Forensic Chemistry 35 (2023) 100523

Contents lists available at ScienceDirect

Forensic Chemistry

journal homepage: www.sciencedirect.com/journal/forensic-chemistry

Synthesis, characterisation and quantification of the new psychoactive substance 1-(1,3-benzodioxol-5-yl)-2-(propylamino)butan-1-one (bk-PBDB, putylone)

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ARTICLE INFO

Keywords:
Novel psychoactive substances
Synthetic cathinones
bk-PBDB
GC-EI-MS
NMR

ABSTRACT

Synthetic cathinones are a continually evolving family of illicit drugs, with novel analogues frequently being detected. This paper reports the detection of 1-(1,3-benzodioxol-5-yl)-2-(propylamino)butan-1-one, bk-PBDB (putylone), within solid dosage forms (tablets) seized by law enforcement for the first time in the United Kingdom. The identity of the compound was confirmed *via* the synthesis of a pure bk-PBDB reference standard and direct spectral comparison by ^{1}H NMR and GC-EI-MS analysis. A full analytical profiling of bk-PBDB by nuclear magnetic resonance (NMR), attenuated total reflection Fourier-transform infrared (FTIR) spectroscopy and gas chromatography-electron ionisation-mass spectrometry (GC-EI-MS) is reported and shows good concordance between the seized sample and the reference standard. A validated GC-EI-MS method for the routine quantification of the cathinone in bulk forensic samples (LOD: 0.09 μg/mL, LOQ: 0.26 μg/mL) was also developed and using this method, the seized tablets were determined to contain a mixture of bk-PBDB (130.6–135.5 mg/tablet) and caffeine (40.2–43.4 mg/tablet) respectively.

1. Introduction

The history, chemistry, and pharmacological action of synthetic cathinones have been the subject of several reviews [1–4]. Synthetic cathinones represent the second largest group of new psychoactive substances (NPS) that are monitored by the United Nations Office on Drugs and Crime (UNODC) [5] and the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) [6]. Specifically, 3,4-methylenedioxy-*N*-alkylcathinones (Scheme 1), which mimic the psychostimulant effects of 3,4-methylenedioxymethamphetamine (MDMA) [1,2,7,8], are characterized by the addition of a 3,4-methylenedioxy-moiety to the aromatic ring and alkyl-substitutions in both the amino group and α-carbon of the side chain (e.g., methylone, **1**; butylone, **2**; ethylone, **3**; eutylone, **4**; pentylone, **5** and *N*-ethylpentylone, **6**) [6]. This sub-family constitutes approximately 18% of the total number of synthetic cathinones recorded within the United Nations Early Warning Advisory on NPS database [5] and many are frequently encountered products sold under a variety of guises including *"research chemicals"*, *"plant food"*, *"bath salts"* or *"glass cleaner"*. These psychostimulants are usually available in several solid-dosage forms including powders or crystals (in colours ranging from white, off-white, beige to brown), tablets (resembling *"Ecstasy"* pills) or capsules.

Once absorbed, 3,4-methylenedioxy-*N*-alkylcathinones produce a variety of behavioural effects, and can affect locomotor activity, thermoregulation, learning and memory, primarily mediated by interactions with the dopamine, noradrenaline and/or serotonin monoamine transporters [1,9]. Short-term adverse effects reported following use are variable and may include loss of appetite, blurred vision, anxiety, post-use depression, confusion, hallucinations, short-term psychosis, and mania [10]. Individuals who have been intoxicated have displayed a

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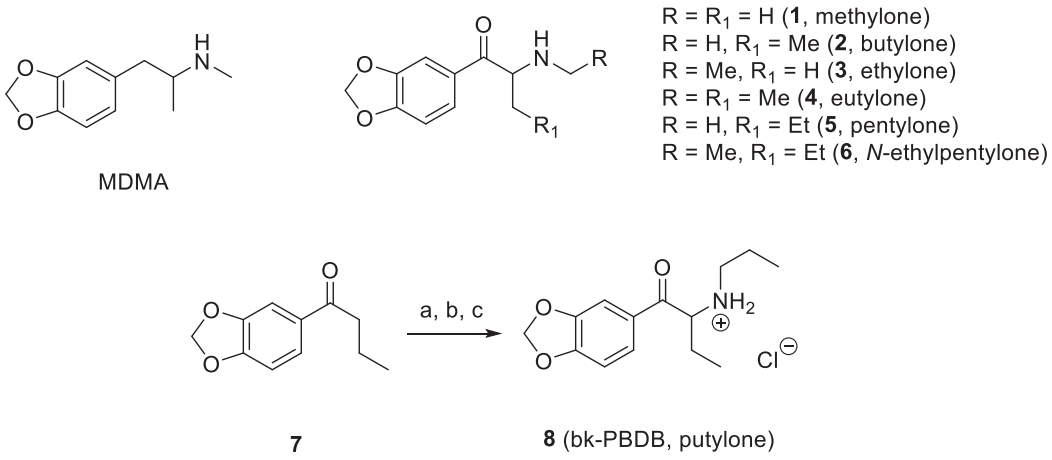
https://doi.org/10.1016/j.forc.2023.100523
Received 16 June 2023; Received in revised form 31 July 2023; Accepted 7 August 2023
Available online 10 August 2023



Scheme 1. *Reagents and Conditions:* (a) Br_2/HBr (48% in water)/CH_2Cl_2/rt/1 h; (b) *n*-propylamine/benzene/Δ/12 h; (c) 3 M HCl in CMPE (23% yield from **7**).

Fig. 1. Photograph of front and reverse sides of blue, *"Donald Trump"* embossed *"Ecstasy"* tablets (**GM443**) suspected to contain MDMA (mean tablet weight = 686.7 mg) obtained in Manchester, UK (20th December 2022). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

variety of symptoms common to sympathomimetic toxicity including palpitations, tachycardia, agitation, aggression, hallucinations, coma and, in some cases, death [11]. Habitual users have also reported the development of tolerance, dependence, or withdrawal symptoms with prolonged use [12]. Between 2008 and 2021, as a result of increasing use of generic scheduling to prohibit the possession, supply, and production of many synthetic cathinone-based stimulants and due to their inherent adverse effects associated with fatal intoxications the Commission on Narcotic Drugs specifically placed several 3,4-methylenedioxy-*N*-alkylcathinones under international control, within schedule II (methylone, 2015 [13]; ethylone, 2017 [14]; *N*-ethylpentylone, 2019 [15] and eutylone, 2022 [16]) of the United Nations Convention on Psychotropic Substances (1971). Despite these control measures, synthetic cathinones continue to be encountered prominently in casework with one review, from early 2019 to mid-2022, reporting 29 novel structures detected for the first time [17]. One recently detected substance is 1-(1,3-benzodioxol-5-yl)-2-(propylamino)butan-1-one (**8**, bk-PBDB, putylone), the *N*-propyl substituted synthetic cathinone analogue of butylone, which was first reported in Czechia (19th January 2015), then subsequently in the USA (21st July 2022) and Canada (30th August 2022) [5]. The published analytical data for this specific compound is limited (GC-EI-MS and LC-qTOF-MS only) [18] and no validated methods for its quantification have been reported. Bk-PBDB has been co-detected with *N, N*-dimethylpentylone (dipentylone) in four toxicological samples, however its pharmacology, pharmacokinetics and/or toxicological profile has not been fully elucidated [18]. Due to the potential health risk associated with (**8**) there is an increased need for new reliable methods of its detection to reduce potential drug-related harms should it become established within the market. This paper presents the synthesis, full structural characterization and development of a validated gas chromatography-electron ionisation-mass spectrometry (GC-EI-MS) approach for quantification of 1-(1,3-benzodioxol-5-yl)-2-(propylamino)butan-1-one (**8**, bk-PBDB, putylone), found in solid dosage forms, mis-sold as MDMA (*"Ecstasy"*) tablets, obtained in Manchester, UK (20th December 2022) (Fig. 1). Analytical features of bk-PBDB were characterized by ^{1}H- and ^{13}C{^{1}H}- nuclear magnetic resonance (NMR) spectroscopy, GC-EI-MS and attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy to provide both a comprehensive analytical profiling and validated chromatographic method for this substance. To the best of our knowledge, this is the first paper detailing the synthesis, comprehensive structural characterization of bk-PBDB and provision of a validated GC-EI-MS method for the routine quantification of the synthetic cathinone within bulk samples, which will be valuable as a reference point for future forensic analysis of this and related compounds.

2. Materials and methods

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 [19]. Reference standards of 1-(1,3-benzodioxol-5-yl)-2-(methylamino)butan-1-one hydrochloride (**2**) and 1-(1,3-benzodioxol-5-yl)-2-(ethylamino)pentan-1-one hydrochloride (**6**) were obtained from LGC Standards (Teddington, UK). The reference standard of 1-(1,3-benzodioxol-5-yl)-2-(propylamino)butan-1-one hydrochloride (**8**) was synthesized, purified and obtained as a stable, colourless powder using an adaptation of the protocols reported by Santali et al. [20] and Fujii et al. [21] (see Supplementary Information for synthetic procedure and characterization data). High-resolution mass spectrometry (HRMS) data was obtained on an Agilent 6540 LC-QToF spectrometer in positive electrospray ionization mode. The suspected *"Ecstasy"* tablets (**GM443**, n = 3, mean tablet weight = 686.7 mg, Fig. 1) were obtained in Manchester, UK and provided, to MANchester DRug Analysis and Knowledge Exchange (MANDRAKE), by Greater Manchester Police on 20th December 2022 in accordance with Manchester Metropolitan University's Home Office license requirements and agreed procedures.

2.1. Nuclear magnetic resonance (NMR) spectroscopy

High-field ^{1}H NMR and ^{13}C{^{1}H} NMR spectra (10.0 mg/mL in DMSO-d_6) were acquired on a JEOL ECA-500 (JEOL, Tokyo, Japan) NMR spectrometer operating at a proton resonance frequency of 500

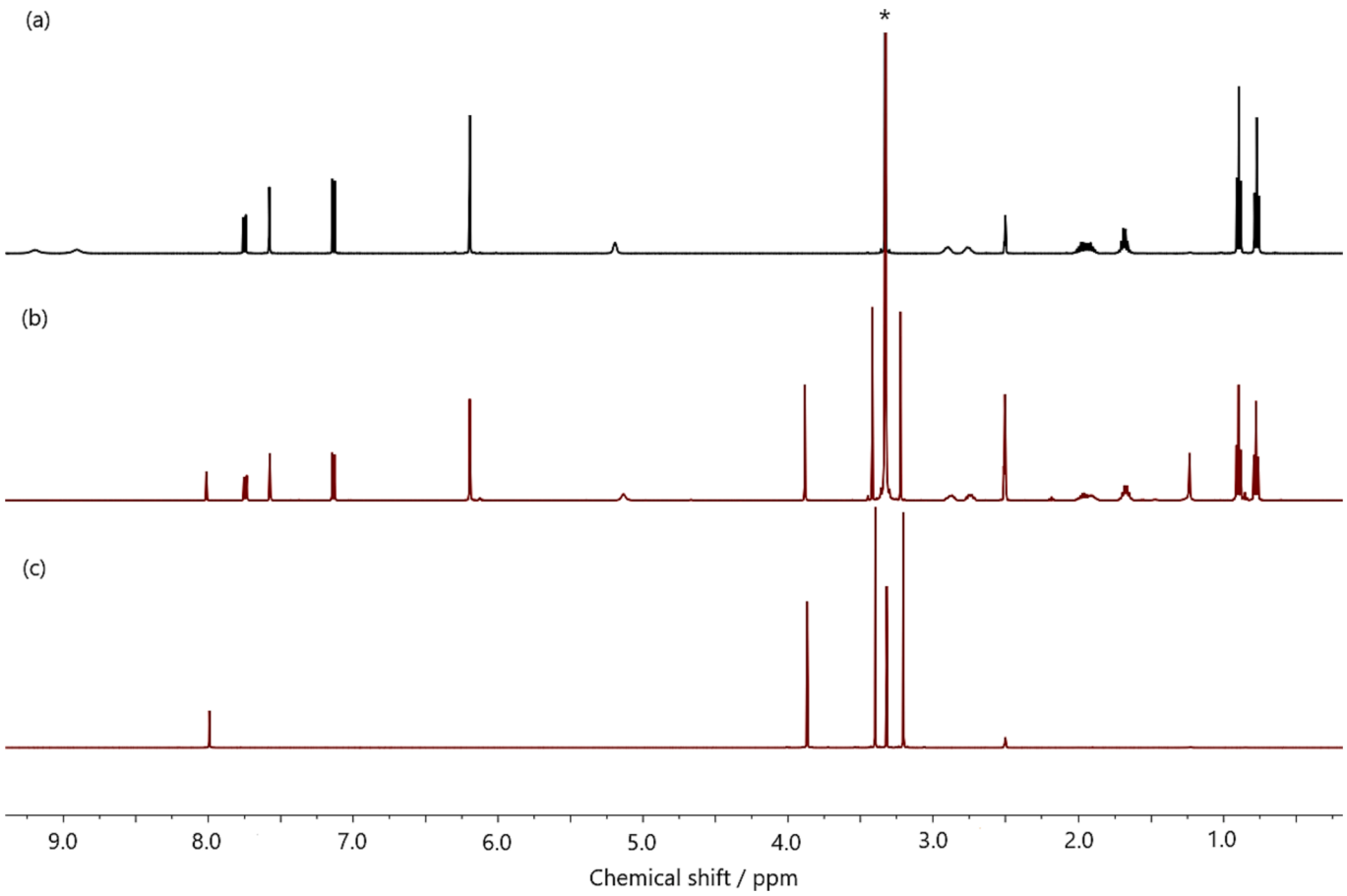


Fig. 2. Representative ^{1}H NMR spectra of (a) putylone (**8**) reference standard, (b) seized blue, *"Donald Trump"* embossed *"Ecstasy"* tablets (**GM443**) suspected to contain MDMA and (c) caffeine reference standard acquired in DMSO-d_6 at 500 MHz. *Note:* * = residual water (δ 3.30). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

MHz, referenced to the residual solvent peak (DMSO-d_6: ^{1}H NMR δ = 2.50 ppm, ^{13}C{^{1}H} NMR δ = 39.52 ppm, respectively) [22]. Low-field ^{1}H NMR spectra were acquired using a Pulsar benchtop NMR spectrometer (Oxford Instruments, Abingdon, UK) operating at a frequency of 59.7 MHz. The temperature of the probe was calculated to be 308.5 K by measuring the separation (in Hz, $\Delta\delta$) between the CH_2 and OH signals of neat ethylene glycol and implementing the equation T [K] = 466.5–102.00 $\Delta\delta$ [23]. A micro-spatula tip of the material (*ca.* 5–10 mg) was dissolved in DMSO-d_6 (0.6 mL). All samples were filtered through a 0.45 μm polyvinylidene difluoride syringe filter directly into an NMR tube. After the sample had been inserted, an automated procedure began whereby the instrument would lock on to the deuterated signature of DMSO (thus used as a chemical shift reference) before acquiring 16 scans [24]. Following acquisition, the data was processed in MNova (Mestrelab Research, Santiago de Compostela, Spain) using an automated script file. The processed free induction decay (FID) was then analyzed by the pattern recognition algorithm, NPS Pattern Match (Oxford Instruments, Abingdon, UK), developed 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 tentative, unreliable, or unknown. Binary mixtures are accommodated by extending the pattern search with synthetically generated mixture spectra of pairwise combinations of the reference library [24,25].

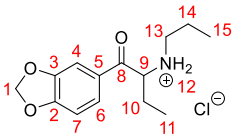
2.2. Attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy

Infrared spectra were obtained in the range 4000–650 cm^{-1} using a Thermo Scientific Nicolet iS10ATR-FTIR instrument (Thermo Scientific, Rochester, USA) equipped with diamond attenuated total reflectance (ATR) accessories. Samples were ground using a pestle and mortar prior to analysis to ensure good sample homogeneity. Sixteen scans were acquired of each sample (*ca.* 5–10 mg) with a resolution of 4 cm^{-1} (line spacing 1.928 cm^{-1}). Qualitative identification of the components present in a sample were performed using OMNIC (Thermo Scientific, Rochester, USA) against defined libraries (Scientific database (version 10.5.3.738) and SWGDRUG IR Library (version 2.1)). Both search platforms utilised a correlation search to determine the component(s) present [25]. The highest match score was used for identification purposes.

2.3. Gas chromatography-Mass spectrometry (GC–MS)

GC–MS analysis was performed using an Agilent 7890B GC and a MS 5977B selective mass detector (Agilent Technologies, Wokingham, UK). The mass spectrometer 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) using helium as the carrier gas at a constant flow rate of 1.0 mL/min. The initial oven temperature was set to 190 °C prior to be ramped to 280 °C in 35 °C/min intervals. A hold time of 0.5 min was used at 280 °C to give a total run time of 10 min. A 0.5 μL aliquot of the sample was injected with a split ratio of 50:1. The injector

Table 1
^{1}H and ^{13}C NMR data (in DMSO-d_6) for the bk-PBDB reference standard (**8**). Analogous ^{1}H NMR data for blue, *"Donald Trump"* embossed *"Ecstasy"* tablets (**GM443**) is also included for comparison purposes. †Additional signals for caffeine observed at δ 8.01 (s, aromatic H), 3.88 (s, CH_3), 3.42 (s, CH_3) and 3.22 (s, CH_3).



8 (bk-PBDB, putylone)

Assignment	Reference Standard (**8**, bk-PBDB)			Sample **GM443**†
	^{1}H	^{13}C {^{1}H}	DEPT-135	^{1}H
1	6.19 (s, 2H)	102.6	−ve	6.19 (s, 2H)
2		152.9	+ve	
3		148.2	+ve	
4	7.57 (d, 1H, $^{4}J_{HH}$ = 1.8 Hz)	107.8	+ve	7.57 (d, 1H, $^{4}J_{HH}$ = 1.7 Hz)
5		128.5	+ve	
6	7.75 (dd, 1H, $^{3}J_{HH}$ = 8.3 Hz, $^{4}J_{HH}$ = 1.8 Hz)	125.9	+ve	7.74 (dd, 1H, $^{3}J_{HH}$ = 8.3 Hz, $^{4}J_{HH}$ = 1.8 Hz)
7	7.14 (d, 1H, $^{3}J_{HH}$ = 8.3 Hz)	108.6	+ve	7.13 (d, 1H, $^{3}J_{HH}$ = 8.2 Hz)
8		194.0	N/A	
9	5.19 (app. quin., 1H, $^{3}J_{HH}$ = 5.1 Hz)	61.3	+ve	5.14 (s, 1H)
10	1.87–2.03 (m, 2H)	23.2	−ve	1.86–2.02 (m, 2H)
11	0.77 (t, 3H, $^{3}J_{HH}$ = 7.6 Hz)	8.4	+ve	0.78 (t, 3H, $^{3}J_{HH}$ = 7.6 Hz)
12	9.20 (s, 1H) and 8.92 (s, 1H)	N/A	N/A	9.23 (s, 1H) and 8.92 (s, 1H)
13	2.90 (app. q, 1H, $^{3}J_{HH}$ = 9.1) and 2.76 (app. q, 1H, $^{3}J_{HH}$ = 9.2)	47.5	−ve	2.85–2.92 (m, 1H) and 2.71–2.77 (m, 1H)
14	1.63–1.74 (m, 2H)	19.1	−ve	1.62–1.73 (m, 2H)
15	0.90 (t, 3H, $^{3}J_{HH}$ = 7.5 Hz)	10.9	+ve	0.90 (t, 3H, $^{3}J_{HH}$ = 7.4 Hz)

was maintained at 280 °C and the GC interface temperature maintained at 300 °C. The MS source and quadrupole temperatures were set at 230 °C and 150 °C. Mass spectra were obtained in full scan mode (50–550 amu; qualitative analysis) and Selected Ion Monitoring (SIM) mode (quantitative analysis) using three specific fragment ions for each analyte. A base ion fragment was used for quantification with the remaining two ions used as qualifiers (Table 2). Stock solutions for quantification were prepared to 0.1 mg/mL and then diluted further to six concentrations between 0 and 12 μg/mL containing methyl stearate (20 μg/mL). Samples were prepared in the same manner, diluted to 10 μg/mL and spiked with methanolic methyl stearate solution, acting as an internal standard, to a final concentration of 20 μg/mL.

Validation was performed using an Agilent 7890B GC and a MS5977B mass selective detector (Agilent Technologies, Wokingham, UK) employing the parameters detailed above. Mass spectra was obtained under Selected Ion Monitoring (SIM) mode. The GC–MS method was validated in accordance with the ICH guidelines [26] using the following parameters: linearity, accuracy, precision, limit of detection (LOQ). *Linearity, precision:* six replicate injections of the calibration standards were performed, and the data analysed under the same conditions. The RSD % was calculated for each replicate test sample. *Accuracy (percentage recovery study):* determined from spiked samples prepared in triplicate at three levels over a range of 80–120% of the target concentration (10 μg/mL). The percentage recovery and RSD % were calculated for each of the replicate samples. *Limits of detection and quantification:* six replicate injections of the calibration standards were performed, and the data analysed under the same conditions. The limits of detection and quantification were determined based on the standard deviation of the response and slope of the calibration curve, where 3.3 and 10.0 times the standard deviation of the response was used to calculate the LOD and LOQ respectively.

Table 2
GC-EI-MS validation data (selective ion monitoring mode) for the quantification of (**2**), caffeine, (**6**) and (**8**). See Fig. 5a for representative total ion chromatogram. *Note:* Methyl stearate: t_R = 9.08 min; SIM ions (base peak indicated in bold) = **74.0**, 87.0 and 143.0.

Parameter	Analyte			
	Butylone (**2**)	Caffeine	*N*-ethylpentylone (**6**)	Putylone (**8**)
SIM ions (for quantification)[a]	**72.1**, 121.0, 148.9	67.0, 109.0, **194.0**	58.0, **100.0,** 149.0	58.0, **100.0,** 149.0
t_R (min)	4.94	5.90	7.12	7.35
RRT[b]	0.67	0.80	0.97	1.00
R_s^c	–	14.2	16.0	3.4
A_s^d	1.2	0.7	1.0	1.2
N (plates)[e]	110,651	96,122	140,006	233,140
H (x10^{-4} mm)[f]	2.71	3.12	21.4	1.29
Linearity (r^2)	0.999 [g]	0.999 [h]	0.999[i]	0.999[j]
LOD (μg/mL)[k]	0.09	0.13	0.09	0.09
LOQ (μg/mL)[l]	0.27	0.39	0.28	0.26
Precision (%RSD, n = 6)				
2.0 μg/mL	1.76	4.61	1.13	0.62
4.0 μg/mL	0.66	3.90	0.86	0.99
6.0 μg/mL	1.38	1.87	1.39	0.89
8.0 μg/mL	0.82	0.74	0.81	1.11
10.0 μg/mL	1.27	1.33	1.30	0.97
12.0 μg/mL	1.27	0.93	1.33	0.81
Assay Recovery (%, n = 3)				
8.0 μg/mL (80%)	97.0	89.7	98.5	99.4
10.0 μg/mL (100%)	96.5	89.5	96.4	99.5
12.0 μg/mL (120%)	98.5	91.0	105.5	100.8
Average recovery (%)	97.3	90.1	100.1	99.9
%RSD	1.64	1.47	4.31	0.95
Relative Error (%)[m]	2.67	9.92	0.14	0.11

Key: [a]Base peak indicated in bold; [b]Relative retention time (with respect to putylone, **8**); [c]Resolution; [d]Asymmetry (or tailing) factor; [e]Number of theoretical plates; [f]Height of a theoretical plate; [g]y = 0.0629x – 0.056; [h]y = 0.0582x – 0.0064; [i]y = 0.0610x – 0.0055; [j]y = 0.0541x – 0.0057; [k]Limit of Detection (determined from the standard deviation of the response and slope of the calibration curve); [l]Limit of Quantification (determined from the standard deviation of the response and slope of the calibration curve); [m]Deviation between the average experimental recovery and a 100% recovery.

3. Results and discussion

3.1. Nuclear magnetic resonance (NMR) spectroscopy

The reference standard of putylone (**8**, bk-PBDB) was synthesized, purified and obtained as a stable, colourless powder (Scheme 1, 23% overall yield from **7**) using an adaptation of previously reported protocols [20,21]. The purity (>99.5%) of (**8**) was calculated by ^{1}H NMR using the relative concentration determination method described by Pauli et al. [27]. The synthesised putylone (**8**) standard was analysed by NMR to facilitate its characterisation. ^{1}H and ^{13}C{^{1}H} NMR spectra of compound (**8**) are shown in Figs. 2a and 3, respectively. The full assignment of ^{1}H and ^{13}C{^{1}H} NMR signals of bk-PBDB (**8**) is presented in Table 1. All spectra including the 2D correlation NMR spectra are available within the Electronic Supplementary Information (Figs. S1–S6). The assignment relied on correlation spectroscopy (COSY;

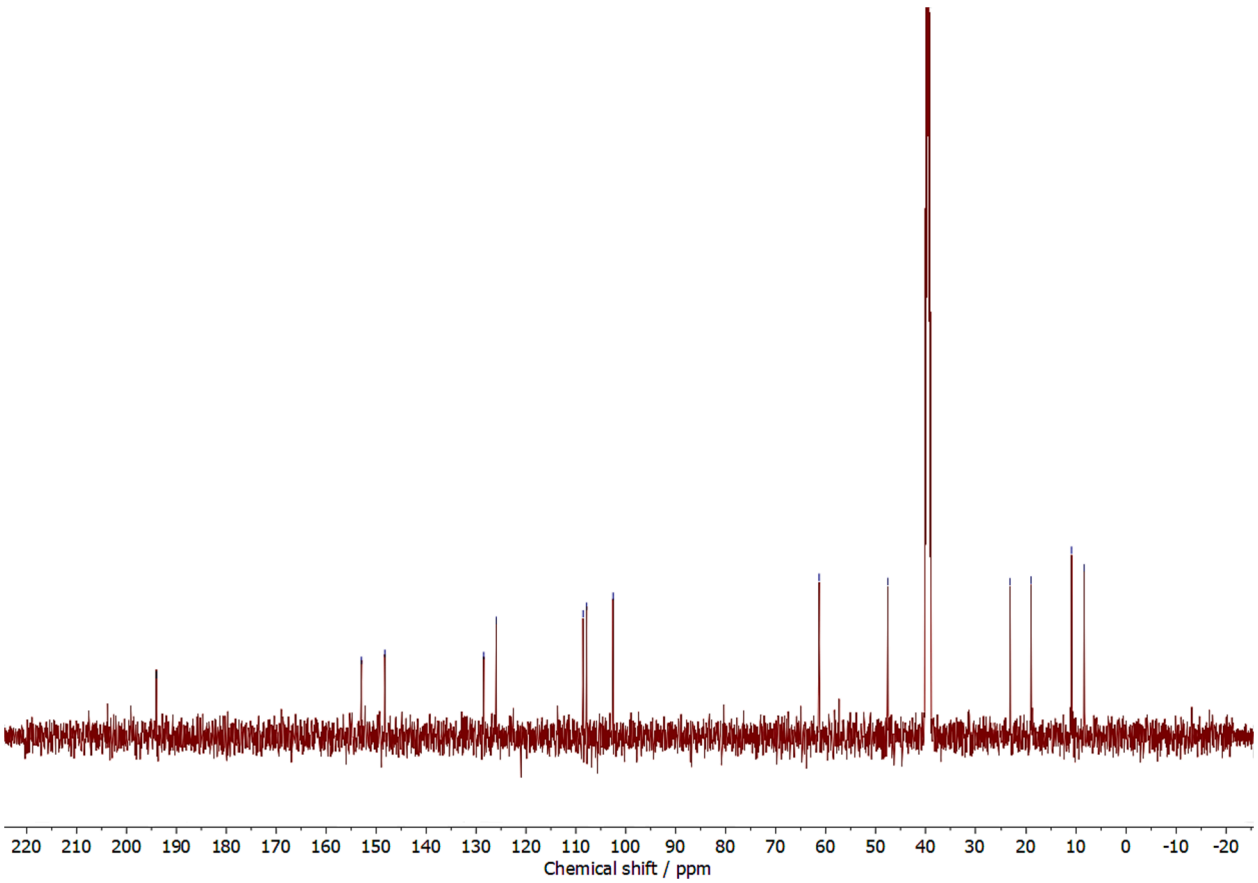


Fig. 3. $^{13}C\{^{1}H\}$ NMR spectrum of putylone (**8**) reference standard acquired in DMSO-d_6 at 125 MHz.

Fig. S3) to observe ^{1}H–^{1}H couplings, heteronuclear multiple quantum coherence (HMQC; Fig. S4) for one-bond ^{1}H–^{13}C couplings, heteronuclear multiple bond correlation (HMBC; Fig. S5) for two- or three-bond ^{1}H–^{13}C couplings (acquired using an evolution period equivalent to $^{2}J_{HC} = 8$ Hz) and Distortionless Enhancement by Polarization Transfer (DEPT, Fig. S6) to determine the multiplicity of carbon atoms.

The high-field (500 MHz) ^{1}H NMR spectra of (**8**) in DMSO-d_6 are shown in Fig. 2a and Fig. S1. The 3,4-methylenedioxy ring aromatic protons are evidenced by the signals at δ 7.75 (doublet of doublets), 7.57 (doublet) and 7.14 (doublet). The signal at δ 7.75 possesses *J*-coupling of 1.8 ($^{4}J_{HH}$) and 8.3 ($^{3}J_{HH}$) Hz. The former is reflected in the signal δ 7.57, whereas the latter is also possessed by the signal at δ 7.14. These couplings are reflective of the 1,3,4-tri-substituted nature of the ring system. Cross-peaks for these interactions are observed in the respective ^{1}H–^{1}H COSY spectrum (Fig. S3). The 3,4-methylenedioxy protons are present as a singlet at δ 6.19. The proton attached to the chiral centre is observed at δ 5.19. This signal shows a cross-peak in the ^{1}H–^{1}H COSY NMR spectrum to a signal at δ 1.95, which presents as a multiplet. This multiplet shows a further cross-peak to a triplet at 0.77 ($^{3}J_{HH} = 7.6$ Hz). This terminates the butyl chain. The *N*-propyl chain consists of signals at δ 2.90, 2.76, 1.69 and 0.90. The latter is a triplet ($^{3}J_{HH} = 7.5$ Hz) whereas the other two signals are best described as multiplets. The signals at δ 2.90 and 2.76 are due to diastereotopic protons and as such they give rise to two unique signals. The two NH protons are observed as two singlets at δ 9.20 and 8.92. Both signals are broad singlets. Therefore, all signals in the reference standard are accounted for in the ^{1}H NMR spectrum.

The reference material (**8**) was further characterised by ^{13}C NMR spectroscopy. The $^{13}C\{^{1}H\}$ NMR spectrum (Fig. 3), possesses 14 signals. The most deshielded signal is that of the carbonyl at δ 194.0. The quaternary nature of this signal was confirmed by DEPT (Fig. S6). There are seven signals in the aromatic region and six in the aliphatic. Of the seven signals in the aromatic region, three of them are quaternary (δ 152.9, 148.2 and 128.5) as shown by their absence in the DEPT-135 spectrum. The remainder are CH environments (δ 125.9, 108.6 and 107.8), with the exception of the signal at 102.6 which is emissive in the DEPT-135 spectrum, and therefore belongs to the methylene carbon that is bonded to two oxygens. The most deshielded aliphatic signal is located δ 61.3 and is absorptive in the DEPT-135 NMR spectrum; this signal is attributed to the chiral carbon. This was confirmed through the use of ^{1}H–^{13}C HMQC. The remaining five signals are methylene carbons (δ 47.5, 23.2 and 19.1) or methyl signals (δ 10.9 and 8.4); the most deshielded methylene environment belongs to the methylene directly bonded to nitrogen.

Fig. 2b shows a representative ^{1}H NMR spectrum of the blue, *"Donald Trump"* embossed *"Ecstasy"* tablets (**GM443**), also collected in DMSO-d_6. The correlation between this spectrum and that of the reference sample is very significant. There are four additional signals at δ 8.01, 3.88, 3.42 and 3.22, which are in a ratio of 1:3:3:3; these all belong to caffeine, which is present as an adulterant in the sample. As well as the GC–MS analysis (Section 3.4), from which caffeine was confirmed, these signals in the ^{1}H NMR spectrum of the seized material correspond very well with Fig. 2c, which is a sample of caffeine dissolved in DMSO-d_6. The tabulated data is shown in Table 1. The biggest change between **GM443** and the reference standard is that of the chiral proton, which shows a 0.05 ppm difference. This environment could be sensitive to changes in the matrix caused by the presence of caffeine.

3.2. Analysis by benchtop NMR in-conjunction with a reference database

Sample **GM443** when subjected to analysis by an automated benchtop (60 MHz) ^{1}H NMR approach, which has been reported previously for the analysis of similar 3,4-methylenedioxy moiety containing

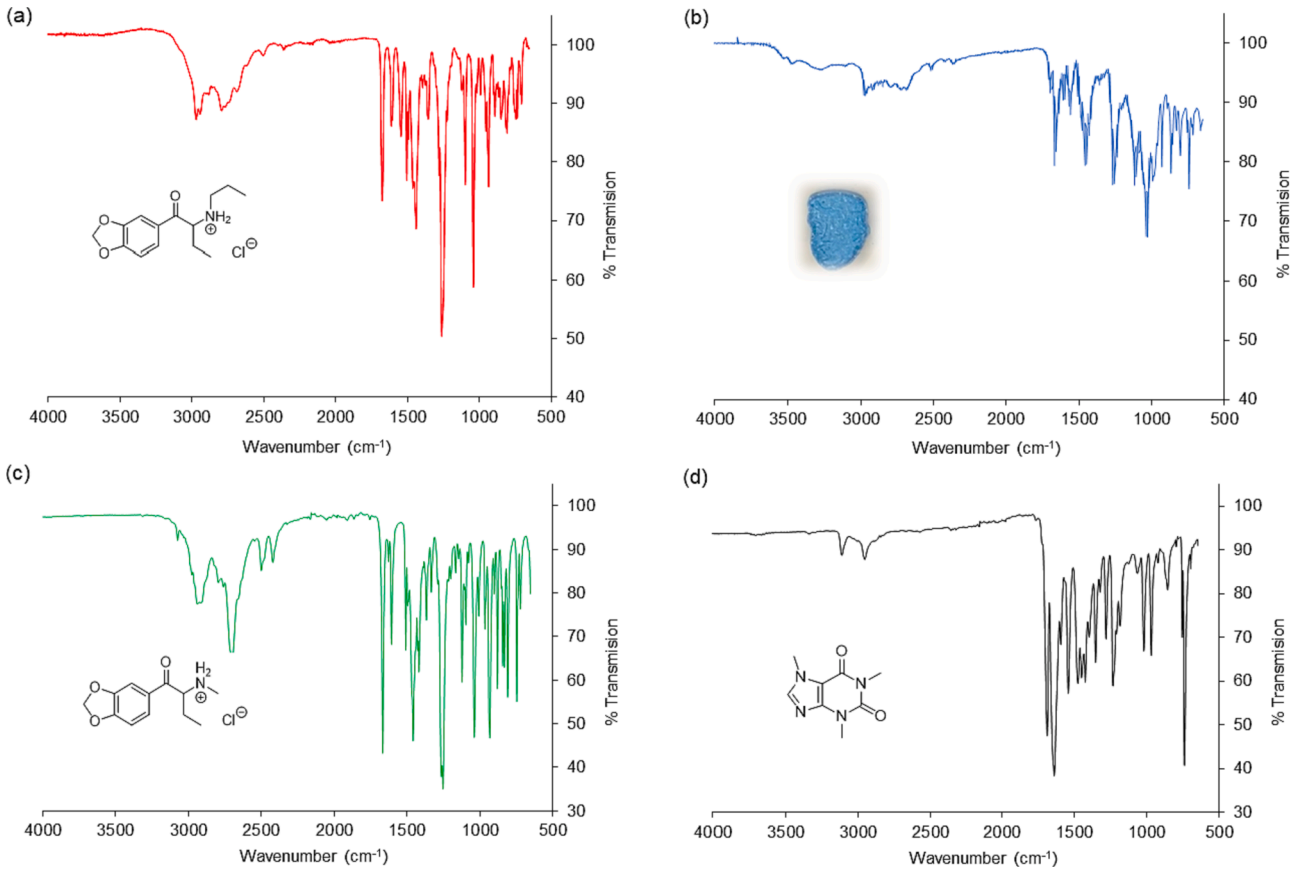


Fig. 4. (a) ATR-FTIR spectrum of synthesised putylone hydrochloride (**8**, bk-PBDB); (b) Representative ATR-FTIR spectrum of blue, *"Donald Trump"* embossed *"Ecstasy"* tablets (**GM443**) suspected to contain MDMA; (c) ATR-FTIR spectrum of butylone hydrochloride (**2**); (d) ATR-FTIR spectrum of caffeine. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

compounds [24], returned butylone (**2**) and oxandrolone (mixture) as the highest rank hit. The hit score was 0.863, indicative of some uncertainty in the returned hit (threshold 0.82). The second highest ranked hit was butylone (hit score = 0.862). However, the structural similarity of putylone and that of the structure of butylone (same 3,4-methylenedioxy moiety as well as butyl chain) showcases that the class of compound could be identified in the seized sample, despite the fact that (**8**) was not present in the database.

The reference sample (**8**) was then added to the database and the seized sample re-ran. Now, the highest hit is that of putylone with a hit score of 0.920. The following hits are those of mixtures, with putylone being the dominant API in each case. The tenth ranked hit is that of putylone and caffeine, with a hit score of 0.894. The second to ninth ranked matches have hit scores of 0.900 to 0.894; the hit scores of the ninth (putylone and harmaline) and tenth ranked matches have identical scores to four decimal places.

3.3. Attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy

The infrared spectrum of putylone (Fig. 4a) collected on an ATR-FTIR spectrometer shows the characteristic bands associated with the hydrochloride salts of 3,4-methylenedioxycathinones such as butylone (Fig. 4c) [28]. The spectrum shows strong, broad bands between 2490 and 2965 cm^{-1} corresponding to a combination of C-H stretches and absorption bands from the ammonium salt, an absorbance at 1672 cm^{-1} for the carbonyl group in conjugation with the 3,4-methylenedioxyphenyl moiety, aromatic C═C ring vibration bands at 1603 cm^{-1}, a strong C-O stretching vibration from the 3,4-methylenedioxy group at 1261 cm^{-1} and a medium C-N stretching band at 1030 cm^{-1}.

The infrared spectra of the seized tablets (Fig. 4b) were acquired, under identical conditions, and automatically compared to the OMNIC (Thermo Scientific, Rochester, USA) against defined libraries (Scientific database (version 10.5.3.738) and SWGDRUG IR Library (version 2.1)). The closest database match was determined based on spectral similarity (highest match score). Visually these tablets are similar to *"Ecstasy"* (MDMA) tablets with each batch being colored and stamped with a particular motif or logo [29]. In each case, infrared analysis confirmed that none of the seized samples described herein contained MDMA and the synthetic cathinone, butylone (match score: 51–53%), was detected. A high level of adulteration can mask active ingredient signals and affect the identification of seized drug samples by infrared analysis [24,25].

3.4. Gas chromatography-electron ionisation-mass spectrometry (GC-EI-MS)

The qualitative GC-EI-MS method (*ca.* 10 min) used required an extremely straightforward solvation of the samples in methanol (10.0 μg/mL containing 20.0 μg/mL methyl stearate as internal standard) followed by direct injection into the instrument. No derivatization step was required. An exemplar total ion chromatogram demonstrating the separation of butylone (**2,** t_R = 4.94 min), caffeine (t_R = 5.90 min), *N*-ethylpentylone (**6,** t_R = 7.12 min) and putylone (**8**, bk-PBDB, t_R = 7.35 min) is presented in Fig. 5a. The use of GC-EI-MS facilitated the visualization of the mass spectral data for (**8**), and this is presented in Fig. 5c. The GC-EI-MS total ion chromatogram of a methanolic extract (containing 20.0 μg/mL methyl stearate as internal standard) of the seized tablets (**GM443**) and the corresponding electron ionisation (EI) mass spectra are presented in Fig. 5b, d and e respectively. The data (scan mode) indicates that the seized sample contains two components:

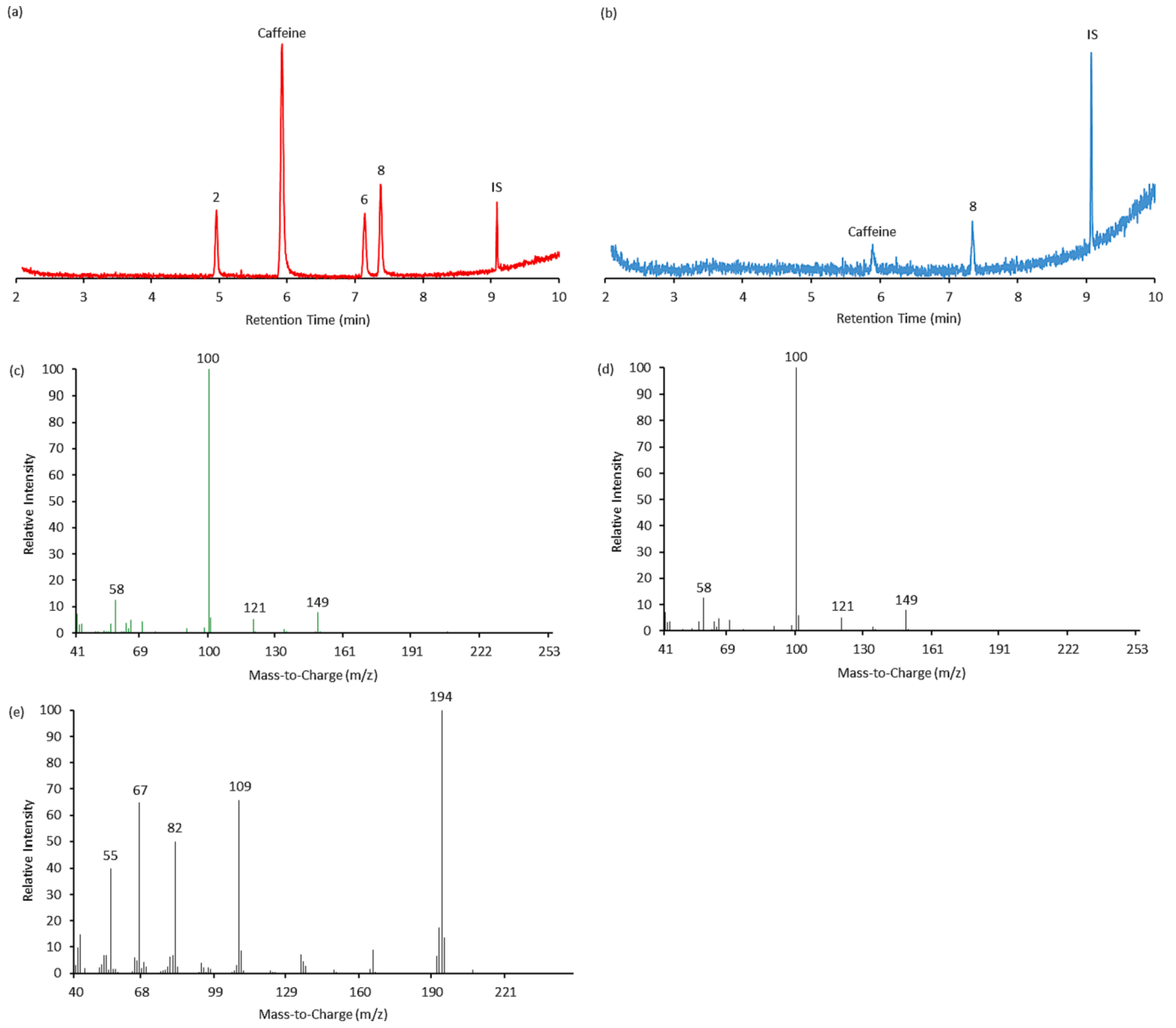
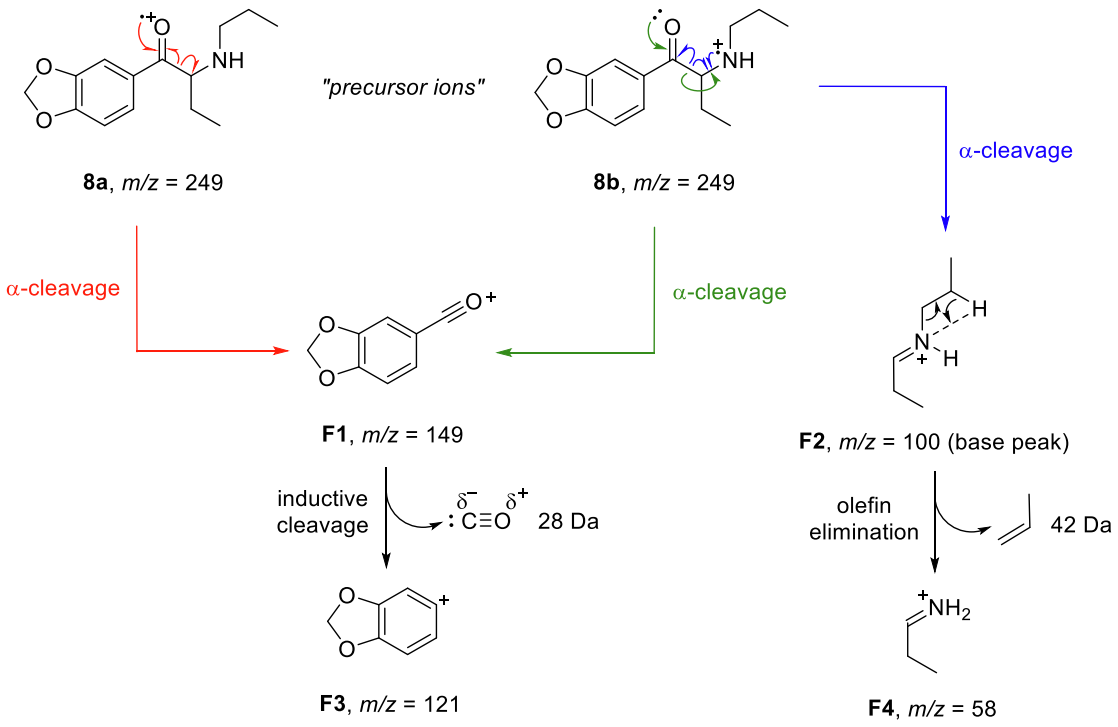


Fig. 5. (a) Exemplar total ion chromatogram demonstrating separation of (**2**), caffeine, (**6**), (**8**) and methyl stearate (internal standard, IS). See materials and methods (Section 2.3) for experimental details; (b) Representative total ion chromatogram of blue, *"Donald Trump"* embossed *"Ecstasy"* tablets (**GM443**); (c) EI-MS spectrum (+ve ion mode) of (**8**) reference standard (t_R = 7.35 min); (d) EI-MS spectrum (+ve ion mode) of (**8**) (t_R = 7.34 min) in seized tablets (**GM443**); (e) EI-MS spectrum (+ve ion mode) of caffeine (t_R = 5.89 min) in seized tablets (**GM443**). *Note:* t_R (methyl stearate) = 9.08 min. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

caffeine (minor component, t_R = 5.89 min, Fig. 5e) and putylone (major component, t_R = 7.34 min) and the EI spectrum (Fig. 5d) is in good agreement with both the synthesised reference standard of (**8**) (Fig. 5b) and the information reported by NPS Discovery [19].

The structures of the molecular ion (*m/z* = 249) and diagnostic fragment ions are presented in Scheme 2. The proposed fragmentation pathway for (**8**) is like those reported for similar synthetic 3,4-methylenedioxycathinones [30]. The 3,4-methylenedioxyphenylacylium (**F1**, *m/z* = 149, red pathway) and iminium (**F2**, *m/z* = 100, blue pathway) ions are the dominant fragmentation pathways for synthetic cathinones. Subsequent inductive cleavage of carbon monoxide (28 Da) from the (**F1**) produces the 3,4-methylenedioxyphenylium ion (**F3**) at *m/z* = 121. The 3,4-methylenedioxyphenylacylium ion (**F1**) can also form through α-cleavage of the bond between the carbonyl carbon and the α-carbon, which is initiated or stabilized by a lone pair of electrons on the oxygen atom (green pathway). The iminium ion (**F2**) is the most intense ion (base peak) in the spectrum and is produced *via* an α-cleavage of the bond between the carbonyl carbon and the α-carbon adjacent to the aminopropyl moiety. The iminium ion pathway subsequently leads to the secondary fragment (**F4**, *m/z* = 58) through a 4-center olefin elimination along the propyl chain.

The quantitative GC–MS method (selective ion monitoring [SIM] mode), using three ions specific to each analyte (Table 2), was developed, and validated in accordance with the ICH guidelines [27]. Calibration standards were prepared, and all four analytes (**2**, **6**, **8** and caffeine) demonstrated a linear response (r^2 = 0.999) over a 2.0–12.0 μg/mL range (containing 20.0 μg/mL methyl stearate) with satisfactory repeatability (RSD = 0.61–4.6%, n = 6). Due to the level of sensitivity required for the detection of the analytes within bulk samples the limits of detection (LOD) and quantification (LOQ) were determined in selective ion monitoring mode. The limits of detection and quantification for the analytes (in bulk samples) were determined (for SIM mode),



Scheme 2. Proposed GC-EI-MS fragmentation pathway for putylone (**8**) adapted from [30].

Table 3
Qualitative and quantitative analysis of seized blue, *"Donald Trump"* embossed *"Ecstasy"* tablets (**GM443**, n = 3) obtained from Greater Manchester Police (Manchester, UK, 20th December 2022). See Fig. 1 for representative image of seized samples.

Sample	Weight (mg)	Qualitative Analysis	Quantitative Analysis	
		GC-EI-MS (scan)	GC-EI-MS (SIM)	qNMR
GM443A	692.8	caffeine (minor, t_R = 5.89 min) and putylone (major, t_R = 7.34 min)	caffeine (minor, 43.0 ± 1.89 mg/tablet) and putylone (major, 135.5 ± 1.16 mg/tablet)	caffeine (minor, 39.1 ± 0.36 mg/tablet) and putylone (major, 133.7 ± 1.63 mg/tablet)
GM443B	681.2	caffeine (minor, t_R = 5.89 min) and putylone (major, t_R = 7.34 min)	caffeine (minor, 40.2 ± 1.63 mg/tablet) and putylone (major, 130.6 ± 0.15 mg/tablet)	caffeine (minor, 36.6 ± 1.14 mg/tablet) and putylone (major, 128.9 ± 1.51 mg/tablet)
GM443C	686.1	caffeine (minor, t_R = 5.89 min) and putylone (major, t_R = 7.34 min)	caffeine (minor, 43.4 ± 0.84 mg/tablet) and putylone (major, 132.5 ± 1.40 mg/tablet)	caffeine (minor, 47.7 ± 3.33 mg/tablet) and putylone (major, 130.8 ± 1.51 mg/tablet)

based on the standard deviation of the response and slope of the calibration curve, as being 0.08–0.13 and 0.26–0.39 μg/mL, respectively.

The accuracy (percentage recovery study) of the assay was determined from spiked samples prepared in triplicate at three levels over a range of 80–120% of the target concentration (10 μg/mL). Experimental concentration is determined using the developed calibration and compared with the theoretical concentration (assay recovery). The relative error shows how the mean assay recovery diverges from an expected 100%. The repeatability (%RSD) of the method and the percentage recovery (% assay) for each of the three replicate samples demonstrated good recoveries (**2**, 97.3 ± 1.64%; caffeine, 90.1 ± 1.47%; **6**, 100.0 ± 4.31% and **8**, 99.9 ± 0.95%) within the desired concentration range.

The GC-EI-MS approach was deemed suitable for the analysis of the street sample (**GM443**). The three tablets individually reanalysed (in triplicate) and quantification of putylone (**8**) and caffeine were performed in SIM mode. The quantitative GC-EI-MS results confirmed that all three tablets contained putylone (t_R = 7.34 min, **8**) at levels ranging between 130.6 and 135.5 mg/tablet, with low levels of caffeine (t_R = 5.89 min, 40.2–43.4 mg/tablet) also present (Table 3). To verify the GC-EI-MS quantitative results, quantitative nuclear magnetic resonance (qNMR) data was also contemporaneously collected for comparison purposes. For the qNMR approach, reference samples consisting of 10 mg/mL of putylone and caffeine in DMSO-d_6 were separately acquired, and the spectra of the samples were normalised to the reference spectrum, in order for quantification to be performed [29]. The qNMR results confirmed that the three surveyed tablets contained (**8**) and caffeine at levels ranging between 128.9 and 133.7 mg/tablet and 36.6–47.7 mg/tablet respectively corroborating the quantitative GC-EI-MS results. It is important to note that due to the small sample size (n = 3), the results presented herein may not truly reflect the typical prevalence or concentrations of samples that contain putylone nationally, however, these results demonstrate that the 10-minute GC-EI-MS method, employing selective ion monitoring described herein is potentially suitable for the routine screening of suspect samples, which may contain this novel cathinone.

4. Conclusion

This study reports the first synthesis and comprehensive analytical profiling (^{1}H, ^{13}C{^{1}H} NMR, ATR-FTIR and GC-EI-MS) of the novel synthetic cathinone: 1-(1,3-benzodioxol-5-yl)-2-(propylamino)butan-1-one (**8**, bk-PBDB, putylone) confirmed within a seized bulk sample. In addition to the synthetic methods and spectral data the paper presents the development of a rapid, validated GC-EI-MS method (employing SIM) for the routine detection (within 10 mins) and quantitative analysis (LOD: 0.09 μg/mL, LOQ: 0.26 μg/mL, respectively) for (**8**) suitable for processing of bulk samples encountered in casework. The seized tablets were determined to contain a mixture of bk-PBDB (130.6–135.5 mg/

tablet) and caffeine (40.2–43.4 mg/tablet) respectively. It is envisaged that the data presented herein will be valuable as a reference point for future analysis of this novel 3,4-methylenedioxy-*N*-alkyl cathinone and any structurally related compounds as they emerge on the illicit drug market.

Funding

This work was supported by the Warehouse Project and Manchester Metropolitan University.

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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.forc.2023.100523.

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