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Review

Confirmatory tests for drugs in the workplace by gas chromatography–mass spectrometry

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Abstract

The Mandatory Guidelines for Federal Workplace Drug Testing Programs require the use of gas chromatography–mass spectrometry (GC–MS) for the confirmation of presumptive positive urine specimens. This review focuses upon GC–MS methods developed specifically for forensic confirmation of amphetamine, methamphetamine, 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-acid), benzoylecgonine, morphine, codeine and phencyclidine in urine for purposes of workplace drug testing. In addition, current laboratory issues pertaining to each drug class are reviewed. Generally, drug assays utilized either liquid–liquid or solid-phase extraction methods, derivatization if necessary, and GC–MS detection operating in the selected ion monitoring mode or by full scan acquisition.

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1. Introduction

The abuse of drugs often leads to medical emergencies as a result of acute toxic reactions, increased susceptibility to potentially life-threatening infections, injury from accidents and violence, other health-related problems and to death [1]. Data collected by the United States Substance Abuse and Mental Health Services Administration, Drug Abuse Warning Network, indicates that abused drugs such as heroin, cocaine and ethanol account for a significant number of drug-related illnesses, injuries and deaths in the USA [2,3].

Concerns regarding the proliferation of urine drug testing in the mid-1980s led to the development of standards by the United States Department of Health and Human Services for laboratories conducting forensic urine drug tests. The final version of the standards was published in guideline format on April 11, 1988 in the *Federal Register*. The Mandatory Guidelines for Federal Workplace Drug Testing Programs require the use of immunoassay techniques for initial drug tests, and a confirmatory test by gas chromatography–mass spectrometry (GC–MS) [4]. The confirmation cutoff concentrations for the applicable drugs are shown in Table 1. Since considerable differences exist between regulated and non-regulated confirmatory testing, cutoff concentrations for both levels of testing are included.

Table 1
Regulated and non-regulated confirmation cutoff concentrations

Analyte	Regulated confirmation cutoff concentration (ng/ml)	Non-regulated confirmation cutoff concentration (ng/ml)
Amphetamines		
Amphetamine	500	300
Methamphetamine	500[a]	300
THC-acid	15	10
Benzoylecgonine	150	75
Opiates		
Morphine	300	200
Codeine	300	200
6-Acetylmorphine	Variable[b]	10
Phencyclidine	25	10

[a] To report a positive methamphetamine result, the sample must also contain amphetamine at a concentration greater than or equal to 200 ng/ml.
[b] Cutoff concentration determined by laboratory.

The purpose of this review is to provide a compilation of GC–MS procedures that can be employed for forensic confirmation of amphetamine, methamphetamine, 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-acid), benzoylecgonine, morphine, codeine and phencyclidine in urine for purposes of workplace drug testing. In addition, current laboratory issues pertaining to each drug class will be discussed.

2. Amphetamines

2.1. Amphetamine and methamphetamine

Amphetamine and methamphetamine are extensively metabolized and can be detected in urine specimens for up to 72 h. Amphetamine and methamphetamine are the primary analytes found in urine following methamphetamine ingestion. The chemical structures of amphetamine and methamphetamine are shown in Fig. 1. Table 2 provides a summary of GC–MS methods

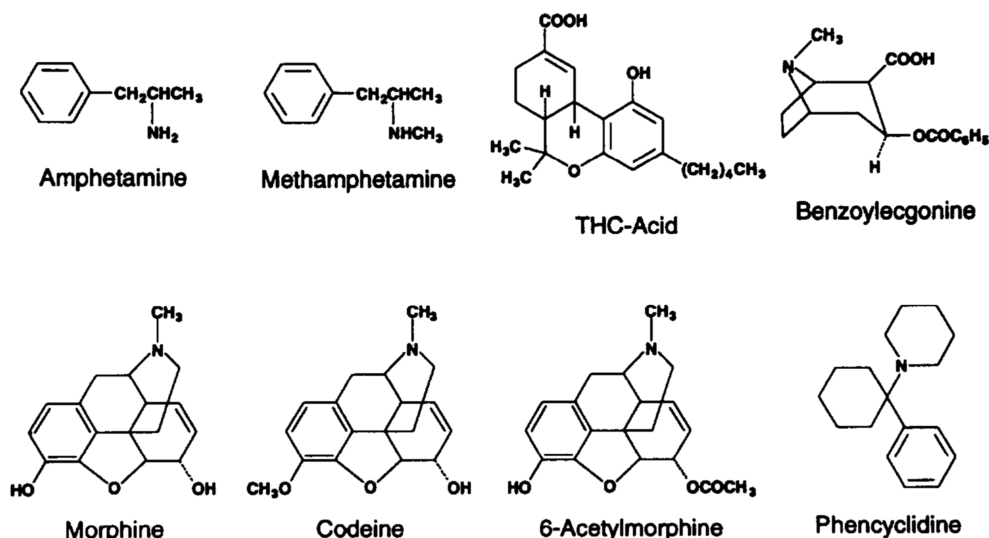

Fig. 1. Chemical structures of abused drugs.

for the analysis of amphetamine and methamphetamine in urine.

Mule and Casella [5] utilized liquid–liquid extraction to isolate amphetamine and methamphetamine prior to GC–MS analysis. Alkalinized samples were extracted with a chloroform–isopropanol solution (9:1, v/v), and the extract was reacted with trifluoroacetic anhydride forming the trifluoroacetyl derivatives of amphetamine and methamphetamine. Phenylcyclohexylamine was utilized as an internal standard. The extraction efficiencies for amphetamine and methamphetamine were approximately 61 and 53%, respectively.

Hornbeck and Czarny [6] compared the trichloroacetyl derivatives of amphetamine and methamphetamine with other common derivatives including trifluoroacetyl and heptafluorobutyryl derivatives for analysis by GC–MS. The extraction procedure utilized an initial liquid–liquid extraction with dichloromethane, a liquid–liquid back-extraction with 1-chlorobutane and derivatization with dimethylaminopyridine and trichloroacetic anhydride. Compared to other

Table 2
GC–MS analysis of amphetamine and methamphetamine in urine

Extraction technique	Derivative	GC column	Detection mode	Detection limit (ng/ml)	Ref.
Liquid–liquid	Trifluoroacetyl	100% Dimethylpolysiloxane	SIM[a]	25	5
Liquid–liquid	Trichloroacetyl	5% Diphenyl/95% dimethylpolysiloxane	SIM	NR[b]	6
Liquid–liquid	Carbethoxy-hexafluorobutyryl	5% Diphenyl/95% dimethylpolysiloxane or 100% dimethylpolysiloxane	SIM	10	7
Solid-phase	Heptafluorobutyryl	5% Methyl/95% dimethylpolysiloxane	SIM	35	8
Solid-phase	Trichloroacetyl	5% Diphenyl/95% dimethylpolysiloxane	SIM	50	9
Solid-phase	Heptafluorobutyryl	5% Diphenyl/95% dimethylpolysiloxane	Scan	50	10

[a] SIM = Selected ion monitoring mode.
[b] NR = Not reported.

derivatives, the trichloroacetyl derivatives demonstrated lower volatility, abundant mass ions and good chromatographic resolution. Utilizing similar extraction conditions, Czarny and Hornbeck [7] also studied the carbethoxyhexafluorobutyryl derivatives of amphetamine and methamphetamine. The carbethoxyhexafluorobutyryl derivatives demonstrated low volatility, high mass fragmentation and good chromatographic resolution at high column temperatures under isothermal conditions.

Taylor *et al.* [8] utilized solid-phase extraction (modified XAD-2 resin) to isolate amphetamine and methamphetamine. Analytes were eluted with a solution of 1% hydrochloric acid in methanol and the final extracts were reacted with heptafluorobutyric anhydride forming the heptafluorobutyryl derivatives of amphetamine and methamphetamine. The extraction efficiencies for amphetamine and methamphetamine were approximately 78 and 87%, respectively. With *n*-propylamphetamine as an internal standard, the assay was linear to 7000 ng/ml.

Gan *et al.* [9] developed a method employing solid-phase extraction (hydrophobic cation exchange) for the isolation of amphetamine and methamphetamine. Analytes were isolated in a solution of 2% ammonium hydroxide in ethyl acetate. The extracts were back-extracted into chlorobutane and reacted with 4-dimethylaminopyridine and trichloroacetic anhydride forming the trichloroacetyl derivatives of amphetamine and methamphetamine. The extraction efficiencies for amphetamine and methamphetamine were approximately 66 and 81%, respectively. The lower recovery obtained for amphetamine was attributed to loss during evaporation. With deuterated internal standards, the assay was linear to 4000 ng/ml.

Wu *et al.* [10] developed an assay for the analysis of amphetamine and methamphetamine employing GC–MS operated in the full scan acquisition mode. Samples were extracted by solid-phase extraction (hydrophobic cation exchange) with $[^2H_8]$methamphetamine as the internal standard. Analytes were isolated in a solution of 2% ammonium hydroxide in ethyl acetate. The extracts were reacted with heptafluorobutyric anhydride forming the heptafluorobutyryl derivatives of amphetamine and methamphetamine.

2.2. Current issues

2.2.1. Other sympathomimetic amines

Because of similarities in chemical structure to amphetamine and methamphetamine, amphetamine assays should be evaluated for interference from other sympathomimetic amines like ephedrine, hydroxynorephedrine, norephedrine, norpseuodephedrine, phentermine, phenylephrine, phenylpropanolamine, propylhexedrine and pseudoephedrine. The evaluation should include the potential for co-elution, and similarity of mass ions and ion ratios to amphetamine and methamphetamine. In addition, chromatograms should be examined for the presence of extraneous chromatographic peaks which may prevent definitive identification.

Thurman *et al.* [11] utilized some of these techniques in a study of the carbethoxyhexafluorobutyryl and heptafluorobutyryl derivatives of amphetamine, methamphetamine and other related sympathomimetic amines. Samples were subjected to liquid–liquid extraction with Toxi-Lab® Toxi-A extraction tubes. Extracts were derivatized with either 4-carbethoxyhexafluorobutyryl chloride or heptafluorobutyric anhydride. $[^2H_3]$Amphetamine and $[^2H_5]$methamphetamine were utilized as internal standards. Based upon the data obtained in full scan acquisition mode, a list of mass ions for selected ion monitoring were developed for all analytes that distinguished amphetamine and methamphetamine from potentially interfering sympathomimetic amines.

2.2.2. Chirality

GC–MS methods utilizing non-chiral derivatives and non-chiral chromatographic liquid phases cannot distinguish between licit (*l*-isomer) and illicit (*d*-isomer and racemic mixtures) forms of methamphetamine. With chiral derivatizing reagents such as N-trifluoroacetyl-l-prolyl chlo-

ride and (−)-menthyl chloroformate, methamphetamine diastereomers can be readily separated with a non-chiral chromatographic system. Typically, isolation of methamphetamine diastereomers is accomplished with routine extraction methods, and extracts are derivatized with the chiral derivatizing reagent. Baseline separation of the *d*- and *l*-isomers is normally achieved allowing identification of the probable source of methamphetamine [12,13].

2.2.3. False positives

Recently, it was discovered that several laboratories engaged in forensic urine drug testing reported false positive methamphetamine results for specimens that contained high concentrations of either ephedrine or pseudoephedrine. Studies performed by Hornbeck *et al.* [14] indicated that methamphetamine was produced by ephedrine or pseudoephedrine derivatized with 4-carbethoxyhexafluorobutyryl chloride, heptafluorobutyric anhydride or N-trifluoroacetyl-l-prolyl chloride. The formation of methamphetamine by thermoconversion was directly related to the temperature of the injection port and the presence of high concentrations of ephedrine or pseudoephedrine. Lowering the injection port temperature, coupled with other preventive measures, eliminates the production of methamphetamine.

To eliminate the possibility of false positive results, ElSohly *et al.* [15] studied the effectiveness of periodate addition to samples containing ephedrine, pseudoephedrine, phenylpropanolamine and norpseudoephedrine. Samples were reacted with a solution of 0.35 *M* sodium periodate for 10 min at room temperature, then subjected to liquid–liquid extraction with chloroform and GC–MS analysis. The data indicated that ephedrine and related compounds are oxidized in the presence of the periodate ion while leaving amphetamine and methamphetamine intact. This process effectively removed the interfering amines and allowed successful analysis of amphetamine and methamphetamine by GC–MS.

3. Cannabinoids

3.1. THC-acid

The major metabolite of tetrahydrocannabinol is THC-acid which is present in urine in both conjugated and unconjugated forms. THC-acid may be detected in urine specimens for variable periods of time depending upon the frequency of use. The chemical structure of THC-acid is shown in Fig. 1. Table 3 provides a summary of GC–MS methods for the analysis of THC-acid in urine.

A comprehensive study of methods for confirmation of THC-acid in urine was reported by Baker *et al.* [16]. The work included an evaluation of hydrolysis, extraction and derivatization procedures. The selected procedure utilized base hydrolysis with 1.0 *M* potassium hydroxide followed by acidification and liquid–liquid extraction with a hexane–ethyl acetate (7:1, v/v) solution. Final extracts were treated with a solution of bis(trimethylsilyl)-trifluoroacetamide and 1% trimethylchlorosilane which formed the trimethylsilyl derivative of THC-acid. The extraction efficiency was 85%, and the procedure was linear to 200 ng/ml.

McCurdy *et al.* [17] developed a method employing C_{18} bonded-phase adsorption columns to isolate THC-acid. Following base hydrolysis, samples were acidified and added to the extraction columns. THC-acid was isolated in methanol, the extract was evaporated and reconstituted in 0.1 *M* hydrochloric acid. This solution was extracted with a hexane–ethyl acetate solution (7:1, v/v), and following evaporation, the final extracts were reacted with a solution of trimethylanilinium hydroxide and iodopropane forming the propyl derivative of THC-acid.

Paul *et al.* [18] developed a method employing a strongly basic anion-exchange resin to isolate THC-acid from base-hydrolyzed urine samples. THC-acid was isolated in ethyl acetate–methanol–acetic acid (90:10:1, v/v/v). Following evaporation, the final extracts were methylated with a mixture of tetramethylammonium hydroxide and iodomethane, acidified and extracted

Table 3
GC–MS analysis of THC-acid in urine

Extraction technique	Derivative	GC Column	Detection mode	Detection limit (ng/ml)	Ref.
Liquid–liquid	Trimethylsilyl	3% SP-2250 on Supelcoport	SIM[a]	10	16
Solid-phase and liquid–liquid	Propyl	100% Dimethylpolysiloxane	SIM	<10	17
Solid-phase	Dimethyl	5% Diphenyl/95% dimethylpolysiloxane	SIM	2	18
Liquid–liquid	Trimethylsilyl	100% Dimethylpolysiloxane	SIM	10	5
Liquid–liquid	Dimethyl	100% Dimethylpolysiloxane	SIM	15	5
Liquid–liquid	Pentafluoropropionyl	5% Diphenyl/95% dimethylpolysiloxane	SIM	1.8	19
Solid-phase	Trimethylsilyl	5% Diphenyl/95% dimethylpolysiloxane	SIM	NR[b]	20
Solid-phase	Dimethyl	100% Dimethylpolysiloxane	Scan	NR	21
Solid-phase	Dimethyl	NR	Scan	2.5	22
Solid-phase	Dimethyl	5% Diphenyl/95% dimethylpolysiloxane	Scan	2.5	10
Liquid–liquid	Dimethyl	5% Diphenyl/95% dimethylpolysiloxane	SIM	2	23
Liquid–liquid	Dimethyl	5% Diphenyl/95% dimethylpolysiloxane	Scan	4	23
Solid-phase	Dimethyl	100% Dimethylpolysiloxane	SIM	NR	24
Liquid–liquid	*tert.*-Butyldimethylsilyl	100% Dimethylpolysiloxane	SIM	1	25
Solid-phase	Trimethylsilyl	5% Diphenyl/95% dimethylpolysiloxane	Scan	1	26

[a] SIM = Selected ion monitoring mode.
[b] NR = Not reported.

into isooctane. The extraction efficiency was approximately 50–60%. The methylated derivative was stable at room temperature for a minimum of 72 h.

Mule and Casella [5] developed a method employing liquid–liquid extraction for the isolation of THC-acid. In addition, two derivatives of THC-acid were studied. Samples were hydrolyzed under basic conditions, acidified and subjected to extraction with a hexane–ethyl acetate solution (7:1, v/v). The final extracts were silylated with N-methyl-N-trimethylsilyl-trifluoroacetamide or methylated with a mixture of tetramethylammonium hydroxide and iodomethane, acidified and subjected to back-extraction in hexane. Mule and Casella [5] noted that both THC-acid derivatives were acceptable; however, the silyl derivative readily contaminated the ion source in the mass spectrometer and was less acceptable than the methyl derivative.

Joern [19] developed a method in which samples were initially hydrolyzed with potassium hydroxide–methanol (1:4, v/v). The hydrolyzed samples were subjected to liquid–liquid extraction with hexane–isoamyl alcohol (98.5:1.5, v/v), followed by acidification and liquid–liquid extraction with hexane–ethyl acetate (5:1, v/v). The final solution was reacted with pentafluoropropionic acid and pentafluoropropanol and evaporated. The extraction efficiency was 70%, and the procedure was linear to 250 ng/ml.

Parry *et al.* [20] developed an assay for THC-acid utilizing hydrophobic anion-exchange chromatography for the isolation step. Following base hydrolysis, the samples were acidified and added to the extraction columns. THC-acid was eluted with methanol. After evaporation, extracts were reacted with bis(trimethylsilyl)-trifluoroacetamide forming the trimethylsilyl derivative of THC-acid. The extraction efficiency was greater than 85%.

Nakamura *et al.* [21] developed a method employing C_{18} bonded-phase adsorption columns to isolate THC-acid. Following base hydrolysis, samples were acidified and added to the ex-

traction columns. THC-acid was isolated in methanol. The final extract was methylated with a mixture of tetramethylammonium hydroxide and iodomethane, acidified and back-extracted into cyclohexane. Meclofenamic acid was employed as a non-isotopic internal standard. The extraction efficiency was greater than 90%, and the methylated derivative was stable at room temperature for several days.

Wimbish and Johnson [22] and Wu *et al.* [10] utilized solid-phase extraction (hydrophobic anion exchange) to isolate THC-acid from base-hydrolyzed urine samples. Following hydrolysis, samples were acidified and transferred to extraction columns. THC-acid was isolated in a solution of hexane–ethyl acetate (75:25, v/v) with 1% acetic acid. The dimethyl derivatives of THC-acid and its corresponding [$^2H_6$]THC-acid internal standard were prepared by reaction with tetramethylammonium hydroxide and iodomethane. The analysis was performed by GC–MS operating in the full scan acquisition mode. Both assays were linear to 1000 ng/ml.

Wimbish and Johnson [22], Joern [23], and ElSohly *et al.* [24] evaluated the use of [$^2H_6$]THC-acid as an internal standard in GC–MS assays for THC-acid. In all reports, final extracts of THC-acid were methylated, acidified and subjected to back-extraction. It was concluded that the use of a highly deuterated internal standard compound increased the dynamic range for GC–MS detection of THC-acid.

Clouette *et al.* [25] modified the method of Baker *et al.* [16] by derivatizing the final extract with N-methyl-N-(*tert.*-butyldimethylsilyl)-trifluoroacetamide in order to form the *tert.*-butyldimethylsilyl derivative of THC-acid. The method provided unusually stable derivatives of THC-acid that demonstrated no apparent deterioration over a period of 10 days.

A newly developed solid-phase C_{18} extraction disc was evaluated by Wu *et al.* [26]. Following base hydrolysis, samples were acidified and applied to the disc. The disc was washed with 20% acetic acid. After drying, the THC-acid was simultaneously eluted and derivatized with N-methyl-N-trimethylsilyl-trifluoroacetamide. The method was linear to 250 ng/ml, and the extraction efficiency was 56%. The new procedure was rapid, produced cleaner extracts compared to ordinary solid-phase extraction minicolumns, and did not require the use of organic solvents.

Two recent reports from the United States Department of Defense described potential sources of interference with the methyl derivative of [$^2H_3$]THC-acid. Depending upon the chromatographic system utilized, ritodrine metabolites [27] and a secondary THC metabolite [28] co-eluted with the prominent mass ions of [$^2H_3$]THC-acid. The presence of these interferences caused the assay to fail quality control criteria; thus, producing false negative results.

3.2. Current issues

3.2.1. Marijuana in foodstuffs

When marijuana is cooked in foodstuffs and ingested, a substantial amount of tetrahydrocannabinol is absorbed and metabolized to THC-acid. The profile of THC-acid excretion in urine is similar to that obtained with a marijuana smoker. Cone *et al.* [29] analyzed consecutive urine samples collected from subjects following the ingestion of one or two marijuana cigarettes cooked in brownie cookies. The samples were extracted with a Prep I extractor (DuPont) and derivatized with tetramethylammonium hydroxide and iodomethane. The analysis was performed by GC–MS operating in the selected ion monitoring mode. Following the ingestion of marijuana in brownies, THC-acid was detectable in urine for 72–346 h.

3.2.2. Passive inhalation

The presence of cannabinoid metabolites in urine specimens due to passive inhalation is a function of environmental conditions, duration and frequency of exposure and tetrahydrocannabinol content of the smoked marijuana. Cone *et al.* [30] studied the effects of passive marijuana smoke in six human subjects who were exposed to smoke of 4 and 16 marijuana cigarettes in a small unventilated room. Consecutive urine samples were collected after passive exposure and analyzed by GC–MS operating in the selected

ion monitoring mode. The GC–MS analysis established that only traces of tetrahydrocannabinol were absorbed by the subjects who were exposed to the smoke of 4 marijuana cigarettes; however, significant amounts of THC-acid were measured after exposure to the smoke of 16 cigarettes. Peak concentrations of THC-acid after the 16 marijuana cigarette exposure ranged from 10 to 87 ng/ml in seven individuals. The results indicated that it was unlikely that individuals exposed to marijuana cigarette smoke would test positive for cannabinoids.

4. Cocaine metabolite

4.1. Benzoylecgonine

Cocaine is rapidly hydrolyzed to benzoylecgonine and ecgonine methyl ester by chemical and metabolic reactions. Benzoylecgonine, the primary cocaine metabolite, may be detected in urine for several days following drug administration. The chemical structure of benzoylecgonine is shown in Fig. 1. Table 4 provides a summary of GC–MS methods for the analysis of benzoylecgonine in urine.

Benzoylecgonine can be extracted readily with either liquid–liquid or solid-phase techniques. Joern [31] adapted the procedure of Graas and Watson [32] to simultaneously extract and derivatize benzoylecgonine employing an extractive alkylation solvent solution of methylene chloride–1-iodopropane (99:1, v/v). Utilizing N-butylbenzoylecgonine as an internal standard, the modified method was linear to approximately 1250 ng/ml.

Taylor *et al.* [33] described a method for the isolation of benzoylecgonine utilizing solid-phase extraction with Amberlite XAD-2 extraction material. Following isolation in *n*-butylchloride–acetonitrile–methanol solution (40:50:10, v/v/v), the final extract was evaporated and reacted with N-methyl-N-trimethylsilyl-trifluoroacetamide forming the trimethylsilyl derivative of benzoylecgonine. Utilizing $[^2H_3]$benzoylecgonine as an internal standard, the method was linear at concentrations ranging from 50 to 4000 ng/ml. The extraction efficiency was approximately 75–80%.

Mule and Casella [34] developed an assay in which an alkalinized sample was extracted with a chloroform–isopropanol (9:1, v/v) solution. Following a water wash, the organic solvent was evaporated, and the extract was reacted with a solution of pentafluoropropionic anhydride and pentafluoropropanol forming the pentafluoropropyl derivative of benzoylecgonine. Ketamine was utilized as an internal standard. The extraction efficiency for benzoylecgonine was 76%.

Gerlits [35] recently described the formation of a unique *tert.*-butyldimethylsilyl derivative of benzoylecgonine. Following extraction, extracts were reacted with a solution of N-methyl-N-*tert.*-butyldimethylsilyl-trifluoroacetamide and 1% *tert.*-butyldimethylchlorosilane.

Wu and co-workers [10,36] developed an assay for the analysis of benzoylecgonine employing

Table 4
GC–MS analysis of benzoylecgonine in urine

Extraction technique	Derivative	GC column	Detection mode	Detection limit (ng/ml)	Ref.
Liquid–liquid	*n*-Propyl	5% Diphenyl/95% dimethylpolysiloxane	SIM[a]	35	31
Solid-phase	Trimethylsilyl	100% Dimethylpolysiloxane	SIM	50	33
Liquid–liquid	Pentafluoropropyl	100% Dimethylpolysiloxane	SIM	12.5	34
Liquid–liquid	*tert.*-Butyldimethylsilyl	100% Dimethylpolysiloxane	SIM	NR[b]	35
Liquid–liquid	Pentafluoropropyl	5% Diphenyl/95% dimethylpolysiloxane	Scan	37	10, 36
Solid-phase	Trimethylsilyl	100% Dimethylpolysiloxane	SIM	5	37

[a] SIM = Selected ion monitoring mode.
[b] NR = Not reported.

GC–MS operated in the full scan acquisition mode. Samples were extracted using solid-phase extraction (hydrophobic cation exchange) with difluorobenzoylecgonine as the internal standard. Benzoylecgonine was isolated in a solution of methylene chloride–isopropanol (80:20, v/v) in 2% ammonium hydroxide. The final extracts were reacted with pentafluoropropionic anhydride and pentafluoropropanol forming the pentafluoropropyl derivative of benzoylecgonine.

Taylor and Le [37] developed a fully automated procedure for the analysis of cocaine and benzoylecgonine utilizing a laboratory robotic system. The system was capable of performing precise sample aliquot transfers, solid-phase extraction and derivatization. The method was linear at concentrations ranging from 5 to 100 000 ng/ml. With this system, within-run and between-run precision studies produced relative standard deviations less than 10%.

4.2. Current issues

4.2.1. Coca tea

Consumption of tea prepared with coca leaf (*e.g.* "Health Inca Tea") can produce detectable concentrations of benzoylecgonine in urine. El-Sohly *et al.* [38] and Jackson *et al.* [39] reported peak urinary benzoylecgonine concentrations exceeding 1000 ng/ml following ingestion of a single cup of tea. Both studies utilized GC–MS analysis to confirm the presence of benzoylecgonine in the urine specimens of tea drinkers.

4.2.2. Passive inhalation

Cocaine base ("crack") is readily vaporized by heating resulting in efficient delivery of drug to the lungs and circulatory system of "crack" smokers. During the smoking process, some of the vaporized cocaine is released into the atmosphere. Individuals in the vicinity of a "crack" smoker could passively breathe in cocaine vapor. Cone *et al.* [40] measured cocaine and benzoylecgonine in urine of persons passively exposed to cocaine vapor by GC–MS analysis. Although benzoylecgonine concentrations were insufficient to be reported positive by the Department of Health and Human Services guidelines, there were sufficient amounts excreted to indicate that significant absorption of cocaine had occurred as a result of passive inhalation.

5. Opiates

5.1. Morphine and codeine

Following administration, heroin is rapidly metabolized to 6-acetylmorphine and then to morphine by chemical and enzymatic processes. Morphine is further metabolized by conjugation to morphine-glucuronide and by demethylation to normorphine. Morphine and conjugated morphine are the primary heroin metabolites found in urine, but heroin and 6-acetylmorphine may also be present for a short period after drug administration. Codeine is metabolized by conjugation to codeine-glucuronide and by demethylation to morphine and norcodeine. Codeine and morphine are the primary analytes found in urine following codeine ingestion. Morphine and codeine may be detected in urine for 2–4 days following drug use. The chemical structures of morphine, codeine and 6-acetylmorphine are shown in Fig. 1. Table 5 provides a summary of GC–MS methods for the analysis of morphine and codeine in urine.

The initial methods developed for the simultaneous analysis of opiates in urine were based upon liquid–liquid extraction techniques. Paul *et al.* [41] developed an assay where an acid-hydrolyzed sample was alkalinized and extracted with methylene chloride–isobutanol (9:1, v/v) solution. This was followed by acid–base extraction and re-extraction into organic solvent. The extract was evaporated and derivatized with acetic anhydride and pyridine. The acetyl derivatives of morphine and codeine were compared to those formed by reaction with trifluoroacetic anhydride, pentafluoropropionic anhydride or heptafluorobutyric anhydride. The acetyl derivative demonstrated the most acceptable chromatographic properties. Nalorphine was utilized as an internal standard. The extraction efficiencies for morphine and codeine were 40 and 58%, respectively.

Table 5
GC–MS analysis of morphine and codeine in urine

Extraction technique	Derivative	GC column	Detection mode	Detection limit (ng/ml)	Ref.
Liquid–liquid	Acetyl	5% Diphenyl/95% dimethylpolysiloxane	SIM[a]	25	41
Liquid–liquid	Perfluoroester	100% Dimethylpolysiloxane	SIM	50	5
Liquid–liquid	Acetyl	100% Dimethylpolysiloxane	SIM	10	42
Solid-phase	Acetyl	5% Diphenyl/95% dimethylpolysiloxane	Scan	10	43
Solid-phase	Perfluoroester	5% Diphenyl/95% dimethylpolysiloxane	Scan	50	10
Solid-phase	Trimethylsilyl	100% Dimethylpolysiloxane	SIM	NR[b]	44

[a] SIM = Selected ion monitoring mode.
[b] NR = Not reported.

Mule and Casella [5] developed an assay in which an acid-hydrolyzed sample was alkalinized and extracted with a chloroform–isopropanol (9:1, v/v) solution. After a water wash, the organic solvent was evaporated, and the extract was reacted with a solution of pentafluoropropanol and pentafluoropropionic anhydride forming the perfluoroester derivative of morphine and codeine. Nalorphine was utilized as an internal standard. The extraction efficiencies for morphine and codeine were 96 and 91%, respectively.

Bowie and Kirkpatrick [42] developed a method for the determination of morphine, codeine, 6-acetylmorphine and other 6-keto-opioids. Following enzymatic hydrolysis, alkalinized samples were extracted with a chloroform–isopropanol (9:1, v/v) solution. After a water wash, the organic solvent was evaporated, and the extract was acetylated by reaction with [$^{2}H_{6}$]acetic anhydride and pyridine. The extraction efficiency for morphine and codeine was greater than 95%.

The recent development of copolymeric bonded-phase extraction cartridges has improved the efficiency and ease of simultaneous opiate extraction. For example, Huang *et al.* [43] developed a method for the analysis of morphine, codeine, hydromorphone, hydrocodone and oxycodone utilizing hydrophobic cation-exchange solid-phase extraction cartridges. Samples were subjected to enzyme hydrolysis, followed by solid-phase extraction. Opiates were isolated in a solution of methylene chloride–isopropanol (80:20, v/v) with 2% ammonium hydroxide. The final extracts were reacted with acetic acid and pyridine forming acetyl derivatives of morphine and codeine. GC–MS was operated in the full scan acquisition mode. Using nalorphine as an internal standard, the assay was linear for all analytes between 50 and 1000 ng/ml. The extraction efficiencies for morphine and codeine were greater than 80%.

Wu *et al.* [10] developed an assay for the analysis of opiates employing GC–MS operated in the full scan acquisition mode. Following enzyme hydrolysis, samples were extracted using solid-phase extraction (hydrophobic cation exchange) with deuterated dihydromorphine and dihydrocodeine as internal standards. Opiates were isolated in a solution of methylene chloride–isopropanol (80:20, v/v) with 2% ammonium hydroxide. The final extracts were reacted with pentafluoropropionic anhydride forming the perfluoroester derivatives of morphine and codeine.

Vidal *et al.* [44] developed a fully automated procedure for the analysis of morphine and codeine utilizing a laboratory robotic system. The system was capable of performing precise sample aliquot transfers, enzyme hydrolysis, solid-phase extraction and derivatization. With this system, the within-run and between-run precision studies produced relative standard deviations of approximately 10%.

The stability of derivatives commonly utilized for the determination of morphine and codeine by GC–MS was evaluated by Chen *et al.* [45].

The study examined derivatives formed by reaction of unextracted morphine and codeine standards with pentafluoropropionic anhydride, heptafluorobutyric anhydride, N-methyl-bis-trifluoroacetamide, bis(trimethylsilyl)-trifluoroacetamide, and acetic anhydride and pyridine. Of the five different derivatives evaluated, the trimethylsilyl and acetyl derivatives produced the most stable mass spectra for GC–MS quantitation.

The stability, chromatographic characteristics, potential interference, and suitability of major fragment ions for GC–MS analysis of morphine and codeine perfluoroester and acetyl derivatives of morphine and codeine were evaluated further by Grinstead [46]. All of the derivatives were found to suffer from some limitations. The perfluoroester derivatives of morphine and codeine showed acceptable stability and lack of interference from other opiates; however, the codeine derivative demonstrated poor chromatography and its mass spectrum consisted of only two ions suitable for analysis by GC–MS operated in the selected ion monitoring mode. The acetyl derivatives were stable, demonstrated acceptable chromatography and produced suitable mass spectra; however, problems with incomplete derivatization and side reactions limited its usefulness. Also, morphine was indistinguishable from 6-acetylmorphine with this derivative since both analytes form diacetylmorphine.

5.2. Current issues

5.2.1. Other opiates

Because of similarities in chemical structure to morphine and codeine, opiate assays should be evaluated for interference from opiate metabolites and semi-synthetic 6-keto-opioids, including commonly prescribed analgesics such as hydromorphone, hydrocodone, oxymorphone and oxycodone. For example, the mass spectra of the trimethylsilyl derivative of hydromorphone resembles the trimethylsilyl derivative of morphine. In addition, the mass spectra of the trimethylsilyl derivative of morphine is similar to the trimethylsilyl derivative of norcodeine.

5.2.2. 6-Acetylmorphine

Recently, assays designed to detect 6-acetylmorphine in urine have been developed to provide information regarding the nature of opiate drug ingestion. The extraction of 6-acetylmorphine in urine can be accomplished readily by liquid–liquid or solid-phase extraction techniques. Mule and Casella [47] utilized a chloroform–isopropanol (9:1, v/v) solution to isolate 6-acetylmorphine. Following derivatization, the trimethylsilyl derivative of 6-acetylmorphine was assayed by GC–MS. The method was sensitive to 10 ng/ml of 6-acetylmorphine with 0.5 ml sample volume.

Paul *et al.* [48] reported a highly sensitive method for 6-acetylmorphine in urine utilizing an initial solvent extraction with 10% isobutanol in methylene chloride, followed by acid–base organic extraction or solid-phase (LC-CN columns) purification. The final extract was reacted with propionic anhydride and pyridine forming propionylated 6-acetylmorphine. The limit of detection of the method was 0.81 ng/ml, and the approximate extraction efficiencies were 80% and greater than 90% for the liquid–liquid and solid-phase extraction methods, respectively. Romberg and Brown [49] subsequently reported an improvement of the methodology of Paul *et al.* [48]. The solid-phase purification step was replaced with an acidic sodium acetate back-extraction, followed by an additional alkaline extraction into 10% isobutanol in methylene chloride. An increase in 6-acetylmorphine recovery, and elimination of extraneous peaks were observed with the modified procedure.

Procedures for the simultaneous determination of 6-acetylmorphine and other opiate analytes have been reported by Bowie and Kirkpatrick [42], Fuller and Anderson [50] and Goldberger *et al.* [51]. The assays utilized either liquid–liquid or solid-phase extraction techniques, followed by derivatization and GC–MS analysis. In order to obtain accurate measurements of 6-acetylmorphine, techniques were developed that avoided chemical and enzymatic hydrolysis. In addition, optimum chemical stability of 6-acetylmorphine was achieved with extraction procedures that utilized neutral pH conditions [51].

5.2.3. Poppy seeds

Morphine and codeine occur naturally in poppy seed. As a result, morphine and codeine are excreted in urine specimens of individuals who have recently ingested foodstuffs prepared with poppy seeds. The concentration of morphine and codeine in urine generally reaches a maximum within 2 to 4 h following ingestion, and declines over a period of 24–48 h. Consequently, urine specimens from poppy seed consumers resemble those obtained from heroin users. In order to differentiate poppy seed ingestion from codeine, morphine and/or heroin use, ElSohly and Jones [52] proposed the following guidelines based upon GC–MS analysis of opiates in urine: morphine concentration greater than 5000 ng/ml; codeine concentration greater than 300 ng/ml with a morphine-to-codeine ratio less than 2; or the presence of 6-acetylmorphine. Presently, these guidelines are being evaluated to determine their validity in differentiating heroin users from poppy seed eaters.

6. Phencyclidine

Phencyclidine undergoes oxidative metabolism forming monohydroxy and dihydroxy metabolites. The hydroxylated metabolites are excreted in urine as glucuronide conjugates. Approximately 10% of a phencyclidine dose is excreted unchanged in urine. Phencyclidine may be detected in urine for several weeks following drug use. The chemical structure of phencyclidine is shown in Fig. 1. Table 6 provides a summary of GC–MS methods for the analysis of phencyclidine in urine.

A limited number of methods have been published describing the analysis of phencyclidine in urine utilizing GC–MS detection. Mule and Casella [5] reported a highly sensitive method employing only 0.2 ml of urine. Urine samples were alkalinized and extracted with a chloroform–isopropanol (9:1, v/v) solution. Ketamine was used as an internal standard. The assay was linear over a range of 10–100 ng/ml with an extraction efficiency of 87%.

Stevenson *et al.* [53] and Chan *et al.* [54] employed solid-phase extraction (hydrophobic cation exchange) for the analysis of phencyclidine. [$^{2}H_{5}$]Phencyclidine was employed as the internal standard in both methods. To isolate phencyclidine, Stevenson *et al.* [53] and Chan *et al.* [54] utilized a solution of 2% ammonium hydroxide in methanol or ethyl acetate, respectively.

Wu *et al.* [10] developed an assay for phencyclidine utilizing solid-phase extraction (hydrophobic cation exchange) with difluorophencyclidine employed as an internal standard. GC–MS was operated in the full scan acquisition mode. The method was linear to 500 ng/ml, and the extraction efficiency was approximately 90%.

7. Conclusions

Although current methods for GC–MS confirmation of abused drugs have been adequate for the certification of approximately ninety laboratories under the Mandatory Guidelines for

Table 6
GC–MS analysis of phencyclidine in urine

Extraction technique	Derivative	GC column	Detection mode	Detection limit (ng/ml)	Ref.
Liquid–liquid	NA[a]	100% Dimethylpolysiloxane	SIM[b]	10	5
Solid-phase	NA	5% Diphenyl/95% dimethylpolysiloxane	SIM	0.5	53
Solid-phase	NA	100% Dimethylpolysiloxane	SIM	10	54
Solid-phase	NA	5% Diphenyl/95% dimethylpolysiloxane	Scan	0.25	10

[a] NA = Not applicable.
[b] SIM = Selected ion monitoring mode.

Federal Workplace Drug Testing, many laboratories continue to improve their methods of extraction and derivatization, and their instrumental techniques. For example, the present trend towards the utilization of solid-phase extraction permits rapid isolation of drug and/or metabolite for GC-MS analysis. This has resulted in cleaner extracts and increased throughput. New isotopic and non-isotopic compounds for use as internal standards have been recently introduced eliminating some of the interferences produced with earlier internal standards. These modifications in chromatographic and spectrometric techniques continue to improve assay specificity and sensitivity. It is expected that future changes in confirmatory tests for drugs in the workplace will focus upon development of new assays for additional drugs of abuse and the use of new hyphenated methods for sample introduction into the mass spectrometer.

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