Coherence (HSQC), Heteronuclear 2-Bond Correlation (H2BC), Heteronuclear Multiple Bond Correlation (HMBC). NMR spectra were processed using Bruker TopSpin 3.5 or Mestralab Mnova 11.2. For quantitative ¹H NMR (q-NMR) analysis, the zg pulse sequence was composed of 3.18 s acquisition time, 16 scans, 50 s delay, 90° pulse angle, phase and baseline corrections were automatic while integration was performed manually.

UHPLC and UHPLC-ESI MS. UHPLC calibration curve for quantitative analysis of samples was constructed on a Dionex Ultimate 3000 UHPLC (Thermo Fisher Scientific, Sunnyvale, CA, USA) with a variable wavelength detector (λ = 210, 254, 280, 285 nm). Liquid chromatographic separation was performed using an Acquity UPLC BEH C18, 1.7 µm, 2.1 × 50 mm RP-column (Waters, Milford, MA, USA) with a flow rate of 0.3 mL min⁻¹, and an injection volume of 10 µL at 25 °C column temperature. Mobile phase A consisted of water 0.1% TFA, mobile phase B was ACN 0.1% TFA. Gradient elution started with 1% B for 1.0 min, followed by a linear increase from 1.0 min to 100% B at 4.0 min and maintained for 1.0 min, followed by a decrease to 1% B at 5.1 min, where it was held for equilibration 2.9 min, total run time of 8.0 min. Data analysis used Bruker Data analysis 4.3, and Excel data analysis tool pack. QTOF-UHPLC-MS analysis was conducted on a MaXis HD quadrupole electrospray time-of-flight (ESI-QTOF) mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany), operated in ESI positive mode. The QTOF was coupled to an Ultimate 3000 UHPLC (Thermo Fisher Scientific, Sunnyvale, CA, USA). The capillary voltage was set to 4500 V, nebulizing gas at 4 bar, drying gas at 12 L min⁻¹ at 220 °C. The TOF scan range was from 75–1000 mass-to-charge ratio (*m/z*). Formic acid (FA) 0.1% was used for the mobile phase instead of TFA and the same solvent gradient and conditions as for the UHPLC were used.

Results and discussion

In this study the association (formulation) of MDMA with other NPS is presented. Quantification using a fast, simple and accurate ¹H q-NMR of each of the components without resorting to tedious preparation and method development assays will



be applied to complex samples. Additionally, seized MDMA tablets were quantified using ^1H q-NMR and cross-method confirmed using UHPLC equipped with a variable wavelength detector (VWD) and UHPLC-MS using MDMA- d_5 as an internal standard (IS). Eqn (1) was used for ^1H q-NMR quantitation:

$$m(x) = P(\text{std}) \frac{M_w(x)}{M_w(\text{std})} \frac{A(x)}{A(\text{std})} m(\text{std}) \frac{N(\text{std})}{N(x)} \frac{m(\text{sample})}{m(\text{sample used})} \quad (1)$$

where x is the analyte, std is the IS, m is the mass in mg, P is the purity, M_w is the molecular weight in g mol^{-1} , A is the integral value of the resonance being investigated, N is the number of protons represented by the signal, $m(\text{sample})$ is the mass of the sample/tablet in mg and $m(\text{sample used})$ is the mass of the extracted sample, *e.g.* 10.00 mg.

¹H q-NMR of MDMA/methylone/trifluoromethylpiperazine (TFMPP) and MDMA/ethylone mixtures using maleic acid (MA) as an IS resonating at $\delta = 6.38$ ppm was performed (Table 1). MA is an ideal IS due to its simple resonance signal (singlet), non-overlapping peak, high purity (99.94%) and high solubility in D₂O. An inversion recovery NMR experiment of MDMA, methylone and TFMPP in the mixture was performed to establish the relaxation time (T_1) of the signals selected for quantification, to satisfy the parameter of adequate relaxation delay. This was found by experiment to be between 1.5–4.4 s, adopting at least $5 \times T_1$ to ensure complete relaxation of the signals between pulses.^{16,18,19} Additionally, no more than 10.0 mg of sample was used for quantitative NMR analysis in D₂O as the high salt concentration affected the broadness of the peaks by affecting the shimming.

In this study, quantitative analysis of different selected samples of MDMA/NPS powder and crystal mixtures was achieved, with simple extraction using D₂O containing MA (2.0 mg mL⁻¹) as an IS (Table 1).^{16,20} Initially the samples were identified and characterized using 1D/2D NMR and LC-ESI/MS. Structural elucidation allowed the selection of signals for quantification of

each component with high confidence. The amounts of MA and TMSP-d₄ were fixed throughout the analyses. The MA olefinic signal (at $\delta = 6.38$ ppm) was used for quantification. It is well separated from the rest of the components in the mixture, even the methylenedioxy peaks of MDMA, ethylone and methylone, and was normalized to the value of 1.00. TMSP-d₄ not only allowed the referencing of the NMR spectra to 0.00 ppm, but also was used to monitor the ratio of MA/TMSP in case of any impurity appearing beneath the MA peak, especially any cutting agents in the maleate form.

In the MDMA/methylone/TFMPP mixtures, the same signal overlapped with the 1' and 2' of TFMPP piperazine ring (Fig. 1), whose signals were also excluded from the integration/quantitation analyses. In all the mixtures, all the signals of MDMA were available for integration with the exception of the chiral centre signal (2) at 3.39 ppm, where in MDMA/ethylone mixture an unknown impurity resulted in a significantly higher integration value compared to the rest of the signals (Fig. 2). Ethylone signals for integration depended on the signal to noise (S/N) ratio. In samples HN26, 31 and 48, all the signals were of an appropriate S/N, while in sample HN154, low S/N allowed only the aromatics and methylenedioxy signal (7') to be integrated. The rest of the signals were too close in proximity to MDMA signals. In methylone spectra, each signal was used for quantitation except the 1'' methyl-amine signal at 2.69 ppm due to its close proximity with the MDMA ¹³C satellite signals of 1'' and 1.

The RSD% is affected by the non-uniformity of the original sample, and even though homogenization was carried out on the powder material some components comprising less than 30% of the sample, and especially less than 10% of the sample, resulted in more than 5% RSD.²⁵ An example of this is the minor components of sample HN157, TFMPP and methylene compared to the major component MDMA (Table 1 and Fig. 1). Furthermore, the total composition of the samples is between 77.9%–87.9%. This is possibly due to the presence of insoluble materials, moisture in the sample, and even an excess of the salt to drug ratio in illicit drug samples as explained by Hays, in which salt and water content have influenced the purity of the samples under investigation by ¹H NMR.¹⁶ Two important parameters were taken into consideration: the S/N ratio was more than 150, and the integration. The latter is believed to be the most significant parameter for error introduction because of it being operator dependent. Therefore, consistency in integration across all the samples is crucial for obtaining accurate quantitative results.^{26,27}

A fast (15 min), accurate and reproducible ^1H q-NMR analysis was performed on many samples ($n = 33$) containing complex mixtures of MDMA and NPS. The extraction step with D_2O was compatible with all the components present in their salt forms due to their high water solubility. NMR allowed the separation of the majority of peaks, the few overlapping signals were excluded from the analysis. The RSD% affected the minor components (<10%) in the samples possibly due to the non-uniformity of the samples. This q-NMR analysis is suitable for an NPS where there is no reference standard available for chromatographic analysis. The analysis results revealed

Table 1 Replicates of ^1H q-NMR analysis of 6 different mixtures of NPS with MDMA

Sample name	Components identified	mg/10 mg of sample $\pm$ SD	RSD%
HN26 $n = 6$	MDMA	1.99 ± 0.2	6.4
	Ethylone	6.54 ± 0.1	0.9
HN31 $n = 6$	MDMA	5.01 ± 0.1	0.3
	Ethylone	3.40 ± 0.2	5.5
HN48 $n = 3$	MDMA	5.85 ± 0.1	1.2
	Ethylone	2.94 ± 0.3	11.0
HN153T $n = 6$	MDMA	3.22 ± 0.2	5.6
	Methylone	2.31 ± 0.2	7.2
	TFMPP	2.50 ± 0.1	3.8
HN154 $n = 6$	MDMA	7.42 ± 0.3	4.2
	Ethylone	0.71 ± 0.1	5.1
HN157 $n = 6$	MDMA	7.08 ± 0.2	2.4
	Methylone	0.53 ± 0.1	8.2
	TFMPP	0.18 ± 0.1	7.5



is outside the scope of this analytical research to determine how such samples are marketed when 2 (or more) illicit compounds are mixed.



Table 2 NMR and UHPLC quantitative results of seized MDMA tablets

No	MDMA tablet	Sample	Weight (mg)	UHPLC		NMR	
				MDMA dose (mg) $\pm$ SD	RSD%	MDMA dose (mg) $\pm$ SD	RSD%
1		a	468.77	82.80 $\pm$ 3.0	3.6	89.08 $\pm$ 0.5	0.6
		b	494.71	108.65 $\pm$ 0.9	0.8	105.26 $\pm$ 0.9	0.8
		c	486.26	93.99 $\pm$ 1.7	1.8	97.16 $\pm$ 0.8	0.8
2		a	449.57	69.76 $\pm$ 1.0	1.4	76.24 $\pm$ 0.3	0.4
		b	449.11	75.64 $\pm$ 1.1	1.5	81.54 $\pm$ 0.3	0.4
		c	443.17	72.94 $\pm$ 2.2	3.1	75.00 $\pm$ 1.1	1.5
3		a	520.37	121.13 $\pm$ 0.6	0.5	121.24 $\pm$ 0.7	0.6
		b	477.08	113.26 $\pm$ 0.4	0.3	112.66 $\pm$ 0.5	0.4
		c	501.74	94.38 $\pm$ 0.6	0.6	92.58 $\pm$ 0.8	0.9
4		a	512.73	160.55 $\pm$ 0.8	0.5	164.21 $\pm$ 0.4	0.3
		b	481.95	150.27 $\pm$ 2.4	1.6	151.92 $\pm$ 2.1	1.4
		c	496.73	160.01 $\pm$ 0.5	0.3	165.13 $\pm$ 0.5	0.3
5		a	513.82	162.88 $\pm$ 3.0	1.8	163.45 $\pm$ 0.3	0.2
		b	520.66	160.95 $\pm$ 3.6	2.2	165.10 $\pm$ 0.9	0.5
		c	514.93	166.54 $\pm$ 1.5	0.9	171.55 $\pm$ 1.4	0.8
6		a	325.00	89.45 $\pm$ 0.2	0.2	89.08 $\pm$ 1.2	1.4
7		a	233.1	87.79 $\pm$ 0.6	0.6	90.98 $\pm$ 0.2	0.2
8		a	211.12	85.63 $\pm$ 0.1	0.1	88.36 $\pm$ 0.7	0.8
		b	209.21	83.31 $\pm$ 0.3	0.4	84.18 $\pm$ 0.3	0.4
		c	187.27	78.38 $\pm$ 0.8	1.0	81.55 $\pm$ 0.5	0.6
9		a	503.50	147.70 $\pm$ 2.0	1.3	151.77 $\pm$ 3.2	2.1
		b	468.77	139.74 $\pm$ 1.7	1.2	144.66 $\pm$ 1.4	1.0
		c	476.81	155.02 $\pm$ 3.2	2.1	153.75 $\pm$ 1.2	0.8
10		a	644.70	87.13 $\pm$ 0.9	1.0	95.08 $\pm$ 0.4	0.5
11		a	273.54	85.34 $\pm$ 1.8	2.2	87.39 $\pm$ 0.7	0.8
		b	288.10	91.57 $\pm$ 0.7	0.8	88.59 $\pm$ 1.5	1.7

26 tablets belonging to 11 different brands of MDMA seized from different dance venues in the Southwest of England were quantified by ^1H q-NMR and confirmed by UHPLC using a Variable Wavelength Detector (VWD) and UHPLC-MS (Table 2).

Both UHPLC using UV and UHPLC-MS quantification were determined first by determining the % of MDMA, followed by calculating the MDMA content using eqn (2) and (3):²⁸

$$\text{MDMA}\% = \frac{\text{concentration of sample } (\mu\text{g mL}^{-1}) \times \text{dilution factor volume used (mL)} \times 100\%}{\text{weight of sample } (\mu\text{g})} \quad (2)$$

For UHPLC analysis, the non-specific (in terms of analyte) UV wavelength of 210 nm was selected to detect impurities present in tablets.

$$\text{mg of MDMA} = \frac{\text{MDMA}\% \times \text{weight of tablet in mg}}{100} \quad (3)$$



A single extraction, using D₂O for NMR analysis and H₂O for UHPLC and UHPLC-MS analysis, was employed. This has been deemed sufficient with 100% recovery according to the literature.^{20,28} Additionally, numerous tablets were qualitatively analysed for cutting agents and types of excipients using NMR. The UHPLC method gave a good linearity on plotting the concentration ($\mu\text{g mL}^{-1}$) against UV response (Fig. 3). Using TFA provided a better buffering capacity for MDMA, with good peak resolution and no peak tailing compared to using FA with UV detection.

For UHPLC-MS, two tablets from the red UPS brand were selected for comparative analysis (Table 3). The calibration curve was run in triplicate, and gave a good linearity by plotting

the concentration (ng mL^{-1}) against response ratio of MDMA to MDMA-d₅. The Extracted Ion Chromatograms (EIC) (Fig. S1†) and spectra (Fig. S2†) of the molecular ions of both MDMA and MDMA-d₅ were used for quantification. Using a deuterated analogue as an IS is optimal, due to it satisfying the IS criteria by having the same chromatography yet with a different mass ion, no possibility of it existing in the targeted analyte, and possessing similar ionization in ESI MS.^{29,30} Furthermore, the RSD% across all methods gave a good RSD% of an acceptable value lower than 5% for powdered samples as set out by the European Network of Forensic Science Institute,²⁵ with the exception of one sample 4b using UHPLC-MS analysis. The results of the UHPLC and NMR analyses were comparable.

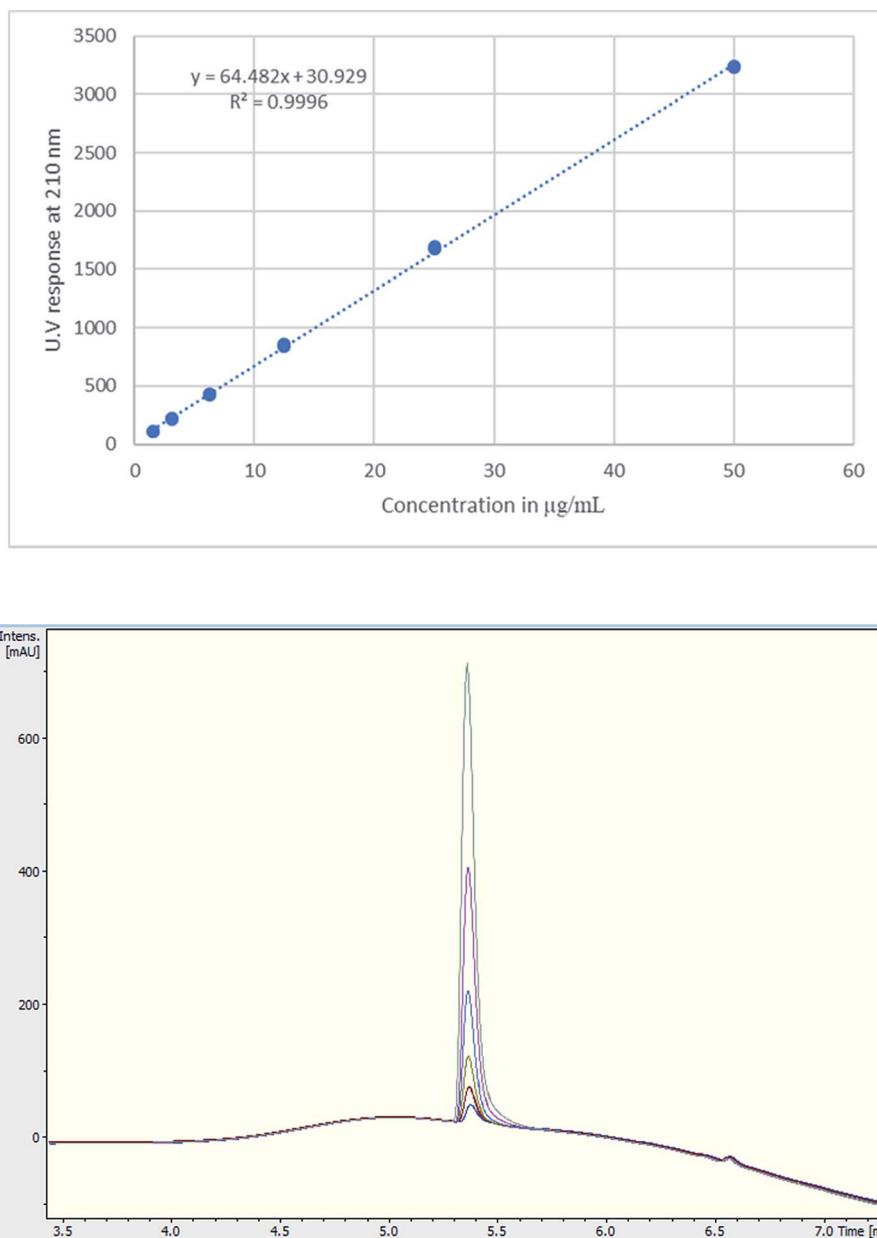


Fig. 3 (Upper) Calibration curve of MDMA between 1.5–50 $\mu\text{g mL}^{-1}$, $R^2 = 0.9996$, (lower) UV chromatogram MDMA RT = 5.4 min of the calibration curve concentrations.

Table 3 UHPLC, NMR and UHPLC-MS quantitative results of MDMA tablet number 4

No	UHPLC				NMR		UHPLC-MS	
4	Entry	Weight (mg)	MDMA dose (mg) $\pm$ SD	RSD%	MDMA dose (mg) $\pm$ SD	RSD%	MDMA dose (mg) $\pm$ SD	RSD%
	a	512.73	160.55 $\pm$ 0.8	0.5	164.21 $\pm$ 0.4	0.3	161.07 $\pm$ 4.7	2.9
	b	481.95	150.27 $\pm$ 2.4	1.6	151.92 $\pm$ 2.1	1.4	153.80 $\pm$ 8.6	5.6

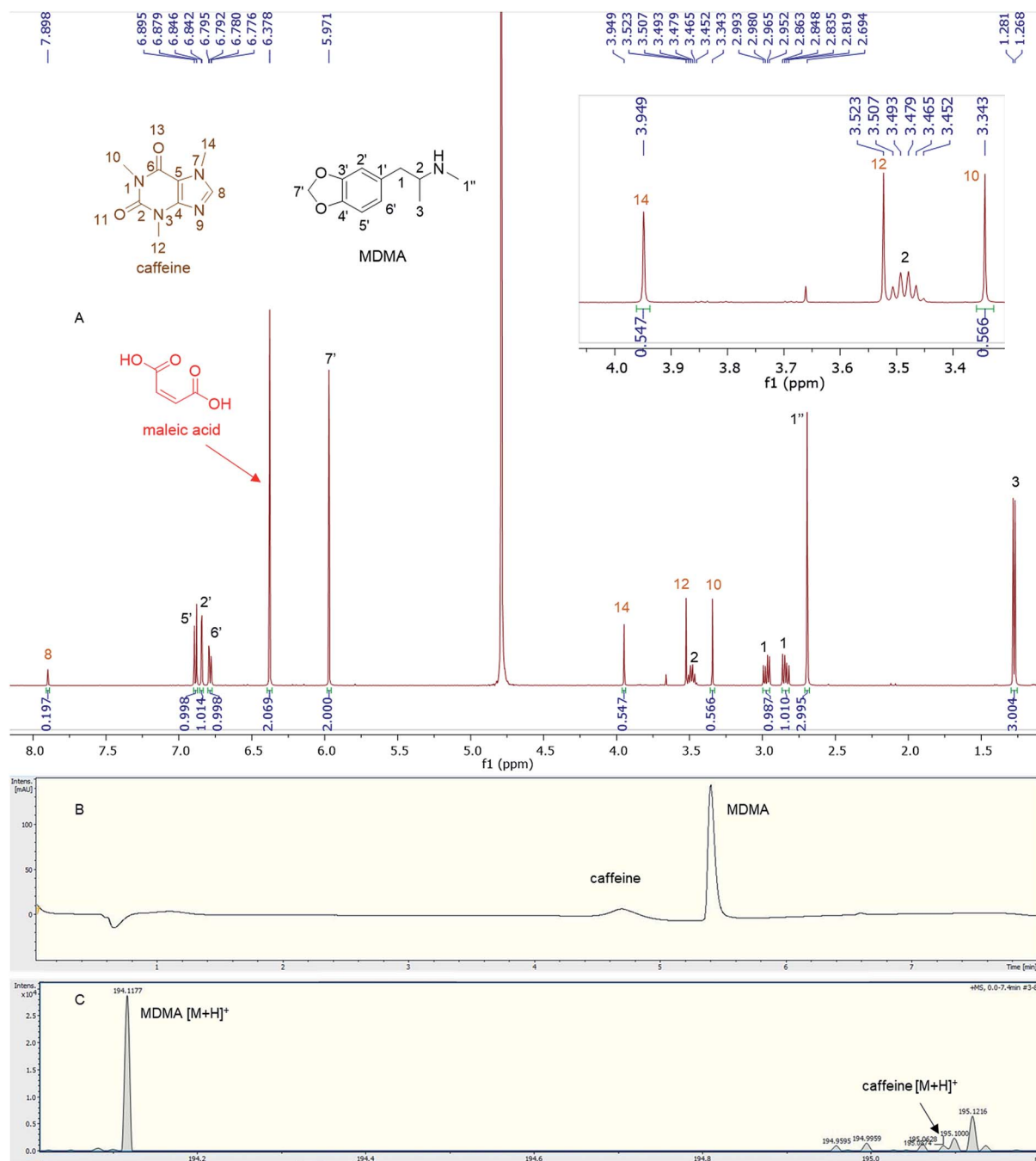


Fig. 4 (A) ^1H NMR (in D_2O) of tablet 11a showing the caffeine peaks with expansion of the region between 3.4–4.5 ppm, (B) UHPLC of tablet 11a showing a broad peak for caffeine at $\text{RT} = 4.7$ min, (C) MS trace of tablet 11a showing MDMA molecular ion and caffeine low intensity molecular ion at $195.0874\text{ }m/z$.

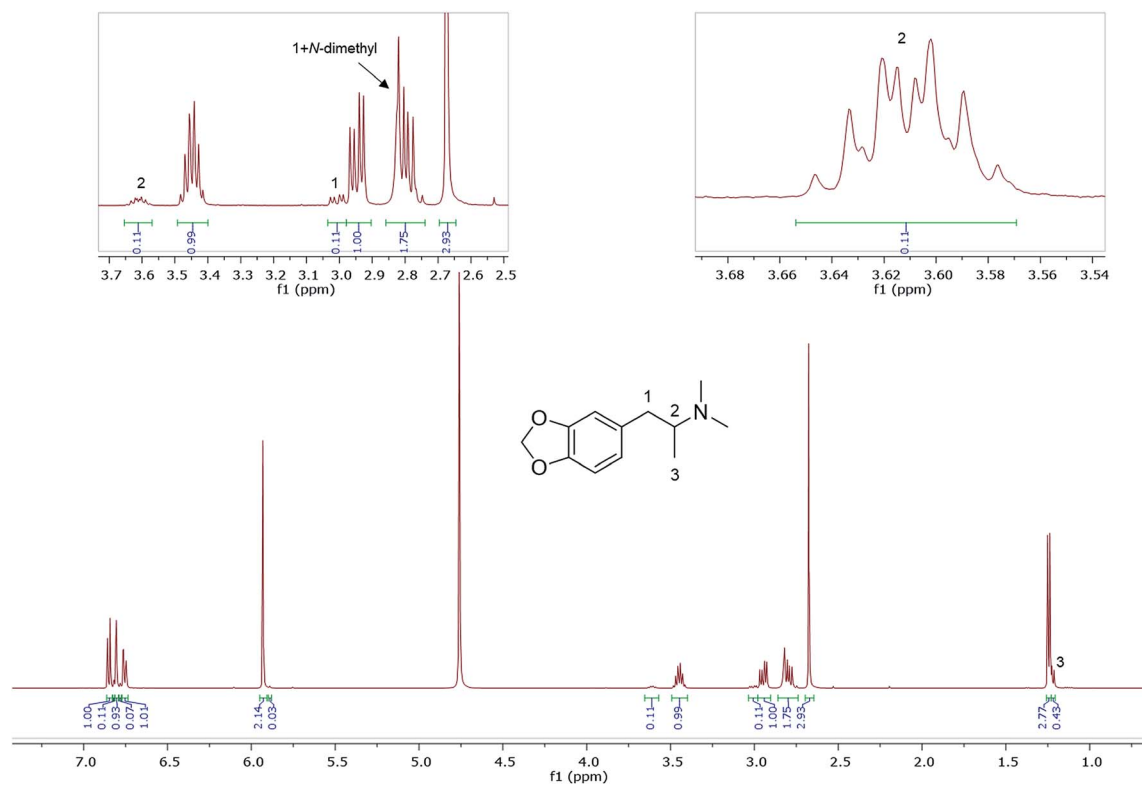


Fig. 5 ^1H NMR (in D_2O) of sample HN165, MDMA containing MDDMA impurity with expansions revealing signals for MDDMA positions 1, 2 and *N*-dimethyl.

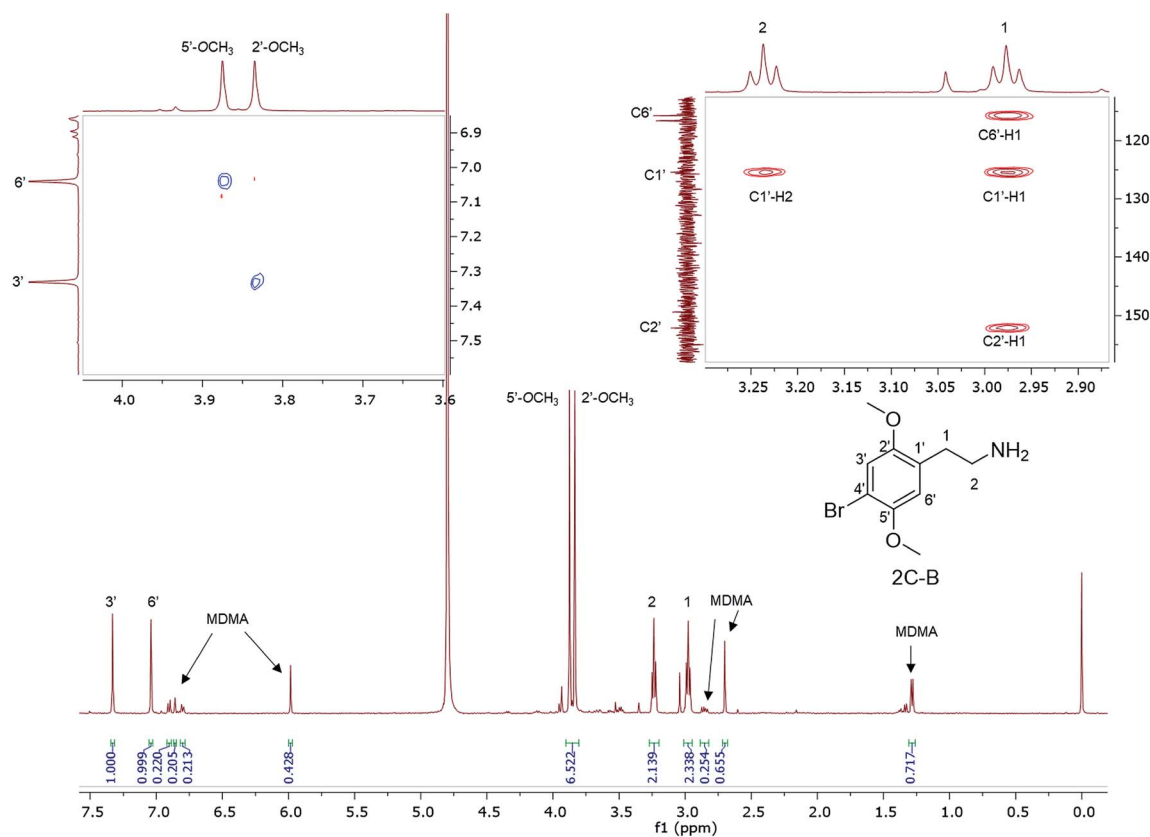
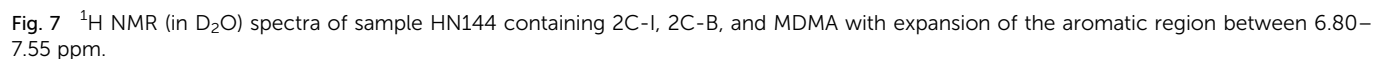


Fig. 6 ^1H NMR (in D_2O) of a 2C-B and MDMA mixture with TMSP 0.00 ppm, (top right) expansion of the HMBC spectra showing key HMBC connectivities, (top left) expansion of NOESY spectra revealing key NOE cross-peak connectivities.

Quantitative analysis revealed variations between different brands of MDMA tablets and within the same brand. In 1990–2009, the average MDMA content of UK tablets was ~50–80 mg, range 20–131 mg, but 96% of tablets contained less than 100 mg MDMA per tablet and with a bimodal distribution of 20–40 mg and 60–80 mg MDMA per tablet, as reported by drug checking services and forensic institutes.³² 53% of all ecstasy tablets tested in 2015 contained over 140 mg of MDMA compared to just 3% in 2009. In 2016, the average MDMA content was

Also shown are ^1H NMR spectra (in D_2O) of other seized MDMA samples cut or contaminated, *e.g.*, with its dimethyl analogue, N,N -dimethyl-3,4-methylenedioxamphetamine (MDDMA), reported to be found in MDMA samples synthesized through a nitropropene and reductive amination route.³⁶ Due to the similar chemical structures, and therefore similar magnetic environments, the NMR spectra displayed multiple



overlapping signals especially in the aromatic region and the methylene protons resonating at 2.73–3.10 ppm (Fig. 5). A total of 7 protons, $2 \times \text{N-CH}_3$ plus one CH from the methylene overlapped with one of the MDMA methylene doublet of doublets at 2.78 ppm. Quantitatively, MDDMA comprised 10% of the sample.

^1H NMR data are reported for MDMA cut with 4-bromo-2,5-dimethoxyphenethylamine (2C-B) (Fig. 6) together with the assignment of the two *p*-methoxy functional groups using NOESY NMR data. 2C-B in combination with MDMA (83% 2C-B, 17% MDMA as molar ratios) was seized as a ground-up orange tablet supplied in a small plastic packet. First synthesized by Shulgin in the mid-1970s,³⁷ the 2C-B pharmacological profile is similar to that of MDMA, it primarily inhibits 5-HT transporters. It also has less potency for dopamine and noradrenalin transporters. There has been a report of tablets sold as MDMA, but rather containing other psychoactive drugs: 3,4-methylenedioxymphetamine (MDA), TFMPP, 2C-B, and caffeine.³⁸

4-Iodo-2,5-dimethoxyphenethylamine (2C-I), the iodo analogue of the 2C family, was detected in sample HN144, a blue powder, in combination with 2C-B and MDMA with a molar percentage of 50% 2C-I, 41% 2C-B and 9% MDMA. The ESI-MS analysis revealed 2C-I $[\text{M} + \text{H}]^+$ 308.0130 *m/z* for $\text{C}_{10}\text{H}_{15}\text{INO}_2$ requires 308.0147. 2C-B $[\text{M} + \text{H}]^+$ 260.0264 *m/z* for $\text{C}_{10}\text{H}_{15}^{79}\text{BrNO}_2$ requires 260.0286, and $[\text{M} + \text{H}]^+$ 262.0253 *m/z* for $\text{C}_{10}\text{H}_{15}^{81}\text{BrNO}_2$ requires 262.0260 (ratio 1 : 1).³⁹ The ^1H NMR of 2C-I and 2C-B overlapped with the exception of the aromatic signals, where the presence of the iodo substituent at the *p*-aromatic position resulted in a greater chemical shift difference in the ^1H spectra between the *meta*- (3') (7.49 ppm) and the *ortho*- (6') (6.96 ppm) protons compared with that found in 2C-B (Fig. 7). This difference is of diagnostic value in determining the type of substituent in the NPS 2C family by ^1H NMR spectroscopy. A survey conducted for self-reporting NPS use on 682 attendees aged between 16–25 at electronic dance music festivals in New York (2015), found that 35% reported the use of NPS including cathinones, with methylone being the most popular cathinone and 2C-I the most popular phenethylamine NPS. The survey also highlighted the multidrug use of MDMA with other NPS and its risk factors for intoxication and possibly death.⁴⁰ In Fig. 7, MDMA is shown to be only a minor component (9%) mixed with 2C-I and 2C-B, but still dangerously sold as “ecstasy”. This is an excellent example that “you do not know what you are buying”, if another one was needed.

Conclusions

In this paper, ^1H q-NMR using the MA IS method was applied successfully to complex samples of MDMA cut with other NPS of closely similar structures seized from night-club venues. A cross-method validation of ^1H q-NMR to analyse seized MDMA tablets is described. ^1H q-NMR proved its versatility in the identification and quantification of the mixing/cutting agents as well as MDMA, with less sample handling (no serial dilution) and better precision (RSD%) compared to chromatographic and MS-based techniques. Comparative analysis with such chromatographic and MS-based methods further corroborated the

results of ^1H q-NMR which provided a fast (15 min), reproducible quantification without the use of a reference standard of the analyte under investigation. MA is a suitable NMR quantitative IS due to its high solubility, simplicity of the peak and non-overlapping signal. Identification of tablets containing such varying amounts of MDMA is a cause of concern to the public. It is also of value to law enforcement officials and health workers. When certain parameters are carefully optimized and considered such as the relaxation delay, number of scans, and S/N, NMR possesses the accuracy and reliability in the quantitative analysis of MDMA in seized tablets with results comparable to other gold standard analytical techniques, *e.g.* GC and LC.

Conflicts of interest

There are no conflicts of interest to declare.

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