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Simultaneous quantification of opiates, amphetamines, cocaine and metabolites and diazepam and metabolite in a single hair sample using GC–MS

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Abstract

A method is described for the simultaneous identification and quantification of opiates, amphetamines, cocainics, diazepam and nordiazepam from one hair extract (typically 10–50 mg hair). After decontamination by washing with shampoo, dichloromethane, isopropanol and acetone, drugs were extracted using 0.1 M HCl followed by SPE clean-up using mixed-mode extraction cartridges. The SPE extracts were submitted to a two-step derivatisation using MBTFA and MSTFA + 1% TCMS and analysis was performed by GC–MS using both SIM and scan modes. Four deuterated standards were used to monitor 14 compounds. The limit of quantification was the total drug detected from the sample. This was 5 ng for amphetamines and 10 ng for remaining drugs which is equivalent to 0.1 and 0.2 ng/mg from a 50 mg sample.

Standard curves for the range 5–400 ng total drug concentration for all drugs had regression coefficients greater than 0.98. An authentic hair sample was used to validate the method and gave R.S.D.s <25% for both inter and intra-day reproducibility.

The results of the analysis of hair taken from four patients attending a drug treatment clinic and six hair samples including head hair, pubic hair, axial hair and beard taken at post-mortem are presented.

Keywords: GC–MS; Hair; Drugs of abuse

1. Introduction

The usual approach to analysis of hair for drugs of abuse is to perform an initial screen using immunoassay and then confirm positives with a more accurate technique such as gas chromatography-mass spectrometry (GC–MS), using a different method for each class of drug of abuse [1]. The methods published outlining simultaneous qualitative analysis of the major drugs of abuse in hair [2,3] and the simultaneous measurement of two or three classes of drugs of abuse [4–6] all use a two-stage procedure for either the extraction or the instrumental analysis. Methods using liquid chromatography–mass spectrometry–mass spectrometry (LC–MS–MS) [3,6] have been developed, but this technique is not routinely available in many laboratories. The following method was developed for GC–MS which is standard equipment in the toxicology laboratory.

The screen/confirmation approach to analysis is efficient when only a small proportion of samples screen positive and confirmation is for just one drug group, for example, in work place drug testing. For the analysis of hair from poly-drug users, including patients in drug treatment programs, where there are several classes of drugs present in each sample, simultaneous screening and quantification of the different classes of drugs of abuse is a more efficient approach. The method described here has been validated for the identification and quantification of the following drug groups: amphetamines including methamphetamine, methylenedioxymethamphetamine (MDMA), methylenedioxyamphetamine (MDA); opiates including morphine, 6-monoacetylmorphine (6MAM), dihydrocodeine (DHC), codeine; cocainics including cocaine, benzoylecgonine (BE), ecgoninemethylester (EME), cocaethylene (CocaEt), the most commonly used/abused benzodiazepine, diazepam and its metabolite nordiazepam (Ndiaz). These compounds were analysed simultaneously from a single extraction which was followed by a single injection onto the GC–MS using selective ion monitoring mode (SIM). This method not only increased

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the efficiency of the analysis but also was useful when sample volume was limited.

A full drug screen for other drugs was possible by re-injecting the same extract onto the GC–MS using full scan mode. In clinical cases this was used for verifying use of co-administered drugs such as antidepressants and for forensic cases it was used to screen for unknowns.

2. Analytical procedure

2.1. Materials

All solvents were of analytical grade and were purchased from VWR (Poole, UK). The deuterated internal standards MDA-d_5, cocaine-d_3, BE-d_3 and 6MAM-d_6 were obtained from LGC Promochem (Teddington, UK). Bakerbond Narc-2 mixed-phase cartridges were from Trichema (Warrington, UK). Derivatising reagent *N*-methyl-bis trifluoroacetamide (MBTFA) was from Sigma–Aldrich (Poole, UK) and *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) + 1% trimethylchlorosilane (TMCS) from Perbio (Cramlington, UK). Ammonium hydroxide, chemicals and all other drug standards were purchased from Sigma–Aldrich (Poole, UK). The 5% phenyl–95% dimethylpolysiloxane capillary column was purchased from Phenomenex (Cheshire, UK).

2.2. Preparation of drug standards

A drugs standard mix was prepared, in methanol, containing amphetamine, methamphetamine, MDMA, MDA, cocaine, BE, EME, CocaEt, codeine, morphine, 6MAM, DHC, diazepam, NDiaz all at a concentration of 2 μg/mL.

Internal standard mix (IS) solution was prepared, in methanol, containing MDA-d_5, cocaine-d_3, BE-d_3 and 6MAM-d_6 all at a concentration of 2 μg/mL.

2.3. Method control material

Authentic hair from post-mortem cases was used as control material. If sufficient sample was not available then laboratory spiked hair (prepared by soaking drug-free hair in drugs solution over several days and excess drug washed off) was used. Where possible the control material contained a compound from each of the drug groups being analysed.

2.4. Sample pre-treatment and extraction of drugs from hair

Hair samples received in the laboratory were noted for length and general appearance. The whole length was stored dry in foil at room temperature until required for extraction.

Hair samples were first segmented and then washed. The number of segments and the length of each are determined by consideration of the case history and amount of hair submitted for analysis. The post-mortem hair samples were washed with shampoo, rinsed thoroughly with water and dried. This was followed by solvent washing with dichloromethane (5 mL), isopropanol (5 mL) and dried with acetone (2 mL). The first two solvent washes were kept for analysis and the acetone was discarded. Clinical hair samples were solvent washed only unless the final solvent wash contained debris or oils. These samples were shampoo washed and the solvent washes repeated. After solvent washing the hair samples were left to dry, then cut into approximately 1 mm clippings using scissors. An aliquot (between 10 and 50 mg) was accurately weighed into a 5 mL screw cap glass tube.

A set of calibrants of spiked drug-free hair (30 mg) was prepared to run with each analysis at concentrations of 0, 5, 10, 50, 200 and 400 ng total drug per tube. Total drug per tube is used as this allows for different weights of hair to be analysed. To each calibrant and sample, IS mix (150 ng) was added. A blank control (without IS) of drug free hair was also analysed.

The extraction of the drugs from the hair matrix was carried out by adding hydrochloric acid (0.1 M, 2 mL) to all tubes. The tubes were heated at 50 °C overnight (approximately 16 h) and then removed from the heating block and allowed to cool. To each tube phosphate buffer (0.1 M, pH 7.0, 2 mL) and potassium hydroxide (1 M, 200 μL) were added which brought the pH to 7.0 ± 0.4.

2.5. Purification/derivatisation procedure

A Bakerbond Narc-2 mixed-mode solid-phase extraction (SPE) cartridge for each tube was conditioned with methanol (2 mL) followed by deionised water (2 mL) followed by phosphate buffer (2 mL, 0.1 M, pH 7.0). On standing the hair settled in the bottom of the tube and the supernatant was applied to the cartridge. When the sample had eluted under gravity the column was washed with deionised water (2 mL) followed by hydrochloric acid (0.1 M, 0.5 mL) followed by methanol (0.5 mL). Vacuum was applied and the cartridges dried for 20–30 min. The analytes were eluted into a single vial with a mix of chloroform:isopropanol (80:20, 2 mL) to elute the acid and neutral drugs followed by chloroform:isopropanol:ammonium hydroxide (80:20:3, 2 mL) to elute the basic drugs. Vacuum was applied for 20–30 s once each solvent had passed through to ensure the column was dry. A dual derivatisation procedure was then used [7]. To the combined extracts, MBTFA (15 μL) was added and then dried down under nitrogen at 80 °C until approximately 1 mL remained. This aliquot was then transferred to labelled microvials and evaporated to dryness under nitrogen at 80 °C. The vial was capped and then MSTFA + 1% TCMS (30 μL) was added and heated to 80 °C for 1 h when further MBTFA (10 μL) was added. After heating for a further 30 min at 80 °C the extract (1 μL) was injected onto the GC–MS using a SIM method. A further 1 μL was also injected onto the GC–MS using full scan.

2.6. Instrumental analysis and data acquisition

A Hewlett-Packard 5973 MSD interfaced with a 6890 series GC fitted with a split-splitless injector and an HP 7683 automatic liquid sampler was used (Agilent, Stockport, UK). The analytical column was a Zebron ZB-5 (30 m × 0.25 mm i.d. × 0.25 μm d.f.) fitted with a retention gap (uncoated, deactivated silica)

Table 1
Retention times (RT) and ions monitored for each analyte

Analyte	RT (min)	Ions monitored (*m/z*)		
Amphetamine-TFA	8.6	140	118*	91
Methamphetamine-TFA	9.9	154*	110	118
EME-TMS	12.1	82*	96	155
MDA-d_5-TFA	12.6	136	166*	167
MDA-TFA	12.6	135	162*	275
MDA-d_5-TFA/TMS	13.0	216*	136	352
MDA-TFA/TMS	13.0	212*	135	347
MDMA-TFA	13.9	154*	135	162
Cocaine-d_3	18.8	85	185*	306
Cocaine	18.8	82	182*	303
CocaEt	19.2	82	196*	317
BE-d_3-TMS	19.3	85	243*	364
BE-TMS	19.3	82	240*	361
Ndiaz-TMS	19.4	341*	327	342
DHC-TMS	20.3	373*	236	315
Diazepam	20.9	283*	284	256
Codeine-TMS	20.9	371*	178	234
Morphine-2TMS	21.3	429*	401	220
6MAM-d_6-TMS	21.8	405*	343	290
6MAM-TMS	21.8	399*	340	287

TFA, trifluoroacetamide derivative; TMS, trimethylsilyl derivative; (*) quantification ion.

(1 m × 0.25 mm i.d.). Temperature conditions were as follows: initial temperature of 80 °C for 1 min, increased to 300 °C at 10 °C/min, held for 6 min giving a total run time of 29 min. The flow of the carrier gas (helium) was maintained at 1.0 mL/min in constant flow mode. The injector port was set at 280 °C. The GC–MS was programmed to perform a 1 μL splitless injection. The samples were analysed using a selected ion monitoring programme (SIM) to monitor the target analytes listed in Table 1. Full scan parameters monitored 55–550 amu. The software used for data acquisition and manipulation was HP Enhanced Chemstation G1701BA Version B.01.00.

2.7. Analysis of decontamination washes

Each solvent wash was left to evaporate at room temperature, the residue was reconstituted in 100 μL methanol and transferred to a microvial. The methanol was left to evaporate at room temperature and the tube was capped when completely dry. This residue was derivatised with MBTFA reagent (10 μL) containing MDA-d_5 as IS (50 ng) and MSTFA + 1% TCMS reagent (30 μL). The extract was injected onto the GC–MS using the SIM program.

2.8. Data analysis

The SIM data were analysed first. In order to report a positive drug result the peak had to match the calibrator ion ratios (±20% of expected value) and RT (±0.15 min) [8]. The limit of detection (LOD) was set to a signal-to-noise ratio equal to or greater than 3 (S/N ≥ 3) and for the limit of quantification (LOQ) the signal-to-noise ratio was equal to or greater than ten (S/N ≥ 10). The LOD was typically lower than the 5 ng calibrator and the LOQ was typically 5 ng for amphetamines and 10 ng for the remaining drugs. Results below the LOQ but above the LOD were reported as positive, less than 5 ng total drug for amphetamines and positive, less than 10 ng total drug for the remaining analytes.

Using cocaine-d_3 as IS, calibrator peaks were integrated to construct $y = mx + c$ curves which were used to calculate total drug content in controls and samples. The rationalization for using cocaine-d_3 is given later. The final result was expressed in ng/mg hair.

The full scan data were analysed after processing the SIM data. The TIC was systematically searched for compounds other than those specifically targeted for quantification. For a full scan spectrum match there had to be at least an 80% match with the ions in the standard spectrum. The drugs were identified by matching full scan spectra using an in-house library (containing underivatised and derivatised compounds), the Pfleger Maurer Webber and the Wiley 275 libraries. All drugs reported were confirmed by matching retention time (±0.15 min) and full scan spectra with a pure drug standard run on the same GC–MS using the same derivitisation procedure.

2.9. Application of the method

Hair samples taken from four patients attending a drug treatment clinic and six post-mortem hair samples were analysed to illustrate the method. The types of hair submitted for analysis in the post-mortem cases included head hair, pubic hair, axial hair and beard. The relevant Research Ethics Committee approved the studies.

2.10. Validation procedure

To demonstrate linearity the regression coefficient was calculated using each IS for all the drugs analysed; the spiked calibrants were analysed in duplicate on five separate occasions at least 3 days apart. For validation two additional spiked calibrants (20 and 100 ng) were added to the routine calibration standards and were included in the calibration lines.

The percentage recovery of the drugs by SPE was determined by comparing the analysis of extracted and non-extracted spiked samples at 100 ng. Recovery data for the extraction of drugs from authentic hair samples cannot be determined by experimentation as there is no way of incorporating a known amount of drug into the hair shaft. However, previous studies analysing the same sample over a series of hours have shown that overnight incubation with various hydrolysis reagents, including dilute HCl, produced efficient extraction of drugs [9].

Reproducibility was examined by analysing a positive authentic sample of hair containing the drugs of interest. The inter-day reproducibility was determined by duplicate analysis on three separate occasions at least 3 days apart and the intra-day precision by analysis of six aliquots of sample. A full set of calibration standards and a blank standard were run with each analysis.

To assess the internal standard recovery, the mean abundance (peak area) of each extracted internal standard in the calibrants was calculated. The abundance of internal standard in a real hair sample was calculated and expressed as a percentage of the

Table 2
Mean regression coefficient (R^2) for standard curves (5–400 ng) for each drug using each internal standard ($n = 5$)

Analyte	Cocaine-d_3	BE-d_3-TMS	6MAM-d_6-TMS	MDA-d_5-TFA	MDA-d_5-TFA (−outliers)	MDA-d_5-TMS/TFA	MDA-d_5-TMS/TFA (−outliers)
Amphetamine-TFA	0.9860	0.9877	0.9807	0.8582	0.9680	0.6969	0.9250
Methamphetamine-TFA	0.9880	0.9857	0.9737	0.9836	0.9684	0.8742	0.9830
MDMA-TFA	0.9924	0.9892	0.9810	0.8931	0.9613	0.8619	0.9767
MDA-TFA	0.9818	0.9814	0.9608	0.9606	0.9699	0.6591	0.9528
MDA-TFA/TMS	0.9807	0.9814	0.9597	0.7750	0.9216	0.9992	0.9992
Cocaine	0.9992	0.9964	0.9864	0.8552	0.9610	0.7938	0.9659
EME-TMS	0.9820	0.9867	0.9697	0.8716	0.9650	0.8612	0.9745
BE-TMS	0.9922	0.9958	0.9870	0.8451	0.9577	0.7749	0.9681
CocaEt	0.9991	0.9968	0.9878	0.8581	0.9525	0.7989	0.9694
DHC-TMS	0.9900	0.9722	0.9913	0.8374	0.9628	0.8611	0.9784
Morphine-2TMS	0.9891	0.9768	0.9902	0.8241	0.9557	0.8427	0.9656
6MAM-TMS	0.9884	0.9738	0.9985	0.7931	0.9324	0.7905	0.9555
Codeine-TMS	0.9941	0.9848	0.9936	0.8097	0.9557	0.8288	0.9720
Ndiaz-TMS	0.9908	0.9877	0.9741	0.7198	0.9530	0.7241	0.9411
Diazepam	0.9973	0.9940	0.9809	0.8013	0.9601	0.7360	0.9661

(−Outliers): Calibration lies calculates leaving out outliers (up to 3 per line).

calibrant mean. The data were obtained from 4 sets of analyses consisting of 14 spiked calibrants and up to 18 real hair samples in each set.

2.11. Validation results

The mean regression coefficient for standard curves (5–400 ng) for each drug using each IS are shown in Table 2. There was good linearity for all drugs using cocaine-d_3, BE-d_3 and 6MAM-d_6 as internal standards. The acceptable value for regression coefficient (R^2) was set to $\geq$0.98 for quantification. As R^2 for the calibration lines calculated using MDA-d_5 were $\leq$0.90 they could not be used for quantification. The calibration lines using MDA-d_5 produced outliers, as many as three in a single calibration line. The figures shown in Table 2 show R^2 for the calibration lines calculated both including and excluding the outliers.

The calculated mean concentration with R.S.D.s (%) for 20 and 200 ng calibrants (duplicates run on five separate occasions giving $n = 10$ in total) using cocaine-d_3 as IS for each drug, SPE recoveries (%) and typical LOQ (ng) are shown in Table 3.

The intra- and inter-day concentrations of drugs detected in the control hair were calculated using each IS. The mean drug concentration ± S.D. calculated for duplicates run on three separate occasions are shown in Table 4a and the mean drug concentration ± S.D. calculated for six replicate analyses are shown in Table 4b. MDA-d_5 results have been calculated with outliers removed from the calibration lines.

The percentage recovery of internal standard in extracted samples in comparison to spiked calibrants is shown in Table 5. As cocaine-d_3 resulted in acceptable recoveries and reproducibility it was used to quantify the drug concentrations in the samples analysed.

Table 3
Calculated mean concentration and R.S.D. (%) for 20 and 200 ng calibrants, SPE recovery (%) and LOQ

Drug	20 ng calibrant ($n = 10$)		200 ng calibrant ($n = 10$)		SPE recovery (%) ($n = 10$)	LOQ (ng)
	Mean concentration	R.S.D. (%)	Mean concentration	R.S.D. (%)		
Amphetamine-TFA	19.3	18.3	209.8	11.2	102	5
Methamphetamine-TFA	18.9	13.1	205.3	8.6	85	5
MDMA-TFA	18.7	15.3	204.8	6.7	84	5
MDA-TFA	20.0	14.8	200.1	14.9	116	5
MDA-TFA/TMS	19.9	17.2	199.4	16.7	92	5
Cocaine	19.6	3.4	204.7	5.5	106	10
EME-TMS	19.0	9.6	214.5	9.6	99	10
BE-TMS	18.7	4.8	210.1	9.9	158	10
CocaEt	20.4	6.1	205.6	5.7	107	10
DHC-TMS	19.8	9.3	191.9	10.3	73	10
Morphine-2TMS	20.4	9.0	197.4	14.3	87	10
6MAM-TMS	21.5	11.4	183.8	9.6	80	10
Codeine-TMS	20.3	11.5	191.8	8.6	91	10
Diazepam	21.4	9.8	199.6	7.4	129	10
Ndiaz-TMS	22.2	10.5	182.7	13.3	156	10

Table 4a
Inter-day reproducibility

IS	Mean drug concentration (ng/mg) ± S.D. (n = 6)								
	MDMA	Cocaine	BE	CocaEt	Morphine	6MAM	Codeine	NDiazepam	Diazepam
Cocaine-d_3	1.5 ± 0.3	20.4 ± 2.7	7.3 ± 1.4	3.5 ± 0.3	10.7 ± 3.1	28.6 ± 13.2	1.9 ± 0.3	0.8 ± 0.2	1.1 ± 0.2
BE-d_3	1.8 ± 0.9	28.8 ± 5.1	9.1 ± 1.5	4.9 ± 1.2	15.5 ± 4.8	44.9 ± 5.7	2.6 ± 0.9	0.9 ± 0.2	1.4 ± 0.5
6MAM-d_6	3.0 ± 1.0	47.8 ± 14.4	14.5 ± 0.5	7.4 ± 1.8	23.9 ± 3.2	71.2 ± 4.2	3.8 ± 0.9	0.7 ± 0.1	1.4 ± 0.5
MDA-d_5-TFA	1.1 ± 0.6	15.4 ± 8.4	5.1 ± 2.2	2.7 ± 1.0	9.0 ± 3.0	32.0 ± 3.7	1.4 ± 0.5	1.0 ± 0.7	1.2 ± 0.5
MDA-d_5-TFA/TMS	1.6 ± 0.5	22.2 ± 11.0	7.6 ± 3.9	3.8 ± 1.6	12.2 ± 8.0	36.2 ± 28.5	2.2 ± 0.9	0.5 ± 0.4	1.3 ± 0.7

Drug concentration ± S.D. in control hair calculated using each IS (duplicates run on three separate occasions giving n = 6 in total).

Table 4b
Intra-day reproducibility

IS	Mean drug concentration (ng/mg) ± S.D. (n = 6)								
	MDMA	Cocaine	BE	CocaEt	Morphine	6MAM	Codeine	NDiazepam	Diazepam
Cocaine-d_3	1.4 ± 0.3	24.6 ± 0.3	9.7 ± 1.2	4.1 ± 1.2	15.6 ± 2.6	30.6 ± 6.1	2.3 ± 0.4	0.8 ± 0.1	1.0 ± 0.1
BE-d_3	1.5 ± 0.4	27.2 ± 2.5	10.7 ± 1.2	4.4 ± 0.5	16.9 ± 3.5	39.8 ± 7.0	2.4 ± 0.5	1.0 ± 0.1	1.2 ± 0.2
6MAM-d_6	2.8 ± 0.3	53.3 ± 7.6	21.0 ± 5.3	8.5 ± 1.3	32.8 ± 1.6	76.9 ± 3.8	4.1 ± 0.3	1.0 ± 0.2	2.2 ± 0.8
MDA-d_5-TFA	0.9 + 0.4	7.0 ± 2.3	3.7 ± 0.9	2.4 ± 0.4	5.6 ± 2.3	10.0 ± 4.3	2.0 ± 0.2	1.9 ± 0.2	1.8 ± 0.2
MDA-d_5-TFA/TMS	1.4 ± 0.2	24.0 ± 2.8	9.5 ± 2.0	3.8 ± 0.5	14.6 ± 1.6	35.3 ± 4.1	2.0 ± 0.3	0.7 ± 0.1	0.9 ± 0.3

Drug concentration ± S.D. in control hair calculated using each IS (n = 6 in total).

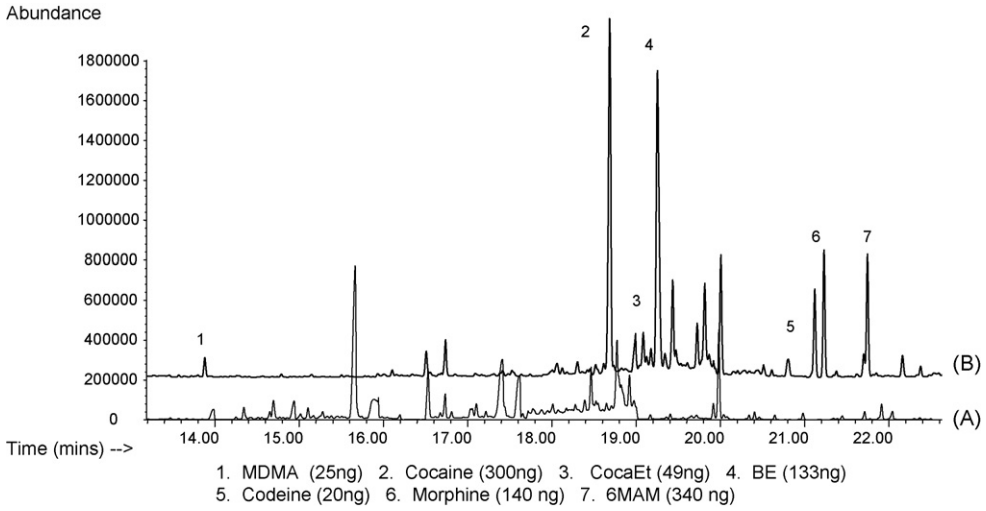

Fig. 1. Total ion chromatogram of: (A) blank hair control and (B) authentic hair sample (case 1) acquired in SIM mode.

3. Results

Fig. 1 shows a total ion chromatogram from (a) a blank control and (b) an authentic hair sample (case 1) acquired using SIM. Fig. 2 shows (a) an extracted ion chromatogram showing papaverine in authentic hair sample (case 1) acquired in scan mode and (b) a full scan spectrum of the papaverine peak.

The results from the analysis of hair samples, all 3 cm in length and weighing from 16 to 31 mg, from four volunteers attending a drug treatment clinic are shown in Table 6; no drugs were detected in the decontamination washes.

Table 5
Recovery (%) of IS in extracted samples in comparison to spiked calibrants

	Cocaine-d_3	BE-d_3	6MAM-d_6	MDA-d_5-TFA	MDA-d_5-TFA/TMS
Run 1	93	82	52	36	42
Run 2	91	68	40	101	75
Run 3	57	51	34	114	65
Run 4	81	74	51	84	90

Volunteer 1 had been on oral methadone maintenance (50 mg) for 4 years and declared crack cocaine and heroin use within the last 3 months. Cocaine, BE, morphine, 6MAM, codeine and methadone were detected in hair. The amount of codeine found indicates codeine use in addition to other prescribed/declared drug use.

Volunteer 2 had been on injectable diamorphine maintenance (210 mg/day) for 3 years. Morphine and 6MAM only were

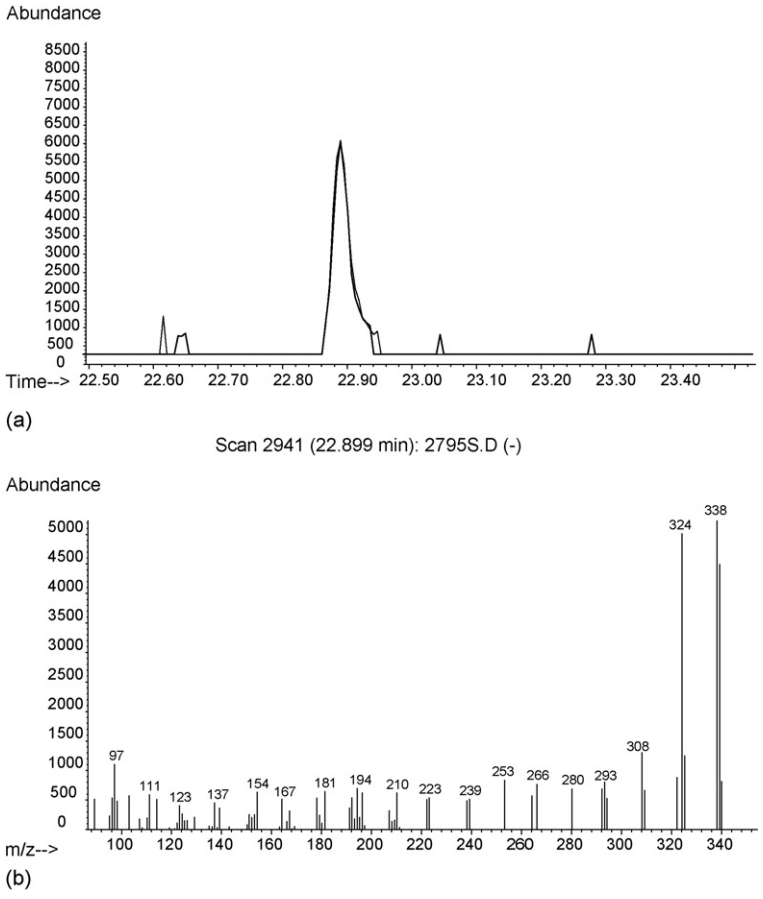

Fig. 2. (a) Extracted ion chromatogram showing papaverine in authentic hair (case 1) acquired in scan mode. (b) Full scan spectrum of the papaverine peak.

found in hair which is consistent with the prescribed medication.

Volunteer 3 had been on oral methadone maintenance (230 mg) for 8 months and was receiving 45 mg mirtazapine daily and declared codeine use. Cocaine, methadone, codeine and mirtazapine were detected in hair showing use of cocaine in addition to the prescribed/declared medication.

Volunteer 4 had been on oral methadone maintenance (55 mg) and was receiving 30 mg of mirtazapine and 2 mg risperidone daily and declared just one hit with heroin within the last 3 months. In hair, methadone, 1,5-dimethyl-3,3-diphenylpyrrolidine, a methadone metabolite (DDP) and mirtazepine were detected which is consistent with prescribed medication but cocaine, BE, morphine and 6MAM were also detected showing use of heroin (more than one hit) and cocaine. Risperidone cannot be detected using GC–MS.

The results from the analysis of hair collected at post-mortem for six cases are shown in Table 7; no drugs were detected in the decontamination washes.

Case 1 was a 41-year-old male found deceased at home address, he was a known drug user who was being prescribed citalopram for depression. The results show that in addition to taking his prescribed citalopram, cocaine, cocaine with ethanol, DHC, heroin, diazepam and methadone were used throughout the 6 months prior to death. The use of MDMA appears to have stopped 2 months prior to death.

Case 2 was a 46-year-old male found unresponsive, was a known heroin user who had recently been discharged from

Table 6
Drugs detected in hair from four volunteers attending a drug treatment centre

	Using SIM (concentration ng/mg hair)					Using scan (ID only)
	Cocaine	BE	Morphine	6MAM	Codeine	
Case 1	5.4	3.5	6.1	13.0	5.6	Methadone
Case 2	–	–	4.7	12.0	–	None
Case 3	1.8	5.4	–	–	4.2	Methadone, mirtazapine
Case 4	1.3	1.8	1.3	3.5	–	Methadone, DDP, mirtazapine

DDP = 1,5-dimethyl-3,3-diphenylpyrrolidine (methadone metabolite).

Table 7
Drugs detected in hair samples taken at post-mortem

Case 1	Using SIM (concentration ng/mg hair)											Using scan (ID only)
	MDMA	Cocaine	BE	EME	CocaET	DHC	Morphine	6MAM	Codeine	Diazepam	Ndiaz	
Section 1	–	5.0	1.8	0.2	2.3	<10*	6.4	11.9	1.9	0.8	<10*	Citalopram, methadone, papaverine
Section 2	2.3	14.7	4.2	0.3	4.6	0.2	16.8	13.8	3.2	0.8	<10*	Citalopram, methadone, papaverine
Section 3	2.5	24.5	5.0	0.3	5.3	0.3	30.1	71.1	4.5	0.7	<10*	Citalopram, methadone, papaverine

Case 2	Using SIM (concentration ng/mg hair)				Using scan (ID only)
	Cocaine	BE	Morphine	6MAM	
Section 1	4.9	3.0	1.4	1.0	Quetiapine, venlafaxine, sertraline
Section 2	6.0	3.3	1.6	1.6	Quetiapine, venlafaxine, sertraline
Section 3	19.4	0.2	2.0	3.2	Quetiapine, venlafaxine, sertraline

Case 3	Using SIM (concentration ng/mg hair)				Using scan (ID only)
	Cocaine	BE	EME	Morphine	
Section 1	1.4	<10*	<10*	2.9	None

Case 4	Using SIM (concentration ng/mg hair)					Using scan (ID only)
	Cocaine	BE	Morphine	6MAM	Codeine	
Section 1	0.6	0.4	2.9	3.0	1.2	Methadone

Case 5	Using SIM (concentration ng/mg hair)							Using scan (ID only)
	MDMA	MDA	Cocaine	BE	EME	CocaEt	Morphine	
Section 1	18.0	0.2	2.9	0.7	0.2	0.21	0.26	None

Case 6	Using SIM (concentration ng/mg hair)						Using scan (ID only)
	MDMA	Cocaine	BE	CocaEt	Morphine	6MAM	
Section 1	0.3	0.6	0.4	0.3	0.2	0.1	None

10* = positive, less than 10 ng total. (Case 1) Head hair, 3 cm × 2 cm sections, each section weighed approximately 50 mg. (Case 2) Head hair, 3 cm × 1 cm section, weighed approximately 50 mg. (Case 3) Head hair, 1 cm × 4 cm section, weighed 8.3 mg (insufficient for segmentation). (Case 4) Pubic hair, 1 cm × 6 cm section, weighed 37.2 mg (insufficient for segmentation). (Case 5) Beard, 1 cm × 4 cm section, weighed 55.3 mg (insufficient for segmentation). (Case 6) Axial hair, 1 cm × 5 cm section, weighed 53.8 mg (insufficient for segmentation).

a psychiatric hospital after an admission for depression. The results show the use of cocaine and heroin in addition to quetiapine, venlafaxine and sertraline during the 3 months prior to death.

Case 3 was a 37-year-old male on a visit to London who was found deceased in his hotel room, there was a suspicion of drug use. The results show previous use of cocaine and a morphine containing preparation.

Case 4 was a 41-year-old male found collapsed in the bathroom. He was an ex-heroin user who was currently on a methadone treatment program. Pubic hair was submitted for analysis. The results show previous use of cocaine and heroin and possibly codeine in addition to methadone.

Case 5 was a 25-year-old male who produced a bag of heroin and said he wanted to try it. His girlfriend assisted with the injection, it appears he did not get a "buzz" so proceeded to inject himself again. Beard hair was submitted for analysis. The results show previous use of MDMA, cocaine, cocaine with ethanol and a morphine containing preparation.

Case 6 was a 49-year-old male found deceased at home address. Known to abuse ethanol and had a recent admission to the hospital emergency department for chest pains. Axial hair was submitted for analysis. The results show previous use of MDMA, cocaine, cocaine with ethanol and heroin.

4. Discussion

Initially the relevant IS was to be used to calculate drug concentrations, i.e. MDA-d_5 for amphetamines (TFA forming compounds), cocaine-d_3 for cocaine, cocaethylene and diazepam (non-derivatised compounds), BE-d_3 for BE and EME and 6MAM-d_6 for opiates (TMS forming compounds). In order to maximise the sensitivity of the assay using one deuterated analogue per group of drugs rather than one for each individual drug allowed the number of ions monitored per time window to be kept to a minimum.

Using MDA-d_5 as the internal standard did not produce acceptable regression coefficients or reproducible results for the amphetamine group; the proportion of TFA to TFA/TMS derivative was not consistent.

During the analysis 6MAM-d_6 broke down to morphine-d_3; the presence of morphine-d_3-2TMS was confirmed by running

extracts in full scan mode. As this breakdown was consistent in the spiked calibrants, linear calibration lines were produced. However, the recovery of 6MAM-d_6 from the real hair samples was approximately 50% or less than from the spiked calibrants. This is shown in Table 5. The recovery of 6MAM-d_6 from real hair samples was lower than the recovery of the other internal standards. The effect of this low recovery of 6MAM-d_6 can be seen by comparing the calculated concentrations of drugs in samples using cocaine-d_3 or BE-d_3 with those using 6MAM-d_6; 6MAM-d_6 values were consistently higher for all drugs. This is shown in Table 4. The breakdown of 6MAM-d_6 to morphine-d_3 and its reduced recovery from real samples highlights that care should to be taken when deuterated analogues are used as IS.

Although the drug concentrations were not in the picogram range for this analysis, the presence of 236 ion from morphine-d_3-2TMS caused interference and distortion of the 236 ion in morphine-2TMS. For this reason for morphine-2TMS an alternative ion, *m/z* 401, rather than *m/z* 236, was monitored for identification purposes. The volume of sample analysed has been noted to have an effect on drug recovery when using other techniques such as solid-phase microextraction (SPME), as the volume of dissolved hair increased so the recovery of some drug decreased [10]. This was thought to be due to the increased amount of organic material released from the hair matrix. Here the relatively low recovery of 6MAM-d_6 from authentic samples was observed in samples ranging from 10 to 55 mg and therefore the IS recovery did not appear to be related to the amount of organic material released from the hair matrix.

The Society of Forensic Toxicologist /American Academy of Forensic Sciences laboratory guidelines state that the minimal acceptable correlation coefficient for a multi-point calibration is a value of at least 0.98. Cocaine-d_3 fulfilled this criteria and it gave acceptable R.S.D.s (<25%) for intra- and inter-day reproducibility for authentic hair samples demonstrating that it was a valid IS to use for quantification for all drugs. The four deuterated internal standards were used to monitor the extraction and derivatisation of the relevant drugs.

The advantage of using acid extraction followed by SPE was more apparent in cases which yielded drug concentrations at the LOD. In the method published previously [2] methanol was used for extraction with no clean-up stage. The chromatograms produced had significant base line noise which could cause difficulty with identification of drugs when they were present in very low concentrations. This is in agreement with the findings of Cirimele et al. [11]. Acid hydrolysis is a straightforward and established extraction for all drugs [12] and was chosen over alkaline hydrolysis as the latter is reported to cause cocaine and heroin degradation [11,13]. Including the solid-phase extraction clean-up stage produced much cleaner chromatograms, which allowed for increased ease of identification and increased sensitivity.

Post-mortem hair samples were segmented in order to produce at least 30 mg of sample for analysis. Using head hair, which grows on average 1 cm per month, the time of drug ingestion, can be deduced [14], but this is not possible with hair from other sites as the growth rates are different [15] and are more likely to have incorporated drugs from sweat, sebum or urine. Pubic hair was not segmented as the growth site is prone to elevated drug content from urine and sweat contamination.

Hair from alternative sites can be used to confirm previous drug use.

In general post-mortem hair was heavily contaminated with oils and debris and required multiple solvent washes. It was noted that when multiple solvent washes were used drugs could be detected in the later washes. This may have been because the solvent was extracting the drugs from the matrix itself. In order to avoid this it was decided to carry out a shampoo wash. Shampoo may wash off slight surface contamination, but in cases with significant surface contamination the solvent washes have contained drugs and elevated concentrations of parent drug were found on extraction of these samples. In cases involving cocaine body packers external contamination has been detected in the solvent washes after three shampoo washes. The most commonly investigated contamination effect is from cocaine. Studies have concluded that examining the parent-to-metabolite ratio proposed by the SOHT (BE/cocaine ≥ 0.05) [16] and/or concentrations of cocaethylene (at $\geq$0.05 ng/mg hair) [17] can exclude contamination. In cases where cocaine is reported near or at the LOD and metabolites are not detected, a statement considering external contamination would be reported.

5. Conclusions

Presented here is a method for the simultaneous quantification of opiates, amphetamines, the cocaine group and diazepam plus its metabolite from one 10–50 mg sample of hair. By injecting a further aliquot from the same extract on to the GC–MS using full scan, the same extract can be also be screened for unknowns. This allows maximum information from a single sample of hair, which is vital when the amount of sample submitted is limited.

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