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Short communication

^{1}H, ^{13}C, ^{15}N HMBC, and ^{19}F NMR spectroscopic characterisation of seized flephedrone, cut with benzocaine

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A R T I C L E   I N F O

A B S T R A C T

Flephedrone (4-fluoromethcathinone, 4-FMC) was analysed using ^{1}H, ^{13}C, ^{15}N HMBC, and ^{19}F observe spectroscopy, gas chromatography-flame ionisation detection (GC-FID), and electrospray ionisation-mass spectrometry (ESI-MS). Analysis of four 4-FMC samples (from a Bristol nightclub in 2013) showed that they all contained benzocaine as the cutting agent present in different amounts from 5 to 12%. Using these methods, we successfully differentiated between flephedrone regioisomers and mephedrone in an analytical method validated for flephedrone as a substituted cathinone. The data show that these now illegal cathinone-derived stimulants (highs) are now being cut; users cannot be certain of the purity of the drug they are taking. Furthermore, there are risks from the pharmaceutically active cutting agents themselves.

1. Introduction

The close similarity between cathinone derivatives (Fig. 1) makes the specific identification of these drugs and their regioisomers challenging and could lead to mislabelling or accidental misidentification or deliberate deception as in the nature of the cutting of illicit (street) drugs. Flephedrone (4-fluoromethcathinone, 4-FMC) (Fig. 1c) was first synthesised in 1952, but surprisingly there is a paucity of published data for this compound. Compared with cathinone (Fig. 1a) and mephedrone (4-methylmethcathinone, 4-MMC) (Fig. 1b), the C—F bond on the aromatic ring of 4-FMC (Fig. 1c) has a specific polarity effect. This may influence receptor binding and therefore influence the biological activity [1,2]. The biological effects of cathinone ring-substituted derivatives are claimed to be similar to cocaine, amphetamine, or MDMA (ecstasy) [3]. In the UK, a question about a decrease in the purity of cathinones has arisen after they were banned as Class B drugs under the Misuse of Drugs Act in April 2010 [4].

As a routine analytical technique, e.g. used by the police, GC–MS [5–7] or GC-FID [6], infrared (FT-IR) [7], and Raman spectroscopy are widely used for the detection of controlled substances [8]. Nevertheless, standard reference materials for detecting what may be present in unknown samples are required.

Using GC–MS alone would potentially not distinguish between the 2-, 3-, and 4-regioisomers of FMC, all the isomers having been reported in elegant synthetic work with full characterisation by Archer [7]. Electron impact-mass spectrometry (EI-MS) also leads to uncertainty in distinguishing between flephedrone isomers [7]. More recently, screening methods for the detection of new (illicit) psychoactive drugs in urine were reported that use liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) [9], HPLC [6], and ultra performance liquid chromatography–quadrupole time-of-flight-mass spectrometry (UPLC–QTOF-MS) [5].

NMR spectroscopy is an established analytical tool for the identification of controlled substances without the need for sample derivatisation or the need to remove any impurity. Also, distinguishing between regioisomers, between salt and free base, determining percentage purity and impurity ratios are successfully and rapidly achieved using NMR spectroscopy. Police or forensic intelligence information may be gained from this kind of analysis to aid law enforcement. The aim of this work is to develop a robust, rapid, and quantitative analytical approach to detect flephedrone as well as the impurities (cutting agents) present in street samples using ^{1}H, ^{13}C, ^{15}N, and ^{19}F NMR spectroscopy together with GC.

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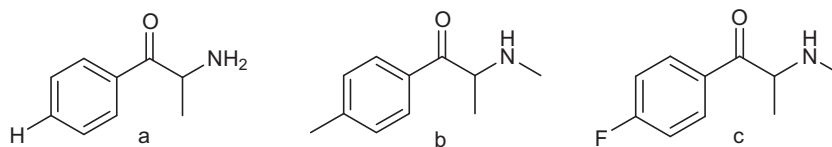E-mail address: prsisb@bath.ac.uk (I.S. Blagbrough).

Fig. 1. (a) Cathinone, (b) mephedrone, and (c) flephedrone.

2. Experimental

2.1. Chemicals and materials

Four creamy coloured crystal samples, as part of an amnesty bin provided in a (Bristol, UK) nightclub, in 2013, were identified as flephedrone. A reference standard of flephedrone hydrochloride salt (4-FMC HCl) was purchased from LGC Standards, UK. An analytical standard of mephedrone hydrochloride salt (4-MMC HCl) was purchased from Aldrich, UK. Deuterium oxide (99.8 atom % D, Aldrich, UK), per-deuterated methanol (99.8 atom % D, Cambridge Isotope Laboratories, USA), and all other solvents were of HPLC grade, $\geq$99.9% purity (Aldrich, UK). Benzocaine B.P. was purchased from J. M. Loveridge plc (Southampton, UK).

2.2. Experimental procedures and instrumentation

Each sample of flephedrone (4-FMC) was weighed, dissolved, and analysed at 20–25 °C without any purification. NMR spectra were recorded on Bruker 400 or 500 MHz spectrometers, referenced to external Me_4Si and/or via residual protons in the deuteriated solvents. Samples ($\sim$30 mg each) were vortexed for 2 min in D_2O (0.6 mL). Identification of flephedrone samples and their impurities followed NMR analysis including: ^1H, ^{13}C, ^{15}N (HMBC), HSQC, HMBC, and COSY. A GC method was optimised to analyse authentic 4-FMC nightclub samples dissolved in methanol (50 mg/mL), and benzocaine B.P. injected directly into the GC-FID without any prior chemical derivatisation. GC separation was performed on a CP-9003 (Chrompack, Middelburg, The Netherlands) using a capillary column ZB-WAX (polyethylene glycol, 10 m × 0.25 mm × 0.25 μm, Phenomenex, UK). An injection volume (1–2 μL) was carried by helium carrier gas at a flow rate of 23–27 mL/s. The oven temperature was programmed with 250 °C injector and detector temperature, 110 °C (2 min) oven initial and 230 °C (4 min) final temperatures with 40 °C/min rate of heating, total run time was 9 min. Positive ion [M+H]$^+$ mode LC/MS was performed, on samples dissolved in methanol, using a Bruker Daltonics micrOTOF mass spectrometer equipped with an electrospray ionisation (ESI) source.

3. Results and discussion

3.1. ^1H, ^{13}C, ^{15}N, and ^{19}F NMR spectroscopy

4-FMC NMR spectra show diagnostic ^{19}F and ^1H coupling (Fig. 2) in the aromatic region; ^1H, ^{13}C, and ^{19}F NMR spectral data are reported in Table 1. NMR spectra are discussed and compared to those of the closely related cathinone derivative mephedrone 4-MMC (Table 1, Fig. 2). The structures were confirmed by comparing the NMR data with those of an authentic sample of 4-FMC and the literature values [7], establishing the regioisomer as para-substituted. Unfortunately, two lines in the literature ^{13}C NMR spectroscopic assignment Table have been transposed [7], and the correct aromatic region assignment is given in Table 1 for δ 116 (d, $^2J_{CF}$ 22 Hz) and 132 (d, $^3J_{CF}$ 10 Hz) ppm, where the coupling constants are diagnostic for ortho to F (C3, C5) and meta to F (C2, C6) respectively.

While the ^1H NMR spectrum of 4-FMC is similar to that of 4-MMC (Fig. 2), the absence of a 4-methyl aromatic substituent peak, and the coupling from the ^{19}F atom are diagnostic. The ^{13}C-^{19}F splitting patterns around the entire aromatic ring (e.g. 167 ppm, d, $^1J_{CF}$ 255 Hz; 129 ppm, d, $^4J_{CF}$ 3 Hz) in the ^{13}C spectra (Table 1) are also significant. ^{15}N NMR HMBC was run to detect the number, kind, and position of the nitrogen atoms proving the presence of a secondary amine coupled to the two N- and α-methyl groups (Fig. 3). ^{19}F NMR spectroscopy proves the presence and position of a fluorine atom in such samples. Comparing with the literature data [7], the ^{19}F NMR chemical shift values for the each of the four 4-FMC samples show para-substitution: ^{19}F NMR showed −102.1 ppm (tt, $^3J_{HF}$ 8.4 Hz, $^4J_{HF}$ 5.2 Hz) (Fig. 3) with the d $^1J_{CF}$ 255 Hz easily visible only in the ^{13}C NMR spectrum, not in the ^{19}F NMR spectrum due to the low (1.1%) ^{13}C natural abundance.

3.2. ESI-MS and impurity profiling

The ESI-MS gave [M+H]$^+$ at 182.0976 and 182.1006 for the samples and the authentic hydrochloride salt of flephedrone respectively, $C_{10}H_{13}FNO$ requires 182.0981 thus confirming the identity of a regioisomer of flephedrone. ESI-MS spectra also gave [M+H]$^+$ 166.0874. Benzocaine $C_9H_{12}NO_2$ [M+H]$^+$ requires 166.0868; [M+Na]$^+$ $C_9H_{11}NO_2Na$ found 188.0689, requires 188.0687.

Analysis of the 4-FMC samples by NMR and ESI-MS showed that they were impure. The ^1H and ^{13}C NMR data of the impurity are: ^1H NMR (D_2O): δ 1.36 (t, $^3J_{HH}$ 7 Hz), 4.33 (q, $^3J_{HH}$ 7 Hz), 6.83 (H3 and H5, t, $^3J_{HH}$ 7.6 Hz), 7.86 (H2 and H6, d, $^3J_{HH}$ 7.6 Hz) [10]; ^{13}C NMR (D_2O): δ 15 (C7), 61 (C6), 114 (C3, C5), 119 (C1), 132 (C2, C6), 155 (C4), 169 (C5) ppm [11]. A GC-FID method was then developed to resolve flephedrone and benzocaine in mixed samples, achieved with retention times of R_t = 2.8 min and 4.9 min respectively (Fig. 4). The benzocaine levels in these 4-FMC samples were quantified by GC (5, 6, 7, and 12%), impurity levels which are in agreement with those determined from the (more approximate) ^1H NMR integration data, 4, 6, 7, and 10%. Benzocaine has previously also been quantified by HPLC [12].

4-FMC is a designed cathinone derivative either designed to side-step the banning of the legal high 4-MMC and/or with one eye on the potential pharmacological enhancement from incorporating a fluorine substituent in a biologically active small molecule as "there is also evidence that single, carbon-bound fluorine substituents, particularly when on an aromatic ring, can exhibit specific polarity influences, including H-bonding, that can strongly influence binding" [1]. The human toxicology of flephedrone is not well established [13]. Lipophilicity is significant as it often controls the absorption, transport, or receptor binding of a compound and so can enhance its bioavailability. The effect on the lipophilicity of fluorine substituents on an aromatic ring was calculated by Dolbier showing that CF_3 ($\pi_x = 0.88$) is greater than CH_3 ($\pi_x = 0.56$) following from the respective n-octanol/water partition coefficients [1]. So, perhaps counter-intuitively, the presence of the most powerful electronegative atom, a fluorine substituent, gives rise to enhanced lipophilicity.

Benzocaine is a well-known topical anaesthetic. However, the FDA has reported that using a gel or spray containing
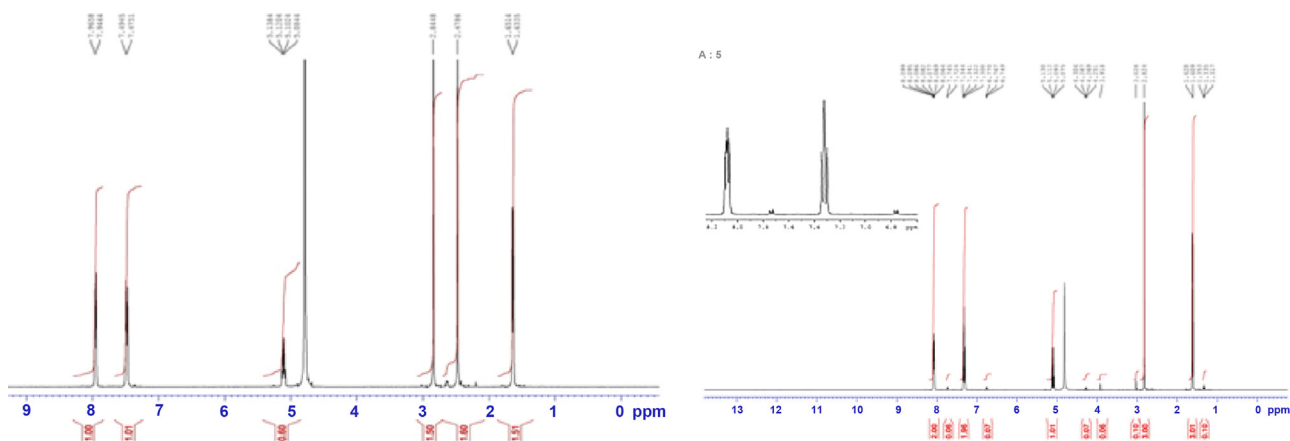
Fig. 2. ^1H NMR of mephedrone (left) and flephedrone (right) (D_2O) with aromatic region expansion showing ^{19}F couplings and the ~7% benzocaine signals here are clearly visible.

Table 1
^1H (400 MHz), ^{13}C (100 MHz), and ^{19}F (470 MHz) spectral data of flephedrone (D_2O).

Position	Flephedrone			Mephedrone	
	^1H	^{13}C	^{19}F	^1H	^{13}C
1	–	129 (d, $^4J_{CF}$ 3 Hz)		–	129.7
2,6	8.07 (dd, $^3J_{HH}$ 8.4 Hz, $^4J_{HF}$ 5.2 Hz)	132 (d, $^3J_{CF}$ 10 Hz)		7.96 (d, $^3J_{HH}$ 8 Hz)	129.1
3,5	7.31 (t, $^3J_{HH} = ^3J_{HF}$ 8.4 Hz)	116 (d, $^2J_{CF}$ 22 Hz)		7.48 (d, $^3J_{HH}$ 8 Hz)	129.9
4	–	167 (d, $^1J_{CF}$ 255 Hz)	–102.1, tt, ^3J 8.4, ^4J 5.2 Hz	–	147
7	–	196		–	197
8	5.09 (q, $^3J_{HH}$ 7 Hz)	60		5.11 (q, $^3J_{HH}$ 7 Hz)	60
9	1.60 (d, $^3J_{HH}$ 7 Hz)	15		1.64 (d, $^3J_{HH}$ 7 Hz)	15
N–CH$_3$	2.81, s	31		2.84, s	31
4-Me	–	–		2.48, s	21

benzocaine could cause a serious blood disorder, methemoglobinemia [14,15]. Similar concerns are raised elsewhere, e.g. Taleb and co-workers review the evidence where even following topical application of benzocaine, oxidised haemoglobin might increase causing methemoglobinemia [16]. Even more recently, a few weeks ago, a case of acquired methemoglobinemia due to the frequent use of the legal high "Pink Panthers" was reported in parallel with the increasing prevalence of legal highs particularly those containing added benzocaine [17]. Benzocaine is currently being used in the illicit drug market as a common adulterant (cutting agent) to increase profit [18], indeed it is now commonly found in cocaine hydrochloride [18–20], which might link the supply of such adulterated flephedrone to that of other commonly cut illicit drugs.

Drug dealers may use benzocaine as it is easily accessible and similar in physical appearance to flephedrone. In sufficient quantity, benzocaine might be added to flephedrone in order to mimic the initial numbing (anaesthetic) effect of cocaine [21] and possibly even to sell the mixture not as flephedrone but as cocaine.

Our rapid analysis and quantification of 4-FMC and benzocaine compare well with the literature methods [7,12]. Research is also on-going to replace aspects of wet-chemistry based quantitative analysis on minimal trace amounts of illegal drugs with rapid in situ (e.g. potentially on-site analysis in a nightclub) screening using handheld (therefore portable) spectrophotometers [22,23]. The use of Raman spectroscopy to detect counterfeit medicines [22] may be extended to the analysis of street drugs [23], whilst
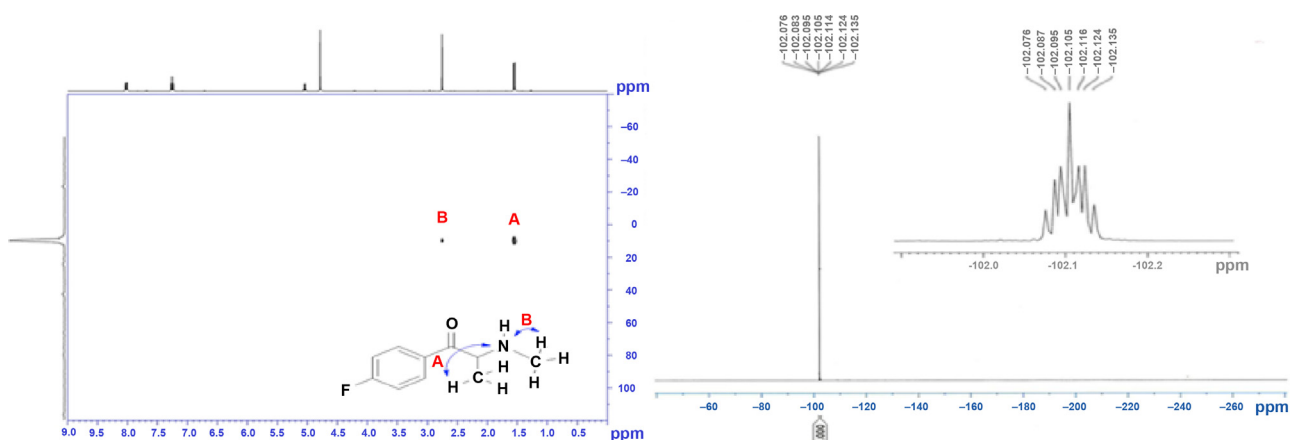
Fig. 3. ^{15}N-HMBC (500 MHz) spectrum of flephedrone (D_2O) (left) showing two ^{15}N–^1H couplings (with peak assignments) and ^{19}F NMR spectrum (^1H coupled) of flephedrone (D_2O) (right) (in the presence of ~7% benzocaine).
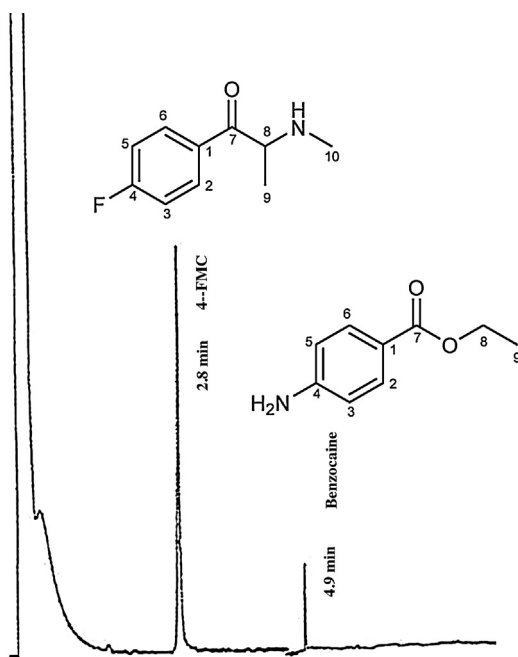
Fig. 4. GC-FID of flephedrone samples impure with benzocaine (showing ring numbering systems).

discrimination between simple drug mixtures has been achieved using a deep ultraviolet-visible (DUV–vis) reflected optical fibre sensor [24]. Both techniques can detect and possibly quantify known drugs for which standard spectra are available, but are not suitable for the analysis of new drugs.

4. Conclusions

The combination of ^{1}H, ^{13}C, ^{15}N, and ^{19}F NMR spectroscopy was used to identify and decipher between flephedrone regioisomers and other cathinones such as mephedrone. We have separated flephedrone and benzocaine with base-line resolution by GC-FID and quantified the amount of benzocaine adulterant in the four (nightclub) flephedrone samples by GC, the ^{1}H NMR integration was in broad agreement. These data show that this now illegal cathinone-derived stimulant (a high) is now being cut by 5–12% with benzocaine, so drug users cannot be certain of the purity of the drug they are taking. Benzocaine as an adulterant can cause serious health problems, potentially more harmful than flephedrone. Therefore, there are risks from the pharmaceutically active cutting agents themselves.

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