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

Gavin McLaughlin[1,2], Noreen Morris[1], Pierce V. Kavanagh[2], Geraldine Dowling[2], John D. Power[2,3], Brendan Twamley[4], John O'Brien[4], Brian Talbot[5], Harald H. Sitte[6,7] and Simon D. Brandt[8]

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 2015 as a total of 100 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 European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) to more than 560.[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 & Fluorophenmetrazine

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) were developed within the pharmaceutical setting as sympathomimetic weight-control medications considered to show less abuse liability compared to other amphetamine anorexiants (Figure 1).[3-6]

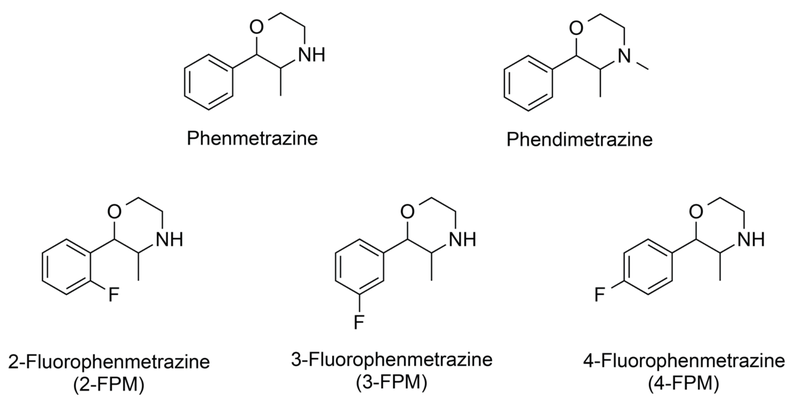

Figure 1. Chemical structures of phenmetrazine, its *N*-methyl derivative phendimetrazine and the fluorophenmetrazine (FPM) positional isomers.

Phenmetrazine is a potent substrate for norepinephrine and dopamine transporters and displays stimulants 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] One of the latest NPS to appear on the recreational drug market is 3-fluorophenmetrazine, a fluorinated analogue of the anorectic drug phenmetrazine. A 2011 patent application featured the synthesis and pharmacological evaluation of 3- and 4-FPM and concluded that both isomers were substrate-type monoamine releasers with higher selectivity toward catecholamines.

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 (Figure 1). 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.

Synthesis

The synthesis procedure (Figure 2) employed for the preparations of 2-, 3- and 4-FPM was adapted from Blough *et al*.[3]

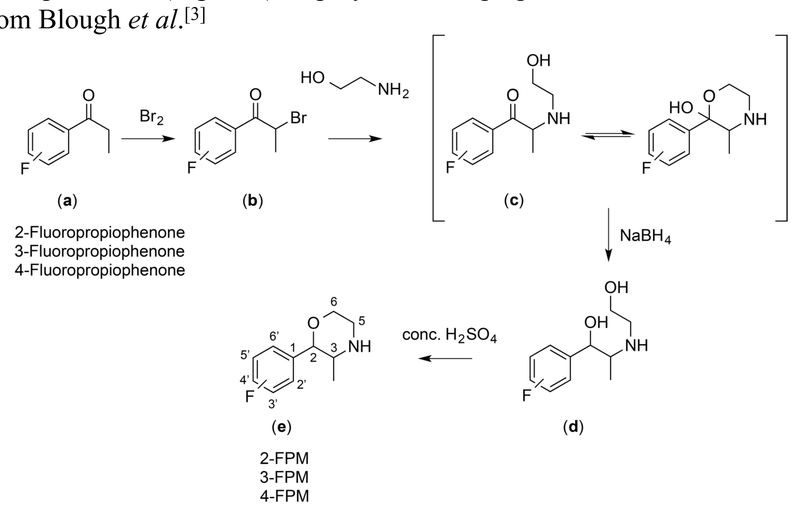

Figure 2. Synthesis procedure for the fluorophenmetrazine (FPM) positional isomers.

Characterization

Initial analysis of the underivatized isomers by Gas Chromatography-Mass Spectrometry (GC-MS) failed to obtain separation between *meta*- and *para*- substituted 3- and 4-FPM isomers although separation from the 2-FPM isomer was feasible. Derivatization with trifluoroacetic anhydride (TFAA) improved the chromatography results and provided mass spectra with diagnostically useful information. 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 (Figures 3A).

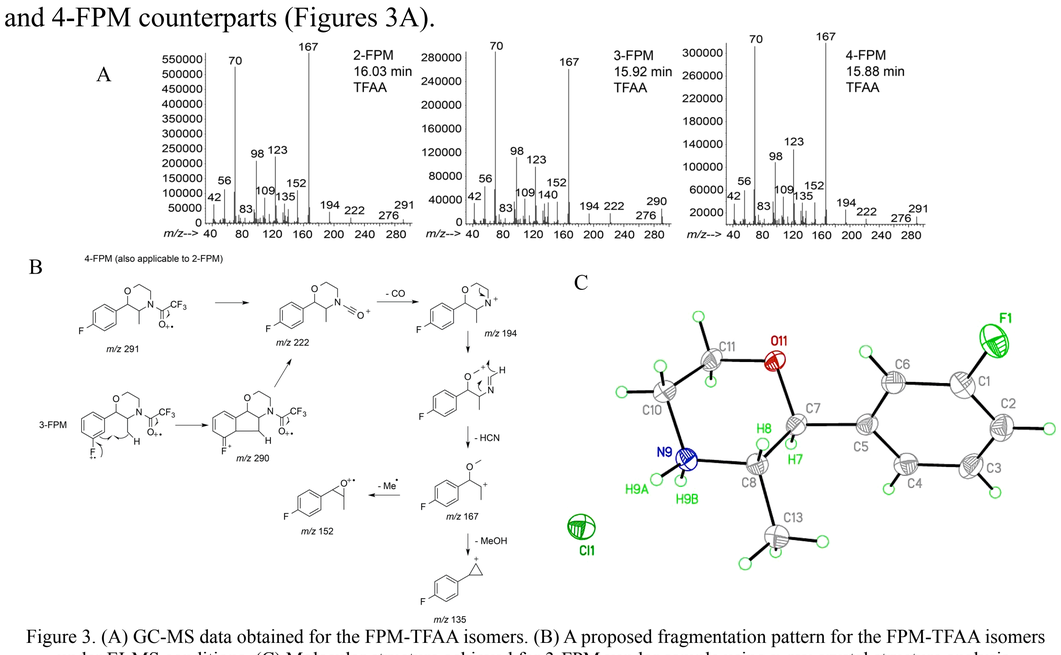

Figure 3. (A) GC-MS data obtained for the FPM-TFAA isomers. (B) A proposed fragmentation pattern for the FPM-TFAA isomers under EI-MS conditions. (C) Molecular structure achieved for 3-FPM vendor sample using x-ray crystal structure analysis.

The molecular ion at *m/z* 291 was detectable in all three mass spectra, however an additional ion at *m/z* 290 was observed in the spectrum of 3-FPM-TFAA. 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 3B). The proposed fragmentation pattern for the FPM-TFAA isomers is outlined in Figure 3B. 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-methylazetidine 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. The x-ray crystal structure of the 3-FPM vendor sample is shown in Figure 3C.

Analysis by High Performance Liquid Chromatography-Mass Spectrometry (LC-MS) obtained satisfactory separation of all three fluorophenmetrazine isomers 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.

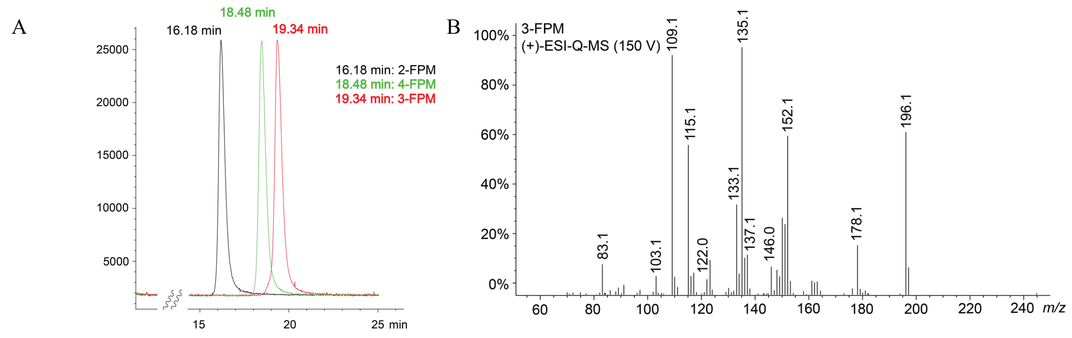

Figure 4. (A) HPLC separation achieved for all three FPM positional isomers. (B) The product ion spectra obtained from in-source collision induced dissociation (CID) at increased fragmentor voltage (150V).

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 5A, respectively. 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 5A).

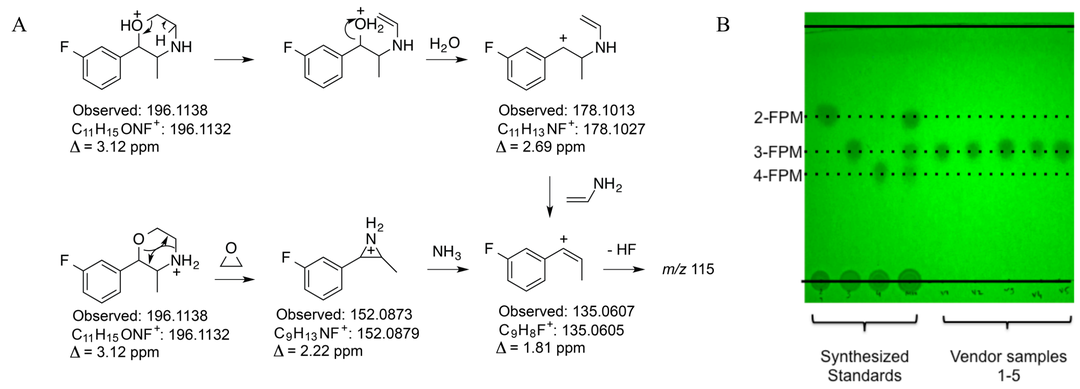

Figure 5. (A) Suggested dissociation pathway with HR-MS providing elemental compositions with acceptable mass accuracies consistent with the proposed structures. (B) TLC separation of the three FPM isomers.

It was encouraging to observe that thin layer chromatography (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 5B). 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. Pharmacological evaluation of the FPM compounds concluded that all three isomers are substrate-like releasing agents at monoamine transporters.[9]

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 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.

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Affililations : **1.** Department of Life and Physical Sciences, School of Science, Athlone Institute of Technology, Co. Westmeath, Ireland. **2.** Department of Pharmacology & Therapeutics, School of Medicine, Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8, Ireland. **3.** Forensic Science Laboratory, Garda HQ, Dublin 8, Ireland. **4.** School of Chemistry, Trinity College Dublin, Dublin 2, Ireland. **5.** School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Dublin 2, Ireland. **6.** Institute of Pharmacology, Center of Physiology and Pharmacology, Medical University Vienna, Vienna, Austria. **7.** Center for Addiction Research and Science, Medical University Vienna, Vienna, Austria. **8.** School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool L33, UK.