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Detection of mephedrone and its metabolites in fingerprints from a controlled human administration study by liquid chromatography-tandem mass spectrometry and paper spray-mass spectrometry

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1 Abstract

The use of synthetic stimulants, including designer cathinones, remains a significant concern worldwide. Thus, the detection and identification of synthetic cathinones in biological matrices is of paramount importance for clinical and forensic laboratories. In this study, distribution of mephedrone and its metabolites was investigated in fingerprints. Following a controlled human mephedrone administration (100 mg nasally insufflated), two mass spectrometry-based methods for fingerprint analysis have been evaluated. The samples deposited on triangular pieces of chromatography paper were directly analysed under ambient conditions by paper spray-mass spectrometry (PS-MS) while those deposited on glass cover slips were extracted and analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The LC-MS/MS method was 5-6 times more sensitive than PS-MS but required sample preparation and longer analysis time. Mephedrone was detected in 62% and in 38% of all post-administration samples analysed by LC-MS/MS and PS-MS, respectively. Nor-mephedrone was the only metabolite detected in 3.8% of all samples analysed by LC-MS/MS. A large inter- and intra-subject variation was observed for mephedrone which may be due to several factors, such as the applied finger pressure, angle and duration of contact with the deposition surface and inability to control the 'amount' of collected fingerprint deposits. Until these limitations are addressed, we suggest that the sole use of fingerprints can be a useful diagnostic tool in qualitative rather than quantitative analysis, and requires a confirmatory analysis in a different biological matrix.

2 Introduction

Over the last decade there has been an unprecedented rise in the number of new psychoactive substances (NPS) [1,2], which has been associated with an increase in NPS related deaths in the United Kingdom (UK) [3]. Mephedrone (4-methylmethcathinone) is a synthetic cathinone known for its psychostimulant properties [4–6]. Synthetic or substituted cathinones, which are naturally present in the leaves of *Catha edulis* (Khat), are one of the biggest groups of NPS. Even though mephedrone use has declined since its ban in April 2010 in the UK, it constitutes an important prototype designer drug which has paved the way for the

development of many new stimulant analogues. Mephedrone has been previously detected in human plasma and urine samples collected from controlled administration studies and analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) [7,8] and gas chromatography-mass spectrometry [9]. However, to date no studies have been conducted to evaluate the distribution of mephedrone and its metabolites in fingerprints.

Fingerprints are an attractive matrix in drug testing because its collection is non-invasive, easy and quick. In addition, storage, transportation and disposal of fingerprints is relatively simple compared with the more traditional matrices (blood and urine), which need to be treated as biological hazards. However, fingerprints are potentially more susceptible to environmental contamination making proof of administration difficult unless unique biotransformative products are detected. Fingerprint deposits contain a mixture of external contaminants (e.g. dirt, make-up, moisturisers), sebum (from touching the face where sebaceous glands are present) and eccrine sweat [10]. Drugs can be detected in sweat but the exact mechanism describing their incorporation into this matrix is poorly understood. The mechanism is thought to take place via passive diffusion (from blood into sweat glands) or via transdermal migration of drugs across the skin [11–13].

A number of studies have previously reported the detection of illicit drugs (either parent, Phase I or Phase II metabolites) in fingerprints by several different methods, including the use of spectroscopy [14,15], immunoassays [16–18] and LC-MS/MS [19,20]. However, spectroscopic- and antibody-based techniques lack analytical selectivity while LC-MS/MS requires lengthy sample preparation. Drug metabolites have also been detected in fingerprints using surface mass spectrometry methods, including desorption ionisation, matrix assisted ionisation and liquid extraction surface analysis [21,22]. Compared to LC-MS/MS, these techniques allow rapid sampling of a fingerprint without prior sample preparation. A drawback of these approaches is the fact that to carry out a rapid analysis, only a small area of the fingerprint is sampled. In contrast, paper spray-mass spectrometry (PS-MS) is a direct mass spectrometry technique that samples a whole fingerprint. Like surface mass spectrometry methods, PS-MS does not require any sample preparation or chromatography prior to analysis, making the total analysis time shorter which greatly reduces costs. PS-MS, first described by *Wang et al.* [23], uses a paper

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substrate where the sample is deposited and onto which a spray solvent and voltage is applied. This causes analytes to be swept from the paper and ionised at the tip of the paper before being transferred into the mass spectrometer. In a recent study, cocaine and its metabolites were detected for the first-time in fingerprints using PS-MS [24]. The authors also showed that it was feasible to develop the fingerprint ridge detail prior to the PS-MS analysis [24]. The same authors have recently demonstrated feasibility for a two-step process whereby a fingerprint sample is first screened using PS-MS and then confirmed using LC-MS/MS [25].

Herein, we evaluate for the first-time two methods developed for the detection of mephedrone and its Phase I metabolites (Figure 1) in fingerprints: Quadrupole-Orbitrap PS-MS and triple quadrupole LC-MS/MS. To our knowledge, this is also the first reported study describing the detection of mephedrone and its metabolites in fingerprints following a controlled human administration.

[FIGURE 1. Mephedrone and five of its Phase I metabolites]

3 Experimental

3.1 Reagents

Mephedrone hydrochloride, dihydro-mephedrone hydrochloride (DHM), mephedrone-d_3 hydrochloride (MEPH-d_3; deuterium labels present on the N-methyl moiety), dihydro-mephedrone-d_3 hydrochloride (DHM-d_3; deuterium labels present on the N-methyl moiety) were purchased from Sigma-Aldrich (Dorset, UK). Nor-mephedrone hydrochloride (NOR) and mephedrone hydrochloride (MEPH) in powder form used for the human administration were purchased from Chiron (Trondheim, Norway). Mephedrone hydrochloride was supplied as a racemic mixture with reported purity of 96.3 ± 0.5%. Hydroxytolyl-mephedrone hydrochloride (HYDROXY), 4-carboxy-mephedrone hydrochloride (4-CARBOXY) were purchased from LGC Standards (Bury, UK). Mephedrone, MEPH-d_3, DHM,

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DHM-d_3 were purchased as certified reference materials. All reference standards were analysed in-house to verify their chemical structure. The synthesis of dihydro-nor-mephedrone (DHNM) has been described in the literature before [26].

All solvents were HPLC grade unless stated otherwise. Methanol (MeOH), acetonitrile (ACN; LC-MS grade for the preparation of the mobile phase on the LC-MS/MS and HPLC grade for other uses), hydrochloric acid, formic acid and acetic acid were purchased from Fisher Scientific (Loughborough, UK). Ultrapure water (18 MΩcm) was prepared on an ELGA Purelab Maxima HPLC water purification system (High Wycombe, UK). For the PS-MS work, ultrapure water was purchased from Fisher Scientific (Loughborough, UK).

3.2 Blank matrix collection

'Blank' fingerprints were collected from drug-free volunteers according to the ethical approval granted by the Research Ethics Committee at King's College London (HR 16/17 4237). Fingertips were wiped with an ethanol wipe and allowed to dry for 10 min. Glass cover slips (15 mm in diameter) were placed inside clean weighing boats (used as a support aid) and fingerprints were deposited on the surface of the cover slips.

For the paper spray analysis, fingerprint samples were collected onto a paper substrate (Whatman Grade 1 chromatography paper). The paper substrate was cut into a triangular area (1.6 cm base and 2.1 cm height) with a rectangular extension used to tape the samples to a support slide and removed prior to analysis as described before [24]. The substrates were washed with 0.1% hydrochloric acid followed by MeOH:water (50:50 v/v), air dried and flattened before the analysis. To test for isobaric interferences from other components present in the fingerprints of non-drug users, fingerprints were collected as presented (without hand washing) from 40 volunteers from the University of Surrey, who claimed to be non-drug users.

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3.3 Volunteer administration study and sample collection

Fingerprints were collected as part of the mephedrone administration study which was fully approved by the Riverside National Research Ethics Service (16/LO/1342). Six healthy male volunteers were recruited into the study where they nasally insufflated 100 mg of mephedrone hydrochloride (informed consent was obtained from all human subjects). The volunteers attended a screening assessment to ensure they met the inclusion criteria and provided a urine sample to confirm that they were drug free before the mephedrone administration day. The urine sample was analysed using a standard stimulant (including mephedrone) screen at Abbott (see Section 5 in Supplementary Information for further details). On the administration day, participants were asked to wear a disposable gown over their clothes, a pair of gloves and a hair cap while they self-administered a single dose of 100 mg mephedrone powder by nasal insufflation. This was done in a different room to the main study room used for subsequent sample collection to minimise contamination. Participants were then asked to wash their hands and face with ethanol wipes and were taken back to the main study room where fingerprints were collected.

Fingerprints were collected from each finger (labelled as F1-F5, where F1 was the thumb and F5 was the little finger) of the right hand from the 6 participants (referred to here as M1-M6) at -10 min (before administration), 10 min, 20 min, 45 min, 90 min, 3 h, 5 h, Day 2 and Day 3 post drug administration (fingers were not rubbed together). Before the -10 min and 10 min sample collection, fingertips were wiped with an ethanol wipe and allowed to dry. Fingertips were only cleaned at these two timepoints to remove external contamination at the -10 min sample collection and at 10 min to wash off any residual mephedrone powder that might have been accidently picked up by the participants (e.g. by wiping their nose) after nasal insufflation. Natural sweat excretions were collected at the other timepoints. Participants stayed in the room from the beginning of the study until 5 h (except for toilet breaks) and conducted normal daily tasks, such as reading, eating and working on their laptops.

Fingerprints were deposited on clean glass cover slips (as described in 3.2), which were then stored in individual 20 mL scintillation vials. On three occasions when glass cover slips broke

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under applied pressure, broken cover slips were transferred into scintillation vials and extracted. At each timepoint an additional fingerprint was collected onto triangular pieces of chromatography paper placed on top of a scale. Participants were asked to push down on the piece of paper for 10 s to give pressure between 800-1200 g, similarly to the method described before [24]. These samples were only collected from M4-M6 from the thumb and the index finger. All sample were stored dessicated at -20°C for approximately 1 week before analysis.

3.4 Calibration standards and quality control (QC) samples

For the LC-MS/MS method, calibration standards containing MEPH, DHNM and NOR at 0.2, 1, 5, 10, 20, 40, 50 ng/mL; HYDROXY and 4-CARBOXY at 0.1, 1, 5, 10, 20, 40, 50 ng/mL; and DHM at 0.16, 0.5, 1, 5, 10, 25, 50 ng/mL were prepared in MeOH. Fingerprints from drug-free volunteers were deposited on glass cover slips as described above. One hundred microliters of each calibration standard was aliquoted onto cover slips and was allowed to dry. QC Low (0.8 ng/mL for MEPH, DHNM, NOR; 0.5 ng/mL for HYDROXY and 4-CARBOXY; and 0.4 ng/mL for DHM), QC Med (10 ng/mL for MEPH, DHNM, NOR, HYDROXY, 4-CARBOXY; 5 ng/mL for DHM) and QC High (40 ng/mL for all analytes) were prepared in the same way as calibration standards. Internal standard (IS) containing MEPH-d_3 and DHM-d_3 at 250 ng/mL was prepared in MeOH.

For the PS-MS method, calibration standards containing MEPH and DHM at 5, 10, 20, 40, 50, 60, 80, 100, 200, 300 ng/mL were prepared in MeOH. IS containing MEPH-d_3 and DHM-d_3 at 50 ng/mL was also prepared in MeOH. Five microliters of each calibration standard were spotted on the paper substrate and were dried for 2 min. Two quality control samples (corresponding to 500 pg and 1500 pg per fingerprint) were prepared in the same way as calibration standards.

3.5 Sample preparation – glass cover slips

Fifty microliters of IS was aliquoted on the cover slips and was allowed to evaporate. Glass cover slips were then transferred into scintillation vials and 300 µL of 0.2% formic acid in

ACN:Water (90:10 v/v) was added. Vials were sonicated for 7 min at 35 kHz and vortex mixed for 30 s at 1200 rpm on a Thermomixer Comfort (Eppendorf, UK). The solvent was transferred into clean 1.5 mL Eppendorf tubes and was evaporated under vacuum at 45˚C. Samples were reconstituted with 100 µL of 0.1% formic acid in ACN:Water (10:90 v/v).

Due to the nature of fingerprint collection, dilution was impractical at the beginning of an extraction. Therefore, dilution was performed after sample reconstitution when an appropriate volume of the reconstituted sample was diluted 1 in 100 in the reconstitution solvent. Where dilution was required, 3 additional QCs were extracted and diluted in the same manner.

3.6 Sample preparation – paper spray

PS-MS analysis was carried out using a custom-made paper spray source built at the Surrey Ion Beam Centre as described before [24]. A sample (a fingerprint, 5 µL of a calibration standard or a QC) was loaded onto the paper substrate followed by the addition of 5 µL of the IS solution. The moist paper was then air dried for 2 min before being placed on top of a glass slide and under a folded piece of aluminium foil. Both the glass slide and aluminium foil were replaced for each consecutive measurement to avoid carryover. Fifty microliters of 0.1% formic acid in ACN was then aliquoted onto the paper and a voltage of 4 kV was applied to the paper spray source.

3.7 LC-MS/MS conditions

The analysis was performed by LC-MS/MS using a Waters Acquity ultra performance liquid chromatograph system equipped with a CTC 2777 open architecture autosampler (Waters, UK) and coupled to a Waters Xevo TQ-S triple quadrupole mass spectrometer (Manchester, UK).

Chromatographic separation was performed on a 2.1 mm x 150 mm Selectra® column containing a 1.8 µm pentafluorophenylpropyl phase (UCT, US). The column was held at 60°C. The strong needle wash was 0.3% formic acid in MeOH and the weak needle wash was 0.01%

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formic acid in acetonitrile:water (10:90 v/v). The flow rate was 0.5 mL/min with 0.3% formic acid in water as mobile phase A and 0.3% formic acid in acetonitrile as mobile phase B. The start of the gradient was at 85% mobile phase A. Mobile phase B was then increased to 55% over 11 min and was held for 2 min. Over the next 0.5 min the gradient returned to the starting condition and the column was re-equilibrated at 85% mobile phase A for the remaining 1.5 min. The total run time was 15 min. The injection volume was 20 µL and the data was acquired using MassLynx software (version 4.1). TargetLynx (version 4.1) was used for data processing and quantification.

Extracted samples were analysed using electrospray ionisation (ESI) operated in positive ion mode. The source temperature was set to 150°C. The desolvation gas flow rate was 1000 L/h at a temperature of 500˚C, capillary voltage was set to 2.22 kV, cone voltage was 45 V and source offset was 84 V. The cone gas flow rate was set to 150 L/h, the nebuliser gas flow was 7.00 bar and the collision gas flow rate was 0.25 mL/min. Mephedrone metabolites and deuterated internal standards were monitored using selected reaction monitoring (SRM) as detailed in Table 1. In order to maximise sensitivity, all analytes except for 4-CARBOXY and HYDROXY had their dehydration products chosen as target precursor ions due to significant in-source fragmentation which is commonly observed in synthetic cathinones [26–28].

[TABLE 1.]

3.8 PS-MS conditions

The paper spray source was coupled to a Q-Exactive Plus Orbitrap hybrid mass spectrometer (Thermo Fisher Scientific, UK). The capillary temperature (300°C) and S-lens RF level (50.0) were optimised using ESI based on the parameters that produced the highest protonated ion counts of analytes. The mass spectrometry parameters are further documented in the Supplementary Information (Section 3). Data acquisition was carried out for 0.5 min in full scan mode (m/z 66.7-500) for quantitative analysis, followed by 0.5 min in MS/MS mode to qualitatively confirm peak assignment with a stepped normalised collision energy of 30, 60, and 90. Fragment ions used for confirmation of each analyte are presented in Table 2. All

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spectra were analysed using Xcalibur software (version 4.1, Thermo Fisher Scientific). Example mass spectra of each analyte and their respective fragment ions are presented in the Supplementary Information (Section 4).

[Table 2.]

3.9 Method validation

Validation experiments determined selectivity, linearity, inter- and intra-day precision and accuracy, limit of detection (LOD), lower limit of quantification (LLOQ), recovery (only LC-MS/MS method), matrix effect, dilution integrity (only LC-MS/MS method) and carryover. The LC-MS/MS method was validated for all analytes presented in Figure 1 according to the Food and Drug Administration guidelines [29] and recommendations published by *Peters et al*. [30] The PS-MS method was validated only for mephedrone and DHM because the only two matching deuterated internal standards available at the time of analysis were MEPH-d_3 and DHM-d_3. Moreover, PS-MS validation assessed initial method capabilities based on 3 replicate measurements and an allowed deviation of ± 20% as published before [24,25].

3.9.1 Selectivity

For the LC-MS/MS method, selectivity was assessed by analysing 6 blank fingerprints collected from 3 drug-free female and 3 drug-free male donors. For the PS-MS method, selectivity was assessed by analysing 40 fingerprint samples collected from non-drug users as described in 3.2.

3.9.2 Linearity

For the LC-MS/MS method, matrix-matched calibration curve was prepared by aliquoting appropriate solutions on the cover slips containing drug-free matrix. Each calibration standard was required to be within ± 15% of its target concentration, except at the lowest level of quantification (LLOQ) where ± 20% variation was allowed. The correlation coefficient (r^2) of the line had to be at least 0.990. A linear regression model with a weighing of 1/x was applied to all calibration curves.

For the PS-MS method, each QC was required to be within ± 20% of its target concentration. A linear regression model without a weighing factor was applied to all calibration curves.

3.9.3 LOD and LLOQ

For the LC-MS/MS method, the LOD for each analyte in a matrix was defined as the lowest concentration where all three ions (two qualifiers and one quantifier) were present with a signal-to-noise equal to or greater than 3. The LLOQ was defined as the lowest concentration at which analytes (detected in the full scan mode) could be quantified with an acceptable precision and accuracy. The upper limit of quantification was defined as the highest concentration of a calibration standard, which could be determined with an acceptable accuracy and precision without saturating the instrument signal.

For the PS-MS method, LOD and LLOQ were established by running calibration standards from the highest concentration to the lowest concentration until the signal-to-noise was equal or greater than 3 and 10, respectively. Blank measurements were taken between calibration standards and no carry-over was observed.

3.9.4 Precision and accuracy

For the LC-MS/MS method, intra-day (n=6) and inter-day (n=3) precision and accuracy was determined by employing QC samples spiked at low (Low), medium (Med), and high (High) concentrations. Intra-day precision was calculated using six replicates obtained on the same day and expressed as a coefficient of variation (%CV). Accuracy was calculated by dividing the mean measured concentration at each QC level by the theoretical spiked concentration and expressed as a percentage of the theoretical spiked concentration. Inter-day precision was evaluated for each QC level on three different days and expressed as %CV. According to the validation guidelines [29,30] the mean value should be within 15% of the true value, except for the LLOQ where it should be within 20% of the true value.

For the PS-MS method, two QCs were prepared at 100 ng/mL (QC Low) and 300 ng/mL (QC High) and were analysed in triplicate on each day (n=3). Values of ± 20% were deemed acceptable.

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3.9.5 Recovery

For the LC-MS/MS method, blank matrix samples (n=6) were spiked at QC Low and QC High level and were taken through the extraction. In parallel, another set of blank matrix samples (n=6) was extracted and spiked after the evaporation step at QC Low and QC High level. Recovery was expressed as a percentage by comparing the absolute peak areas of the samples spiked before extraction with samples spiked after extraction.

For the PS-MS method, recovery was not calculated because the method does not involve a sample preparation step.

3.9.6 Matrix effect

For the LC-MS/MS method, the IS-corrected matrix effect was evaluated. A set of blank matrix samples (n=6 from three female and three male individuals) and a set of clean cover slips (no matrix, n=6) was taken through the extraction. All samples were reconstituted with a solution containing known amounts of the IS and analytes at QC Low and QC High levels. Matrix effect was evaluated by comparing peak area ratios in blank matrix samples spiked after extraction with peak area ratios in clean cover slips spiked after extraction.

For the PS-MS method, fingerprints from right thumb (RT), right index (RI) and 5 overlaid fingerprints from a male and a female donor were compared to a standard prepared at 50 ng/mL in MeOH (n=3) and analysed in the absence of a fingerprint.

3.9.7 Carryover

For the LC-MS/MS method, carryover was assessed by injecting methanol blanks after the highest calibration standard.

For the PS-MS method, carryover was assessed by spotting the extracting solvent, 0.1% formic acid in ACN, on paper and analysing it after each set of calibration standards.

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3.9.8 Dilution integrity

Dilution integrity was only assessed for mephedrone (LC-MS/MS method only). IS was prepared at 250 ng/mL to allow 1 in 100 dilution after sample reconstitution.

3.9.9 Stability

Stability of mephedrone and DHM on a paper substrate and in the presence of a fingerprint was assessed. A solution prepared at 50 ng/mL in MeOH was spotted onto paper containing a single fingerprint. Samples (n=3 per condition) were stored in the fridge at +5°C and in the freezer at -20°C for 1 week and 4 weeks and were compared to a freshly made solution.

4 Results

4.1 Method validation

Method validation results are presented in the Supplementary Information, except for the LOD and LLOQ results which are shown in Table 3. Briefly, the intra- and inter-day precision were within ± 15% and ± 20% of the true value when analysed by LC-MS/MS and PS-MS, respectively. Linearity was also assessed on both instruments. On PS-MS calibration line gave $r^2 > 0.990$ while on LC-MS/MS mean linearity of $r^2 > 0.996$ was achieved for all analytes. Carryover was not observed while matrix effect was within ± 7.3% and ± 13% on LC-MS/MS and PS-MS, respectively.

On PS-MS, LOD was found to be 25 pg/fingerprint for MPEH and DHM. On LC-MS/MS, LOD of 5 pg/fingerprint (LLOQ of 20 pg/fingerprint) was achieved for mephedrone, NOR and DHNM. LOD of 4 pg/fingerprint and LLOQ of 16 pg/fingerprint were achieved for DHM. The lowest LOD of 2.5 pg/fingerprint and LLOQ of 10 pg/fingerprint were achieved for HYDROXY and 4-CARBOXY. Table 3 compares the LOD, LLOQ and calibration range between the two methods. LOD and LLOQ achieved on PS-MS are only shown for mephedrone and DHM because other analytes were not analysed by his method.

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[TABLE 3.]

4.2 Detection of mephedrone and its metabolites in fingerprints by LC-MS/MS

Mephedrone was detected in 163 (62%) of the 264 fingerprints collected from 10 min until Day 3 and in the fingerprint from at least one fingerprint in all six participants (Figure 2). Mephedrone concentrations detected in fingerprints ranged from 23.5 pg/fingerprint to 479 ng/fingerprint. Mephedrone was first detected at 10 min in M1 in all fingers except F5, in M2 and M3 in all fingers, in M5 in all fingers except F2 and in M6 in F1 and F5 only. In M4 mephedrone was detected after 10 min in F3, after 90 min in F2 and F4, and after 3 h in F1. In F5 mephedrone was only detected at 3 h. The last detected concentration was determined at the assay LOD of 5 pg/fingerprint and was observed between 5 h and Day 3 in M1-M6, except for F5 in M4 where it was observed at 3 h. Mephedrone was detected above the LOD in 11 (37%) fingerprints collected on Day 2 and in 7 (23%) fingerprints collected on Day 3. The analyte was present in 29 out of 30 (97%) fingerprints collected at 5 h, the exception being F5 in M4.

[FIGURE 2. Concentration of mephedrone in A) M1, B) M2, C) M3, D) M4, E) M5, F) M6 in fingerprints (F1-F5) collected onto glass cover slips]

NOR was detected in 7 (2.7%) fingerprints above the LLOQ (20 pg/fingerprint) and in 10 (3.8%) fingerprints above the LOD of 5 pg/fingerprint in M1 and M2 only (Table 4). HYDROXY was only detected in M1 F1 at 20 min at 39.9 pg/fingerprint (LLOQ of 10 pg/fingerprint). Other analytes were not detected.

[TABLE 4.]

4.3 Detection of mephedrone and its metabolites in fingerprints by PS-MS

Following the analysis of fingerprints from 40 non-drug users, interferences were not observed (data not shown). Figure 3 shows the mass per fingerprint of mephedrone detected at different timepoints in fingerprints collected from three participants (M4-M6). Mephedrone was detected between 0.03 ng and 0.4 ng in fingerprints collected from each participant, with the time window of detection having some degree of inter-subject variability.

[FIGURE 3. Concentration of mephedrone in A) M4, B) M5, C) M6 in fingerprints collected onto triangular piece of chromatography paper from each fingerprint (F1-F2; note that 10 min and 20 min samples were not collected from M5)]

5 Discussion

5.1 Method comparison

Both methods were successfully validated for quantification of mephedrone and its Phase I metabolites in fingerprints. LC-MS/MS was 5-6 times more sensitive than PS-MS, with the biggest sensitivity difference observed for DHM. However, PS-MS offered faster analysis than LC-MS/MS which required sample preparation and a 15 min chromatographic run.

A direct comparison of the sensitivity offered by the two methods is complex because they differ in sample preparation, ionisation sources, modes of operation and employed mass analysers. Nevertheless, it is expected that PS-MS will offer advantages to laboratories wishing to specialise in rapid screening of fingerprints. The sensitivity of PS-MS may be further enhanced by coupling a PS source (now commercially available) to a triple quadrupole mass analyser.

Even though the PS-MS methodology will require more extensive validation to be fully implemented in clinical and forensic laboratories, we have shown its potential feasibility in

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detecting low levels of mephedrone in fingerprints under ambient ionisation conditions and without the need for sample preparation.

5.2 Detected analytes

Mephedrone and NOR were found above the LOD in 62% and 3.8%, respectively, of all fingerprints collected on glass cover slips. Mephedrone was detected above the LOD in 42% of all samples analysed by PS-MS but the metabolites were not detected. Fingerprints collected at the same timepoints by both methods resulted in considerably different concentrations. In general, samples collected on glass cover slips and analysed by LC-MS/MS showed greater mephedrone concentrations and were detected in samples, such as M6 3 h in the thumb, where the analyte was not detected on paper analysed by PS-MS.

The parent drug is expected to be found in fingerprints but some of its more polar metabolites may not effectively incorporate into sweat. This has been reported in other studies where the analysis of fingerprints from drug users (methamphetamine) [31] or from a controlled administration (cocaine, codeine) [32] resulted in the parent drug being detected at high concentrations in the samples whereas the metabolite(s) were not detected or were detected at much lower concentrations. The evidential value of fingerprints where metabolites are not detected is questionable because it does not exclude the possibility of external contamination. However, in this study the possibility of environmental contamination was assessed based on 40 fingerprints collected from non-drug users which demonstrated the absence of notable interferences. This shows that a fingerprint test for mephedrone use might still be an effective screening tool, backed up by a whole blood, urine or oral fluid test if a confirmatory analysis is required. Moreover, we hypothesise that a relatively low dose of administrated mephedrone contributed to the low detectability of its metabolites in the samples. Mephedrone users report taking 100-200 mg every hour or two hours, such that they use up to 1 g or more per "session" [4,33]. It is therefore likely that in high-dose clinical or forensic cases of mephedrone abuse our method would demonstrate the presence of both parent and one (or potentially more) of its metabolites. This is particularly important with respect to the less sensitive PS-MS method, presumably due to the lack of sample

preparation, and the use of an Orbitrap rather than a triple quadrupole mass analyser in SRM mode.

Mephedrone was not detectable in Day 2 fingerprints analysed by PS-MS but was present in one sample from the index finger on Day 3 of donor M6. It is unclear whether this is the result of external contamination (the hands were not immediately cleaned prior to sample collection on Days 2 or Day 3 to avoid loss of excreted analytes). Previous work by *Ismail et al.*, *Costa et al.* and *Jang et al.* have demonstrated the need for a sampling strategy that reduces external contamination for fingerprint collection [34–36]. Glass cover slips offered better detectability on Day 2 and Day 3 where 37% and 23% of all samples, respectively, were detected above the LOD. Low detectability of the analytes on Day 2 and Day 3 by PS-MS might be due to the instrument not being as sensitive as LC-MS/MS. The LOD achieved for mephedrone on LC-MS/MS was 5 pg/fingerprint which is five times lower than the LOD on PS-MS. Additionally, analyte instability described in Supplementary Information (Section 2.7) might have affected detectability of the analytes and so faster analysis time would be recommended in the future.

5.3 Inter- and intra-subject variability

A large inter- and intra-subject variability in mephedrone concentrations was observed. There are several factors which could have contributed to the variability in this study. The difference in the pressure applied during fingerprint deposition (glass cover slips only), the angle and duration of contact with glass cover slips were not controlled but have been shown to greatly vary between individuals or even between samples collected from the same individual on different occasions [10,37].

Several strategies could be employed to help overcome these limitations in future studies as well as in clinical or forensic practice. The pressure at which fingerprints were deposited on the glass cover slips was not controlled in this study, and even in the case of the triangular pieces of chromatography paper where it was controlled it was difficult to maintain constant pressure within the 400-1000 g range. A more robust method was developed by *Fieldhouse* who designed a device where a finger is placed on a slide and is pressed down with constant

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pressure from above for a required duration. The device produced reproducible and consistent results within and between participants and improved the quality of the deposited marks [37,38]. Moreover, compounds found in high abundance in fingerprint-derived sweat, such as creatinine or serine, could be measured alongside analytes of interest as demonstrated by *Goucher et al* [20]. For example, concentration of creatinine in human sweat has been reported to be directly proportional to the concentration in plasma. Therefore, when a drug or its metabolite passes from whole blood into sweat, the ratio of drug to creatinine would be independent of the amount of fingerprint deposits deposited on a glass cover slip. Targeting endogenous compounds present in sweat would also demonstrate that sweat was deposited on the collection device, which could help explain situations like the one observed in this study for M3 F5 where the mephedrone concentration dropped at 90 min to 0.110 ng/fingerprint and increased to 0.351 ng/fingerprint at 3 h (Figure 2C).

Interestingly, the highest mephedrone concentration between 10 min and 5 h from the glass cover slips was detected in the thumbs (F1). This might be linked to the 'amount' of fingerprint deposits collected due to their larger surface area and/or to the higher pressure applied [34]. Mephedrone concentrations were lower in index fingers (F2) compared to thumbs but were considerably higher than those in middle (F3), ring (F4) and little fingers (F5), which could be due to the index finger being a more dominant finger with more muscle strength. We did not observe higher mephedrone concentrations in the thumbs compared to the index fingers from the samples collected on the triangular pieces of chromatography paper, which suggests that the deposition surface may also play a role in analyte retention.

Excessive sweating following drug use can influence the volume of excreted sweat which may impact the results of fingerprint analysis. Mephedrone is a sympathomimetic drug which is expected to increase sweating. Sweating was reported in the first published case of analytically confirmed acute mephedrone toxicity [39] and in 5-10% of 150 acute mephedrone toxicity calls to the Swedish Poisons Information Centre [40,41]. However, it is not known what the overall effect of mephedrone or other sympathomimetic drugs is on the sweat gland function and how it impacts their concentration in this matrix, which may ultimately impact the analysis of fingerprints, too.

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5.4 Comparison of the collection devices

Even though fingerprints collection onto glass cover slips is an easy and quick process, some problems were encountered during the study. Glass cover slips are very thin and fragile. They broke on three occasions under excessive pressure applied during fingerprint deposition. Moreover, glass cover slips had to be transferred from the weighing boats into scintillation vials which made them likely to be dropped in the process. On the other hand, fingerprint collection on paper was more convenient but the preparation of the triangular pieces of chromatography paper was more time consuming. In addition, a direct imaging of the fingerprint ridge detail cannot be performed on paper without the use of chemical reagents.

6 Conclusion

To our knowledge this is the first study which evaluates two methods (Quadrupole-Orbitrap PS-MS and triple quadrupole LC-MS/MS) developed for the detection of mephedrone and its Phase I metabolites in fingerprints collected from a controlled human administration. A relatively small dose of administered mephedrone coupled with analyte detection in individual fingerprints resulted in only mephedrone and NOR being detected above the LOD by LC-MS/MS. Fingerprints analysed by PS-MS were positive for mephedrone, however, its metabolites were not detected.

Inter- and intra-subject variability was observed which can be attributed to the differences in pressure applied during fingerprint deposition, the angle and duration of contact with the deposition surface coupled with the inability to control the 'amount' of collected fingerprint deposits.

Fingerprint deposits are an attractive matrix for use in clinical and forensic settings but given its current limitations we suggest they should be used for qualitative rather than quantitative analysis until practical solutions to the problems discussed in the paper are found.

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7 Conflicts of interest

There are no conflicts to declare.

8 Acknowledgments

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DOI: 10.1039/C9AN02477H

9 References

1 European Monitoring Centre for Drugs and Drug Addiction, *European Drug Report 2016: Trends and Developments*, European Monitoring Centre for Drugs and Drug Addiction, 2016.

2 G. Stephenson and A. Richardson, *New Psychoactive Substances in England: A review of the evidence*, Home Office, 2014.

3 C. Wells, *Deaths related to drug poisoning in England and Wales, 2018.*, 2019.

4 J. P. Kelly, *Drug Test. Anal.*, 2011, **3**, 439–453.

5 F. Pantano, R. Tittarelli, G. Mannocchi, R. Pacifici, A. di Luca, F. P. Busardò and E. Marinelli, *Curr. Neuropharmacol.*, 2017, **15**, 738–749.

6 F. Schifano, A. Albanese, S. Fergus, J. L. Stair, P. Deluca, O. Corazza, Z. Davey, J. Corkery, H. Siemann, N. Scherbaum, M. Farre', M. Torrens, Z. Demetrovics and A. H. Ghodse, *Psychopharmacology (Berl).*, 2011, **214**, 593–602.

7 E. Papaseit, C. Pérez-Mañá, J.-A. Mateus, M. Pujadas, F. Fonseca, M. Torrens, E. Olesti, R. de la Torre and M. Farré, *Neuropsychopharmacology*, 2016, **41**, 2704–2713.

8 E. Olesti, M. Farré, E. Papaseit, A. Krotonoulas, M. Pujadas, R. de la Torre and Ó. J. Pozo, *AAPS J.*, 2017, **19**, 1767–1778.

9 E. Olesti, M. Pujadas, E. Papaseit, C. Pérez-Mañá, Ó. J. Pozo, M. Farré and R. de la Torre, *J. Anal. Toxicol.*, 2016, **41**, 100–106.

10 S. Cadd, M. Islam, P. Manson and S. Bleay, *Sci. Justice*, 2015, **55**, 219–238.

11 S. L. Kacinko, A. J. Barnes, E. W. Schwilke, E. J. Cone, E. T. Moolchan and M. A. Huestis, *Clin. Chem.*, 2005, **51**, 2085–94.

12 N. Fucci, N. De Giovanni and V. L. Pascali, *Ski. Res. Technol.*, 2015, **21**, 129–30.

13 N. De Giovanni and N. Fucci, *Curr. Med. Chem.*, 2013, **20**, 545–561.

14 M. J. West and M. J. Went, *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.*, 2009, **71**, 1984–1988.

15 J. S. Day, H. G. M. Edwards, S. A. Dobrowski and A. M. Voice, *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.*, 2004, **60**, 563–568.

16 P. Hazarika, S. M. Jickells, K. Wolff and D. A. Russell, *Anal. Chem.*, 2010, **82**, 9150–4.

17 P. Hazarika, S. M. Jickells and D. A. Russell, *Analyst*, 2009, **134**, 93–6.

18 P. Hazarika, S. M. Jickells, K. Wolff and D. A. Russell, *Angew. Chemie Int. Ed.*, 2008, **47**, 10167–10170.

19 S. Jacob, S. Jickells, K. Wolff and N. Smith, *Drug Metab. Lett.*, 2008, **2**, 245–7.

20 E. Goucher, A. Kicman, N. Smith and S. Jickells, *J. Sep. Sci.*, 2009, **32**, 2266–72.

21 M. Bailey, E. C. Randall, C. Costa, T. L. Salter, A. M. Race, M. de Puit, M. Koeberg, M. Baumert and J. Bunch, *Anal. Methods*, 2016, **16**, 3373–3382.

22 M. J. Bailey, R. Bradshaw, S. Francese, T. L. Salter, C. Costa, M. Ismail, R. P. Webb, I. Bosman, K. Wolff and M. de Puit, *Analyst*, 2015, **140**, 6254–6259.

23 H. Wang, J. Liu, R. G. Cooks and Z. Ouyang, *Angew. Chemie Int. Ed.*, 2010, **49**, 877–880.

24 C. Costa, R. Webb, V. Palitsin, M. Ismail, M. de Puit, S. Atkinson and M. J. Bailey, *Clin. Chem.*, 2017, **63**, 1745–1752.

25 C. Costa, C. Frampas, K. A. Longman, V. Palitsin, M. Ismail, P. Sears, R. Nilforooshan and M. J. Bailey, *Rapid Commun. Mass Spectrom.*, 2019, rcm.8553.

26 J. Czerwinska, M. C. Parkin, P. I. Dargan, C. George, A. T. Kicman and V. Abbate, *Drug Test. Anal.*, 2018, **11**, 586–594.

27 M. Majchrzak, R. Celiński, P. Kuś, T. Kowalska and M. Sajewicz, *Forensic Toxicol.*, 2018, **36**, 33–50.

28 O. J. Pozo, M. Ibanez, J. V. Sancho, J. Lahoz-Beneytez, M. Farre, E. Papaseit, R. de la Torre and F. Hernandez, *Drug Metab. Dispos.*, 2014, **43**, 248–257.

29 *Bioanalytical Method Validation Guidance for Industry Biopharmaceutics Bioanalytical*, 2018.

30 F. T. Peters, O. H. Drummer and F. Musshoff, *Forensic Sci. Int.*, 2007, **165**, 216–224.

31 T. Zhang, X. Chen, R. Yang and Y. Xu, *Forensic Sci. Int.*, 2015, **248**, 10–14.

32 M. A. Huestis, J. M. Oyler, E. J. Cone, A. T. Wstadik, D. Schoendorfer and R. E. Joseph, *J. Chromatogr. B Biomed. Sci. Appl.*, 1999, **733**, 247–264.

33 H. Sumnall and O. Wooding, *Mephedrone – an update on current knowledge*, Centre for Public Health, Liverpool John Moores University, 2009.

34 M. Ismail, D. Stevenson, C. Costa, R. Webb, M. de Puit and M. Bailey, *Clin. Chem.*, 2018, **64**, 909–917.

35 C. Costa, M. Ismail, D. Stevenson, B. Gibson, R. Webb and M. Bailey, *J. Anal. Toxicol.*

36 M. Jang, C. Costa, J. Bunch, B. Gibson, M. Ismail, V. Palitsin, R. Webb, M. Hudson and

View Article Online
DOI: 10.1039/C9AN02477H

M. J. Bailey, *Sci. Rep.*, 2020, **10**, 1974.

37 S. J. Fieldhouse, *J. Forensic Sci.*, 2015, **60**, 422–427.

38 S. Fieldhouse, *Forensic Sci. Int.*, 2011, **207**, 96–100.

39 D. M. Wood, S. Davies, M. Puchnarewicz, J. Button, R. Archer, H. Ovaska, J. Ramsey, T. Lee, D. W. Holt and P. I. Dargan, *J. Med. Toxicol.*, 2010, **6**, 327–30.

40 D. M. Wood and P. I. Dargan, *Prog. Neuro-Psychopharmacology Biol. Psychiatry*, 2012, **39**, 227–233.

41 D. James, R. D. Adams, R. Spears, G. Cooper, D. J. Lupton, J. P. Thompson and S. H. L. Thomas, *Emerg. Med. J.*, 2010, **28**, 686–689.

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Table 1. The retention time, SRM transitions and collision energy for each ion on Xevo TQ-S; * denotes a quantifying transition

Table 2. Target analytes with their protonated mass (m/z) and their respective main fragment ions (m/z) on Q-Exactive

Table 3. Comparison of the LOD (in pg/fingerprint), LLOQ (in pg/fingerprint) and calibration range (in ng/fingerprint) between LC-MS/MS and PS-MS; ND – not determined

Table 4. NOR concentrations (in pg/fingerprint) detected above the LLOQ of 20 pg/fingerprint and LOD of 5 pg/fingerprint (ticks) in fingerprints (F1-F5) collected from M1 and M2; ND – not detected

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Table 1. The retention time, SRM transitions and collision energy for each ion on Xevo TQ-S;

** denotes a quantifying transition*

Analyte	Retention time (min)	Precursor ion (*m/z*)	Product ion (*m/z*)	Collision energy (V)	Internal standard
MEPH	5.85	160.4	145.1*	15	MEPH-d_3
			144.1	33	
			91.1	28	
MEPH-d_3	5.85	163.4	148.4	19	
DHM	5.38	162.4	147.3*	19	DHM-d_3
			131.4	17	
			91.3	26	
DHM-d_3	5.38	165.4	150.3	18	
NOR	5.00	146.0	131.1	25	MEPH-d_3
			130.1*	25	
			119.0	15	
HYDROXY	1.98	194.1	158.1	17	DHM-d_3
			146.0*	17	
			131.1	23	
4-CARBOXY	2.06	208.0	146.0*	13	DHM-d_3
			144.1	28	
			130.1	31	
DHNM	4.45	148.1	131.1*	13	DHM-d_3
			116.2	23	
			91.1	25	

Table 2. Target analytes with their protonated mass (m/z) and their respective main fragment ions (m/z) on Q-Exactive

Analyte	Precursor ion (*m/z*)	Product ion (*m/z*)	Internal standard
MEPH	178.1	160.11 145.09	MEPH-d_3
MEPH-d_3	181.1	162.13	-

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DHM	180.1	146.10 131.09	DHM-d_3
DHM-d_3	183.2	131.07	-

Table 3. Comparison of the LOD (in pg/fingerprint), LLOQ (in pg/fingerprint) and calibration range (in ng/fingerprint) between LC-MS/MS and PS-MS; ND – not determined

	LC-MS/MS			PS-MS		
Analyte	**LOD**	**LLOQ**	**Range**	**LOD**	**LLOQ**	**Range**
MEPH	5	20	0.020-50	25	50	0.025-1.5
DHM	4	16	0.016-50	25	50	0.025-1.5
NOR	5	20	0.020-50	ND	ND	ND
HYDROXY	2.5	10	0.010-50	ND	ND	ND
4-CARBOXY	2.5	10	0.010-50	ND	ND	ND
DHNM	5	20	0.020-50	ND	ND	ND

Table4. NOR concentrations (in pg/fingerprint) detected above the LLOQ of 20 pg/fingerprint and LOD of 5 pg/fingerprint (ticks) in fingerprints (F1-F5) collected from M1 and M2; ND – not detected

	M1					M2				
	F1	**F2**	**F3**	**F4**	**F5**	**F1**	**F2**	**F3**	**F4**	**F5**
-10 min	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
10 min	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
20 min	**164**	ND	ND	ND	ND	ND	ND	ND	ND	ND
45 min	**62.2**	ND	ND	ND	ND	ND	ND	ND	ND	ND
90 min	**20.9**	ND	ND	ND	ND	✓	**21.4**	ND	ND	ND
3 h	ND	ND	ND	ND	ND	✓	**21.7**	26.0	ND	ND
5 h	ND	ND	ND	ND	ND	29.6	ND	✓	ND	ND
Day 2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Day 3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

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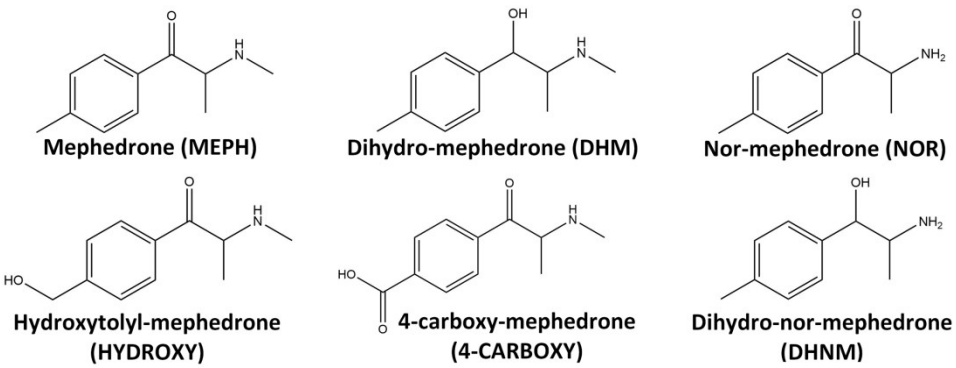

Mephedrone and five of its Phase I metabolites

119x48mm (600 x 600 DPI)

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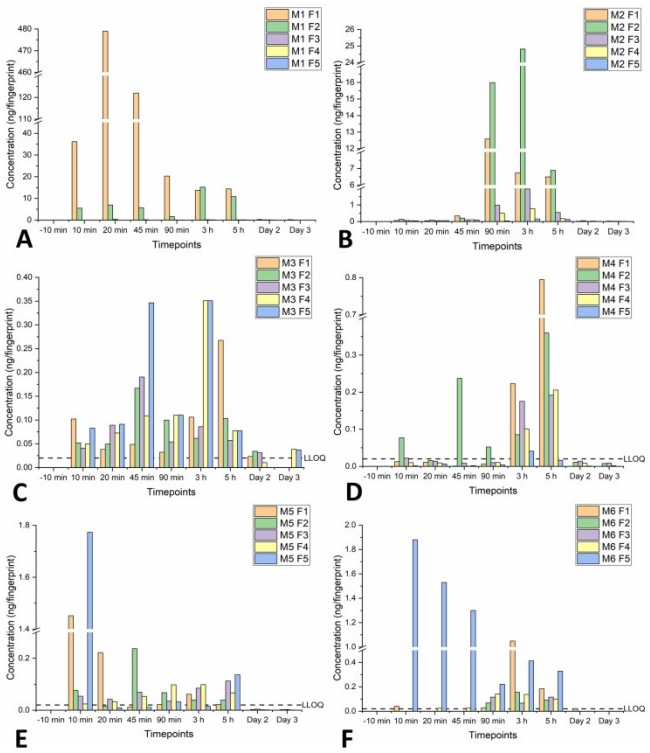

Concentration of mephedrone in A) M1, B) M2, C) M3, D) M4, E) M5, F) M6 in fingerprints (F1-F5) collected onto glass cover slips

254x190mm (300 x 300 DPI)

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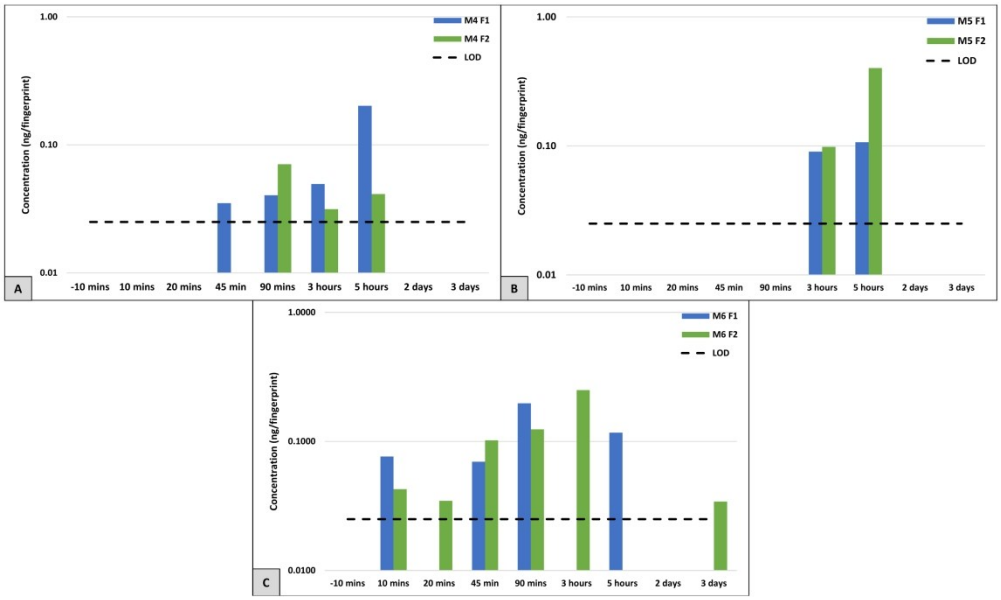
DOI: 10.1039/C9AN02477H



Concentration of mephedrone in A) M4, B) M5, C) M6 in fingerprints collected onto triangular piece of chromatography paper from each fingerprint (F1-F2; note that 10 min and 20 min samples were not collected from M5)

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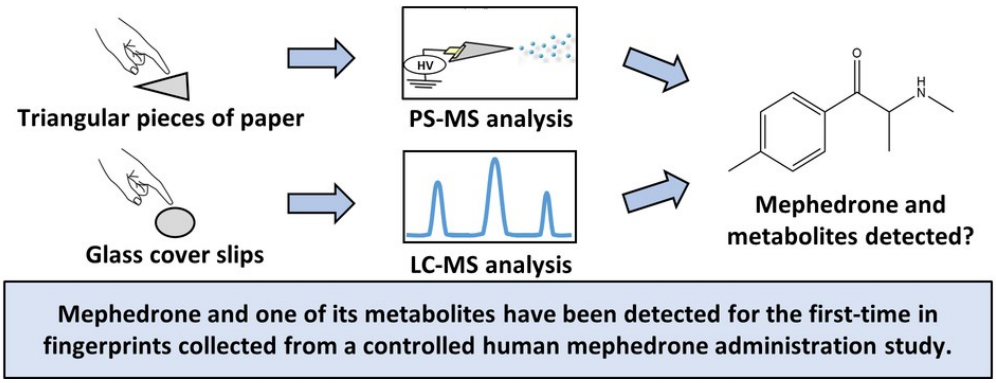
DOI: 10.1039/C9AN02477H



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