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Simultaneous quantitation of 14 drugs of abuse from a single hair sample, using GC-MS detection

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Introduction

•Hair analysis is being increasingly requested for clinical and post mortem analysis. As the growth rate in hair is fairly uniform (~ 1 cm/month) and once incorporated drugs are detectable for long periods a greater window of detection than for urine or blood may be found.

•Drugs may be incorporated into the hair through three mechanisms:

- •drugs present in the blood stream are incorporated into the hair in the follicle
- •transfer may occur from sweat into the hair
- •environmental contamination may occur through exposure to smoke or powdered drugs

•Useful in post mortem cases where longer term drug history is unknown

•Usual screening method is radio immunoassay (RIA), followed by GC-MS confirmation. This increases costs due to multiple tests

•A single test detailed here has been devised for simultaneous extraction of a variety of drugs of abuse and metabolites in hair, useful were sample size is limited

•Metabolites analysed to show ingestion of drug (and hence metabolism), presence not due to environmental contamination

•Method validated for the analysis of amphetamines, opiates, cocaine (and metabolites) and some benzodiazepines.

Method

A hair sample of approximately 1 cm in diameter taken from the rear of the head was requested for analysis, then segmented into the required lengths (i.e. 1 cm/month). The method was essentially as follows:

•Hair washed (shampoo and solvent) then cut into small sections (~1 mm)

- •Washing should remove external contamination

•Calibration line prepared using a standard solution for all drugs (0, 5, 10, 50, 100, 200 ng/sample)

•Internal standard added (D_3-cocaine, D_5-MDA, D_3-BE)

•HCl added (0.1 M, 2 mL), left overnight @ 50 °C

•Neutralised with phosphate buffer (pH 7.2, 2 mL) and NaOH (1 M, 200 µL)

•Extracted using mixed mode SPE columns (narc-2, Baker bond)

•Eluted with:

- •Choloroform : isopropanol (80:20, 2mL) then
- •Choloroform : isopropanol: ammonium hydroxide (80:20:3, 2mL)

•Derivatised with MSTFA:TCMS (99:1, 30 µL) then MBTFA (10 µL)

•Injected onto GC-MS (1 µL) and analysed in SIM and scan modes

(modified from *(1)*)

TABLE 1 – Validation Data

Drug	R^2 (typical)	QC A (ng/mg)	QC B (ng/mg)	Intraday %CV N=6		Interday %CV N/8	
				QC A	QC B	QC A	QC B
Cocaine	0.996	6.36	3.16	2.5	2.0	3.8	7.0
BE	0.993	16.33	3.93	1.6	3.9	6.7	21.5
EME	0.999	0.66	3.12	5.1	7.9	18.1	6.7
Cocaethylene	0.999	6.33	3.40	3.5	6.0	5.5	7.0
DHC	0.991	1.59	-	21.5	-	21.5	-
Codeine	0.992	-	1.83	-	21.14	-	18.28
Morphine	0.997	4.57	-	22.1	-	19.6	-
6-MAM	0.998	1.84	-	11.8	-	17.8	-
Amphetamine	0.996	-	2.61	-	19.57	-	17.0
Methamphetamine	0.994	8.55	2.62	13.7	13.2	11.8	15.7
MDMA	0.990	30.54	2.71	15.5	13.5	12.6	12.2
MDA (TFA)	0.996	20.88	2.55	6.8	12.7	11.0	11.0
Diazepam	0.996	130.88	2.65	12.7	17.6	38.7	24.0
Desmethyl diazepam	0.991	-	0.91	-	5.0	-	9.2

Validation

•To validate the assay two quality control samples were used

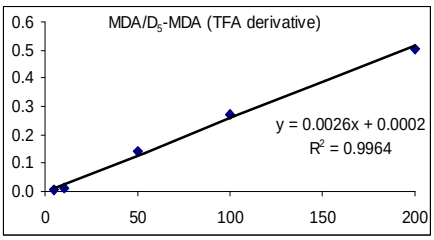
•QCA was a hair soaked in standard solution, dried and washed. QC B was a blank hair spiked with a known amount of standard solution

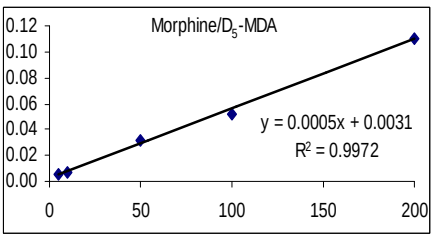
•Limit of quantification (LOQ) was the lowest calibrant point. Limit of detection was set as a signal to noise ratio of greater than 3:1.

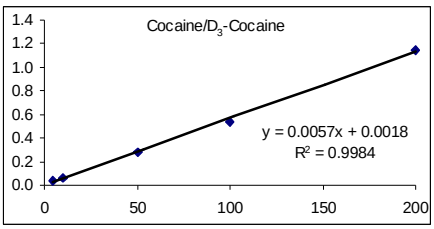
•The LOQ was therefore 5 ng/sample, which is 0.01 ng/ mg hair for an ideal sample of 50 mg.

•Validation data can be seen in Table 1.

Example Calibration Lines







Summary

- •Simultaneous analysis and quantitation of several classes of compounds achievable

•Validation results within acceptable criteria (linearity, %CV)

•Good limits of detection for all compounds

•Also picks up (in scan) nicotine, methadone, anti-depressants, anti-pyschotics, ketamine…

•Method will save time & money compared to by RIA with GC-MS confirmation

•Single assay for multiple drugs, useful when limited sample is available

•Possible uses:

- •Post mortem analysis of hair alongside other available samples may be routinely requested, or even where no other suitable sample is available
- •Clinical applications, drug used per month may be analysed for compliance with regimen
- •Detection of illicit drug use during pregnancy or during other treatment

•Dose response relationship not seen in methadone users *(2)* but possibly further study will show relationships for other drugs.

References

1. Paterson *et al*, J Anal Toxicol 25 (3):203-208
2. Paterson *et al*, J Anal Toxicol 27 (1):20-23