

Test purchase, synthesis and characterization of 3-fluorophenmetrazine (3-FPM) and differentiation from its *ortho*- and *para*-substituted isomers

Gavin McLaughlin,^{a,b*} Noreen Morris,^a Pierce V. Kavanagh,^b Geraldine Dowling,^b John D. Power,^{b,c} Brendan Twamley,^d John O'Brien,^d Brian Talbot,^e Harald H. Sitte,^{f,g} and Simon D. Brandt^h

^a *Department of Life and Physical Sciences, School of Science, Athlone Institute of Technology, Dublin Road, Westmeath, Ireland*

^b *Department of Pharmacology and Therapeutics, School of Medicine, Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8, Ireland*

^c *Forensic Science Laboratory, Garda HQ, Dublin 8, Ireland*

^d *School of Chemistry, Trinity College Dublin, Dublin 2, Ireland*

^e *School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Dublin 2, Ireland*

^f *Institute of Pharmacology, Center of Physiology and Pharmacology, Medical University Vienna, Vienna, Austria*

^g *Center for Addiction Research and Science, Medical University Vienna, Waehringstrasse 13A, 1090 Vienna, Austria*

^h *School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK*

*Correspondence to: Gavin McLaughlin, Department of Pharmacology & Therapeutics, School of Medicine, Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8, Ireland. Email: gmclaug@tcd.ie or gavinmclaughlin@research.ait.ie

Running title: Synthesis and characterization of fluorophenmetrazine positional isomers

Keywords: New psychoactive substances; psychostimulants; positional isomers; phenmetrazine; chemistry.

Abstract

The knowledge captured in patent and scientific research literature stimulates new ideas and fosters new drug development efforts. Manufacturers and entrepreneurs dedicated to the sale of 'research chemicals' and/or new psychoactive substances (NPS) also make use of the access of information to identify, prepare and launch a range of new substances. One of the most recent compounds to appear on the NPS market was the phenmetrazine analog 3-fluorophenmetrazine (3-FPM) that represented one of many phenylmorpholines designed to explore treatment options in areas such as obesity and drug dependence. The anorectic drug analogs phenmetrazine and phendimetrazine, used as prescription medicines before they were withdrawn, feature amphetamine-like properties associated with monoamine release and available data on 3-FPM suggest that the effects might show mechanistic overlaps. This study describes the synthesis and extensive analytical characterization of 3-FPM and its differentiation from synthesized *ortho*- and *para*- substituted isomers, 2-FPM and 4-FPM, respectively. This study was triggered by the purchase of five powdered samples advertised as 3-FPM by five different Internet vendors based in the United Kingdom. The analytical data obtained for the vendor samples were consistent with the synthesized 3-FPM standard and differentiation between all three isomers was possible. The presence of positional isomers and the absence of suitable reference material can cause difficulties in the day-to-day operation of forensic work and given the rate at which many of the newly emerging NPS appear on the market, a comprehensive approach is needed when attempting to decipher the identity of NPS arriving onto the drug market.

Introduction

Over the past number of years in Europe, there has been an unprecedented increase in the number, types and seizures of chemicals frequently referred to as new psychoactive substances (NPS). There was no change to this trend in 2014 as a total of 101 new substances was detected and reported for the first time by the European Union Early Warning System, which brings the total number of substances being monitored by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) to more than 450 substances.^[1] The nature of substances available for purchase is not limited to compounds derived from illicit drugs as increasing ranges of compounds derived from medicinal products have also joined the catalogs of NPS suppliers.^[2]

Phenmetrazine (3-methyl-2-phenylmorpholine) is a synthetic morpholine derivative of amphetamine that includes a phenylisopropylamine skeleton where the terminal amine is incorporated into a morpholine ring.^[3] In the 1950s, phenmetrazine and its *N*-methyl derivative phendimetrazine (3,4-dimethyl-2-phenylmorpholine) (Figure 1A) were developed within the pharmaceutical setting as sympathomimetic weight-control medications considered to show less abuse liability compared to other amphetamine anorexiant.^[3-6] Phenmetrazine is a potent substrate for norepinephrine and dopamine transporters and displays stimulant properties similar to those of amphetamine, whereas phendimetrazine is classified as a pro-drug and exerts its pharmacological effect through biotransformation to phenmetrazine.^[3, 7-8] Following the observation of adverse drug reactions relating to phenmetrazine use, it was withdrawn from the market and classified as a Schedule II drug under the United Nations Convention on Psychotropic Substances 1971. Similarly, phendimetrazine was listed in Schedule IV of the Convention.^[9-10] In the 1970s, phenmetrazine (street name 'Prellies') reportedly replaced amphetamine after it became known in popular culture due to its association with The Beatles.^[11-13]

Recent studies indicate that phendimetrazine may be a suitable candidate for the treatment of cocaine addiction.^[14-19] Phendimetrazine has also been suggested as an effective and safer alternative to *d*-amphetamine as it may simultaneously function as a monoamine uptake inhibitor (via the parent drug) and as a monoamine releaser (via the active metabolite).^[14] However in recent years, it has also been observed that NPS manufacturers are also finding new uses for established (and/or withdrawn) medicines since several derivatives of these medicinal products can be found as 'research chemicals' on the NPS market.

Similar to that of other anorectic agents such as aminorex,^[20,21] the chemical structure of phenmetrazine may be open to structural manipulation through

substitution of the phenyl and/or morpholine ring. In 2011, a study by Blough *et al.* featured the synthesis and pharmacological evaluation of many phenylmorpholine analogs for potential therapeutic applications.^[3] For example, 2-(3-fluorophenyl)-3-methylmorpholine (3-fluorophenmetrazine, 3-FPM, PAL-593) and 2-(4-fluorophenyl)-3-methylmorpholine (4-fluorophenmetrazine, 4-FPM, PAL-748) (Figure 1A) were synthesized and pharmacological evaluations revealed that both isomers were substrate-type monoamine releasers with higher selectivity toward catecholamines.^[3] In the synaptosomal preparations used, 3-FPM showed higher potency than 4-FPM in the ability to induce dopamine and norepinephrine release and further investigations related to the 2-FPM isomer are needed for further comparisons.^[3] Studies such as these can be exploited by manufacturers of NPS who are examining the scientific and patent literature for compounds that are considered potentially suitable as NPS products. In the United Kingdom, one of the psychostimulants available for purchase, either individually or as a component in a mixture present in range of branded products, was *N*-methyl-1-(thiophen-2-yl)propan-2-amine (methiopropamine, MPA). A recent change in legislation placed this substance under control^[22] which makes it possible that 3-FPM might act as a replacement in such products.

This study reports on the first phenmetrazine analog to be encountered on the NPS drug market, namely 3-fluorophenmetrazine (3-FPM). The study describes the synthesis and analytical characterization of 3-FPM and differentiation from its *ortho*- and *para*- substituted isomers, 2-FPM and 4-FPM, respectively, that have also been prepared for comparison (Figure 1A). This was triggered by the purchase of five powdered samples advertised as 3-FPM by five different Internet vendors based in the United Kingdom. Various chromatographic, spectroscopic and mass spectrometric platforms were employed followed by structural investigations using X-ray crystal structure analysis.

Reagents and standards

All reagents and dry solvents used in the syntheses were obtained from Sigma Aldrich Ltd (Arklow, Co. Wicklow, Ireland). 2-, 3- and 4-fluoropropiophenone were obtained from Alfa Aesar (Heysham, United Kingdom). Preparative silica gel thin layer chromatography plates (UV254, GF 20 x 20 cm, 2000 microns) were obtained from Analtech (Newark, United States). LC-MS grade solvents were obtained from Fisher Scientific (Dublin, Ireland). Five samples, advertised as 3-fluorophenmetrazine (3-FPM), were purchased from five different online vendors based in the United Kingdom.

Syntheses

2-(2-Fluorophenyl)-3-methylmorpholine (2-fluorophenmetrazine, 2-FPM)

A solution of bromine (0.05 mol, 4.34 g, 1.40 mL) in dichloromethane (26.5 mL) was added slowly to a solution of 2-fluoropropiophenone (26.3 mmol, 4.0 g) in dichloromethane (26.5 mL). The mixture was stirred for 1 h, dried (anhydrous magnesium sulfate) and the solvent was removed to afford a colorless oil (21.6 mmol, 5.0 g). This oil was dissolved in acetonitrile (66.25 mL) and the addition of ethanolamine followed (0.055 mol, 3.33 g, 3.30 mL). The reaction mixture was heated for 3 h at 40 °C and then left to stir overnight at room temperature. To this mixture, a solution of sodium borohydride (0.1 mol, 4.0 g) in water (100 mL) was added and allowed to stir at room temperature overnight. The reaction mixture was partitioned between dichloromethane and water, the organic layer collected, dried (anhydrous magnesium sulfate), and the solvent removed to give a bright-yellow, viscous oil (15.3 mmol, 3.257g). This oil was then purified by flash chromatography (ethanol:ethyl acetate, 6:4) to afford a yellow oil (3.5 mmol, 0.739 g, 23%). The oil was dissolved in dichloromethane (5 mL) and conc. sulfuric acid (0.41 mol, 40.48 g, 22 mL) was added to the mixture (reaction vessels kept on ice) and allowed to stir overnight. Water (250 mL) was added and the reaction mixture was made alkaline with 10 M sodium hydroxide and extracted into dichloromethane. Drying with magnesium sulfate and removal of solvent yielded crude 2-fluorophenmetrazine (3.1 mmol, 0.608 g) as a cloudy, yellow oil. Purification was conducted by preparative thin layer chromatography using dichloromethane/methanol (8:2) as the mobile phase. The purified band was removed from the TLC plate, dissolved in ethanol, centrifuged and isolated as a white solid (1.20 mmol, 0.233 g, 5%).

Preparation of 2-fluorophenmetrazine fumarate

To the purified sample, dissolved in 0.5 mL methanol, was added fumaric acid (1.20 mmol, 0.139 g) dissolved in 2 mL methanol. The solvent was removed and the addition of *tert.*-butyl methyl ether (TBME) afforded a white solid powder (1.0 mmol, 0.332 g): m.p. 150 °C (148–152 °C). ¹H NMR (DMSO) δ ppm 7.48 (t; *J* = 7.4 Hz; 1H; Ar-CH; H-2'), 7.42–7.35 (m; 1H; Ar-CH; H-4'), 7.27–7.17 (m; 2H; Ar-CH; H-1 and H-3'), 6.55 (s; 2H; CH; fumarate), 4.50–4.46 (m; 1H; CH; H-2), 3.97–3.68 (m; 2H; CH₂; H-6), 3.06–3.02 (m; 3H; 1 x CH, 1 x CH₂; H-3, H-5), 0.85 (d; *J* = 6.4 Hz; 3H; CH₃). ¹³C NMR (DMSO) δ ppm 167.0526 (C=O; fumarate), 159.51 (*J*_{CF} = 243.6 Hz; Ar-CF; C-6'), 134.60 (CH; fumarate), 130.49 (*J*_{CF} = 8.2 Hz; Ar-CH; C-2'), 128.86 (*J*_{CF} = 3.5 Hz; Ar-CH; C-3'), 125.88 (*J*_{CF} = 11.5 Hz; Ar-CH; C-5'), 124.73 (*J*_{CF} = 3.3 Hz; Ar-CH; C-4'), 115.12 (*J*_{CF} = 22.1 Hz; Ar-CH; C-1), 75.85 (CH; C-2), 65.63 (CH₂; C-6), 54.54 (CH; C-3), 44.10 (CH₂; C-5), 15.60 (CH₃); ¹⁹F NMR (d₆-DMSO) δ ppm –

118.64; HR-ESIMS observed m/z 196.1143 (theory $[M + H]^+$: $C_{11}H_{15}FNO^+$ m/z 196.1132 ($\Delta = 5.61$ ppm).

2-(3-Fluorophenyl)-3-methylmorpholine (3-fluorophenmetrazine, 3-FPM)

The reaction was carried out as described above using 3-fluoropropiophenone instead yielding 3-fluorophenmetrazine free base as a colorless oil (0.5 mmol, 0.093 g, 2%). The free base was converted to the fumarate salt yielding a white solid (0.5 mmol, 0.128 g): m.p.178 °C (176–180 °C). 1H NMR (DMSO) δ ppm 7.43–7.36 (m; 1H; Ar-CH; H-3'), 7.21–7.12 (m; 3H; Ar-CH; H-2', H-4', H-6'), 6.55 (s; 2H; CH; fumarate), 4.13 (d; $J = 6.0$ Hz; 1H; CH; H-2), 3.94–3.62 (m; 2H; CH₂; H-6), 3.05–2.95 (m; 2H; CH₂; H-5), 2.93–2.86 (m; 1H; CH; H-3), 0.80 (d; $J = 6.5$ Hz; 3H; CH₃); ^{13}C NMR (DMSO) δ ppm 167.29 (C=O, fumarate), 161.94 ($J_{CF} = 241.9$ Hz; Ar-CF; C-1), 142.23 ($J_{CF} = 6.5$ Hz; Ar-CH; C-5'), 134.72 (CH; fumarate), 130.10 ($J_{CF} = 8.4$ Hz; Ar-CH; C-3'), 123.68 ($J_{CF} = 1.2$ Hz; Ar-CH; C-4'), 114.86 ($J_{CF} = 20.8$ Hz; Ar-CH; C-2'), 114.11 ($J_{CF} = 21.7$ Hz; Ar-CH; C-6'), 82.76 (CH; C-2), 65.76 (CH₂; C-6), 54.89 (CH; C-3), 44.44 (CH₂; C-5), 16.40 (CH₃); ^{19}F NMR (d₆-DMSO) δ ppm –113.73; HR-ESIMS observed m/z 196.1138 (theory $[M + H]^+$: $C_{11}H_{15}FNO^+$ m/z 196.1132 ($\Delta = 3.06$ ppm).

2-(4-Fluorophenyl)-3-methylmorpholine (4-fluorophenmetrazine, 4-FPM)

The reaction was carried out as described above using 4-fluoropropiophenone instead yielding the 4-fluorophenmetrazine free base as a white solid (0.8 mmol, 0.162 g, 3%). The free base was converted to the fumarate salt yielding a white solid powder (0.8 mmol, 0.240 g): m.p.170–172 °C. 1H NMR (DMSO) δ ppm 7.43–7.34 (m; 2H; Ar-CH; H-4', H-6'), 7.22–7.11 (m; 2H; Ar-CH; H-1, H-3'), 6.55 (s; 2H; CH; fumarate), 4.19 (d; $J = 9.4$ Hz; 1H; CH; H-2), 3.96–3.67 (m; 2H; CH₂; H-6), 3.10–2.96 (m; 3H; 1 x CH, 1 x CH₂; H-3; H-5), 0.82 (d; $J = 6.6$ Hz; 3H; CH₃). ^{13}C NMR (DMSO) δ ppm 167.13 (C=O; fumarate), 161.83 ($J_{CF} = 242.3$ Hz; Ar-CF; C-2'), 135.11 ($J_{CF} = 2.8$ Hz; Ar-CH, C-5'), 134.63 (CH; fumarate), 129.56 ($J_{CF} = 8.1$ Hz; Ar-CH; C-6'), 129.51 ($J_{CF} = 8.1$ Hz; Ar-CH; C-4'), 115.11 ($J_{CF} = 21.2$ Hz; Ar-CH; C-1), 114.97 ($J_{CF} = 21.2$ Hz; Ar-CH; C-3'), 82.04 (CH; C-2), 65.21 (CH₂; C-6), 54.67 (CH; C-3), 43.96 (CH₂; C-5), 15.81 (CH₃); ^{19}F NMR (d₆-DMSO) δ –114.28 ppm; HR-ESIMS observed m/z 196.1134 (theory $[M + H]^+$: $C_{11}H_{15}FNO^+$ m/z 196.1132 ($\Delta = 1.02$ ppm).

Instrumentation

Gas chromatography mass spectrometry

The gas chromatography system consisted of an Agilent 6980 GC coupled to an Agilent 5973 MSD (Agilent, Little island, Cork) equipped with a HP-5ms column (30 m x 0.25 mm x 0.25 μ m). The helium carrier gas flow rate was set at a constant flow of 1 mL/min in splitless mode. Injection port and transfer line temperatures were set at 250 °C and 280 °C. Oven temperature: 40 °C held for 1 min, ramped at 10 °C/min to 250 °C, within 22 minutes, then ramped again at 20 °C/min to 300 °C and held for 3 min. The total run time was 27.5 min.

Liquid chromatography electrospray mass spectrometry

LC-MS analyses were performed on an Agilent 1100 HPLC system equipped with a G13795 degasser, G1312A BinPump, a G1313A ALS and G1316A column oven (COLCOM) (Agilent, Little Island, Cork). Separation was obtained on a Kinetex phenyl-hexyl column (2.6 μ m, 100 x 2.10 mm) Phenomenex (Cheshire, United Kingdom). The analytes were eluted under isocratic conditions using a mobile phase of 97% water and 3% acetonitrile (both containing 0.1% formic acid). The Agilent LC-MSD settings were as follows: positive electrospray mode, capillary voltage 3500 V, drying gas (N₂) 12 L/min at 350 °C, nebulizer gas (N₂) pressure 50 psi, SIM *m/z* 196, fragmentor voltage 50 V. Samples for LC-MS analysis were dissolved in acetonitrile/water (1:1, containing 0.1% formic acid) at a concentration of 10 μ g/mL. The injection volume was 0.5 μ L, flow rate was 0.4 mL/min and the column temperature was 30 °C. Total run time was 25 min.

High-resolution electrospray mass spectrometry

HR-ESI mass spectra were recorded by direct injection into a LTQ Orbitrap Discovery (Thermo Fisher Scientific, Bremen, Germany). Samples were dissolved in acetonitrile/water (1:1, containing 0.1% formic acid) and infused at a rate of 5 μ L/min. Full accurate high-resolution (30000) mass scans were performed in positive electrospray mode. Measured accurate masses were within \pm 5 ppm of the theoretical masses. The following conditions were used: drying gas (N₂) 10 L/min, capillary temperature 310 °C, spray voltage 4 V, capillary voltage 22 V and tube lens 77 V. The mass calibration procedure was performed in both positive and negative mode using solutions of caffeine, L-methionyl-arginyl-phenylalanyl-alanine acetate \times H₂O (MRFA), Ultramark 1621[®], sodium docecyl sulfate and sodium taurocholate.

Thin layer chromatography

Separations were conducted using TLC silica gel 60 (F254, 20 x 20 cm) aluminium sheets (Merck, Germany). The mobile phase used was ethyl acetate/methanol (8:2) with 0.1% ammonia (7N in methanol). All standards and vendor samples were dissolved in the mobile phase and vortex mixed before spotting onto the TLC plate. UV light at 254 nm was used for detection.

Nuclear magnetic resonance spectroscopy

All analytes were prepared in deuterated dimethyl sulfoxide (DMSO- d_6) at a concentration of 20 mg/mL. ^1H (600 MHz) and ^{13}C (150 MHz) spectra were recorded on a Bruker AV600 NMR spectrometer using a 5 mm TCI cryoprobe. ^1H NMR spectra were referenced to an external TMS reference at $\delta = 0$ ppm. ^{19}F (376 MHz) spectra were recorded on a Bruker DPX400 NMR spectrometer and the external reference was trifluorotoluene set at $\delta = -64$ ppm.

X-ray crystallography

Data were obtained for the synthesized 3-FPM standard and one representative vendor sample on a Bruker APEX DUO with Cu K α and Mo K α radiation ($\lambda = 1.54178$ and 0.71073 Å) using a MiTeGen micromount and at 100(2) K (Oxford Cobra Cryosystem). The Bruker APEX2 software^[23] was used to collect and reduce data, determine the space group, solve and refine the structure. Absorption corrections were applied using SADABS.^[24] All final refinements were performed with SHELXL.^[25] All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were assigned to calculated positions using a riding model. Donor hydrogen atoms were refined for the 3-FPM standard and refined with restraints for the 3-FPM vendor sample. CCDC 1418406 and 1418407 contains the supplementary crystallographic data for this investigation. Crystal data and structure refinement parameters were as follows:

Synthesized 3-FPM standard: $\text{C}_{13}\text{H}_{16}\text{FNO}_3$, $M = 253.27$, $T = 100(2)$ K, Monoclinic, $P2_1/c$, $a = 18.7821(8)$, $b = 7.7948(3)$, $c = 8.7467(4)$ Å, $\beta = 91.8726(14)^\circ$, $V = 1279.86(9)$ Å³, $Z = 4$, μ (Cu K α) = 0.863 mm⁻¹, $\rho = 1.314$ Mg/cm³, 14670 reflections collected, 2421 independent ($R_{\text{int}} = 0.0367$), $^aR_1 = 0.0516$, $wR2 = 0.1338$ ($I > 2\sigma(I)$), $S = 1.081$. CCDC 1418406.

Representative 3-FPM vendor sample: $\text{C}_{11}\text{H}_{15}\text{ClFNO}$, $M = 231.69$, $T = 100(2)$ K, Monoclinic, Cc , $a = 6.1009(4)$, $b = 29.1650(18)$, $c = 7.0747(5)$ Å, $\beta = 111.322(2)^\circ$, $V = 1172.66(14)$ Å³, $Z = 4$, μ (Mo K α) = 0.313 mm⁻¹, $\rho = 1.312$ Mg/cm³, 15349 reflections collected, 2125 independent ($R_{\text{int}} = 0.0568$), $^aR_1 =$

0.0339, $wR_2 = 0.0653$ ($I > 2\sigma(I)$), $S = 1.028$. CCDC 1418407. $^aR_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$, $wR_2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{1/2}$.

Results and discussion

Five powdered samples, advertised as 3-fluorophenmetrazine (3-FPM), were obtained from different online vendors in the United Kingdom and subjected to extensive analytical characterizations. The vendor samples were characterized as 3-FPM and consistent with the information given on the product labels which was verified by organic synthesis of this material. Given the challenges associated with the ability to correctly identify a suspected positional isomer, further investigations were carried out by syntheses and characterizations of the 2-, 3- and 4-fluorophenmetrazine isomers (2-, 3- and 4-FPM). Currently, extensive analytical data on 3-FPM and its positional isomers appear to be lacking in the reported literature.

The synthesis employed for the preparations of 2-, 3- and 4-FPM was adapted from Blough et al. (Figure 1B).^[3] The synthesis involved bromination of the fluoropropiophenone starting material (a), yielding α -bromo-fluoropropiophenone (b). This was reacted with ethanolamine to give the intermediate 1-(3-fluorophenyl)-2-((2-hydroxyethyl)amino)propan-1-one (c). Reduction to the alcohol (d) was achieved by reaction with sodium borohydride followed by reaction with concentrated sulfuric acid to aid cyclization and formation of the morpholine ring (e). It was found most practical to generate the fumarate salts to induce crystallization. The formation of hydrochloride salts was attempted for each FPM isomer, however sufficient yields were not obtained for analytical characterization.

Similar to the aminorex analogs previously detected on the NPS market,^[20,21] the fluorinated analogs of phenmetrazine contain two chiral centres which yield the potential for four stereoisomers and two racemic mixtures (i.e., *cis*- and *trans*-racemates) (Figure 1C). The results observed for the preparation of the FPM isomers were consistent with the reported synthesis of phenmetrazine where the formation of the more *trans*- isomer was reported.^[26,27] Analytical characterization of the 3-FPM reference standard and vendor sample using X-ray crystallography concluded that both were consistent with the *trans*- isomeric form. The analyses of the five 3-FPM products also revealed high purity as judged by chromatographic characterizations and nuclear magnetic resonance spectroscopy (NMR) (supplemental data).

[Insert Figure 1 here]

Analytical features

Gas chromatography mass spectrometry (GC-MS)

Initial analysis of the underivatized isomers by GC-MS failed to obtain separation between *meta*- and *para*- substituted 3- and 4-FPM isomers although separation for 2-FPM was feasible (Figure 2A). Derivatization with heptafluorobutyric anhydride (HFBA) (Figure 2C) and pentafluoropropionic anhydride (PFBA) (Figure 2E) improved the chromatography results dramatically whereas reaction with trifluoroacetic anhydride (TFAA) (Figure 3A – 3C) retained some overlap between 3-FPM and 4-FPM. The electron ionization (EI) mass spectra recorded for underivatized 3-FPM, and its HFBA and PFBA derivatives are shown in Figures 2B, 2D and 2F, respectively. A comparison of mass spectral data acquired for all three isomers were identical (supplemental information). A proposed fragmentation pathway for underivatized 3-FPM under EI-MS conditions is shown in Figure 3D.

As mentioned above, derivatization of FPM isomers with TFAA gave incomplete separation but diagnostically useful information was obtained based on their mass spectra. The spectra obtained for FPM-TFAA isomers provided distinctive differences that facilitated differentiation of the 3-FPM isomer from its 2- and 4-FPM counterparts (Figure 3A – 3C). In addition to the molecular ion at m/z 291 that was detectable in all three mass spectra, an ion at m/z 290 was observed in the spectrum of 3-FPM-TFAA that was not detected in the other two instances. The detection of this ion was rationalized by a possible rearrangement that might have involved the loss of a hydrogen radical and the formation of a thermodynamically stable five membered ring. In this case, a bond would have been formed between the carbon at the *ortho*- position of the phenyl ring and the methyl group on the morpholine ring (Figure 3E). The proposed fragmentation pattern for the FPM-TFAA isomers is outlined in Figure 3E. The fragment observed at m/z 222 might have been the result of radical loss of CF_3 via cleavage of the nitrogen in the morpholine ring. A further loss of carbon monoxide would be consistent with m/z 194. These fragment arrangements are similar to that obtained for TFAA derivatives of amphetamine-based designer drugs discussed in the literature.^[28] Two dominant fragments were noticed at m/z 70 and m/z 167. The m/z 70 indicated a potential loss of the ring-substituted fluorobenzyl alcohol, which is suggested to give rise to a 2-methylazetidinium ion ($C_4H_8N^+$). The base peak at m/z 167 could have been accounted for by a loss of hydrogen cyanide from the m/z 194 ion, thus, resulting in a fragment with a formula of $C_{10}H_{12}FO^+$, possibly consistent with ring-substituted (1-methoxypropyl)benzene ion. Further cleavage of a methyl radical could then

result in the detection of a methyloxirane species at m/z 152. The loss of a hydroxyl group then leads to the formation of a cyclopropylium species at m/z 135. The GC-MS analysis of vendor samples confirmed that all five test purchases were consistent with the identity of the 3-FPM isomer as claimed on the product label (supplemental information).

[Insert Figures 2 and 3 here]

Liquid chromatography mass spectrometry (HPLC-MS)

Separation of all three fluorophenmetrazine isomers was deemed satisfactory with retention times of 16.18 min, 18.48 min and 19.34 min for 2-FPM, 4-FPM and 3-FPM, respectively (Figure 4A) and analysis of all five purchased 3-FPM samples revealed identical retention times. During initial method development, the reproducibility of retention times was found to be challenging but improved when exploring a mobile phase composition of 97% water and 3% acetonitrile (both containing 0.1% formic acid).

The product ion spectra obtained from in-source collision-induced dissociation (CID) at increased fragmentor voltage (150 V) and the suggested dissociation pathways are shown in Figure 4B and 4C. The formation of m/z 178 might have represented a loss of methanol from the protonated molecule (m/z 196), consistent with $C_{11}H_{13}FN^+$. The m/z 152 ion was consistent with a loss of ethylene oxide from $[M + H]^+$ to form an aziridine species. The product ion at m/z 135 might have formed following the loss of ethenamine (C_2H_5N) from m/z 178 and/or the loss of NH_3 from the aziridine species at m/z 152. A loss of HF from the m/z 135 ion might have resulted in the detection of m/z 115. Implementation of high-resolution mass spectrometry provided elemental compositions with acceptable mass accuracies consistent with the proposed structures (Figure 4C).

[Insert Figure 4 here]

Thin layer chromatography (TLC)

It was encouraging to observe that TLC analysis also facilitated a successful separation of the three isomers as evidenced by distinct retardation factors of 0.65, 0.51 and 0.43 for 2-, 3- and 4-FPM, respectively (Figure 5). This served as a valuable reminder that a seemingly basic separation technique should not be discounted when facing the challenge of dealing with the presence of isomers, particularly when operating within a forensic context where time and financial constraints can be significant. Indeed, previous investigations on

three methoxydiphenidine isomers (2-, 3-, and 4-MXP) have shown how useful TLC can be for the differentiation of NPS isomers.^[29]

[Insert Figure 5 here]

X-ray crystallography

Consistent with reports on phenmetrazine encountered in the literature,^[26,27] it was observed that the adopted synthetic pathway used for the fluorophenmetrazine isomers led to the formation of the more stable *trans*-racemate. X-ray crystal structure analysis was employed to investigate the structural features of the synthesized 3-FPM reference standard, in addition to one representative 3-FPM vendor sample (Figure 6). The 3-FPM reference standard was prepared as the fumarate salt, crystallized from ethyl acetate:methanol, and found to crystallize in the centrosymmetric space group P2(1)/c where one half of the fumarate was generated by symmetry (supplemental information). The crystal structure of the 3-FPM vendor sample is shown in Figure 6, and confirmed that it was manufactured as the hydrochloride salt, which crystallized in the polar space group Cc as the (*S*)-enantiomer, i.e. showing (*S*)-conformation at C7 and C8. In both structures, the 3-FPM cation displayed the chair conformation consistent with earlier reports on phenmetrazine hydrochloride^[30] and 3,5-dimethyl-2-(3-chlorophenyl)-2-morpholinol hydrochloride, respectively.^[31,32] An overlay of both 3-FPM phenmetrazinium cations shows that there was negligible impact on conformation when changing the counter ion with a weighted RMS deviation of 0.09 Å (F1–C14 of both moieties used, see supplemental information). The hydrogen bonding network in the 3-FPM vendor sample was similar to that in phenmetrazine hydrochloride with NH₂⁺...Cl⁻ hydrogen bonds of ca. 3.06 and 3.08 Å forming a ion pair dimer array parallel to the *a*-axis. In the synthesized 3-FPM reference standard hydrogen bonding network was more complex. The fumarate ion contained more H-bonding acceptors and formed a 3D network and a much stronger association in comparison to the Cl⁻ salt with hydrogen bonds ~2.69 Å between the morpholinium NH₂⁺...O of the fumarate anion.

[Insert Figure 6]

A key challenge encountered in the development of new medicines that target monoamine transporters is the potential for abuse liability, which triggered an interest in the exploration of dual dopamine/serotonin releasers with low abuse potential.^[33-36] At the same time, the knowledge generated by these

important research endeavors have also opened the door to unregulated and globalized markets that provide access to the general public, for example in the form of 'research chemicals' and NPS.^[2] It is hoped that the increasing challenges associated with these developments do not interfere with the capacity of developing potentially promising medicines.

Conclusion

The release of new psychoactive substances onto the recreational drug market continues to create challenges for scientists in the forensic, clinical and toxicology fields. The appearance of substances such as 3-fluorophenmetrazine reflects the gathering of information from the patent literature concerned with the development of potential medicines. A corollary of this approach is that the appearance of positional isomers has to be considered when facing the correct identification of substances considered as new psychoactive substances (NPS). The combination of test purchases, analytical characterization and confirmation by organic synthesis was found to be a useful and proactive approach for the generation of analytical data that may be of interest to a range of stakeholders. Given the rate at which many of newly emerging NPS appear on the market, increasing collaborations are needed between forensic science laboratories and academic institutions when attempting to actively engage in the challenges linked to the NPS phenomenon.

References

1. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). New psychoactive substances in Europe. An update from the EU Early Warning System (March 2015). Lisbon, 2015. Available at: http://www.emcdda.europa.eu/attachements.cfm/att_235958_EN_TD0415135ENN.pdf [22 August 2015]
2. S.D. Brandt, L.A. King, M. Evans-Brown. The new drug phenomenon. *Drug Test. Anal.* **2014**, *6*, 587.
3. B.E. Blough, R. Rothman, A. Landavazo, K.M. Page, A.M. Decker. Preparation of phenylmorpholines and analogs thereof. Patent No. WO 2011/146850 A1. Research Triangle Institute, USA, **2011**.
4. Hampson, J. Phemetrazine and dexamphetamine in the management of obesity. *Lancet.* **1960**, *275*, 1265.

5. A. Boehringer, E. Boehringer, I. Liebrecht, J. Liebrecht. Improvements in or relating to the preparation of substituted morpholines. Patent No. GB 773780. C. H. Boehringer Sohn. Ingelheim, Germany, **1957**.
6. A. Boehringer, E. Boehringer, I. Liebrecht, J. Liebrecht, W. Mayer-List. Improvements in or relating to the production of substituted tetrahydrooxazines. Patent No. GB 791416. C. H. Boehringer Sohn. Ingelheim, Germany, **1958**.
7. G.R. Rudolph, J.R. Miksic, M. J. Levitt. GLC determination of phedimetrazine in human plasma, serum, or urine. *J. Pharm. Sci.* **1983**, *72*, 519.
8. A.H. Beckett, M.A. Salami. A note on the identification N-hydroxyphenmetrazine and phenmetrazine. *J. Pharm. Pharmacol.* **1974**, *24*, 900.
9. D.G. Barceloux. Medical toxicology of drug abuse – synthesized chemicals and psychoactive plants. John Wiley & Sons, New Jersey. **2012**.
10. United Nations Convention on Psychotropic Substances 1971. Available at: https://www.unodc.org/pdf/convention_1971_en.pdf [23 October 2015].
11. J. Mellar, L.E. Hollister. Phenmetrazine: an obsolete problem drug. *Clin. Pharmacol. Ther.* **1982**, *32*, 671.
12. B. Miles, P. McCartney. Many years from now. London: Secker and Warburg. **1997**.
13. H. Davies. The beatles: the authorized biography. McGraw-Hill, United States. **1968**.
14. M.L. Banks, B.E. Blough, T.R. Fennell, R.W. Snyder, S.S. Negus. Effects of phendimetrazine treatment on cocaine vs food choice and extended-access cocaine consumption in rhesus monkeys. *Neuropsychopharmacology* **2013**, *38*, 2698.
15. M.L. Banks, B.E. Blough, T.R. Fennell, R.W. Snyder, S.S. Negus. Role of phenmetrazine as an active metabolite of phendimetrazine: evidence from studies of drug discrimination and pharmacokinetics in rhesus monkeys. *Drug Alcohol Depend.* **2013**, *130*, 158.

16. M.L. Banks, B.E. Blough, S.S. Negus. Effects of 14-day treatment with the schedule III anorectic phendimetrazine on choice between cocaine and food in rhesus monkeys. *Drug Alcohol Depend.* **2013**, 131, 204.
17. M.L. Banks, B.E. Blough, S. Stevens Negus. Interaction between behavioral and pharmacological treatment strategies to decrease cocaine choice in rhesus monkeys. *Neuropsychopharmacology* **2013**, 38, 395.
18. C.T. Bauer, M.L. Banks, B.E. Blough, S.S. Negus. Rate-dependent effects of monoamine releasers on intracranial self-stimulation in rats: implications for abuse liability assessment. *Behav. Pharmacol.* **2013**, 24, 448.
19. P.W. Czoty, P. Tran, L.N. Thomas, T.J. Martin, A. Grigg, B.E. Blough, T.J.R. Beveridge. Effects of the dopamine/norepinephrine releaser phenmetrazine on cocaine self-administration and cocaine-primed reinstatement in rats. *Psychopharmacology* **2015**, 232, 2405.
20. S.D. Brandt, M.H. Baumann, J.S. Partilla, P.V. Kavanagh, J.D. Power, B. Talbot, B. Twamley, O. Mahony, J. O'Brien, S.P. Elliott, R.P. Archer, J. Patrick, K. Singh, N.M. Dempster, S.H. Cosbey. Characterization of a novel and potentially lethal designer drug (\pm)-*cis-para*-methyl-4-methylaminorex (4,4-DMAR, or 'Serotoni'). *Drug. Test. Anal.* **2014**, 6, 684.
21. G. McLaughlin, N. Morris, P.V. Kavanagh, J.D. Power, B. Twamley, J. O'Brien, B. Talbot, G. Dowling, O. Mahony, S.D. Brandt, J. Patrick, R.P. Archer, J.S. Partilla, M.H. Baumann. Synthesis, characterization and monoamine transporter activity of the new psychoactive substance 3',4'-methylenedioxy-4-methylaminorex (MDMAR). *Drug. Test. Anal.* **2015**, 7, 555.
22. The Misuse of Drugs Act 1971 (Temporary Class Drug) (No. 3) Order 2015 No. 1929. Available at:
http://www.legislation.gov.uk/ukxi/2015/1929/pdfs/ukxi_20151929_en.pdf [05 December 2015].
23. APEX2 v2012.12-0, Bruker AXS Inc., Madison, Wisconsin, USA.
24. SADABS (2012) Bruker AXS Inc., Madison, Wisconsin, USA.
25. G. Sheldrick. A short history of SHELX. *Acta Cryst. A* **2008**, 64, 112.

26. C.J. Tschärner, R.I. Warwick. Process for the conversion of cis-2-phenyl-3-methylmorpholine to trans-2-phenyl-3-methylmorpholine. Patent No. US 3282936A. Geigy Chemical Corporation. New York, USA, **1966**.
27. F.H. Clarke, cis- and trans-3-methyl-2-phenylmorpholine. *J. Org. Chem.* **1962**, 27, 3251.
28. T. Kumazawa, K. Hara, C. Hasegawa, S. Uchigasaki, X.P. Lee, H. Seno, O. Suzuki, K. Sato. Fragmentation pathways of trifluoroacetyl derivatives of methamphetamine, amphetamine and methylenedioxyphenylalkylamine designer drugs by gas chromatography/mass spectrometry. *Int. J. Spectrosc.* **2011**. DOI: 10.1155/2011/318148
29. G. McLaughlin, N. Morris, P.V. Kavanagh, J.D. Power, J. O'Brien, B. Talbot, S.P. Elliott, J. Wallach, K. Hoang, H. Morris, S.D. Brandt. Test purchase, synthesis, and characterization of 2-methoxydiphenidine (MXP) and differentiation from its meta- and para-substituted isomers. *Drug Test. Anal.* **2015**, DOI: 10.1002/dta.1800.
30. D. Carlström, I. Hacksell. The crystal structure of phenmetrazine hydrochloride. *Acta Cryst.* **1974**, 30, 2477.
31. A.X. Hu, G. Cao, X.R. Xiao, H.Y. Wu. (2S,3S,5R)-2-(3-Chlorophenyl)-2-hydroxy-3,5-dimethyl-2-morpholinium chloride. *Acta Cryst.* **2006**, 62, 1584.
32. X.X. R., Y.L. Yaun, N. Zhu, Y., M.H. Chen. Synthesis and crystal structure of chiral 2-aryl-3,5-dimethyl-2-morpholinol hydrochloride. *Chin. J. Org. Chem.* **2007**, 27, 989.
33. R.B. Rothman, M.H. Baumann. Therapeutic potential of monoamine transporter substrates. *Curr. Top. Med. Chem.* **2006**, 6, 1845.
34. R.B. Rothman, B.E. Blough, M.H. Baumann, Dual dopamine/serotonin releasers as potential medications for stimulant and alcohol addictions. In *Drug Addiction. From Basic Research to Therapy*, (Ed.: R.S. Rapaka, W. Sadée), Springer New York, **2008**, 311.
35. L.L. Howell, S.S. Negus. Monoamine transporter inhibitors and substrates as treatments for stimulant abuse. *Adv. Pharmacol.* **2014**, 69, 129.

36. H.H. Sitte, M. Freissmuth. Amphetamines, new psychoactive drugs and the monoamine transporter cycle. *Trends Pharmacol. Sci.* 2015, 36: 41.

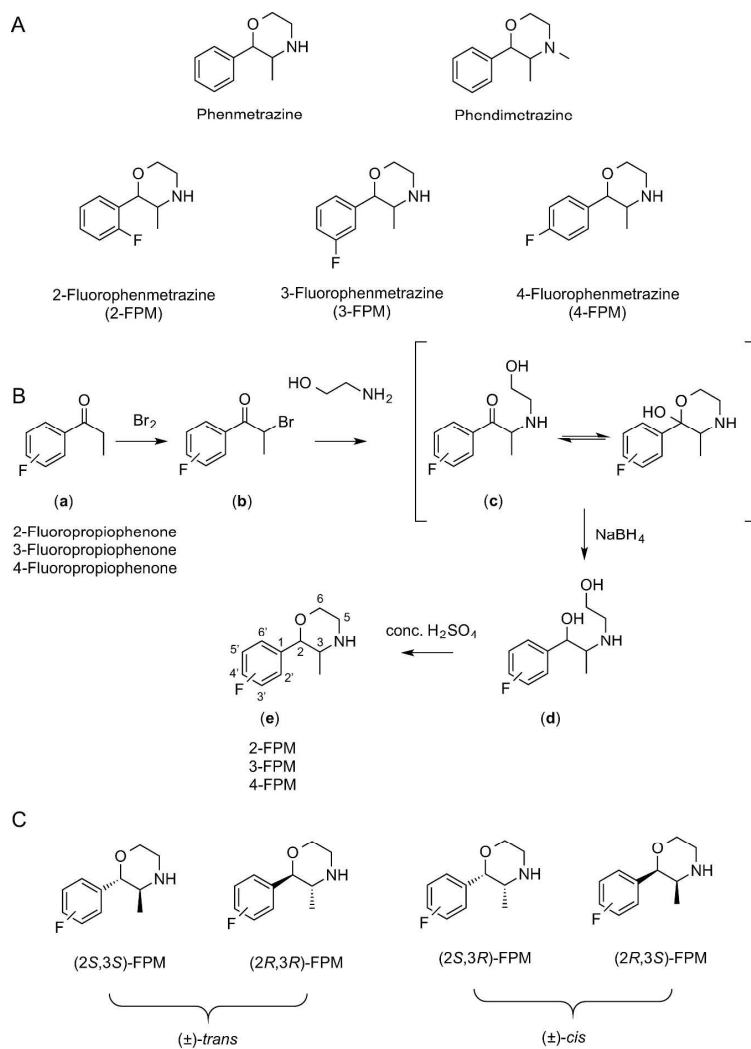


Figure 1. A. Chemical structures of phenmetrazine, its N-methyl derivative phendimetrazine and the fluorophenmetrazine (FPM) positional isomers. B. The synthesis pathway employed for the preparation of 2-, 3- and 4-FPM isomers. C. Structural representation of all four FPM enantiomers.

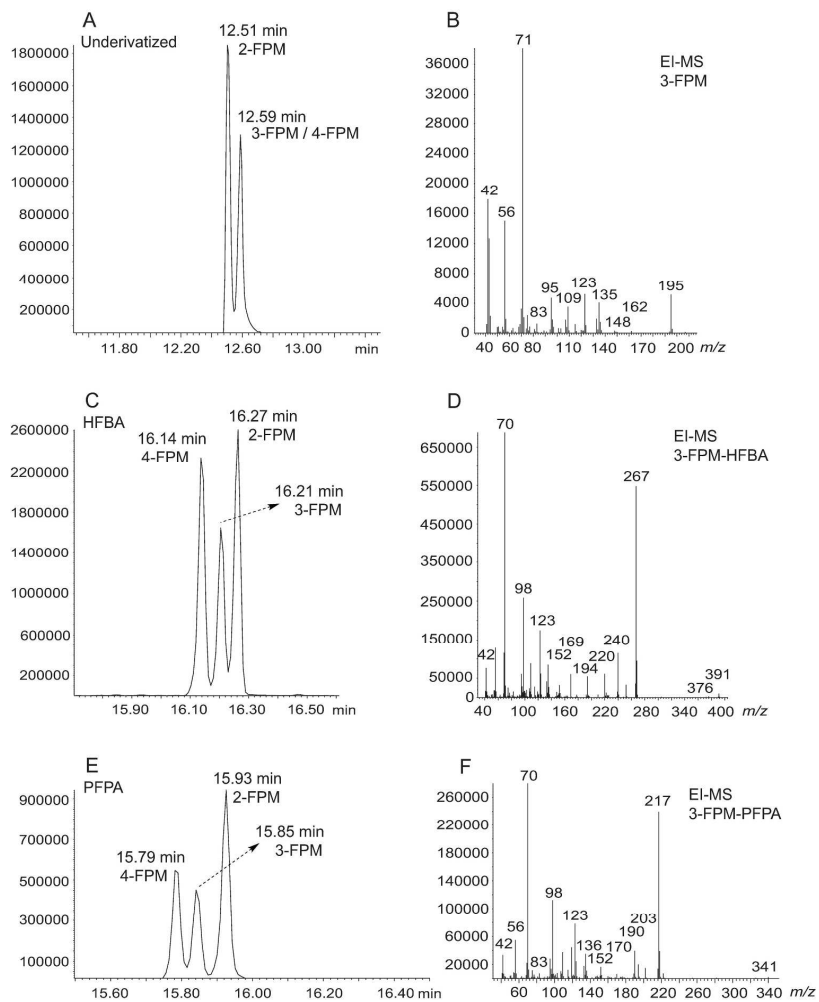


Figure 2. Gas chromatographic (GC) separation achieved for the underivatized FPM isomers (A), the HFBA-FPM derivatives (C) and the PFBA-FPM derivatives (E). The electron ionization (EI) mass spectra recorded for underivatized 3-FPM, and its HFBA and PFBA derivatives are shown in Figure 2B, 2D and 2F, respectively.

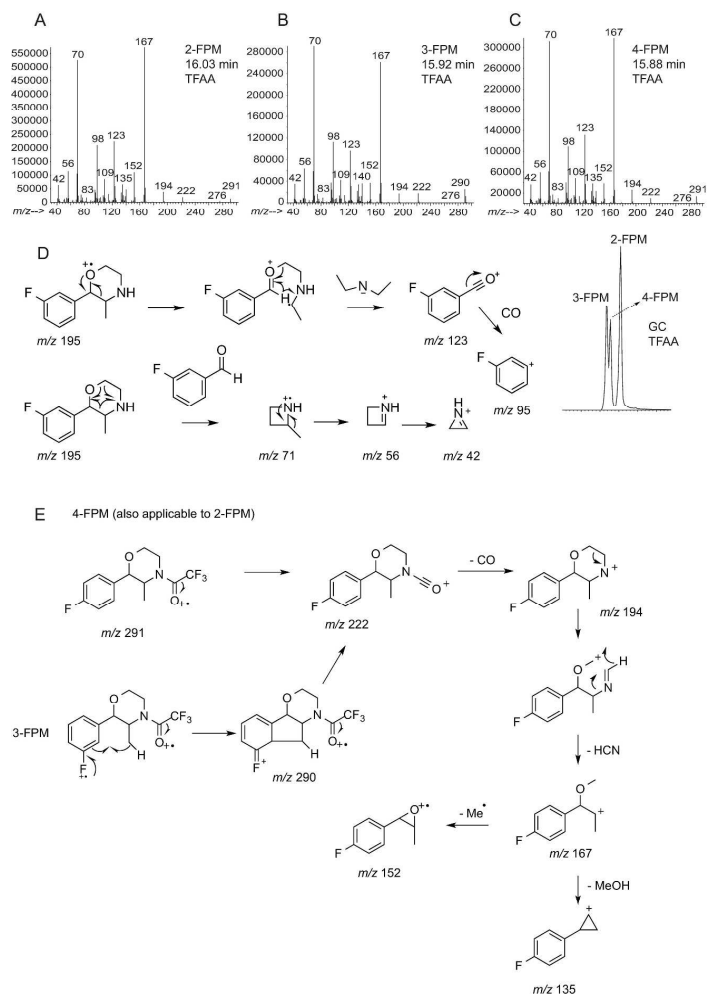


Figure 3. A-C. GC-MS data obtained for the FPM-TFAA isomers. D. A proposed fragmentation pathway for underivatized 3-FPM under EI-MS conditions. E. A proposed fragmentation pattern for the FPM-TFAA isomers under EI-MS conditions.

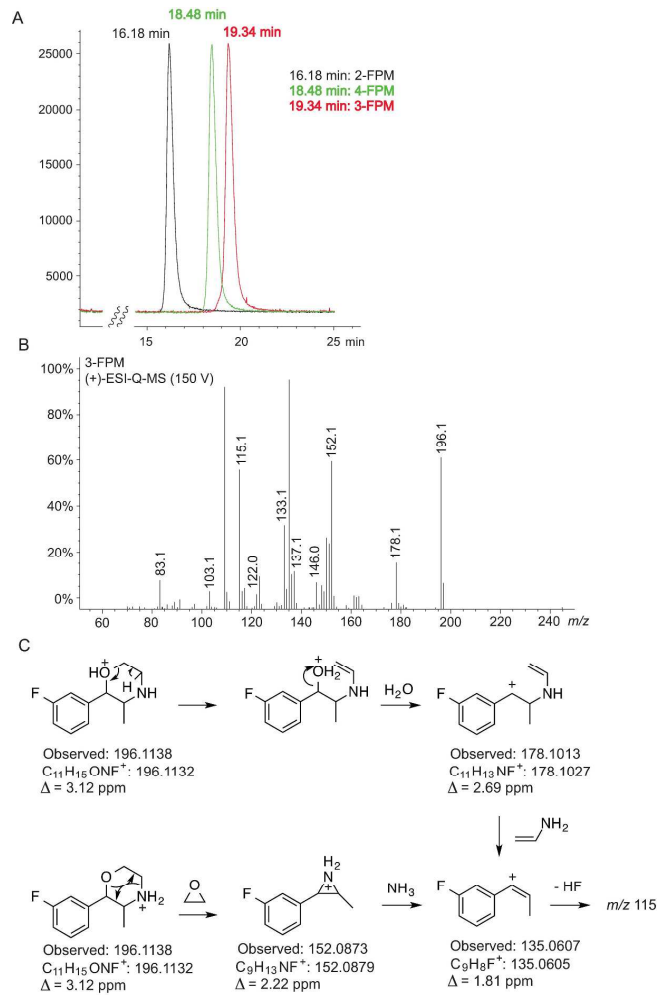


Figure 4. A. HPLC separation achieved for all three fluorophenmetrazine isomers. B-C. The product ion spectra obtained from in-source collision-induced dissociation (CID) at increased fragmentor voltage (150 V) and the suggested dissociation pathways with HR-MS providing elemental compositions with acceptable mass accuracies consistent with the proposed structures.

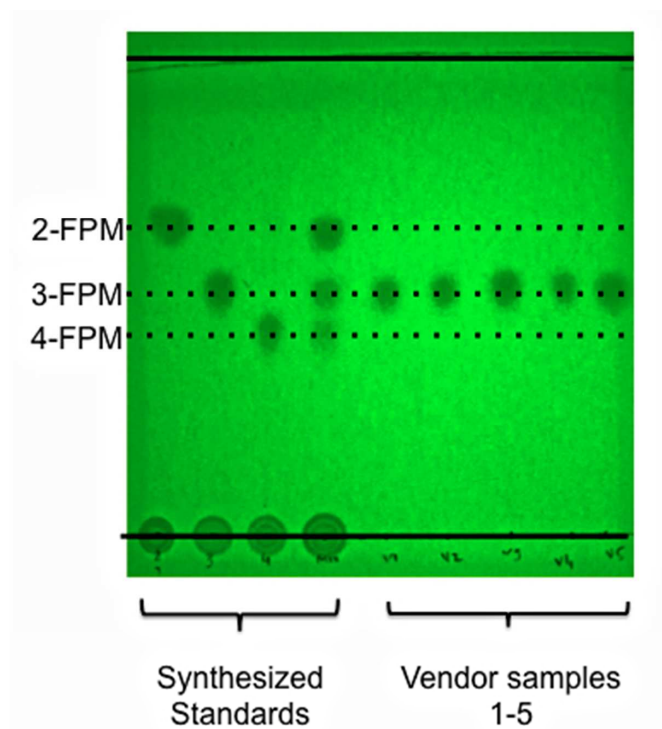


Figure 5. TLC separation of the three isomers provided retardation factors of 0.65, 0.51 and 0.43 for 2-, 3- and 4-FPM, respectively.

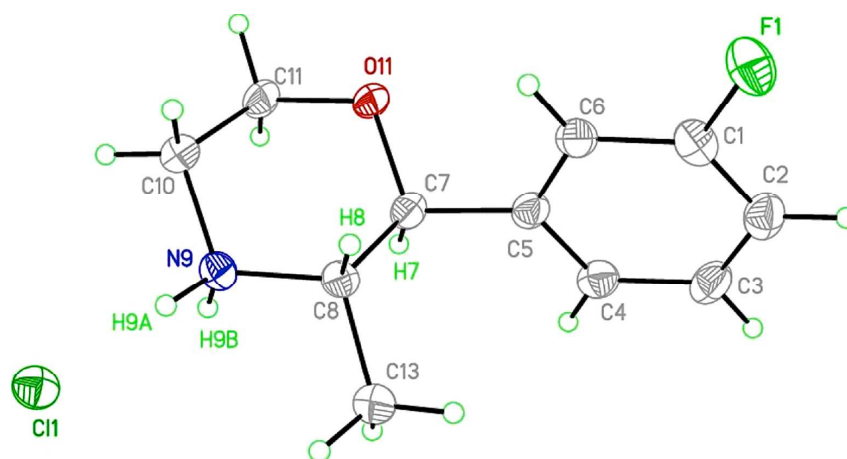


Figure 6. Molecular structure achieved for 3-FPM vendor sample using x-ray crystal structure analysis.