A method for the confirmation and identification of drugs of misuse in urine using solid phase extraction and gas–liquid chromatography with mass spectrometry

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Abstract

A method is described for the confirmation/identification of a range of commonly misused drugs in urine samples. The method has been used for two years without problems for a range of purposes including hospital/clinic drugs of misuse screening and for toxicology in coroner's cases. Urine samples which have given a positive result on immunochemical screening for a particular drug group or groups (for example, opiates) can be processed with identification of the drugs present using a single procedure. Bond ElutCertify columns are used for the extraction of drugs from the samples followed by propionylation and gas chromatography with mass selective detection.
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Keywords: drugs of misuse; gas chromatography/mass spectrometry, urine

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The misuse of drugs has continued to grow since the 1960s. This has had significant implications for the workload of clinical laboratories: over the last five years our laboratory workload for drug screening of urine samples has increased by an average of 49% each year. Commercial automated or semiautomated immunoassay systems for screening urine samples for commonly misused drugs are available and have enabled many laboratories, including our own, to cope with this increase in work. However, these systems yield putative results only, the definitive identification of the drugs or other compounds in the sample causing the putative positive result requires additional confirmatory procedures. Also, in many cases the clinical significance of a positive result for a particular group of drugs can only be ascertained if the drugs present in the sample can be identified. For example, a positive opiate result by immunoassay may be caused by the misuse of diamorphine (heroin), the innocent use of pholcodine (an antitussive), or even the ingestion of food containing poppy seeds.[1] An additional complicating factor in the case of heroin is its short plasma half life (about five minutes) and general lack of stability.[2 3] The confirmed presence of morphine in a sample cannot be necessarily attributed to heroin use, as morphine is also a metabolite of codeine. Heroin is rapidly metabolised to 6-monoacetylmorphine (6-MAM), which has a somewhat longer half life (about 45 minutes) and provides a reliable if short lived marker for recent heroin use when it is present in urine. Thus any confirmatory method for opiates should be able to detect the presence of 6-MAM.

Similarly, a positive amphetamine class result could result from the presence of amphetamine, or methylenedioxymethamphetamine (MDMA—often described along with other drugs as "Ecstasy"); or it could reflect the use of ephedrine or pseudoephedrine.

Numerous approaches to the problem of confirming and identifying drugs and other compounds present in urine sample that yield positive screening tests for misused drugs have been described. Some investigators use liquid chromatographic techniques[4] but most have used gas–liquid chromatography with mass spectrometry (GC/MS).[5 6] In preparing samples for gas chromatography the method of choice seems to have been derivatisation to trimethylsilyl derivatives,[5] though alternative approaches have been described.[6 7]

The published methods generally address the identification and confirmation of a particular group of misused drugs, though we have found an occasional exception,[8] or they require multiple analyses.[4] We present here a single system that facilitates the identification and unequivocal confirmation of many of the com-

Table 1 Retention data and major ions for the propionylated derivatives of commonly misused drugs and drug metabolites*

Name	*Retention time (min)*	*Relative retention time*	*Major ions (m/z)*
Amphetamines	6.659	0.563	44, 100, 118
Methamphetamine	7.100	0.600	58, 114, 91
Methylecgonine	7.361	0.622	82, 182, 96
MDA	9.267	0.783	162, 44, 135
Ephedrine	9.312	0.787	58, 114
Pseudoephedrine	9.353	0.790	58, 114
MDMA	9.651	0.816	58, 162, 114
MDEA	9.885	0.835	72, 162, 128
Cyclizine *	10.030	0.848	99, 56, 194
Methadone met*	10.148	0.858	277, 276, 262
Methadone *	10.887	0.920	72, 73, 91
Cocaine *	11.122	0.940	82, 182, 96
SKF 525a *	11.832	1.000	86
Dihydrocodeine	13.361	1.129	357, 300, 284
Codeine	13.720	1.159	355, 282, 229
6-MAM	14.391	1.216	327, 383, 268
Morphine	14.905	1.259	341, 397, 268
Pholcodine	18.629	1.574	114, 100

Compounds marked * are not derivatised using this system. The major ions in the mass spectra of the underivatised compounds are shown.

MDA, 3,4-methylenedioxyamphetamine; MDMA, 3,4-methylenedioxy-N-methylamphetamine; MDEA, 3,4-methylenedioxy-N-ethylamphetamine; met, metabolite; 6-MAM, 6-monoacetylmorphine.

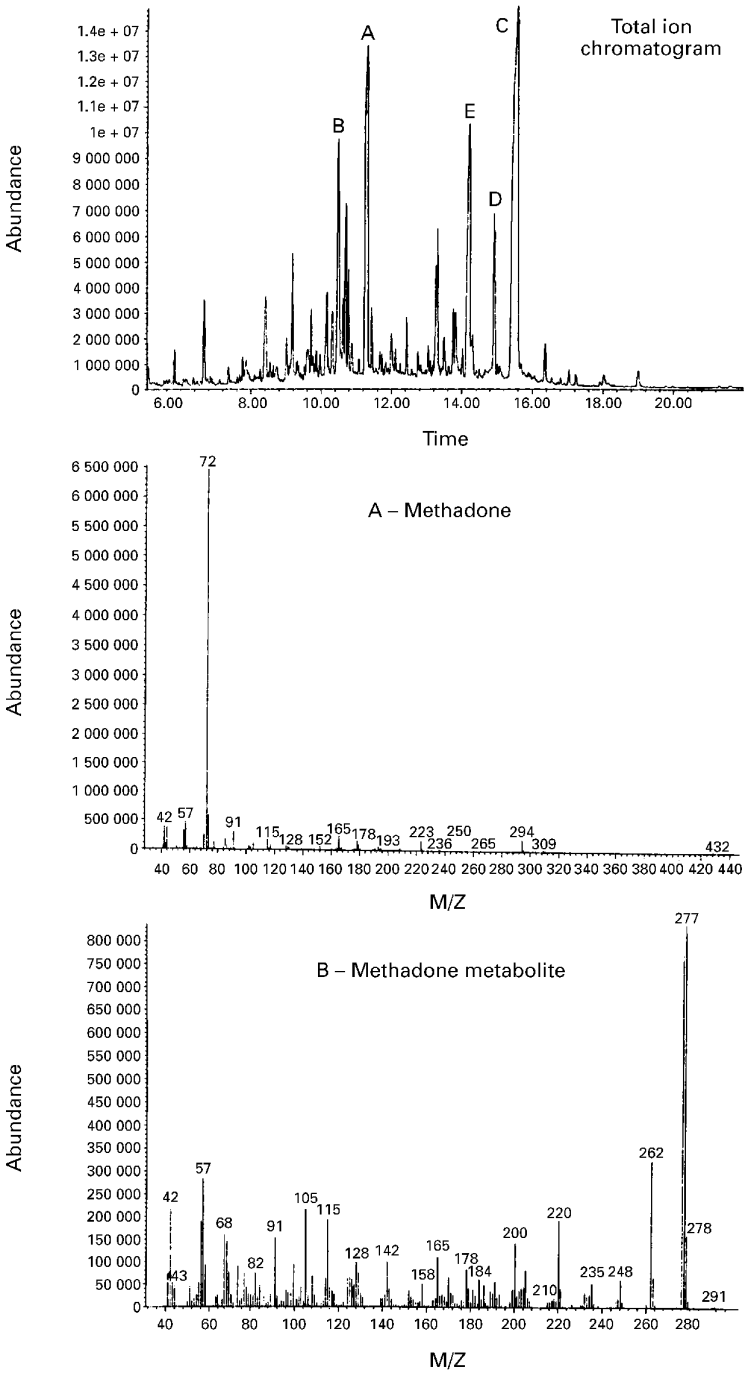

Figure 1 Example of a total ion current chromatogram obtained from a sample provided by a heroin user who was also taking prescribed methadone. Also shown are mass spectra for methadone and a major metabolite (EDDP : 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine).

monly misused drugs and their metabolites that are encountered in urine samples.

Methods

REAGENTS

Methylenedioxyethylamphetamine (MDEA) was a gift from Andrew Greenfield of the Forensic Science Service, Birmingham, UK. The internal standard used for establishing relative retention times on GC/MS was SKF 525a (Proadifen), a gift from SmithKline Beecham (Welwyn Garden City, UK). All other drug standards were purchased from the Sigma Chemical Company, Poole, Dorset, UK.

β-Glucuronidase (type H-1) used for the deconjugation of drugs in urine samples was obtained from Sigma.

The columns used were Varian Bond Elut Certify b, purchased from Phenomenex (Macclesfield, UK). The solvents used in the methods described were obtained from Fisher Scientific UK (Loughborough, UK). All other reagents were purchased from BDH Chemicals (Merck, Lutterworth, UK).

DIGESTION AND EXTRACTION

Urine samples which screened positive and for abused drugs using the Syva ETS system (Behring Diagnostics, Milton Keynes, UK) and required confirmation were digested to deconjugate the drugs present. A 10 ml aliquot of the sample was taken and the pH adjusted using a pH meter to about 5.0 by dropwise addition of either 1 M hydrochloric acid or 1 M ammonium hydroxide; 1 ml of 0.1 M acetate buffer (pH 5.0) was then added, together with approximately 2500 units of glucuronidase. The aliquot was then incubated overnight at 20°C.

The pH of the digested samples was adjusted to between 8.0 and 9.0 by addition of 1 M potassium hydroxide.

The manual solid phase extraction technique was similar to the method recommended by the manufacturer. Without allowing the column to dry between additions, Bond Elut Certify columns were solvated by the addition of 2 ml of methanol followed by 2 ml of water; 5 ml of sample were then passed through the column at a rate of about 2 ml/min, followed by 2 ml of water and 1 ml of 0.1 M acetate buffer, pH 4.0; 2 ml of methanol were added and the column dried under vacuum; 2 ml of eluting solvent (dichloromethane : propan-2-ol : concentrated ammonia (specific gravity 0.88), 80:20:2) were then added to the column and the eluate collected. The solvent was evaporated from the eluate at 50°C under a stream of nitrogen. Care was taken at this stage to avoid excessive loss of more volatile compounds (for example, amphetamine) that would result from extended incubation at 50°C after the solvent was evaporated.

DERIVATISATION

The method chosen for derivatising our extracts has been described previously in a method for opiate confirmation.[6] To the dried residue, 100 µl of pyridine : propionic anhydride (1:2 vol/vol) were added. The contents of the tube were mixed, the tubes were sealed, and they were incubated on a heated block at 75°C for 30 minutes.

Following incubation the reagent was evaporated to dryness at 50°C under a stream of nitrogen and reconstituted with 100 µl of butyl acetate. Three microlitres were injected into the GC/MS.

CHROMATOGRAPHY

The GC/MS system used was a Hewlett-Packard 5890 Series II GC with a 5971A mass

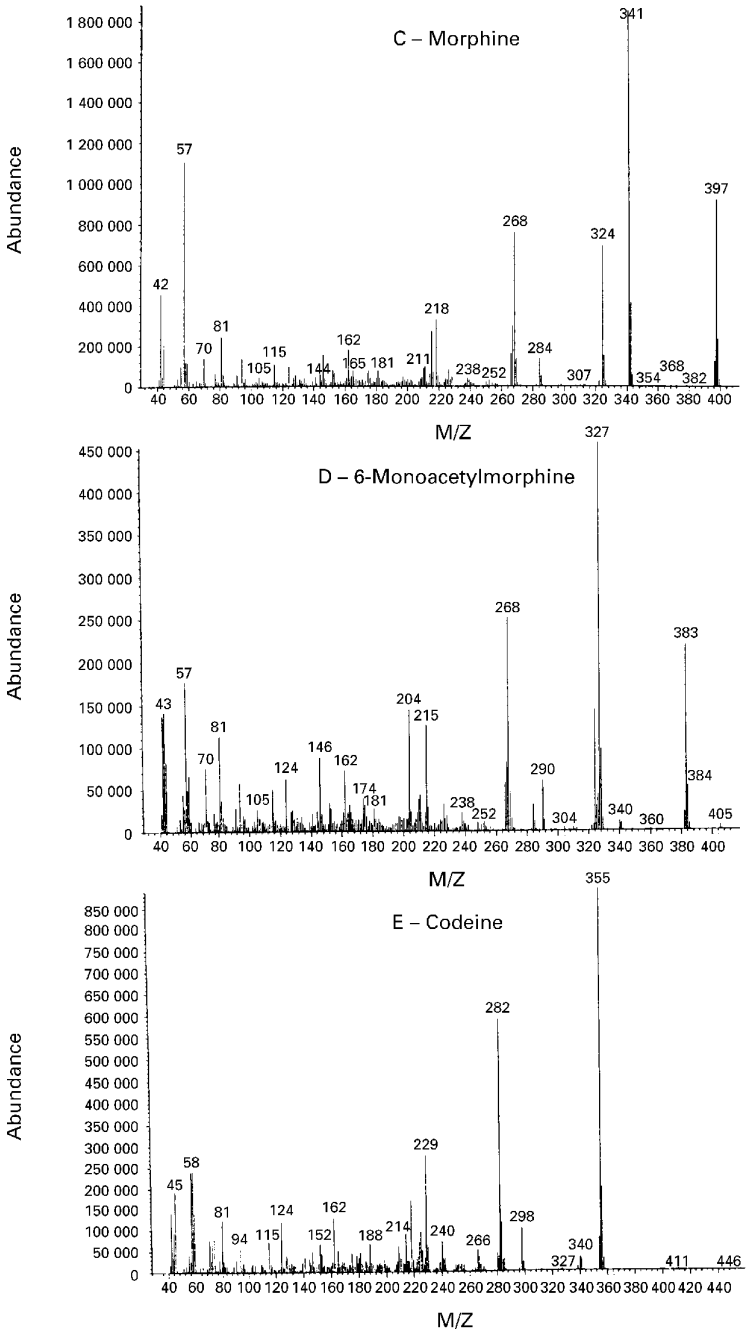

Figure 2 Mass spectra of derivatised morphine, 6-monoacetylmorphine. and codeine.

selective detector, operated using a standard autotune. The column used was a Hewlett-Packard HP-5 capillary 25 metres in length, with an internal diameter 0.32 mm and 0.17 μm film thickness.

GC conditions were initial temperature 85°C, initial time 0.70 minutes, rate 14°C/min, final temperature 285°C, and final time five minutes. This gave a run time of 22 minutes.

MS conditions were scan range 40–500 and solvent delay 2.5 minutes.

Results

RETENTION DATA AND MAJOR IONS

The retention data for a range of drugs of interest in the toxicology laboratory are presented in table 1, relative retention time (RRT) being expressed against SKF 525a. We express the retention data against SKF 525a as a widely used marker, though it is not routinely included for this qualitative analysis. An indication of major ions associated with each compound is also given.

Figure 1 presents an example of a total ion current chromatogram obtained from a sample provided by a heroin user who was also taking prescribed methadone. Also shown are mass spectra for methadone and a major metabolite (EDDP: 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine). In fig 2 the mass spectra of derivatised morphine, 6-monoacetylmorphine, and codeine are also presented. The presence of codeine is a common finding in heroin users, since illicit preparations of heroin usually contain codeine, and codeine is produced during the metabolism of diamorphine.[9]

SENSITIVITY

We assessed the sensitivity of the assay using urine samples spiked with known concentrations of standards and were readily able to detect opiates at a concentration of 50 μg/litre and amphetamines at 100 μg/litre using this system. This is more than adequate given the cut-off values for the immunoassay of 300 μg/litre for opiates and 1000 μg/litre for amphetamines.

Discussion

There are limitations to this system, as we have implied, and we should address these. Using this system we are unable to detect benzoylecgonine as it is lost at the SPE stage; additionally, confirmation of barbiturates and cannabinoids was not possible using this method. We do not normally have a requirement for confirmation of methadone, cocaine, cannabinoids, benzodiazepines, and barbiturates. As was indicated in the introduction, identification of opiates and amphetamines is of primary importance in the majority of routine situations for the reasons described. A range of other abused drugs present at very low concentrations or having physical properties which cause particular difficulties in analysis (that is, buprenorphine, fentanyl, and so on) would not be found using this system and they require various specific immunoassays, or designated methodologies.

We have described a system for the confirmation of drugs from urine samples which have given putative positives by immunoassay. We have found this method to be robust and reliable in routine use over two years. Particular advantages have been the stability of the reagents, especially the derivatisation reagents, and the simple protocol which has enabled staff to perform the assay after a very short training period. The full range of drugs detected using this system has not been given in the table. It is hoped that anyone using the system will add to their lists according to experience and interest. As the system facilitates the detection of opiates, amphetamines, and a range of other drugs within a relatively short GC/MS run time it has proved an invaluable aid at a time of increasing workload.

1 Mule SJ, Casella GA. Rendering the "poppy-seed defense" defenseless: identification of 6-monoacetylmorphine in urine by gas chromatography/mass spectroscopy. *Clin Chem* 1988;**34**:1427–30.
2 Barrett DA, Dyssegaard AL, Shaw PN. The effect of temperature and pH on the deacetylation of diamorphine in aqueous solution and in human plasma. *J Pharm Pharmacol* 1992;**44**:606–8.
3 Goldberger BA, Cone EJ, Grant TM, *et al.* Disposition of heroin and its metabolites in heroin-related deaths. *J Anal Toxicol* 1994;**18**:22–8.
4 Ferrara SD, Tedeschi L, Frison G, *et al.* Solid-phase extraction and HPLC-UV confirmation of drugs of abuse in urine. *J Anal Toxicol* 1992;**16**:217–22.
5 Kintz P, Mangin P. Simultaneous determination of opiates, cocaine and major metabolites of cocaine in human hair by gas chromatography/mass spectrometry (GC/MS). *Forensic Sci Int* 1995;**73**:93–100.
6 Zezulak M, Snyder JJ, Needleman SB. Simultaneous analysis of codeine, morphine, and heroin after B-glucuronidase hydrolysis. *J Forensic Sci* 1993;**38**:1275–85.
7 Saady JJ, Narasimhachari N, Blanke RV. Rapid, simultaneous quantification of morphine, codeine, and hydromorphone by GC/MS. *J Anal Toxicol* 1982;**6**:235–7.
8 Moeller MR, Fey P, Wennig R. Simultaneous determination of drugs of abuse (opiates, cocaine and amphetamine) in human hair by GC/MS and its application to a methadone treatment program. *Forensic Sci Int* 1993;**63**:185–206.
9 Boerner B, Abbott S. New observation on the metabolism of morphine, the formation of codeine from morphine in man. *Experimentia* 1973;**28**:180–1.