Short communication

Drug Testing and Analysis

Received: 21 September 2012 Revised: 7 December 2012 Accepted: 7 December 2012 Published online in Wiley Online Library: 24 January 2013

(www.drugtestinganalysis.com) DOI 10.1002/dta.1451

Identification of (2-aminopropyl)benzofuran (APB) phenyl ring positional isomers in Internet purchased products

Andrzej Stanczuk,[a] Noreen Morris,[a] Elizabeth A. Gardner[b] and Pierce Kavanagh[c]*

5-(2-Aminopropyl)benzofuran (5-APB), a 'research chemical' that was first reported by UK authorities to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) in 2010, is anecdotally reported to produce a combination of stimulant and entactogenic effects. More recently, in 2011, 6-(2-aminopropyl)benzofuran (6-APB) was identified by Hungarian authorities. To confirm positional isomer identity in Internet purchased products, 4- 5- 6- and 7-APBs were synthesized and found to be separable by gas chromatography (as heptafluorobutyramide derivatives) and liquid chromatography. The analyses of products purchased from online vendors of 'research chemicals' identified the presence of 5- or 6-APBs. These findings were further confirmed by liquid chromatography-mass spectrometry and ^{1}H nuclear magnetic resonance spectroscopy. In products containing 6-APB, the 4- positional isomer was also identified and this may have arisen during the manufacturing process. Copyright © 2013 John Wiley & Sons, Ltd.

Supporting information may be found in the online version of this article.

Keywords: APB; aminopropylbenzofuran; Benzo Fury; research chemical; forensic chemistry; isomer

Introduction

5-(2-Aminopropyl)benzofuran (1-benzofuran-5-ylpropan-2-amine, 5-APB) (Table 1) was first reported by UK authorities to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) via their Early Warning System (EWS) in 2010.[1] In 2011, another positional isomer, 6-(2-aminopropyl)benzofuran (6-APB), was identified by Hungarian authorities.[2] Some websites selling 'research chemicals' refer to 6-APB as Benzo Fury, although the UK Home Office drugs information website, Talk to Frank, states that products sold as Benzo Fury have also been found to contain 5-APB or diphenyl-2-pyrrolidinemethanol (D2PM).[3–5] In one instance, toxicological screening identified D2PM in the urine of a patient following the self-reported ingestion of Benzo Fury.[6] Both 5 and 6-APBs have also been mistakenly reported to be 1-(2,3-dihydrobenzofuran-5-yl)propan-2-amine (5-APDB) and 1-(2,3-dihydrobenzofuran-6-yl)propan-2-amine (6-APDB), respectively.[7,8]

Phenyl ring substituted (2-aminopropyl)benzofurans are structurally similar to methylenedioxyamphetamine (MDA) with the dioxole moiety replaced by a furan ring; it has been claimed that they possess 5-HT_{2C} receptor agonist activities.[9] Anecdotally, recreational drugs users have reported that 5-APB and 6-APB either alone or in combination possess stimulant and entactogenic activities.[10,11] Both 2- and 3- substituted β–aminoalkyl benzofuran derivatives have also been reported and it has been claimed that they possess calming and 'psychic energizer' activities.[12] The 2-APB isomer has been reported be a monamine oxidase-A (MAO-A) inhibitor.[13] Considering that the phenyl ring substituted isomers have been reported to be 'preferred' classes of the compounds as regards pharmacological activity[9] and that they are structurally similar to MDA, it is most likely that manufacturers would synthesize them for sale to recreational drugs users.

The occurrence of positional isomerism on aromatic rings in recreational drugs has been previously reported and necessitates the need for the syntheses of multiple standards to facilitate adequate discrimination for forensic purposes.[14,15] With mono-substituted amphetamines, three isomers (ortho, meta, and para) exist[16–18] but, with the addition of the furan ring in APBs, six are possible. Varying the position of the aminopropyl group on the phenyl ring may be expected to be the most likely to be chosen by manufacturers and thus it would be envisaged that the discrimination of 4-, 5-, 6- and 7-APBs (Table 1) may be required for forensic purposes. To identify the positional isomer(s) present in products purchased from Internet vendors, the four phenyl ring isomers were synthesized and characterized for use as reference standards. Analyses by gas chromatography–mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) and ^{1}H NMR (^{1}H nuclear magnetic resonance) spectroscopy showed that these isomers could be readily discriminated.

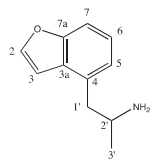
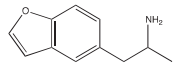
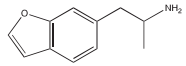
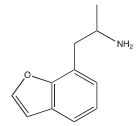
* *Correspondence to: Pierce Kavanagh, Department of Pharmacology and Therapeutics, Trinity Centre for Health Sciences, St James's Hospital, Dublin 8, Ireland. E-mail: pierce.kavanagh@tcd.ie*

a *Department of Life and Physical Science, Athlone Institute of Technology, Athlone, Co. Westmeath, Ireland*

b *Department of Justice Sciences, University of Alabama, Birmingham, Alabama, USA*

c *Department of Pharmacology and Therapeutics, Trinity Centre for Health Sciences, St James's Hospital, Dublin 8, Ireland*

Table 1. Analytical data for the APB standards (hydrochloride salts)

	4-APB		5-APB		6-APB		7-APB	
								
No.	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
2	145.1	8.03 (d, J = 2.2 Hz, 1H)	145.3	7.99 (d, J = 2.2 Hz, 1H)	145.2	7.97 (d, J = 2.2 Hz, 1H)	145.2	8.03 (d, J = 2.2 Hz, 1H)
3	105.3	7.23 - 7.18 (m, 1H)	105.3	6.94 (dd, J = 2.2, 1.0 Hz, 1H)	106.9	6.94 (d, J = 2.1, 1H)	107.3	6.98 (d, J = 2.2 Hz, 1H)
3a	130.0	-	131.3	-	133.5	-	130.0	-
4	127.1	-	127.4	7.20 (dd, J = 8.4, 1.9 Hz, 1H)	126.3	7.15 (dd, J = 7.8, 1.4 Hz, 1H)	120.0	7.57 (dd, J = 7.5, 1.5 Hz, 1H)
5	123.6	7.13 (d, J = 7.3 Hz, 1H)	121.7	-	121.5	7.62 (d, J = 7.9 Hz, 1H)	123.7	7.23 (t, J = 7.4 Hz, 1H),
6	124.3	7.29 (t, J = 7.8 Hz, 1H)	125.6	7.53 (d, J = 1.8 Hz, 1H)	124.6	-	125.2	7.19 (dd, J = 7.3, 1.4 Hz, 1H),
7	110.0	7.52 (d, J = 8.2 Hz, 1H)	111.2	7.56 (d, J = 8.4 Hz, 1H)	112.2	7.52 (d, J = 1.8 Hz, 1H)	110.0	-
7a	154.3	-	153.4	-	154.9	-	153.3	-
1′	37.6	2.93 (dd, J = 13.2, 9.2 Hz, 1H)						
3.32 (m, 1H)	39.9	3.13 (dd, J = 13.4, 5.5 Hz, 1H) 2.79 (dd, J = 13.4, 8.7 Hz, 1H)	40.4	3.13 (dd, J = 13.5, 5.6 Hz, 1H) 2.82 (dd, J = 13.4, 8.5 Hz, 1H)	34.7	3.02 (dd, J = 13.6, 9.4 Hz, 1H)		
3.31 (dd, J = 13.6, 5.2 Hz, 1H)								
2′	47.7	3.44 (m, 1H)	48.4	3.42 (m, 1H)	48.5	3.51 – 3.37 (m, 1H)	47.1	3.59 (tq, J = 11.5, 5.9 Hz, 1H)
3′	17.7	1.14 (d, J = 6.5 Hz, 3H)	17.6	1.15 (d, J = 6.5 Hz, 3H)	18.0	1.15 (d, J = 6.5 Hz, 3H)	18.1	1.12 (d, J = 6.5 Hz, 3H)
NH_3^+	-	8.21 (vbs, 3H)	-	8.17 (vbs, 3H)	-	8.11 (vbs, 3H)	-	8.29 (vbs, 3H)
Melting point (°C)		199-201		161-163		140-142		157-159
ESI-HRMS (found) (calc. for $[M+H]^+$, $C_{11}H_{14}NO$, 176.1070)		176.1079		176.1068		176.1078		176.1069
% Yield (from bromobenzofuran starting material)		18		25		22		20

Experimental

Reagents and chemicals

Heptafluorobutyric anhydride (HFBA), methanol for LC-MS and dimethyl sulfoxide-d_6 for NMR were purchased from Sigma-Aldrich (Arklow, Co. Wicklow, Ireland). Water for LC-MS and ethyl acetate were purchased from Fisher Scientific (Loughborough, UK). 4-Bromobenzofuran (97%) and 6-bromobenzofuran (95%) were obtained from KaïronKem (Marseille, France), 5-bromobenzofuran from Fluorochem Ltd (Derbyshire, UK), and 7-bromobenzofuran (90%) from Maybridge (Tintagel, Cornwall, UK).

Standards

The four APB isomers were synthesized as by a previously described protocol.[9] The free bases were converted to their hydrochloride salts using ethereal hydrogen chloride. Analytical data for the compounds (^{1}H and ^{13}C NMR, ESI-HRMS, melting point and yield) are shown in Table 1.

Products purchased from Internet vendors

Three samples, one sold as 5-APB (IP1) and two as 6-APB (IP2-3), were purchased from online vendors of legal highs.

Gas chromatography–mass spectrometry (GC-MS)

This was performed on an Agilent 5973 mass selective detector (MSD; EI mode, 70 eV; m/z 40–450; source temp. 260°C) coupled to an Agilent 6890 GC (injector temperature 250°C; 1 μl injected in splitless mode; carrier gas, helium 1 ml/min) fitted with a 30 m x 0.25 mm HP-5MS capillary column coated with 0.25 μm bonded 5% phenyl, 95% dimethylpolysiloxane (Agilent HP-5ms Capillary GC Column, Agilent Technologies 19091S-433). The temperature program was as follows: initial temperature, 100°C, initial hold, 3.0 min; ramp rate, 20°C/min, temperature 140°C, hold 2.0 min; ramp rate, 5°C/min, temperature 150°C, hold 2.0 min; ramp rate, 5°C/min, temperature 160°C, hold 5.0 min; ramp rate, 20°C/min, final temperature, 250°C.

Liquid chromatography/electrospray ionization mass spectrometry (LC-EIMS)

LC-ESIMS was performed on Agilent 1100 LC system coupled to an Agilent LC-MSD (positive electrospray mode, capillary voltage 3000 V, drying gas (N_2) 10 l/min at 330°C, nebulizer gas (N_2) pressure 50 psig, SIM m/z 176, fragmentor voltage 50 V). Separations were performed using a phenylhexyl column (100 mm x 4.6 mm, 2.6 μm; Phenomenex, Macclesfield, UK) coupled to an Allure PFP column (50 mm x 2.1 mm, 5.0 μm; Restek, Bellefonte, CA, USA) using gradient elution (A – methanol containing 0.1% formic acid, B – water containing 0.1% formic acid; 3 % A (0–1 min.) followed by a linear gradient up to 95 % A at 50 min. and then down to 3 % A at 51 min.). The flow rate was 200 μl/min and 0.5 μl was injected. Samples were prepared in methanol/water (1/1) containing 0.1 % formic acid.

NMR spectroscopy

^{1}H (600 MHz) and ^{13}C (150 MHz) spectra were recorded on a Bruker AV600 NMR spectrometer using a 5 mm TCI cryoprobe. Approximately 5.0 mg of each sample was dissolved in 1 ml DMSO-d_6.

Infra-red spectroscopy

Infra-red spectra were recorded on a PerkinElmer Spectrum 100 with ATR (Diamond/ZnSe crystal, 4000–600 cm^{-1}, 16 scans, 4 cm^{-1} resolution).

High-resolution mass spectrometry

High-resolution mass spectra were recorded on an LTQ/Orbitrap™ Discovery mass spectrometer (Thermo Scientific, Bremen,

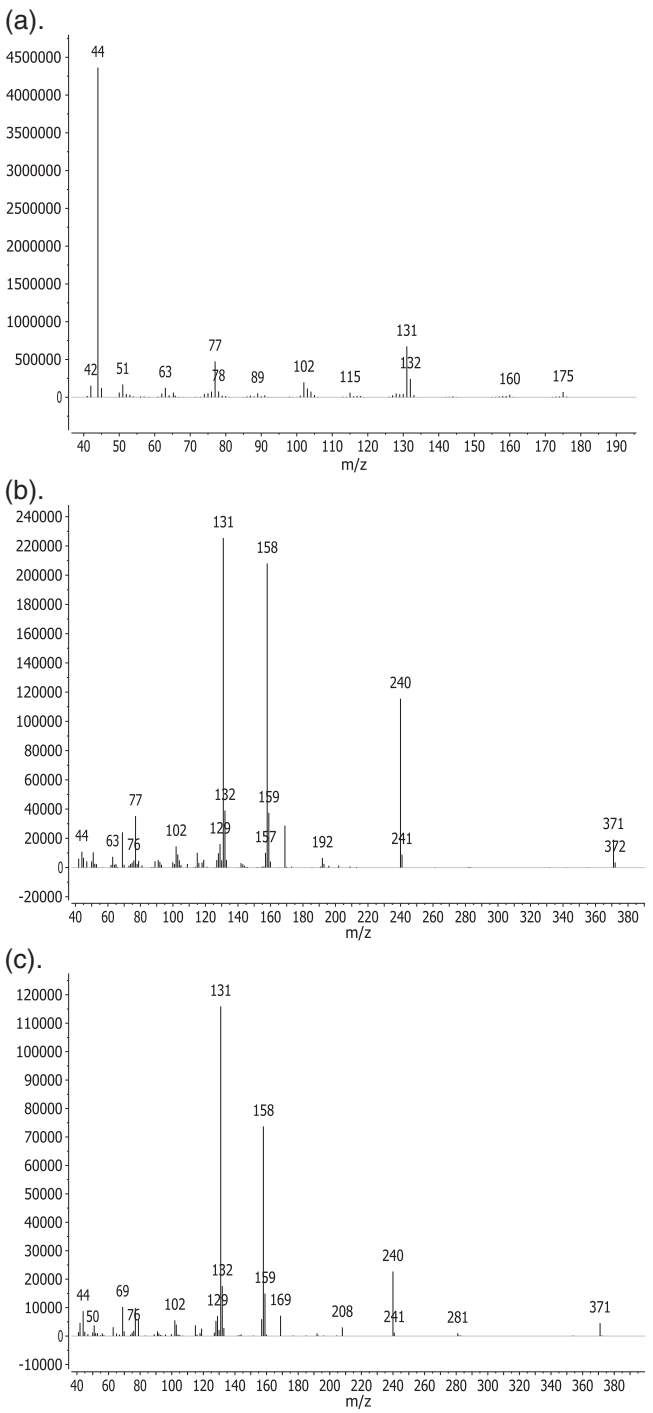


Figure 1. Electron ionization (EI) mass spectra for (a). 4-APB (underivatized), (b). 4-APB (HFB), (c). 5-APB (HFB), (d). 6-APB (HFB) and (e). 7-APB (HFB) and (f). possible fragmentation mechanisms for the HFB derivatives (5- isomer shown as an example).

Germany). Samples were dissolved in acetonitrile/water (1/1) containing 0.1 % formic acid), were infused at a rate of 5 μl/min. Full accurate high-resolution (30 000) mass scans were performed in positive electrospray mode. The following conditions were used: drying gas – nitrogen (10 l/min); capillary temp., 310°C; spray voltage, 4 V; capillary voltage, 22 V, tube lens, 77 V.

Heptafluorobutyamide (HFB) derivatization

A solution (1 mg/ml in ethanol) of each standard, mixture of standards or purchased product (50 μl) was evaporated to dryness, HFBA (50 μl) was added and the mixture was heated at 75°C for 30 min.[19] The mixture was evaporated to dryness using N_2 at 30°C and the residue was reconstituted in ethyl acetate (1 ml).

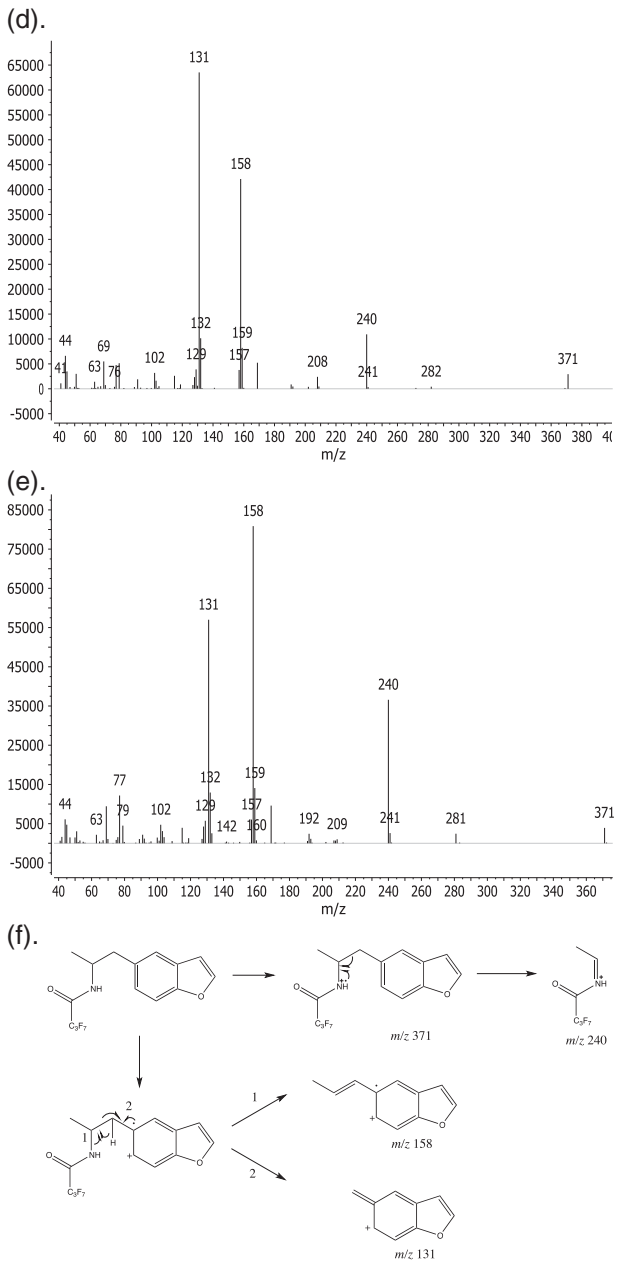


Figure 1. (Continuation)

Results and discussion

The synthetic protocol for the four APB phenyl ring positional isomers consisted of a two-step sequence starting with the respective bromobenzofuran (synthetic route shown in supplemental data).[9] This was cross-coupled with iso-propenyl acetate in the presence of palladium (II) chloride, tri(o-tolyl)phosphine and tributyl tin methoxide to produce a ketone intermediate[20,21] which was then reductively aminated with ammonium acetate and sodium cyanoborohydride to afford the amine. The yields of final products, from their respective bromobenzofuran starting materials, were found to reasonably low (around 20 %) and flash column chromatography was required for purification at each stage of the protocol. The syntheses of the 2- and 3- isomers were also considered but it was not possible to utilize this cross-coupling/reductive amination route for these. The synthesis of the 2- isomer has been previously reported and this involves the formation of a nitropropene derivative from benzofuran-2-carboxaldehyde followed by reduction with lithium aluminium hydride.[22,23] Considering the extra difficulty involved in the syntheses of the 2- and 3- isomers, and that the four phenyl ring substituted isomers have

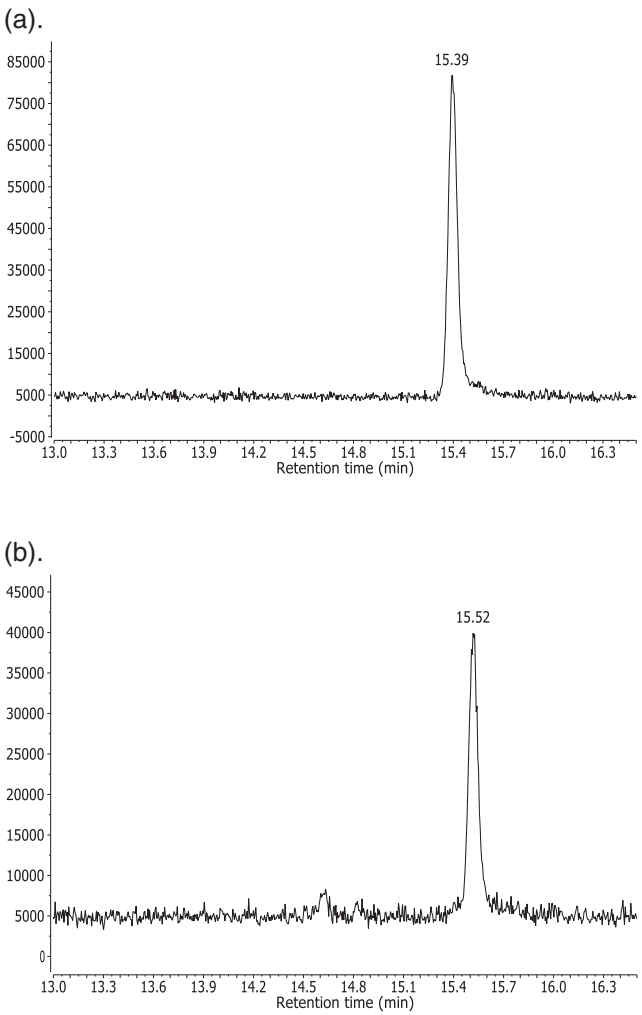


Figure 2. GCMS TICs (HFB derivatives) for (a). IP1, (b). IP2, (c). IP3 and (d). APBs standards: 4- (14.624 min.), 5- (15.390 min.), 6-(15.513 min.) and 7-APB (13.968 min.)

been noted as being the preferred ones for pharmacological activity, it was felt that the discrimination of 4-, 5-, 6- and 7-APBs would be sufficient to provide forensically sound and acceptable data.

The analysis of the four underivatized standards by GC failed to resolve the 5- and 6- isomers. Their electron ionization (EI) mass spectra were found to be similar and contained a weak m/z 175 molecular ion along with an iminium ion base peak of m/z 44, a fragmentation pattern typical of amphetamines.[24] As an example, the EI mass spectrum of 4-APB is shown in Figure 1 (see supplemental data for the EI mass spectra of all four isomers). Formation of heptafluorobutyramide (HFB) derivatives resulted in discrimination of the isomers by GC and this was used for analysis of the Internet purchased products (Figure 2). Some differences were noted in their EI mass spectra (Figure 1), all of which contained a molecular ion of m/z 371, with 4-, 5- and 6-APBs displaying a base peak base peak, m/z 131. However, the mass spectrum of 7-APB was found to contain a base peak of m/z 158 formed by loss of heptafluorobutyramide (Figure 1). The iminium ion, m/z 240, was found to be common to the four isomers.

High performance liquid chromatography (HPLC) was also utilized for the discrimination of the isomers. The use of a single column (C-18, naphthalene, phenylhexyl or pentafluorophenyl) for LC-MS analysis failed to resolve the isomers. However, coupling phenylhexyl and pentafluorophenyl columns together facilitated sufficient resolution (Figure 3) for discrimination although this resulted in longer retention times. It was not possible to obtain full base-line resolution of 5- and 7-APBs and, following identification of the isomer in the Internet purchased products, based upon retention times, the samples were spiked with the other APB isomers to confirm the initial finding. Although not explored here, it may be possible to utilize in-source collision induced dissociation (CID) to provide further structural information and offer better discrimination.[25] The use of UV detection was also explored and the 4 isomers were found to produce very similar and indistinguishable spectra (see supplemental data).

The standards and samples were also analysed by ^{1}H and ^{13}C NMR spectroscopies (Table 1). In the ^{1}H spectra, the two hydrogens on the furan ring in (H-2 and H-3) have very similar shifts and the largest differences were seen in the phenyl ring hydrogens from which it was possible to discriminate the isomers (Figure 4). The occurrence of 4-APB in the samples of the 6- isomer products (IP2-3) was also noticeable with the presence of its H-2 doublet and the H-6 triplet. The infra-red spectra of the four isomers were also recorded (see supplemental data) but their usefulness for the analysis of the Internet

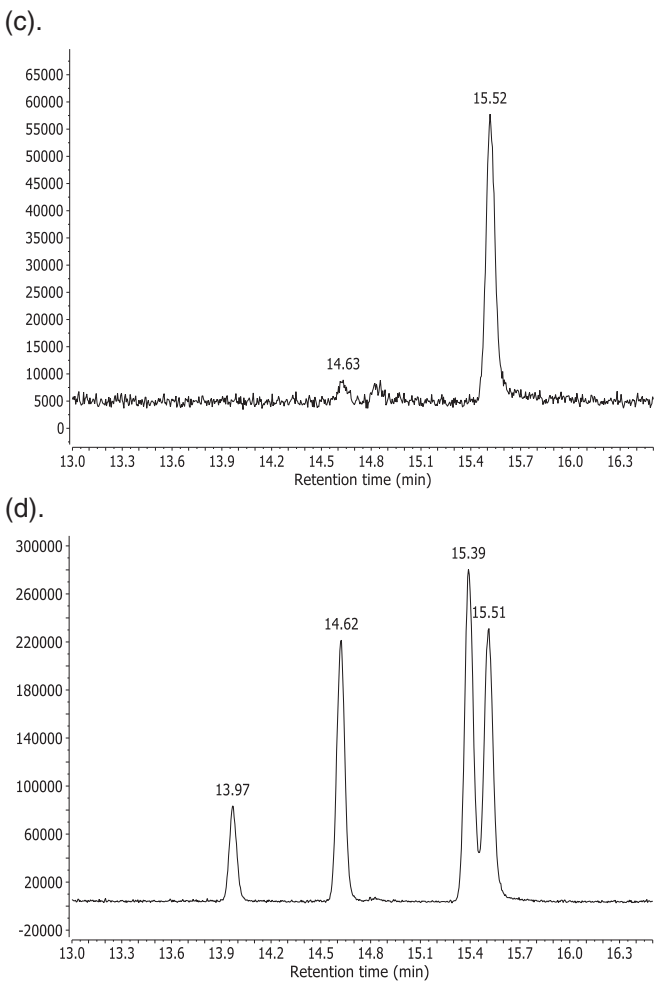


Figure 2. (Continuation)

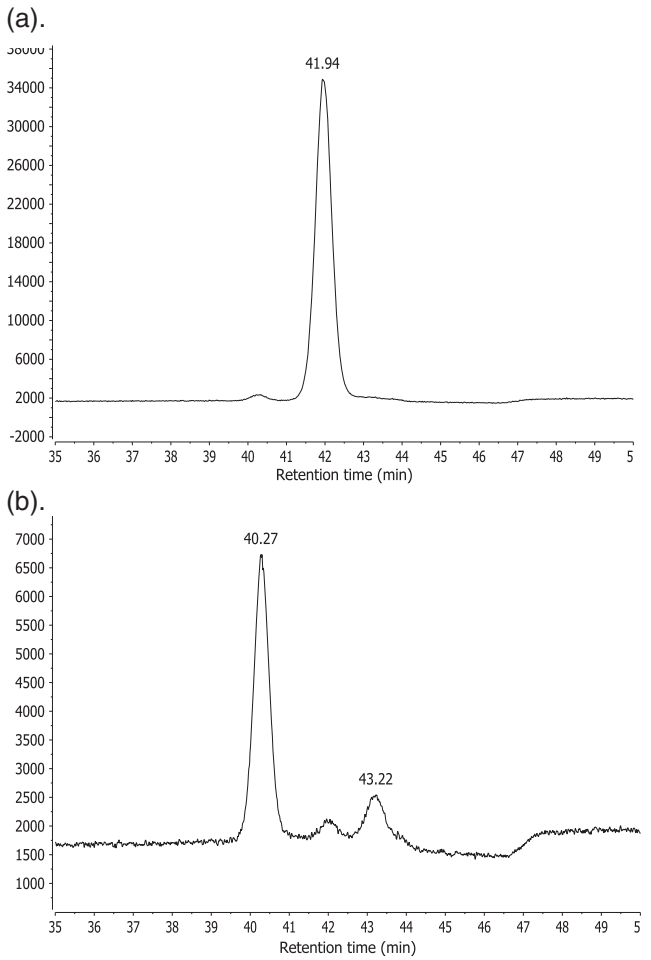


Figure 3. LCMS chromatograms for (a). IP1, (b). IP2, (c). IP3 and (d). APB standards: 6- (40.19 min.), 7- (41.28 min.), 5-(41.87 min.) and 4-APB (43.10 min.).

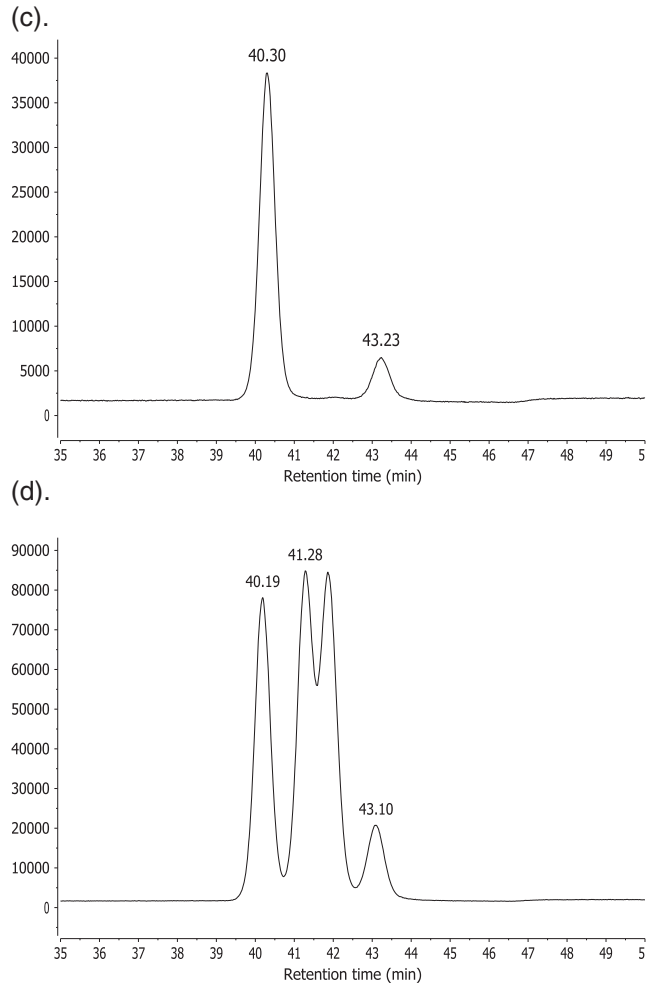


Figure 3. (Continuation)

purchased products was found to be of limited value possibly due the presence of diluents in the samples.

The Internet purchased product IP1 was sold as 5-APB and products IP2-3 as 6-APB, and analyses revealed that these isomers were indeed present in the products. However, the presence of some 4-APB in the 6-APB products (IP2-3) was noted and this may have arisen from the presence of 4-bromobenzofuran as a contaminant in 6-bromobenzofuran used in the manufacturing process (see supplemental data). The cyclization of 1-bromo-3-(2,2-diethoxyethoxy)benzene, an intermediate, results in the formation of both 4- and 6-bromobenzofuran isomers, and these reportedly require chromatography separation.[9] The percentage of 4-APB isomer, relative to the 6- isomer, present in products IP2 and 3 was determined to be 12%, and 7 % in the reference standard. The 6-bromobenzofuran used to prepare this standard was found to contain approximately 8% 4-bromobenzofuran. The LC chromatogram for product IP1 (5-APB) was noted to contain a small peak with a retention time similar to that of 6-APB but this could not be confirmed from the NMR data.

Conclusions

GC and LC separations of 4-, 5-, 6- and 7-APB were achieved and, coupled with mass spectrometry, both techniques allowed isomeric identification in products purchased from 'research chemicals' websites. ^{1}H NMR spectroscopy was also found to be useful to confirm identity. Products sold as containing 5- or 6-APB were found to contain the stated ingredient although those containing 6-APB were also found to contain some of the 4-isomer, presumably present as an artefact from the manufacturing process.

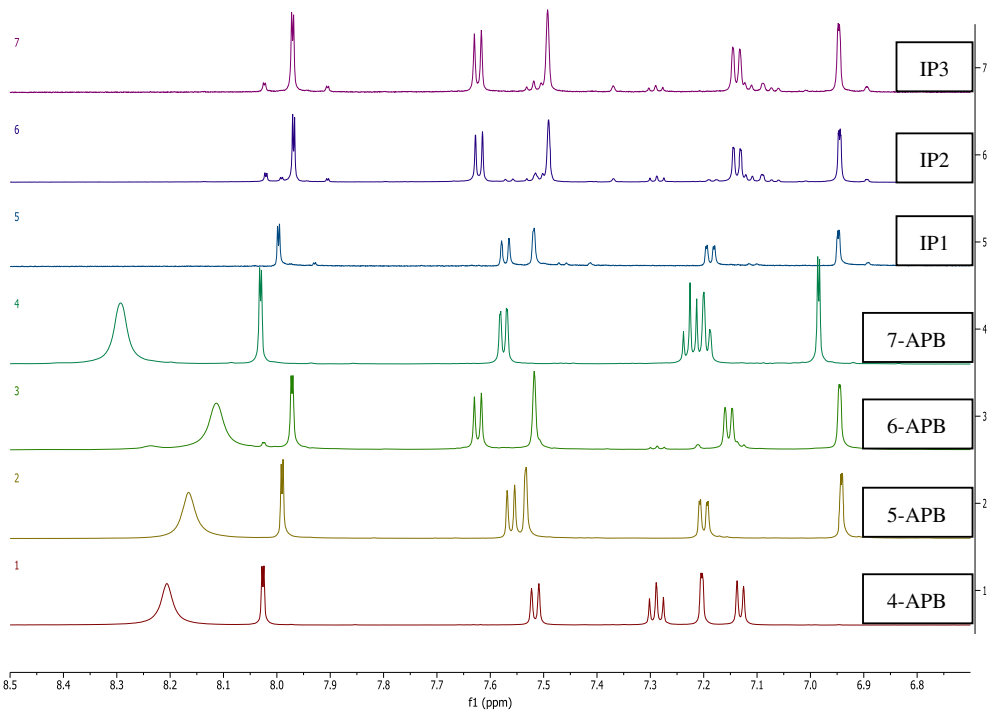


Figure 4. ^{1}H NMR spectra (aromatic region) for the four APB phenyl-ring positional isomers and Internet purchased products IP1–3.

Supporting information

Supporting information may be found in the online version of this article.

References

[1] EMCDDA–Europol. **2010**, Annual Report on the implementation of Council Decision 2005/387/JHA. In accordance with Article 10 of Council Decision 2005/387/JHA on the information exchange, risk assessment and control of new psychoactive substances.
[2] EMCDDA–Europol. **2011**, Annual Report on the implementation of Council Decision 2005/387/JHA. In accordance with Article 10 of Council Decision 2005/387/JHA on the information exchange, risk assessment and control of new psychoactive substances.
[3] Benzofury.com Available at: http://www.benzofury.com [22 October 2012].
[4] Buckledbonsi.co.uk Available at: http://www.buckledbonzi.co.uk [22 October 2012].
[5] Talktofrank.co.uk Available at: http://www.talktofrank.com/news/what-'benzo-fury' [22 October 2012].
[6] D.M. Wood, S. Davies, M. Puchnarewicz, A. Johnston, P.I. Dargan. Acute toxicity associated with the recreational use of the ketamine derivative methoxetamine. *Eur. J. Clin. Pharmacol.* **2012**, *68*, 853.
[7] J.F. Casale, P.A. Hays. The Characterization of 5- and 6-(2-Aminopropyl) 2,3-dihydrobenzofuran. *Microgram J.* **2011**, *8*, 62.
[8] J.F. Casale. Letter to the Editor regarding: Abbreviations for 5- and 6-(2-Aminopropyl)-2,3-dihydrobenzofuran vs. 5- and 6-(2-Aminopropyl) benzofuran: A Clarification of "APB" and "APDB". *Microgram J.* **2012**, *9*, 46.
[9] K. Briner, J.P. Burkhart, T.P. Burkholder, M.J. Fisher, W.H. Gritton, D.T. Kohlman, *et al*. Aminoalkylbenzofurans as Serotonin (5-HT (2C)) Agonists. Eli Lilly and Company, USA. International Patent No. 2000044737, **2000**.
[10] Drugsforum.com Available at: www.drugsforum.com [22 October 2012].
[11] Bluelight.ru Available at: www.bluelight.ru [22 October 2012].
[12] Smith, Kline & French Laboratories. No Inventor data available. β-Aminoalkylthianaphthene and β-aminoalkylbenzofuran derivatives. UK Patent No. 855115, **1960**.
[13] G. Vallejos, A. Fierro, M.C. Rezende, S. Sepulveda-Boza, M. Reyes-Parada. Heteroarylisopropylamines as MAO inhibitors. *Bioorg. Med. Chem.* **2005**, *13*, 4450.
[14] J.D. Power, P. McGlynn, K. Clarke, S.D. McDermott, P. Kavanagh, J. O'Brien. The analysis of substituted cathinones. Part 1: Chemical analysis of 2-, 3- and 4-methylmethcathinone. *Forensic Sci. Int.* **2011**, *212*, 6.
[15] P. Kavanagh, J. O'Brien, J. Fox, C. O'Donnell, R. Christie, J.D. Power, et al. The analysis of substituted cathinones. Part 3. Synthesis and characterisation of 2,3-methylenedioxy substituted cathinones. *Forensic Sci. Int.* **2012**, *216*, 19.
[16] S. Davis, K. Blakey, K. Rands-Trevor. GC-MS and GC-IRD analysis of 2-, 3- and 4-methylmethamphetamine and 2-, 3- and 4-methylamphetamine. *Forensic Sci. Int.* **2012**, *220*, 67.
[17] F. Westphal, P. Rosner, T. Junge. Differentiation of regioisomeric ring-substituted fluorophenethylamines with product ion spectrometry. *Forensic Sci. Int.* **2010**, *194*, 53.
[18] T.A. Dal Cason. A re-examination of the mono-methoxy positional ring isomers of amphetamine, methamphetamine and phenyl-2-propanone . *Forensic Sci. Int.* **2001**, *119*, 168.
[19] S.-M Wang, S.-M. Chye, R.H. Liu, R.J. Lewis, D.V. Canfield. Mass spectrometric data characteristics of commonly abused amphetamines with sequential derivatization at two active sites. *Forensic Sci. Int.* **2006**, *161*, 97.
[20] M. Kosugi, M. Suzuki, I. Hagiwara, K. Goto, K. Saitoh, T. Migita. A new palladium catalysed aromatic acetonylation by acetonyltributyltin. *Chem. Lett.* **1982**, *939*.
[21] M. Kosugi, I. Hagiwara, T. Sumiya, T. Migita. Arylation and 1-alkenylation on α–position of ketones via tributyltin enolates catalysed by palladium complex. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 242.
[22] C.J. Cattanach, R.G. Rees. Preparation of 4a-alkoxy-1,2,3,4,4a,9b-hexahydro- and −1,2,3,4-tetra-hydro-benzofuro[3,2-c]pyridines. *J. Chem. Soc. C* **1971**, *53*.
[23] J. Zhou, T.P. Maduskuie, D-Q Qian, W. Yao. Preparation of substituted pyrimidine derivatives as antagonists of the histamine H4 receptor. Incyte Corporation, USA. International Patent No. 2010-US27904, **2010**.
[24] M. Takahashi, M. Nagashima, J. Suzuki, T. Seto, I. Yasuda, T. Yoshida. Analysis of phenethylamines and tryptamines in designer drugs using gas chromatography mass spectrometry. *J. Health Sci.* **2008**, *54*, 89.
[25] J.D. Power, S.D. McDermott, B. Talbot, J.E. O'Brien, P. Kavanagh. The analysis of amphetamine-like cathinone derivatives using positive electrospray ionization with in-source collision-induced dissociation. *Rapid Commun. Mass Spectrom.* **2012**, *26*, 2601.