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Detection, discrimination and quantification of amphetamine, cathinone and *nor*-ephedrine regioisomers using benchtop ^1H and ^{19}F NMR spectroscopy

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Abstract

Amphetamine and cathinone derivatives are abused recreationally due to the sense of euphoria they provide to the user. Methodologies for the rapid detection of the drug derivative present in a seized sample, or an indication of the drug class, is beneficial to law-enforcement and healthcare providers. Identifying the drug class is prudent because derivatisation of these drugs, to produce regioisomers for example, occurs frequently to circumvent global and local drug laws. Thus, newly encountered derivatives might not be present in a spectral library. Employment of benchtop NMR could be used to provide rapid analysis of seized samples as well as identifying the class of drug present. Discrimination of individual amphetamine-, methcathinone-, *N*-ethylcathinone and *nor*-ephedrine-derived fluorinated and methylated regioisomers is achieved herein using qualitative automated ^1H NMR analysis and compared to GC-MS data. Two seized drug samples, SS1 and SS2, were identified to contain 4-fluoroamphetamine by ^1H NMR (match score median = 0.9933) and GC-MS ($\text{RR}_t = 5.42\text{-}5.43$ min). The amount of 4-fluoroamphetamine present was 42.8 – 43.4% w/w and 48.7 – 49.2% w/w for SS1 and SS2 respectively from quantitative ^{19}F NMR analysis, which is in agreement with the amount determined by GC-MS (39.9 – 41.4% w/w and 49.0 – 49.3% w/w). The total time for the qualitative ^1H NMR and quantitative ^{19}F NMR analysis is *ca.* 10 min. This contrasts to *ca.* 40 min for the GC-MS method. The NMR method also benefits from minimal sample preparation. Thus, benchtop NMR affords rapid, and discriminatory, analysis of the drug present in a seized sample.

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Introduction

Novel Psychoactive Substances (NPS) continue to feature heavily on the recreational drug scene, leading to drug intoxication that requires treatment.^[1] The structures of prohibited drugs are chemically modified by clandestine laboratories to circumvent laws restricting their use, or for medical research under appropriate licences. Many of the NPS that are encountered or seized in criminal cases can be classified as synthetic cathinones, piperidines and pyrrolidines, benzodiazepines, piperazines, aminoindanes or phenethylamines.^[2] The number of drugs reported in each classifier to monitoring bodies,^[3] such as the European Union early warning system,^[4] continues to increase year on year. GC-MS is currently the accepted gold standard in forensic drug analysis,^[5] and is routinely used for the analysis of drug samples.

Amphetamine (Figure 1) is a substance that belongs to the phenethylamine class of psychoactive drugs. Many controlled substances, such as methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA), are derived from the amphetamine structure and are described as “substituted amphetamines”. Amphetamines are abused due to the stimulating and physiological effects they generate. For example, MDMA is known for its entactogenic effects that induce a pleasant and relaxed feeling of happiness that results in consumers becoming addicted to the substance.^[6] Similarly, methamphetamine also provides stimulating effects as well as sympathomimetic effects through interaction with the sympathetic nervous system receptors.^[7] In addition to cocaine, amphetamines are one of the most commonly used illicit substances and this prevalence along with abuse of methamphetamine has led to a dramatic increase in the number of emergency department visits for amphetamine intoxication.^[8] GC-MS is again the preferred analytical technique for the qualitative, and quantitative, analysis of amphetamines.^[9]

In recent years the mono-fluorinated substituted amphetamine derivatives, fluoroamphetamines, have been discovered in forensic cases and on the recreational drug market, mainly around Europe, incorrectly sold as amphetamine and MDMA.^[10] 4-Fluoroamphetamine has been detected in both urine and serum using validated GC-MS methods.^[11] Fluorinated NPS are often encountered as fluorination influences the lipophilicity, electronegativity, basicity and bioavailability of drug molecules.^[12]

Cathinone is the β -keto-analogue of amphetamine. Cathinone is the naturally occurring active ingredient in the leaves of the shrub *Catha edulis*, often referred to as Khat, and is responsible for the euphoric effect.^[13] The euphoric effects are similar to amphetamine.^[14] Initially, Khat consumption was limited to inhabitants of East Africa and the Arab peninsula as a stimulant, but its use is now widespread in Western countries due to increased air transportation and loosening of customs restrictions.^[15]

A number of derivatives of cathinone exist, which have largely been synthesised to circumvent international laws curtailing NPS use^[16] or represent pro-active efforts to characterise derivatives that may in the future become targets for clandestine laboratories.^[17] In 2018, 36% of seizures reported to the EU's early warning system (EWS) were cathinones.^[4] In the period 2016 – 2018, *ca.* 100 cathinone derivatives were detected each year; cathinones were the most prevalent drug class detected alongside cannabinoids over this period. In 2019, ten new cathinone derivatives were reported to the EU's EWS for the first time. Thus, cathinone derivatives are prevalent in the EU. As such, chromatographic and

electrophoretic techniques have been developed to separate and analyse cathinone derivatives^[18] e.g. mass spectrometry,^[19] Raman spectroscopy,^[20] and ultra-high performance liquid chromatography.^[21]

One such derivative of cathinone is methcathinone (ephedrone), a synthetic cathinone. Ephedrone demonstrates amphetamine-like effects, although these are less potent than amphetamine.^[22] Fluoromethcathinones (**1a** – **1c**) are a further example of cathinone derivatization. Capsules marketed as “plant feeders” were identified to contain 3-fluoromethcathinone in a report by Archer in 2009.^[23] At this time it was clear from internet forums that these plant feeders were being used as recreational drugs, although online vendors were only thought to be selling 4-fluoromethcathinone (flephedrone, **1c**). Elucidation of the isomer present was achieved after synthesising reference samples of 2-, 3- and 4-fluoromethcathinone and comparing them to the material in the capsule. Discriminatory evidence was gained principally from ¹H and ¹⁹F NMR studies, along with analysis of the fingerprint region of IR spectra. Due to the similarity of retention times of the regioisomers, even when derivatized as their respective acetamides, GC-MS data did not provide satisfactory evidence to discriminate the isomers.

To further aid in the detection and screening of NPS containing samples, we detail in this paper a fully automated NMR system that, after acquiring and processing a ¹H NMR spectrum of a sample, returns the identity of the amphetamine, methcathinone, *N*-ethylcathinone or *nor*-ephedrine regioisomer present. A pattern recognition algorithm is utilised to automatically compare the acquired spectrum with a reference library to produce a match score. Six sets of regioisomers are probed which includes a range of fluorinated and non-fluorinated amphetamine and cathinone regioisomers and potential metabolite (*nor*-ephedrine) products. Qualitative and quantitative analysis using ¹H and ¹⁹F NMR spectroscopy of two street samples is reported and validated against contemporaneously acquired GC-MS data

Results and Discussion

Reference standards of the regioisomers, **1a–6c**, depicted in Figure 2, were synthesised using previously reported methods,^[23-24] as the corresponding hydrochloride salts and characterised by NMR spectroscopy (400 MHz), mass spectrometry and IR analysis. Subsequently, these regioisomers were analysed using benchtop ¹H NMR spectroscopy (60 MHz). These regioisomers belong to the amphetamine, methcathinone, *N*-ethylcathinone and *nor*-ephedrine classes of NPS. These regioisomers were chosen for their prevalence with respect to drugs seizures, potential future targets for clandestine laboratories or they represent metabolites of other regioisomers studied. For example, a study conducted in the Netherlands between 2013-2017 reported that **1c** was the most frequently detected phenethylamine throughout this time-period (20–65% of all phenethylamines sampled).^[25] This study also reported that **2c** was the most prevalent cathinone in 2015-2016 whilst in 2017 **2b** was the most reported cathinone identified. Furthermore, **3a** – **3c** have been found in several seizures in Italy over the period 2013-2015. Of these seizures, 22% were crystalline **2b** whilst a further 20% were **2c**.^[26] In 2013, several tablets were analysed from a Bristol (UK) night-club and were found to contain **3c**.^[27]

In effects to understand cathinone pharmacology, **4b** and **4c** have been shown to be 10-fold more potent than methcathinone as uptake inhibitors and as release inhibitors at the serotonin

transporter.^[28] **4a** was a weaker inhibitor and releaser than methcathinone. Compared to methcathinone, **4a** – **4c** might provide access to drugs with therapeutic value alongside diminished abuse potential. However, **4a** – **4c** are included here for structural similarity to the other regioisomers in order to rigorously test the algorithm employed.

Lastly, **5a** – **5c** represent cathinone derivatives that could be synthesised by clandestine laboratories and could, therefore, be encountered in the future. *Nor*-ephedrine **6a** – **6c** are the metabolite products of **5a** – **5c** that are formed via a keto-reduction, which is the metabolic pathway through which cathinone is metabolised.^[29]

The ¹H NMR spectra of these regioisomers were combined with those of other NPS, narcotics, and other controlled substances, as well as commonly encountered non-controlled substances and adulterants to produce a ¹H NMR spectral library. In total, there were 21 classes of compound and 283 compounds in the reference library.

A previously reported algorithm^[30] was utilised to analyse an acquired ¹H NMR spectrum and, following analysis, return the name and drug class of the compound(s) present. In order to do this, the ¹H NMR spectra in the library were truncated into two discrete sections: the “class” region (0.46 – 1.94 ppm) and a “fingerprint” region (3.90 – 12.50 ppm). As our focus here is on only three drug classes, the ability to distinguish compounds largely revolved around differences in the fingerprint region rather than in the class region. However, the pattern recognition process utilised both sections in the analysis (Figure S9). In addition to the name and class of compound(s) present, a match score is produced by the algorithm. The match score is the largest of the Pearson’s correlations between the sample spectrum and each of the library spectra; a value of unity represents a perfect match.

Firstly, “technical replicate” ¹H NMR spectra of the reference samples used to generate the library were analysed using the algorithm. These comprised spectral acquisitions from independent preparations of the reference compounds, chronologically separated by some weeks from the reference data collection, providing a level of challenge to the pattern recognition algorithm. These samples returned a 100% success rate in terms of identifying the single regioisomer present in the sample. The median match score was 0.9906.

Crucially, the algorithm was successfully able to differentiate compounds **1a** – **6c** from similar compounds. For example **2a** – **2c** were differentiated from 2-, 3- and 4-methyl-*N*-ethylcathinone, and methcathinone; the latter four compounds were all included in the spectral library and so could have been potential hits. Furthermore, **3a** – **3c** were differentiated from **5a** – **5c**; these compounds only differ in the alkyl chain attached to the amine group. This is notable, as it demonstrates that the algorithm can distinguish between *N*-ethylcathinone and methcathinone derivatives readily.

To highlight the similarity of the regioisomers investigated and the importance of the class and fingerprint regions analysed, the ¹H NMR spectra of **3c**, **4c** and **5c** are shown in figure 3. The similarity between **3c** and **4c** is apparent in the aliphatic region in that the doublet at 1.46 ppm, the singlet at 2.60 ppm and the quartet due to the proton on the chiral centre located at 5.16 ppm overlap. The aromatic region, however, differs significantly due to the reduced influence of fluorine coupling in **4c** compared to **3c**. Better overlap in the aromatic region is shown between **3c** and **5c**, as they both possess the same substituted benzene ring, whereas variation is observed in the aliphatic region due to the longer *N*-ethyl chain present in **5c**

(additional peaks at 2.92 and 1.27 ppm, as well as the loss of the singlet at 2.60 ppm) compared to the *N*-methyl present in **3c**. It should be noted that the chemical shift region between 1.54 and 3.90 ppm is not utilised by the algorithm (it is neither part of the class or fingerprint region) and so variations here are not accounted for in the match score reported. This means that the variable amount of water present at 3.33 ppm, and the quintet of the d_6 -DMSO peak at 2.50 ppm, does not affect the fitting process.

The algorithm employed returns more than one match score, which it ranks appropriately. The highest match score is indicative of the compound(s) present in the sample. Analysis of the second and third match scores is often only required for samples that may be tertiary in nature. Previously, it has been reported that this approach has been used to determine the constituents of a tertiary mixture consisting of cocaine, ketamine, and benzocaine in a 35.6:35.7:28.7 ratio by appraising both the first and second hit scores.^[30] This approach has limited use here as the technical replicates are known to consist of a single regioisomer. However, the analysis of these match scores does indicate consistency in terms of the regioisomer being detected. For example, the highest match score for the technical replicate of **1c** is 0.9930; the second highest match score (0.9798) is for a combination of **1c** and **1b** whilst the third highest match score (also 0.9798) is **1c** and **1b** (see Table S4). It should be noted that all three of the highest hit scores involve **1c**, with the highest-ranking hit score consisting solely of **1c**.

The second and third match scores represent the spectral residual that remains after the best match library spectrum has been subtracted. Because the residual is so small (and often very noisy), the assignments obtained from the second and third match scores should be considered somewhat unreliable, especially if the first match score has a large Pearson correlation i.e. close to 1.

For all six sets of regioisomers tested, the regioisomer present in the sample was always the sole hit for the highest match score, further, for the second and third matches, it was observed in combination with one other compound from the library (see SI). The only notable exception is **2c**; analysis returned solely 4-methylcathinone as the second hit score and **2c** and 4-methylcathinone as the third hit score. Given the difference is solely a *N*-methyl group at 2.58 ppm that is present in **2c** whilst it is absent for 4-methylcathinone (see Figure S4), this result is perhaps not surprising. However, it does highlight that the algorithm returns the correct compound even when the structures are highly similar, especially when considering the substitution pattern of the aromatic ring.

Analysis of a subset of the regioisomers examined here by GC-MS highlights the significance of these results. Regioisomers **1a – 1c**, **3a – 3c** and **4a – 4c** were analysed by GC-MS, using eicosane as an internal standard. The relative retention times (RR_t) for **1a–1c** were 0.17-0.18 whilst the RR_t for **3a – 3c** and **4a – 4c** were 0.33 – 0.36. Thus, although the amphetamine regioisomers (**1a – 1c**) could be distinguished from the methcathinone regioisomers (**3a – 3c** and **4a – 4c**), the amphetamine regioisomers could not be discriminated from one another successfully using this technique. This is highlighted in Figure S1 in that the chromatograms of **1b** and **1c** overlap significantly. The chromatograms of **3a – 3c** and **4a – 4c** highlight the same problem in that the regioisomers are not resolved from one another and they possess very similar RR_t . Retention times for **4a – 4c** have been reported previously to range from

7.38-7.61 mins.^[31] The problematic separation by GC-MS of **3a** – **3c** has been reported previously,^[23] and it was noted that derivatisation to their respective acetamides did not improve retention times, in that they again eluted with similar retention times. Furthermore, in order to achieve separation, a long run time is required in that eicosane elutes at 30.85 min. Conversely, the NMR approach showcased herein takes only 5 min to acquire the spectrum and report the regioisomer present, in addition to minimal sample preparation compared to the GC-MS method.

Due to the presence of ¹⁹F nuclei in five out of the six groups of regioisomers studied, qualitative ¹⁹F NMR (56 MHz) analysis to determine the identity of the regioisomer present was also possible. However, as Table 1 showcases, the similarity of the single ¹⁹F chemical shift for some of the regioisomers would be problematic for the qualitative analysis by the algorithm, due to the fact that there is only one ¹⁹F peak for analysis. Consequently, the analysis might not be satisfactorily robust for the complete qualitative determination of the regioisomer present. For example, the ¹⁹F chemical shifts of **1b** and **5b** differ by 0.41 ppm. The ¹⁹F NMR chemical shifts of **1c** and **6c** are even more similar in that they differ by only 0.09 ppm. Furthermore, the similarity of the ¹⁹F chemical shifts for **3a** – **3c** and **5a** – **5c** demonstrates that the *N*-methyl or *N*-ethyl substituent has little effect on the observed chemical shifts. Notably, only **4a** – **4c** are effectively easily distinguished using these data as they possess a benzene ring substituted with a trifluoromethyl group; all other regioisomers possess fluorinated benzene rings and this is reflected in the similarity of the chemical shifts for the single ¹⁹F nucleus present (range –102.02 ppm to –117.58 ppm).

Analysis and quantification of seized samples

Two street samples, SS1 and SS2, were supplied by Greater Manchester police via the MANchester DRug Analysis and Knowledge Exchange (MANDRAKE) partnership on 25th May 2018. They were purported to contain MDMA, however, preliminary analysis by low-field ¹H NMR confirmed the principle component in both samples was **1c** (SS1, match score = 0.9979; SS2, match score = 0.9887). Both samples were confirmed to contain **1c** solely; this conclusion can be drawn from the very high (i.e. almost 1) match scores obtained. The ¹H NMR spectrum of SS1 is shown in Figure S8 and is compared to the ¹H NMR spectrum of the **1c** standard to exemplify the similarity of the two spectra. Gas chromatography validated the NMR analysis as each sample possessed retention times of 5.43 and 5.42 mins, which agrees with the value of 5.42 mins for a reference standard of **1c**. It should be noted here that **1b** has a retention time of 5.39 mins, so there is a possibility of peaks being poorly resolved from one another and hence misidentified. As regioisomers **1a** – **1c** all possess a base peak of *m/z* = 44.1 (CH₃CHNH₂⁺), along with a secondary peak of *m/z* = 109.0 (fluorine substituted tropylium cation), the mass spectra cannot be used to distinguish between regioisomers of the same class. Having validated their identity, the amount of **1c** in SS1 and SS2 was quantitatively determined. Each sample was analysed in duplicate by both GC-MS and benchtop ¹⁹F NMR spectroscopy.

¹⁹F NMR was chosen to quantify the amount of fluoroamphetamine present due to the simplistic nature of the spectrum relative to the ¹H NMR spectrum. This approach is not applicable to many drugs, such as MDMA (quantified prior to this study using benchtop NMR^[9f]), due to the lack of ¹⁹F nuclei, although here it provides a convenient method for

quantification. Prior to analysis, ^{19}F NMR calibration series for **1a** – **1c** were obtained. Calibration standards were prepared over the concentration range 5 mg mL^{-1} – 15 mg mL^{-1} , with trifluoroacetic acid (TFA) added as an internal standard at a concentration of 0.1% v/v of DMSO used. Each ^{19}F NMR spectrum was collected using 16 transients with a 5 second relaxation delay. Calibration graphs were then produced by calculating the integrated area ratio between sample peaks and TFA peaks and plotting against concentration (Figure 4).

The three isomers show acceptable correlation (>0.99) meaning the method can be used to perform quantitative analysis. The LOD and LOQ for the three regioisomers are reported in Table 2 and reports that the highest LOQ is below 2 mg mL^{-1} . This is acceptable as the majority of street samples will have active components greater than 2 mg per sample. Comparatively, the GC-MS analysis gave LOD and LOQ values of 20 and $68\text{ }\mu\text{g mL}^{-1}$ respectively for **1a** and 25 – $23\text{ }\mu\text{g mL}^{-1}$ and 80 – $75\text{ }\mu\text{g mL}^{-1}$ for **1b** and **1c**. The lower threshold of detection and quantification is expected for GC-MS given the much greater levels of sensitivity relative to NMR. The LODs and LOQs determined by NMR are sufficient for the routine detection of active components in a tablet. By way of example, the amount of MDMA, a substituted amphetamine, in tablets have been reported to be 20 mg to 131 mg per tablet in 2006,^[32] a median of 105 mg over the period 2001-2018^[33] and 133 – 223 mg for tablets seized in between August 2018 and March 2019.^[9f] The LOD and LOQs for the ^{19}F NMR quantitative method are an order of magnitude lower than these reported values thus showcasing the applicability of this approach for routine analysis.

GC-MS reported that SS1 contained 39.9 – 41.4% w/w of **1c**. SS2 contained 49.0 – 49.3% w/w. This equates to 109 – 122 mg of **1c** being present in the two samples. ^{19}F NMR quantitative analysis reported that SS1 contained 42.8 – 43.4% w/w whereas SS2 contained 48.7 – 49.2% w/w. This equates to 117 – 122 mg of **1c** being present. The two approaches are therefore in agreement. Notably, the collection of the GC trace takes *ca.* 40 min whereas acquiring the ^{19}F NMR spectrum takes *ca.* 5 min . Based on the results reported herein, specific regioisomeric detection of **1c** is readily performed using ^1H NMR analysis followed by quantitation by ^{19}F NMR in a total time of *ca.* 10 minutes .

Conclusion

This paper has demonstrated regioisomer discrimination of fluoroamphetamines, methyl- and fluoro-methcathinones, trifluoromethylmethcathinones, fluoro-*N*-ethylcathinones and fluoro-*N*-ethyl-*nor*-ephedrine. The analysis of single component samples was performed using an automated database whereby a Pearson score is generated based on the similarity of the ^1H NMR spectrum to a reference library consisting of 283 spectra of NPS, pharmaceuticals and cutting-agents. For the six sets of regioisomers analysed (**1a** – **6c**), technical replicates were analysed and in 100% of cases, the correct regioisomer was returned. The median match score was 0.9906. Evidence for how well the automated process was able to differentiate regioisomers from similar regioisomers and distinguish between similar classes was obtained from the analysis of the second and third highest hit scores. In all but one case (**2c**), the second and third hit scores were always a combination of the regioisomer present and that of a similar regioisomer from the database.

The difficulty of distinguishing between regioisomers and similar classes of drugs using GC-MS was highlighted using **1a – 1c**, **3a – 3c** and **4a – 4c**. RR_t for **1a – 1c** were 0.17 – 0.18 whilst for **3a – 3c** and **4a – 4c** they were 0.33 – 0.36. The similarity of RR_t for regioisomers using the GC-MS method herein meant that regioisomers, particularly the 2- and 3- isomers were not baseline resolved.

Two seized drug samples, SS1 and SS2, were analysed by both GC-MS and ^1H NMR. Both methods confirmed the presence of **1c** (^1H NMR match score median = 0.9933, RR_t = 5.42–5.43 min), although the RR_t obtained from GC-MS analysis could also suggest the presence of **1b** (5.39 mins). Both **1c** and **1b** possess the same mass spectra as they produce identical fragment ions, and as such, this highlights the problematic use of GC-MS here compared to the use of the automated qualitative ^1H NMR approach.

^{19}F NMR quantification was performed on SS1 and SS2. Calibration plots for **1a – 1c** gave LODs and LOQs of 0.29 – 0.49 and 0.93 – 1.63 mg mL⁻¹ respectively. Comparatively, the GC-MS analysis gave LOD and LOQ values of 20 and 68 µg mL⁻¹ respectively for **1a** and 25 – 23 µg mL⁻¹ and 80 – 75 µg mL⁻¹ for **1b** and **1c**. The amount of **1c** present in the two tablets was 42.8 – 43.4% w/w and 48.7 – 49.2% w/w for SS1 and SS2 respectively following ^{19}F NMR analysis. Quantification by GC-MS returned similar values (39.9 – 41.4% w/w and 49.0 – 49.3% w/w respectively).

The qualitative and quantitative NMR analysis takes *ca.* 10 min whereas GC-MS analysis takes *ca.* 40 min. In addition to the shorter analysis time, the NMR method benefits from greater regioisomer differentiation compared to the GC-MS method as well as minimal sample preparation. The rapid analysis afforded by qualitative ^1H and quantitative ^{19}F NMR spectroscopy, coupled with minimal sample preparation, may be beneficial to legal entities and healthcare providers in determining the exact regioisomers present in a sample. This is important as the toxicological profile of regioisomers can vary significantly.

Experimental

The reference compounds **1a – 6c** were synthesised, as their corresponding hydrochloride salts, under UK Home Office licence. **1a – 5c** were synthesised according to, or adapted from the literature references cited: **1a-2c**,^[24b] **3a-3c**,^[23] **4a-4c**,^[24a] and **5a-5c**.^[23] The synthesis of **5a-6c** are detailed in the SI.

^1H and ^{19}F NMR spectra were acquired of all samples using a Pulsar benchtop NMR spectrometer (Oxford Instruments, Abingdon, UK) operating at a ^1H frequency of 59.7 MHz. For qualitative ^1H NMR analysis, after the sample had been inserted, an automated procedure began whereby the instrument would lock on to the deuterated signature of d₆-DMSO (thus used as a chemical shift reference) before acquiring 16 scans.

Following acquisition, the data were processed in MNova (Mestrelab Research, Santiago de Compostela, Spain) using an automated script file. The processed FID was then analyzed by the pattern recognition algorithm, NPS Pattern Match (Oxford Instrument, Abingdon),^[30, 34] 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 considered to be tentative, unreliable, or unknown.

SS1 and SS2 were obtained from Greater Manchester Police via the MANchester DRug Analysis & Knowledge Exchange (MANDRAKE) partnership, on 25th May 2018, and were stored and analysed in accordance with the UK Misuse of Drugs Act (1971) and Misuse of Drugs Regulations (2001). Both samples were supplied in their solid, bulk forms. For the NMR qualitative analysis of the seized materials, a micro-spatula tip of the material (ca. 5–10 mg) was dissolved in 0.6 mL of deuterated DMSO and a ¹H NMR spectrum acquired using 16 scans.

For the quantitative ¹⁹F NMR, SS1 and SS2 were prepared in d₆-DMSO at a concentration of 15 mg mL⁻¹. 0.1% v/v TFA was used as an internal standard. Calibration standards were prepared over the concentration range 5 mg mL⁻¹ – 15 mg mL⁻¹, with TFA added as an internal standard at a concentration of 0.1% v/v of d₆-DMSO used. Each ¹⁹F NMR spectrum was collected using 16 transients with a 5-second relaxation delay.

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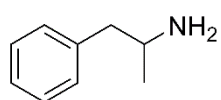
References

- [1] a) J. Neicun, J. C. Yang, H. Shih, P. Nadella, R. van Kessel, A. Negri, K. Czabanowska, C. Brayne, A. Roman-Urrestarazu, *PLoS One* **2020**, *15*, DOI: 10.1371/journal.pone.0241056; b) A. M. Dines, D. M. Wood, C. Yates, F. Heyerdahl, K. E. Hovda, I. Giraudon, R. Sedefov, P. I. Dargan, D. E. N. R. G. Euro, *Clin. Toxicol.* **2015**, *53*, 893-900.
- [2] A. Shafi, A. J. Berry, H. Sumnall, D. M. Wood, D. K. Tracy, *Ther. Adv. Psychopharmacol.* **2020**, *10*, DOI: 10.1177/2045125320967197.
- [3] a) C. Guillou, F. Reniero, J. L. Vicente, M. Holland, K. Kolar, H. Chassaigne, S. Tirendi, H. Schepers, *Curr. Pharm. Biotechnol.* **2018**, *19*, 91-98; b) F. Pantano, S. Graziano, R. Pacifici, F. P. Busardo, S. Pichini, *Curr. Neuropharmacol.* **2019**, *17*, 818-822; c) S. Zaami, *Eur. Rev. Med. Pharmacol. Sci.* **2019**, *23*, 9681-9690.
- [4] European Monitoring Centre for Drugs and Drug Addiction, European Drug Report Trends and Developments, https://www.emcdda.europa.eu/system/files/publications/13236/TDAT20001ENN_web.pdf, 2020 (accessed 13/10/2020).
- [5] a) C. Feliu, A. Fouley, H. Millart, C. Gozalo, H. Marty, Z. Djerada, *Ann. Biol. Clin.* **2015**, *73*, 54-69; b) L. Harper, J. Powell, E. M. Pijl, *Harm Reduct. J.* **2017**, *14*, 52-52.
- [6] E. Gouzoulis-Mayfrank, L. Hermle, K. A. Kovar, H. Sass, *Nervenarzt* **1996**, *67*, 369-380.
- [7] B. K. Logan, *Forensic Sci. Rev.* **2002**, *14*, 133-151.
- [8] a) T. F. Borders, B. M. Booth, X. Han, P. Wright, C. Leukefeld, R. S. Falck, R. G. Carlson, *Addiction* **2008**, *103*, 800-808; b) K. C. Lan, Y. F. Lin, F. C. Yu, C. S. Lin, P. Chu, *J. Formos. Med. Assoc.* **1998**, *97*, 528-533.
- [9] a) Y. A. Bin Jardan, K. Mohamed, N. Abbas, M. El-Gendy, N. Alsaif, M. Alanazi, M. Mohammed, M. Abounassif, M. Hefnawy, *J. Pharm. Biomed.* **2021**, *194*; b) Y. S. X.

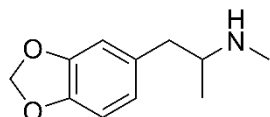
- Liu, Y. L. Fan, Z. P. Huang, H. J. Liu, L. L. Wang, Z. L. Shen, I. Watanabe, *J. Chromatogr. B* **2020**, 1153; c) J. W. Shi, J. F. Zhou, X. He, Y. Zhang, *J. Chromatogr. Sci.* **2020**, 58, 569-575; d) Z. Turkmen, M. Kuloglu, T. Tekin, S. Mercan, I. Bavunoglu, *J. Chem. Metrol.* **2019**, 13, 61-67; e) M. K. Wozniak, L. Banaszkiwicz, M. Wiergowski, E. Tomczak, M. Kata, B. Szpiech, J. Namiesnik, M. Biziuk, *Forensic Toxicol.* **2020**, 38, 42-58; f) J. H. Hussain, N. Gilbert, A. Costello, C. J. Schofield, E. K. Kemsley, O. B. Sutcliffe, R. E. Mewis, *Forensic Chem.* **2020**, 20, DOI: 10.1016/j.forc.2020.100263.
- [10] a) L. Bijlsma, J. V. Sancho, E. Pitarch, M. Ibáñez, F. Hernández, *J. Chromatogr. A* **2009**, 1216, 3078-3089; b) F. Linsen, R. P. J. Koning, M. van Laar, R. J. M. Niesink, M. W. Koeter, T. M. Brunt, *Addiction* **2015**, 110, 1138-1143.
- [11] a) J. Rohrich, J. Becker, T. Kaufmann, S. Zorntlein, R. Urban, *Forensic Sci. Int.* **2012**, 215, 3-7; b) S. Al-Abri, K. H. Meier, J. M. Colby, C. G. Smollin, N. L. Benowitz, *Clin. Toxicol.* **2014**, 52, 1292-1295.
- [12] D. E. Yerien, S. Bonesi, A. Postigo, *Org. Biomol. Chem.* **2016**, 14, 8398-8427.
- [13] A. F. Lo Faro, A. Di Trana, N. La Maida, A. Tagliabracci, R. Giorgetti, F. P. Busardo, *J. Pharm. Biomed.* **2020**, 179, DOI: 10.1016/j.jpba.2019.112945.
- [14] a) R. Brenneisen, H. U. Fisch, U. Koelbing, S. Geisshusler, P. Kalix, *Br. J. Clin. Pharmacol.* **1990**, 30, 825-828; b) P. Kalix, S. Geisshusler, R. Brenneisen, U. Koelbing, H. U. Fisch, *Eur. J. Pharmacol.* **1990**, 183, 457-458.
- [15] A. M. Feyissa, J. P. Kelly, *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2008**, 32, 1147-1166.
- [16] J. L. Goncalves, V. L. Alves, J. Aguiar, H. M. Teixeira, J. S. Camara, *Crit. Rev. Toxicol.* **2019**, 49, 549-566.
- [17] H. Gaspar, S. Bronze, C. Oliveira, B. L. Victor, M. Machuqueiro, R. Pacheco, M. J. Caldeira, S. Santos, *Forensic Sci. Int.* **2018**, 290, 146-156.
- [18] M. G. Schmid, J. S. Hagele, *J. Chromatogr. A* **2020**, 1624, DOI: 10.1016/j.chroma.2020.461256.
- [19] a) J. T. Davidson, Z. J. Sasiene, Y. Abiedalla, J. DeRuiter, C. R. Clark, G. P. Jackson, *Int. J. Mass spectrom.* **2020**, 453, DOI: 10.1016/j.ijms.2020.116343; b) J. T. Davidson, Z. J. Sasiene, G. P. Jackson, *Int. J. Mass spectrom.* **2020**, 453, DOI: 10.1016/j.ijms.2020.116354.
- [20] S. Metternich, S. Fischmann, S. Munster-Muller, M. Putz, F. Westphal, T. Schonberger, M. Lyczkowski, S. Zorntlein, C. Huhn, *Forensic Chem.* **2020**, 19, DOI: 10.1016/j.forc.2020.100241.
- [21] C. Ploumen, I. Marginean, I. S. Lurie, *J. Sep. Sci.* **2020**, 43, 3449-3457.
- [22] J. P. Kelly, *Drug Test. Anal.* **2011**, 3, 439-453.
- [23] R. P. Archer, *Forensic Sci. Int.* **2009**, 185, 10-20.
- [24] a) O. I. G. Khreit, M. H. Grant, T. Zhang, C. Henderson, D. G. Watson, O. B. Sutcliffe, *J. Pharm. Biomed.* **2013**, 72, 177-185; b) A. Plenevaux, S. L. Dewey, J. S. Fowler, M. Guillaume, A. P. Wolf, *J. Med. Chem.* **1990**, 33, 2015-2019.
- [25] L. Hondebrink, J. J. Nugteren-van Lonkhuyzen, C. C. Hunault, J. van den Berg, D. van der Gouwe, A. van Riel, *Addiction* **2020**, 115, 716-725.
- [26] S. Odoardi, F. S. Romolo, S. Strano-Rossi, *Forensic Sci. Int.* **2016**, 265, 116-120.
- [27] M. R. Alotaibi, S. M. Husbans, I. S. Blagbrough, *J. Pharm. Biomed.* **2015**, 107, 535-538.
- [28] N. V. Cozzi, S. D. Brandt, P. F. Daley, J. S. Partilla, R. B. Rothman, A. Tulzer, H. H. Sitte, M. H. Baumann, *Eur. J. Pharmacol.* **2013**, 699, 180-187.
- [29] R. Brenneisen, S. Geisshüsler, X. Schorno, *J. Pharm. Pharmacol.* **1986**, 38, 298-300.

- [30] L. H. Antonides, R. M. Brignall, A. Costello, J. Ellison, S. E. Firth, N. Gilbert, B. J. Groom, S. J. Hudson, M. C. Hulme, J. Marron, Z. A. Pullen, T. B. R. Robertson, C. J. Schofield, D. C. Williamson, E. K. Kemsley, O. B. Sutcliffe, R. E. Mewis, *ACS Omega* **2019**, *4*, 7103-7112.
- [31] S. D. Brandt, P. F. Daley, N. V. Cozzi, *Drug Test. Anal.* **2012**, *4*, 525-529.
- [32] D. M. Wood, V. Stribley, P. I. Dargan, S. Davies, D. W. Holt, J. Ramsey, *Emerg. Med. J.* **2011**, *28*, 764.
- [33] L. Couchman, A. Frinculescu, C. Sobreira, T. Shine, J. Ramsey, M. Hecht, K. Kipper, D. Holt, A. Johnston, *Drug Test. Anal.* **2019**, *11*, 1172-1182.
- [34] D. C. Williamson, E. K. Kemsley, O. B. Sutcliffe, R. E. Mewis, GB Patent 2571817A, 2019

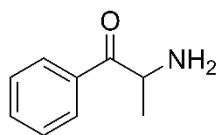
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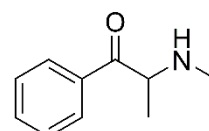
Amphetamine



MDMA



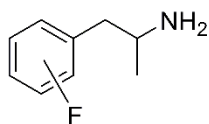
Cathinone



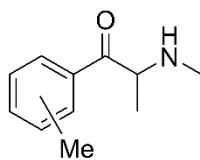
Methcathinone

Figure 1: Chemical structures of amphetamine, MDMA, cathinone and methcathinone

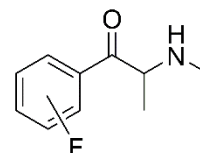
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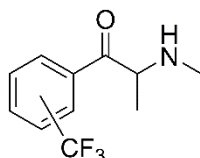
1a 2-fluoroamphetamine
1b 3-fluoroamphetamine
1c 4-fluoroamphetamine



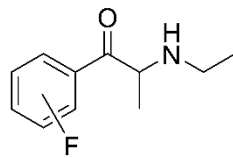
2a 2-methylmethcathinone
2b 3-methylmethcathinone
2c 4-methylmethcathinone



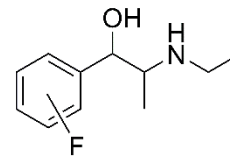
3a 2-fluoromethcathinone
3b 3-fluoromethcathinone
3c 4-fluoromethcathinone



4a 2-trifluoromethylmethcathinone
4b 3-trifluoromethylmethcathinone
4c 4-trifluoromethylmethcathinone



5a 2-fluoroethcathinone
5b 3-fluoroethcathinone
5c 4-fluoroethcathinone



6a 2-fluoro-*N*-ethyl-*nor*-ephedrine
6b 3-fluoro-*N*-ethyl-*nor*-ephedrine
6c 4-fluoro-*N*-ethyl-*nor*-ephedrine

Figure 2: Chemical structures of the amphetamine-, methcathinone-, *N*-ethylcathinone- and *nor*-ephedrine-based regioisomers explored in this study. All regioisomers were analysed in their hydrochloride forms.

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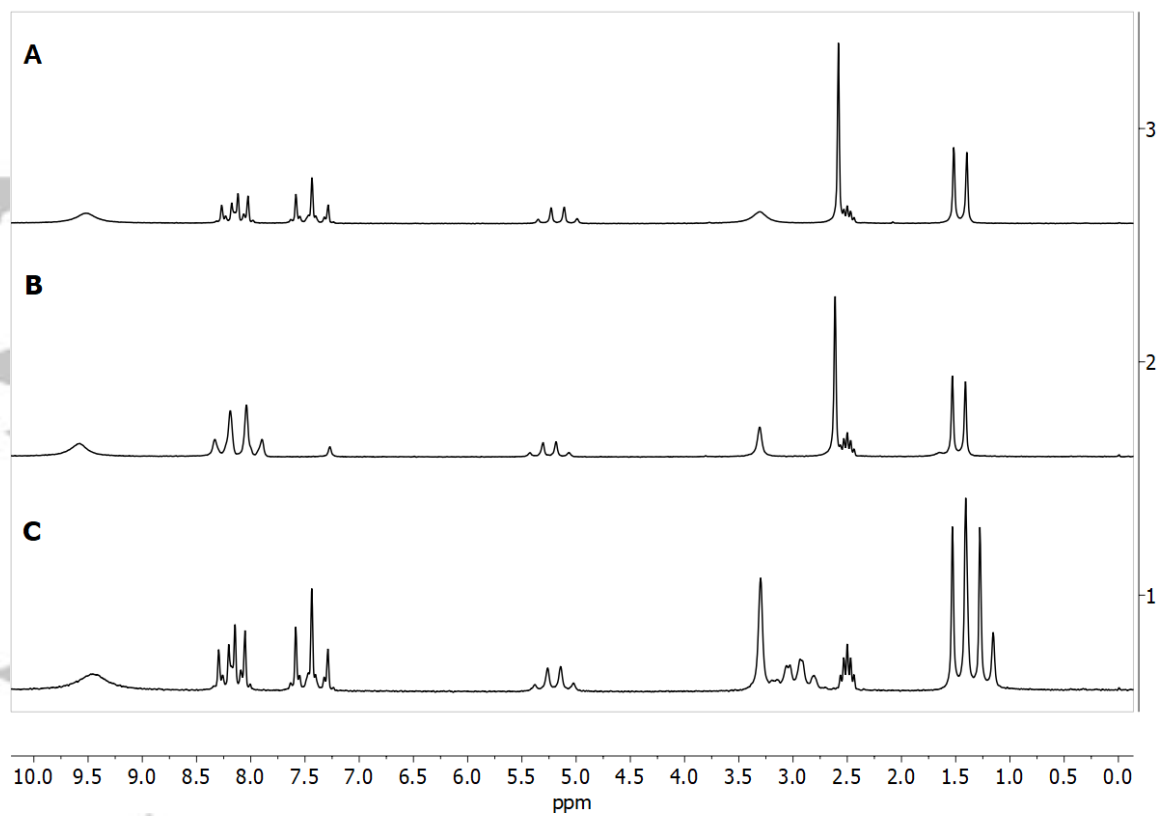


Figure 3: ^1H NMR spectra of **3c** (A), **4c** (B) and **5c** (C) collected at 60 MHz. All spectra were collected in d_6 -DMSO.

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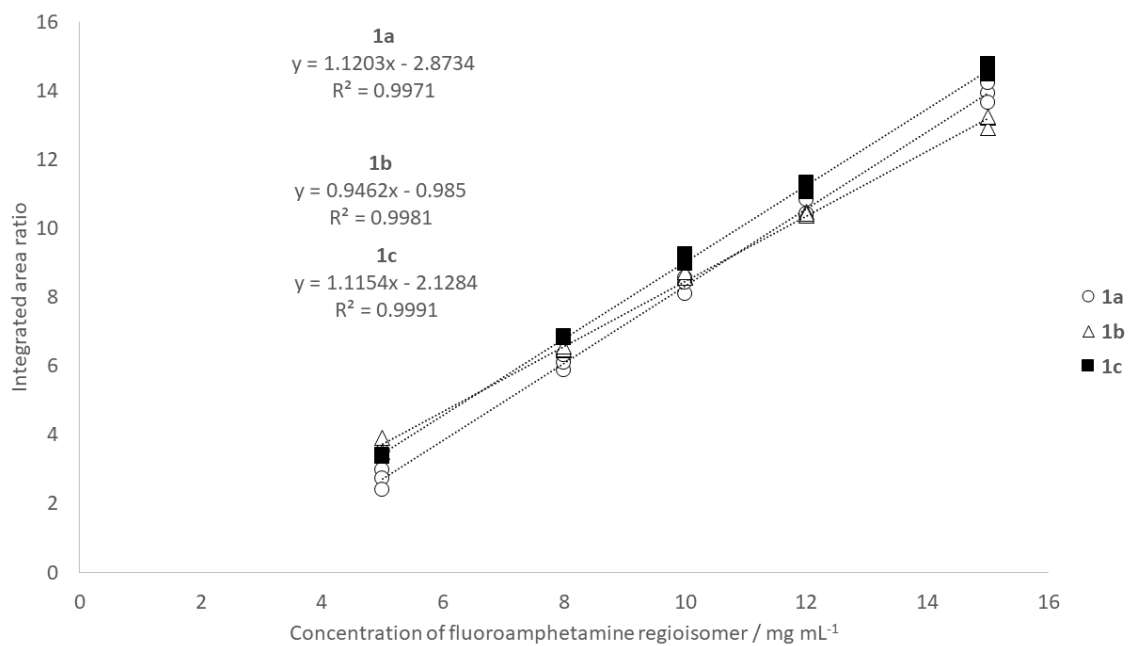


Figure 4: ¹⁹F NMR calibration plots for **1a-1c** over the concentration range 5 mg mL⁻¹ – 15 mg mL⁻¹. All data collected on a 60 MHz NMR spectrometer

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Table 1: ^{19}F NMR chemical shifts for **1a** – **1c** and **3a** – **6c**. Data collected in D_2O . Chemical shifts referenced to TFA ($\delta -76.55$)

Regioisomer	^{19}F chemical shift (ppm)
1a	-117.58
1b	-113.03
1c	-115.69
3a	-110.35
3b	-111.70
3c	-102.02
4a	-58.34
4b	-63.65
4c	-64.17
5a	-111.75
5b	-113.44
5c	-105.42
6a	-110.57
6b	-114.04
6c	-115.78

Table 2: LOD and LOQ values for **1a–1c** from ^{19}F NMR and GC-MS analysis

Regioisomer	^{19}F NMR		GC-MS	
	LOD (mg mL^{-1})	LOQ (mg mL^{-1})	LOD ($\mu\text{g mL}^{-1}$)	LOQ ($\mu\text{g mL}^{-1}$)
1a	0.49	1.63	20	68
1b	0.33	1.10	25	80
1c	0.29	0.93	23	75

Graphical Abstract



GC-MS ANALYSIS
109-122 mg of 4-fluoroamphetamine
Total time for analysis = ca. 40 min

AUTOMATED ¹H QUAL. NMR ANALYSIS

4-fluoroamphetamine detected

¹⁹F QUANT. NMR ANALYSIS
117-122 mg of 4-fluoroamphetamine
Total time for analysis = ca. 10 min

Detection, discrimination and quantification of amphetamine, cathinone and *nor*-ephedrine regioisomers using benchtop ¹H and ¹⁹F NMR spectroscopy

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