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Review

Rapid assay of cocaine, opiates and metabolites by gas chromatography–mass spectrometry

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ABSTRACT

The simultaneous assay of cocaine, opiates and metabolites in small biological samples continues to be a difficult task. This report focuses upon tabulation of important techniques (extraction, derivatization, chromatographic conditions, detection mode, data acquisition) reported over the last decade that were used in the development of assays for these analytes. The most prevalent procedures for extraction of cocaine, opiates and metabolites were liquid–liquid and solid-phase extraction isolation methods. Following extraction analytes were derivatized and analyzed by gas chromatography–mass spectrometry. The technique most often used for chromatographic separation was fused-silica capillary column gas chromatography. Detection generally was performed by selected ion monitoring in the positive-ion electron-impact ionization mode, although full-scan acquisition and positive- and negative-ion chemical ionization methods have been used. It was apparent from the review that there is a continuing need for greater sensitivity and selectivity in the assay of highly potent opiates and for cocaine and metabolites.

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1. INTRODUCTION

Although overall drug abuse appears to be decreasing in the United States, cocaine and heroin continue to cause significant health problems. Data from the Drug Abuse Warning Network (DAWN) for the year 1990 indicate that cocaine and heroin/morphine rank as the second and third most frequently mentioned drugs after alcohol by emergency rooms in the United States [1]. Other opiates such as oxycodone, codeine and hydromorphone are cited less frequently. Cocaine, heroin/morphine and codeine also are the first, third and fourth most frequently cited drugs by medical examiners [2]. These data further reveal that when alcohol is excluded, cocaine in combination with heroin is the most frequently cited drug combination by both emergency rooms and medical examiners.

Analytical toxicology has much to offer in terms of combatting the problems of drug abuse. Detection of drugs of abuse in biological fluids remains the most objective means of diagnosis and monitoring drug abuse problems. This is accomplished by collection of biological specimens, *e.g.*, urine, blood, saliva, with subsequent assay for parent drug and/or metabolite.

For reasons of speed and simplicity, initial testing of specimens is often performed by immunoassay. These tests provide valuable leads in the drug identification process. Unfortunately, due to the lack of specificity of most immunoassays, the tests provide only qualitative or semi-quantitative information. Further, the legal defensibility of test results based solely on immunoassay is questionable. A recent survey of scientific experts and arbitrators indicated that two different analytical test methods are required and that immunoassay followed by gas chromatography–mass spectrometry (GC–MS) is rated as fully defensible against legal challenge [3]. When the experts were asked to rate a single most defensible assay, they choose GC–MS.

Unquestionably, the use of GC–MS in forensic testing has evolved to the point where it has become the "gold standard" technique. Indeed, the Mandatory Guidelines for Federal Workplace Drug Testing requires the use of GC–MS for confirmation of all specimens that initially test positive by immunoassay [4].

The purpose of this review is to provide a compilation of GC–MS methodologies employed for the analysis of cocaine, opiates and metabolites primarily in body fluids and tissues. Since a complete review was published in 1980 [5], the major focus of this report is on literature published after 1980.

2. ANALYTICAL CONSIDERATIONS

The development of GC–MS assays for drugs of abuse must encompass both pharmacologic and chemical factors. Fig. 1 is a general representation of factors which lead to increased difficulty in assay development. Obviously if only small amounts of drug/metabolite are present in a sample due to the drug's potency, the difficulty of the assay will increase. In addition, high rates of metabolism, distribution and excretion diminish analyte concentration, thus demanding greater assay sensitivity. Fortunately, most GC MS detectors demonstrate a wide dynamic range in

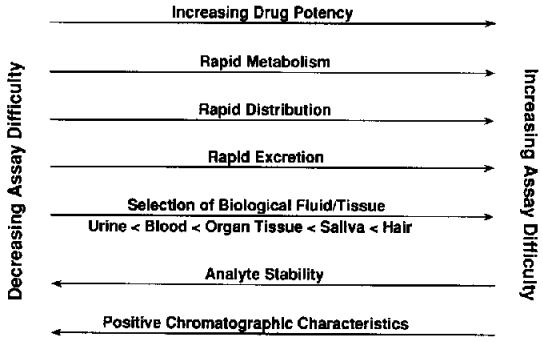


Fig. 1. Pharmacologic and chemical factors which increase the difficulty of assay development.

response to analyte concentration; hence, it is only when the analyte concentration approaches the limit of sensitivity of the instrument that precision and accuracy deteriorate. Chemical factors such as analyte stability and molecular structure also influence assay outcome. If the analyte decomposes in the biological specimen during collection, storage or during sample work-up, the assay results may not be valid. Also, the chemical structure and analytic utility of the drug molecule impact upon its detectability [6].

If the molecule contains numerous polar groups, it may be difficult to isolate from the biological specimen and/or it may exhibit poor chromatographic properties. Biological samples contain a vast array of organic and inorganic compounds. In comparison, drug analytes will be found in very low concentrations. Isolation techniques must be devised that will concentrate the analyte, freeing the sample of the bulk of water, protein and background materials. A recent review by McDowall [7] describes current approaches used for sample preparation for biomedical analysis. The most commonly used methods for sample preparation are liquid–liquid extraction and solid-phase extraction (SPE). Both techniques are in use in forensic urine drug testing (FUDT) laboratories. A survey of 49 FUDT laboratories indicated that SPE and liquid–liquid extraction techniques were used with equal frequency for the analysis of opiate and cocaine metabolites in urine samples [8]. Following extraction, polar analytes generally are derivatized to improve chromatographic properties.

Both cocaine and opiates, particularly heroin, present unique problems in assay development. Table 1 lists some of the factors that must be considered in the development of a GC–MS assay for these drugs. Different strategies must be developed for analytes that exhibit stability problems, extreme water solubility or high potency. Toxicologic analysis of cocaine and opiate specimens presents a continuing challenge to the fo-

TABLE 1

MAJOR CONSIDERATIONS IN THE DEVELOPMENT OF COCAINE AND OPIATE ASSAYS

Drug	Assay development considerations
Cocaine	Unstable in blood specimens and basic solutions Rapid and extensive metabolism Short half-life Forms water-soluble metabolites with longer half-lives
Heroin	Unstable in biological specimens and basic solutions Rapid and extensive metabolism Extremely short half-life Forms water-soluble metabolites with longer half-lives Forms several active metabolites, some more active than parent drug
Morphine, hydromorphone and oxymorphone	Stable analytes Extensive metabolism Forms water-soluble metabolites Forms minor amounts of active metabolites
Codeine, hydrocodone and oxycodone	Stable analytes Substantial amounts of parent drug are excreted unchanged Forms minor amounts of active metabolites, some more active than parent drug Forms water-soluble metabolites
Buprenorphine	High potency Extensive metabolism Forms major amounts of active metabolite Highly lipid-soluble

rensic chemist due to the many different ways in which these substances are metabolized and the varied chemical structures of the opiate compounds. Numerous forensic questions are posed that require accurate identification and quantitation of these drug residues in biological fluids. Their correct analysis may ultimately aid the diagnosis, treatment and prevention of drug abuse.

3. COCAINE AND METABOLITES

3.1. Biotransformation and stability

Cocaine is rapidly hydrolyzed in biological fluids by ester hydrolysis to benzoylecgonine and ecgonine methyl ester (Fig. 2) [9,10]. Further hydrolysis produces ecgonine. The production of ecgonine methyl ester in blood is catalyzed by cholinesterase, whereas benzoylecgonine is produced spontaneously in solution, particularly at pH ≥ 7 [11,12]. Isenschmid *et al.* [13] have shown that these stability problems can be overcome by chemical treatment with preservatives and pH adjustment. They reported that cocaine is stable in blood indefinitely when preserved with cholinesterase inhibitors and the solution is adjusted to pH 5 and maintained at 4°C or lower.

Interestingly, when cocaine free base ("crack") is smoked, a pyrolytic product, anhydroecgonine methyl ester, is produced. Analysis by GC–MS of urine obtained from "crack" smokers indicated that this product was excreted in nearly equivalent amounts as cocaine [14,15]. Identification of anhydroecgonine methyl ester in urine may serve as a marker for the identification of "crack" users.

When cocaine and alcohol are used simultaneously, cocaethylene is formed via transesterifica-

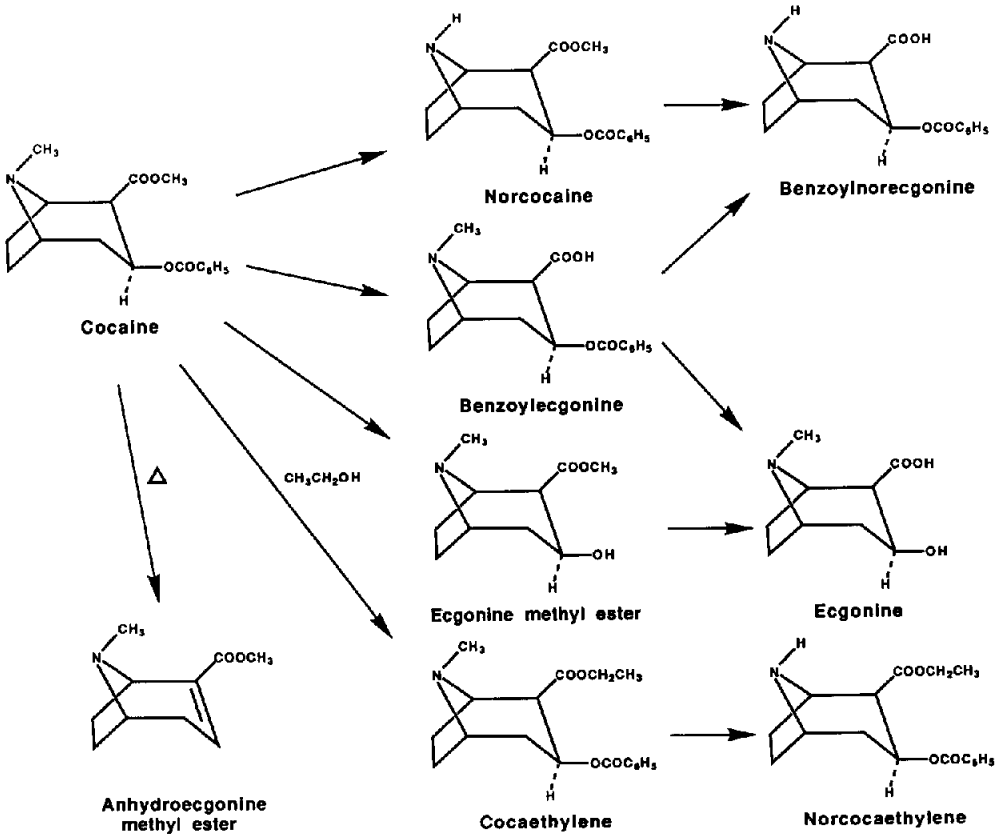


Fig. 2. Biotransformation of cocaine in humans.

tion of the carbomethoxy group of cocaine. Cocaethylene was first identified in the urine of cocaine abusers by GC–MS operated in the chemical ionization (CI) mode [16]. Subsequent studies also indicated that hydroxylated metabolites of cocaethylene are formed metabolically, analogous to those observed for cocaine [17]. Recently, a controlled clinical trial was conducted in which cocaethylene was detected in urine following administration of 100 mg of cocaine hydrochloride (intranasal route) and a vodka-based drink (1 g/kg) [18]. This study established that cocaethylene is formed rapidly *in vivo* during the co-administration of these drugs.

3.2. Cocaine

Cocaine can be readily isolated by liquid–liquid extraction [19] or SPE [20]. Extraction recoveries generally range from 50 to 100%. If liquid–liquid extraction is employed, cocaine exposure to pH >8.0 must be brief due to rapid hydrolysis [10].

Following extraction, cocaine can be identified and quantitated by GC–MS. Normally, it displays excellent chromatographic characteristics both on packed columns and capillary columns due to its non-polar structure. At injection port temperatures of 250°C, cocaine exhibits some thermal lability and degrades to methylecgonidine when injected as cocaine hydrochloride [21].

The analysis of illicit cocaine samples by GC–MS has indicated that cocaine is often impure and may contain a variety of other components. GC MS identification of these impurities is often useful for forensic purposes. Identification of the minor alkaloids of *Erythroxylon coca*, *cis*- and *trans*-cinnamoylcocaine, in cocaine seizures provides indirect proof that the cocaine arises from natural sources rather than synthesis [22]. The presence of these excipients also helps determine whether different samples originated from the same or different illicit sources.

Several methods have been reported for the identification of cocaine at trace levels in biological materials by GC–MS. Ruiz and Gallego [23] used selected ion monitoring (SIM) for the simultaneous identification of amitriptyline and cocaine in human serum of overdose patients. Following identification, both analytes were quantitated in a separate procedure by GC with a nitrogen–phosphorus detector. Thompson *et al.* [24] reported the use of full-scan GC–MS for the confirmation of intact cocaine in human saliva. They reported highly significant correlations of saliva cocaine levels to plasma levels in subjects who had received single intravenous doses of 15 or 40 mg of cocaine. Cone and Weddington [25] utilized SIM for the confirmation of cocaine in human saliva. They found that cocaine was excreted over five to ten days by chronic cocaine addicts during withdrawal. Nowak *et al.* [26] used full-scan GC–MS for the confirmation of cocaine in sheep tissues after lethal and repeated sublethal doses.

Recently, human hair was analyzed successfully for the presence of cocaine. Martz *et al.* [27,28] applied both GC–MS and MS–MS techniques to hair and found conclusive evidence that cocaine was present in hair specimens of known cocaine users. Balabanova and Homoki [29] also reported confirmation of cocaine in hair samples obtained from a cocaine user after death and in sheep after repeated administration of cocaine.

Quantitation of cocaine by GC–MS was reported by Cravey [30] who determined blood and tissue levels in poisoned infants. Concentrations of cocaine in blood, brain and liver ranged from 19 to 122 μg/l. Nicolaysen *et al.* [31] used high resolution SIM for the measurement of cocaine in rat striatum following intraperitoneal cocaine injection. Microdialysis perfusate was dried, reconstituted in toluene and analyzed. The limit of detection for cocaine was 25 pg cocaine on-column with a signal-to-noise ratio of 3. Cocaine was found to reach a maximum *in vivo* concentration of 10.1 μM within 30 min of the injection.

3.3. Cocaethylene

Cocaethylene is the ethyl homologue of cocaine (Fig. 2) and also is known as ethylbenzoylecgonine or homococaine. Cocaethylene can be formed *in vivo* in humans by the co-administra-

tion of cocaine and alcohol. Earlier studies indicated that cocaethylene could be detected and measured by GC–MS [16,18] in human urine following simultaneous use of cocaine and alcohol. Recently, cocaethylene was identified together with cocaine in blood of overdose victims [32]. In five cases, cocaethylene concentrations ranged from 0.03 to 0.53 mg/l and cocaine ranged from 0.03 to 1.4 mg/l. Also, cocaethylene and cocaine have been determined in hair samples of drug abusers [33]. Cocaethylene was detected in eight of ten hair specimens and ranged in concentration from trace amounts to 2.6 ng/mg of hair. Cocaine was detected in all ten specimens in concentration ranging from 6.4 to 19.2 ng/mg.

Cocaethylene also can be formed by simple exposure of cocaine to alcohol, *e.g.*, storage of cocaine hydrochloride in alcoholic solution. Janzen [34] recently reported the detection of cocaethylene in illicit cocaine samples that had been imported dissolved in liquor. In this case, prolonged exposure of cocaine to alcohol resulted in the transesterification of approximately 20% of the cocaine to cocaethylene.

Minor metabolites of cocaethylene have been reported by Smith [17] in the urine of human subjects who used high doses of cocaine. The metabolites were identified as the ethyl esters of arylhydroxy- and arylhydroxymethoxycocaine. Identification was based on comparison of full-scan mass spectra and relative GC retention time data to authentic standards.

3.4. Cocaine metabolites

A variety of metabolites are produced when cocaine is administered to humans (see Fig. 2). The isolation of these metabolites from biological media is more difficult than cocaine due to their greater water solubility. Extraction recoveries generally follow the trend: cocaine > norcocaine ≈ benzoylecgonine > ecgonine methyl ester.

3.4.1. Benzoylecgonine

Benzoylecgonine is the primary metabolite found in blood and urine after cocaine administration. It can be extracted either by liquid–liquid extraction or SPE techniques. Graas and Watson [35] utilized an extractive alkylation solvent, methylene chloride and 1-iodopropane, to extract benzoylecgonine from urine samples with a reported recovery of 70%. Joern [36] adapted this extraction procedure for the confirmation of benzoylecgonine in human urine. The analysis was performed by GC–MS in the SIM mode. The limit of detection of the original method was extended to 35 ng/ml by Joern [36] by use of lower standard curve concentrations and summing SIM peak-area responses. Mule and Casella [37] also used SIM to confirm benzoylecgonine in urine following immunoassay screening. They used chloroform–isopropanol (9:1) as the extraction solvent and obtained a 65% recovery. Benzoylecgonine was derivatized with pentafluoropropionic anhydride. The method was sensitive to 50 ng/ml benzoylecgonine with a 0.2-ml sample volume. Weiss and Gawin [38] also used GC–MS to confirm benzoylecgonine in urine of heavily dependent cocaine users who were in withdrawal. Urine specimens were positive (300 ng/ml cut-off) for cocaine metabolite for up to 22 days in one individual who had a ten-year history of cocaine use and who had smoked up to 30 g of cocaine a day for a year prior to his admission to the clinic.

Confirmation of benzoylecgonine in human urine by GC–MS following ingestion of "Health Inca Tea" was reported by ElSohly *et al.* [39] and Jackson *et al.* [40]. Both authors reported peak urinary concentrations exceeding 1.0 mg/l following ingestion of tea prepared from one tea bag. In the Jackson *et al.* study [40], the four subjects who had consumed the tea were positive for benzoylecgonine at a cut-off of 300 ng/ml for 21 to 26 h.

Dermal application of 5 mg of cocaine hydrochloride or cocaine free base to human subjects resulted in excretion of small amounts of benzoylecgonine in urine. Baselt *et al.* [41] reported finding a total of 58 μg of benzoylecgonine, representing 1.2% of the applied dose excreted in urine over a period of 96 h.

3.4.2. Ecgonine methyl ester

A second major metabolite of cocaine excreted in urine is ecgonine methyl ester. Ambre and coworkers [42,43] developed a full scan GC–MS procedure for the quantitative determination of ecgonine methyl ester in urine. Samples were extracted with methylene chloride–isopropanol (3:1) providing a 51% extraction efficiency for ecgonine methyl ester and 89% for cocaine. Analysis of urine specimens collected after intravenous and intranasal administration of cocaine indicated that ecgonine methyl ester accounted for 26 to 60% of the cocaine dose.

3.4.3. Norcocaine

Norcocaine is a minor metabolite of cocaine and traces are excreted in urine following cocaine administration. Recently, norcocaine also was identified as a trace contaminant in illicit cocaine exhibits and in coca leaves. Full scan mass spectra confirmed the identity of norcocaine at trace levels of approximately 1% in seized samples [44].

3.4.4. Miscellaneous metabolites

A variety of metabolites have been reported for cocaine over the last decade. Smith *et al.* [45,46] obtained urine from human subjects who were chronic users or overdose cases. Arylhydroxy metabolites and hydroxymethoxybenzoylmethylecgonine metabolites of cocaine were identified by GC–MS. Identification was based on comparison of full scan mass spectra and relative GC retention data of extracted metabolites to that of authentic standards. Jindal and Lutz [47,48] described the identification of twelve metabolites of cocaine by GC–MS using stable isotope-labeled cocaine analogues and ion cluster techniques to facilitate metabolite identification. The artificially created isotopic clusters greatly simplified the mass spectral identification of cocaine metabolites in complex biological matrix backgrounds. The identified metabolites arose from five distinct metabolic processes: (1) hydrolysis; (2) N-demethylation; (3) aryl hydroxylation and/or aryl epoxidation followed by hydrolysis, conjugation and alkylation; (4) dehydrobenzoylation; and (5) N-oxidation and/or demethylation followed by N-oxidation [48].

3.5. Simultaneous cocaine and metabolite assays

The need for simultaneous measurement of drugs and metabolites is of vital importance in the assay of biological materials where there is a scarcity of sample or in cases where a potent drug is involved. Clinical and forensic studies of cocaine easily fit into this category. There may be only 1 ml (or 1 g) or less of specimen containing cocaine and multiple metabolites. Because of cocaine's potency, the concentration may vary from low nanogram to microgram amounts. It is essential in these circumstances to develop a sensitive assay which measures multiple analytes.

3.5.1. Urine and blood

Two recent procedures have been reported for the determination of cocaine and benzoylecgonine in urine [49,50]. Both procedures employed SPE and derivatization of benzoylecgonine as the trimethylsilyl ester. In addition, one of the procedures utilized a totally automated robotic system for extraction, derivatization and preparation for GC–MS analysis. Mule and Casella [51] simplified an earlier GC–MS assay [37] which involved separate procedures for benzoylecgonine and ecgonine methyl ester. The new assay measured cocaine, benzoylecgonine and ecgonine methyl ester simultaneously in urine. The procedure employed a liquid–liquid extraction with chloroform–isopropanol (9:1) and derivatization with pentafluoropropionic anhydride and pentafluoropropanol. Recoveries of 82, 76 and 70% efficiency were obtained for cocaine, benzoylecgonine and ecgonine methyl ester, respectively. Isenschmid *et al.* [52] also reported a method for the simultaneous analysis of cocaine, benzoylecgonine and ecgonine methyl ester; however, the method employed a single-extraction procedure followed by division of the extract into two portions. The separated extracts were evaporated and derivatized independently for benzoylecgonine and for ecgonine methyl ester. Matsubara *et al.* [53] reported a SIM CI (isobutane) method for

the determination of cocaine, benzoylecgonine and ecgonine methyl ester in urine. The method employed a separate extraction procedure for ecgonine methyl ester. Both extractions were performed with an Extrelut column (diatomaceous earth). Recoveries were 95, 81 and 97%, respectively, for cocaine, benzoylecgonine and ecgonine methyl ester. Sensitivities of the method were 1 ng/ml for cocaine and benzoylecgonine and 10 ng/ml for ecgonine methyl ester. Both ecgonine methyl ester and benzoylecgonine were identified as major metabolites in animal experiments.

A qualitative GC–MS method utilizing an ion trap detector was developed for the identification of multiple metabolites by Zhang and Foltz [54]. They identified cocaine and eleven metabolites, four of which were reported for the first time: ecgonidine, norecgonidine methyl ester, norecgonine methyl ester and *m*-hydroxybenzoylecgonine. The structures of the new metabolites were identified by comparison of their GC retention times and their electron-impact (EI) and CI mass spectra with those of synthesized standards. The authors reported that use of hexafluoroisopropanol and pentafluoropropionic anhydride was more advantageous than the use of silylation reagents in providing spectra that were easier to interpret. In addition, the CI mass spectra provided valuable supporting information in establishing the molecular mass of the new metabolites. The identifications also were greatly facilitated by the sensitivity of the ion trap detector.

In an unusual approach, Goenechea *et al.* [55] hydrolyzed cocaine, benzoylecgonine and ecgonine methyl ester to ecgonine with sodium hydroxide solution. Extraction was performed with a cation/anion-exchange resin. Ecgonine was silylated and analyzed qualitatively by GC–MS. The method had a detection limit of 20 ng/ml for the combined analytes in urine.

3.5.2. Postmortem tissues

Several methods have been reported for the determination of cocaine and metabolite(s) in postmortem tissues. Chinn *et al.* [56] measured cocaine, benzoylecgonine and norcocaine in blood, liver, urine, gastric contents and vitreous humor by GC–MS in the CI mode. Cocaine and norcocaine were determined in a separate assay from benzoylecgonine. The assay had a sensitivity limit of approximately 5 ng/ml. Griesemer *et al.* [57] also reported a method for determination of cocaine and benzoylecgonine in postmortem tissues. The procedure utilized chloroform–ethanol (9:1) as the extraction solvent. Benzoylecgonine was subsequently converted to the ethyl derivative, cocaethylene, by esterification. Recovery was 91% for cocaine and 65% for benzoylecgonine. Spiehler and Reed [58] used GC MS to determine cocaine and benzoylecgonine in brain, blood and liver tissues of toxic overdose cases and cases in which cocaine was incidental to the cause of death. Cocaine and benzoylecgonine were quantitated by coextraction and formation of the propyl derivative. Findings indicated that in cocaine overdose cases the brain/blood mean ratio for cocaine was 9.6; in incidental cases, the mean was 2.5, indicating that this ratio could be useful in distinguishing between overdose cases and incidental cocaine use.

3.5.3. Hair

The analysis of hair for cocaine and metabolites presents a challenging problem to the analyst because of the uniqueness of specimen material and the lack of information on disposition of drugs in hair. Hair is composed primarily of protein, and must be chemically treated or dissolved to remove drug residue. The use of GC–MS for the analysis has become essential because of the small amount of cocaine and metabolites present in hair specimens.

Two recent methods have been reported for the simultaneous determination of cocaine and metabolites in hair. Harkey *et al.* [59] developed a GC CI-MS method for the determination of cocaine, benzoylecgonine and ecgonine methyl ester in human hair. Samples were digested enzymatically, extracted by SPE and derivatized as silyl derivatives. Recoveries of cocaine, benzoylecgonine and ecgonine methyl ester were essentially quantitative. The analysis was performed with an ion trap detector operating in the full-scan mode. Detection limits of 0.1 ng/mg were

obtained for cocaine and benzoylecgonine and 0.5 ng/mg for ecgonine methyl ester with a signal-to-noise ratio ≥ 3. The method had a reported useful range for measurement of cocaine in hair of 0.1 to 100 ng/mg. Cone *et al.* [33] reported a GC–MS method for the determination of cocaine, benzoylecgonine, ecgonine methyl ester, norcocaine, cocaethylene, norcocaethylene and benzoylnorecgonine in human hair. Hair samples were extracted with 0.05 *M* sulfuric acid. Hair extracts were further purified by SPE and silyl derivatives were formed. The assay had a detection limit of approximately 0.1 ng/mg with a 50-mg hair sample. Analysis of cocaine users' hair indicated that cocaine is the primary analyte found in hair. Amounts of analyte found in hair averaged 10.8 ng/mg for cocaine and 1.2 ng/mg for benzoylecgonine ($n = 10$). Ecgonine methyl ester ranged from 0.6 to 1.9 ng/mg. Norcocaine was detected in four samples in amounts ranging from 0.5 to 0.7 ng/mg and cocaethylene was measured in six samples in amounts ranging from 0.4 to 2.6 ng/mg of hair.

4. OPIATES AND METABOLITES

4.1. Heroin and metabolites

4.1.1. Biotransformation and stability

The detailed review by Way and Adler [60] on the metabolism of opiates identified four major routes of metabolism of morphine and its surrogates: N-dealkylation, O-dealkylation, conjugation and hydrolysis. Although all of these pathways are operative in the metabolism of heroin resulting in the excretion of numerous minor metabolites [61], the primary routes of metabolism are hydrolysis and conjugation (Fig. 3). Like cocaine, heroin undergoes rapid esterase hydrolysis in blood [62,63] and spontaneous hydrolysis in aqueous solution [64,65]. At physiological pH (pH 7.4), heroin was shown to spontaneously hydrolyze to 6-acetylmorphine without further conversion to morphine. The chemical and enzymatic instability of heroin accounts for its extremely short half-life of approximately 2–7 min [66,67]. 6-Acetylmorphine also has a short half-life as a result of rapid metabolic hydrolysis. As a result, the assay of heroin and 6-acetylmorphine in biological fluids and tissues is difficult. Most assay procedures have focused upon the identification and measurement of morphine and conjugated morphine because of their abundance and stability in biological media.

4.1.2. Heroin

Although heroin has been identified in biological fluids by GC [68] and high-performance liquid chromatography (HPLC) [66] there has been only one report of identification in biological materials by GC–MS. Goldberger *et al.* [69] reported identification and measurement of heroin, 6-acetylmorphine, morphine and codeine in the hair of twenty documented heroin users. The hair samples were extracted with methanol, followed by liquid–liquid purification (toluene–heptane–isoamyl alcohol, 7:2:1, pH 8.4) and derivatization as the trifluoroacetate derivative. Heroin and 6-acetylmorphine were identified by comparison of their full-scan mass spectra to authentic standards. Quantitation was performed by SIM utilizing deuterated internal standards for 6-acetylmorphine, morphine and codeine. The limit of sensitivity for the assay for each analyte was approximately 0.05 ng/mg (100-mg sample). Heroin was detected in seven samples in concentrations ranging from 0.23 to 15.46 ng/mg and 6-acetylmorphine was found in all specimens in concentrations ranging from traces to 67.1 ng/mg.

4.1.3. 6-Acetylmorphine

The extraction of 6-acetylmorphine and other opiates from biological fluids can be accomplished by liquid–liquid extraction or SPE methods. Vu-Duc and Vernay [70] reported superior performance by SPE with a hydrophobic, cation-exchange column (Clean Screen DAU, Chemical Technologies, Horsham, PA, USA) than with a C_{18} column or by liquid–liquid extraction.

Several methods have been reported for the identification and measurement of 6-acetylmorphine in biological fluids. Schuberth and Schuberth [71] reported a GC–MS method for the determination of 6-acetylmorphine, morphine and

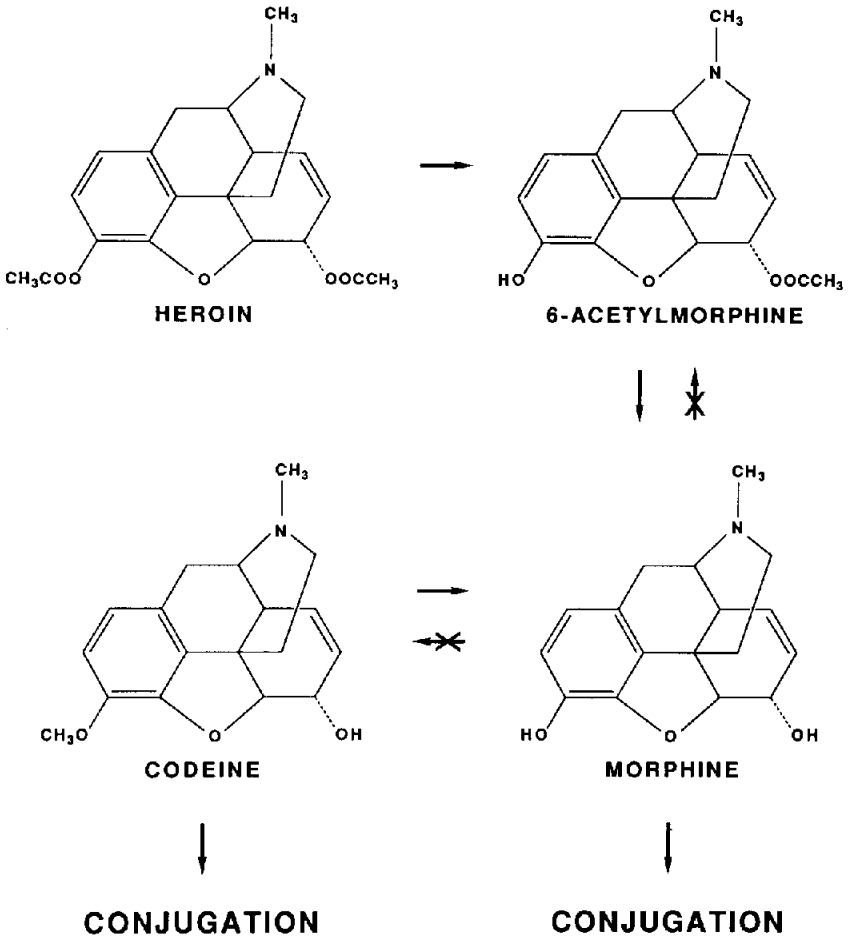


Fig. 3. Biotransformation of heroin in humans.

codeine in blood. The method employed an SPE system with a C_{18} column. Analytes were derivatized with pentafluoropropionic anhydride. Recoveries were 68% for morphine and >90% for the other analytes. Weitz *et al.* [72] used GC–MS in both the CI and EI modes of ionization to identify endogenous 6-acetylmorphine in mammalian brain. Extracts were purified by HPLC and derivatized with trifluoroacetic anhydride. A close match of the EI and CI spectra was obtained with bovine brain extracts. The authors speculate that 6-acetylmorphine is derived in tissues by O-acylation of endogenous morphine.

With the advent of widespread urine testing in the USA, numerous methods have been developed for the confirmation of 6-acetylmorphine in urine. Table 2 provides a summary of the methods targeted specifically for this purpose. One of the key methods that is used by the United States Navy was reported by Paul *et al.* [73] who developed a GC–MS SIM method for 6-acetylmorphine. Specimens were initially extracted with 10% isobutanol in methylene chloride followed by further purification with acid–base/organic extraction or SPE (LC-CN columns). Recoveries were approximately 80% by liquid–liquid extraction and >90% by the SPE method. Derivatives were prepared by treatment with propionic anhydride. The limit of detection of the method was 0.81 ng/ml. Cone *et al.* [74] utilized this methodology to monitor the excretion of 6-acetylmorphine in human urine following the administra-

TABLE 2

GC MS METHODS FOR THE ANALYSIS OF 6-ACETYLMORPHINE IN URINE

Analyte(s)	Ref.	Extraction	Efficiency	Derivative	Detection	Limit of detection (ng/ml)
6-Acetylmorphine	73	Methylene chloride–10% isobutanol, followed by acid–base or SPE (LC-CN)	Liquid–liquid, 80%; SPE, 92–95%	Propionic anhydride	SIM-EI	0.81
6-Acetylmorphine	76	SPE (C_{18})	Not reported	Pentafluoro-propionic anhydride	SIM-EI	2
6-Acetylmorphine, morphine and codeine	77	Chloroform–isopropanol (9:1)	>95%	[$^2H_6$]Acetic anhydride	SIM-EI	10
6-Acetylmorphine, morphine, codeine and heroin	78	Chloroform–isopropanol–*n*-heptane (50:17:33)	81.6%	Trifluoroacetic anhydride	SIM-EI	2
6-Acetylmorphine and morphine	79	Chloroform–isopropanol (9:1)	Not reported	Trimethylsilyl	SIM-EI	10

tion of single doses of heroin. They found that 6-acetylmorphine was excreted rapidly with an average half-life of 0.6 h. This resulted in a very short detection time of 2 to 8 h. Romberg and Brown [75] subsequently reported an improvement on the methodology of Paul *et al.* [73] by replacing the SPE purification step with a back-extraction into pH 1.0 sodium acetate solution followed by an alkaline extraction into 10% isobutanol in methylene chloride. Compared to the SPE method, the method provided improvement in 6-acetylmorphine recovery and, importantly, elimination of extraneous peaks when analyzed by GC–MS.

4.2. Codeine and morphine

Codeine is metabolized in humans by conjugation and by demethylation to morphine (Fig. 3); however, the reverse pathway leading to the production of codeine from morphine does not occur [80]. Both codeine and morphine are generally found in biological fluids after codeine ingestion. This results in the need to assay both codeine and morphine simultaneously. Co-extraction of codeine and morphine can be problematic due to the amphoteric nature of morphine. Recently, development and use of cation exchange SPE columns have greatly improved the efficiency of multiple analyte extraction [20], thus providing recoveries of 96% for morphine and 99% for codeine.

The poor chromatographic characteristics of underivatized morphine analogues necessitate production of stable derivatives. Chen *et al.* [81] evaluated five derivatizing agents for analysis of codeine and morphine by GC–MS and found that the acetyl derivative exhibited the greatest stability of those examined. Acetyl derivatives of codeine and morphine were stable for up to 24 h after preparation without significant hydrolysis.

The stability of the acetyl derivatives of codeine and morphine also was demonstrated by Paul *et al.* [82] who developed a simultaneous assay for these analytes in urine. The extraction was performed with methylene chloride–isobuta-

nol (9:1), followed by acid–base extraction and re-extraction with organic solvent. Recoveries were 58% for codeine and 40% for morphine. The on-column sensitivity for both compounds was 2 ng at a signal-to-noise ratio of 5:1. This procedure was subsequently used by Cone *et al.* [83] to monitor the urinary excretion of free and conjugated codeine and morphine in human subjects following the intramuscular administration of 60- and 120-mg doses of codeine. Urinary data were reported on free and total codeine and morphine levels for periods up to 120 h after codeine administration. Mule and Casella [37] and Wu Chen *et al.* [84] also reported simultaneous assays for codeine and morphine by GC–MS SIM. Both procedures utilized liquid–liquid extractions for the isolation of codeine and morphine from urine and other biological fluids. The sensitivities of the procedures were approximately 1 ng on-column.

Donnerer *et al.* [85] measured endogenous morphine and codeine in arthritic rats' spinal cords. They hydrolyzed tissues in 0.01 *M* hydrochloric acid and performed a prepurification step by SPE followed by HPLC purification. The extracts were prepared as the trifluoroacetyl derivatives and analyzed by GC–MS in the full-scan mode. Good correlation was obtained between the GC–MS method and quantitative analysis by radioimmunoassay (RIA).

Analysis of codeine and/or morphine in hair by various GC–MS techniques has been reported. Pelli *et al.* [86] reported a highly sensitive identification of morphine in hair of heroin addicts by collisional spectroscopy. Hair samples were extracted with 0.1 *M* hydrochloric acid, followed by re-extraction with an organic solvent. Analysis was performed by direct introduction of the residue dissolved in methanol into the ion source. The method had a limit of detection of 1–10 fg, with a signal-to-noise ratio >5. Other investigators have used GC–MS for the determination of both codeine and morphine [87,88] in hair and have concluded that it is possible to distinguish between heroin and codeine addicts by GC–MS analysis, but caution against the use of less specific techniques like RIA.

A number of methods have been developed for assay of morphine in biological specimens. Drost *et al.* [89] reported a GC–MS SIM method with CI, ammonia–methane (1:5), for the determination of free and hydrolyzed morphine in serum and cerebrospinal fluid. Specimens were extracted by SPE and prepared as silyl derivatives. Recovery of morphine at a concentration of 10 ng/ml was 90%. The method was used for determination of the pharmacokinetics of morphine after epidural administration to human subjects undergoing abdominal surgery [90]. Spiehler *et al.* [91] also reported a method for the determination of free and hydrolyzed morphine in blood. Postmortem samples were extracted by organic solvent and derivatized with trifluoroacetic anhydride. Quantitative values obtained by GC–MS for free morphine were found to be comparable to those obtained by RIA analysis. Jones *et al.* [92] developed a highly sensitive method for morphine in urine and body organs. Two liquid–liquid extraction methods were used and the extracts were derivatized with pentafluoropropionic anhydride. The authors compared the use of packed columns with fused-silica capillary columns and found the sensitivity of the assay was increased about ten-fold through the use of capillary columns. Fuller *et al.* [93] also used liquid–liquid extraction for the determination of morphine in specific regions of rat brain. Morphine levels were measured by GC–MS SIM with methane CI. The method provided evidence for dose-dependent accumulation of morphine in different brain regions following incremental morphine administration.

The use of GC–MS for detection of illicit drugs in urine led to the discovery that poppy seed products contain significant amounts of morphine and codeine. A number of studies [94–101] have shown that individuals who ingest poppy seeds in foodstuffs can produce detectable levels of morphine and codeine in urine and blood. The mode of analysis and the maximum concentrations of morphine and codeine measured in poppy seeds and in urine and blood are summarized in Table 3. Typically, concentrations of drug reached a maximum within 2–4 h of poppy seed ingestion and declined over a period of 24–48 h

TABLE 3

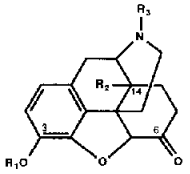
GC–MS ANALYSIS OF POPPY SEED PRODUCTS AND BIOLOGICAL SPECIMENS AFTER POPPY SEED INGESTION

Detection mode	Ref.	Derivative	Maximum morphine/codeine concentrations in seeds/paste	Poppy seed dose (g)	Biological specimen
SIM-EI	94	Pentafluoro-propionic anhydride	1 mg morphine/10 g	100	Urine, 305 ng/ml morphine
EI full scan	95	Silyl	0.2 mg morphine/g	150	Urine, 18 000 ng/ml morphine by GC assay
SIM-EI	96	Acetic anhydride	0.96 mg morphine/g, 0.08 mg codeine/g	5	Urine, 2797 ng/ml morphine, 214 ng/ml codeine
SIM-EI	97	Trifluoroacetic anhydride	Not reported	Not reported	Urine, 1481 ng/ml morphine, 75 ng/ml codeine
Not reported	98	Not reported	18.6 μg morphine/g, 2.5 μg codeine/g	70	Urine, 3.3 μg/ml morphine
SIM-EI	99	Trifluoroacetic anhydride	294 μg morphine/g, 14 μg codeine/g	40	Urine, 2635 μg/l morphine, 45 μg/l codeine; blood, 131 μg/l morphine
SIM-EI	100	Acetic anhydride	106 μg morphine/g, 3.8 μg codeine/g	200	Urine, 860 ng/ml morphine, 358 ng/ml codeine
SIM-EI	101	Silyl	108 μg morphine/g	15	Urine, 2010 ng/ml morphine, 78 ng/ml codeine

to the level of detection of the assay. The studies invariably point out that the confirmed presence of morphine and codeine in urine by GC–MS does not necessarily indicate heroin abuse.

4.3. 6-Keto-opioids

The 6-keto-opioids constitute a group of semi-synthetic compounds chemically related to morphine. As shown in Fig. 4, their prominent structural feature is a 6-keto substituent. Other substitutions occur at C-3, C-14 and the N-substituent. Those drugs with substitutions at C-3 and C-14 are potent analgesic substances. In contrast, substitution of the N-methyl group with a cyclopropylmethyl or allyl group produces an opioid antagonist with no analgesic activity. Despite the



Drug	R_1	R_2	R_3
Hydromorphone	H-	H-	$-CH_3$
Hydrocodone	CH_3-	H-	$-CH_3$
Oxymorphone	H-	HO-	$-CH_3$
Oxycodone	CH_3-	HO-	$-CH_3$
Naloxone	H-	HO-	$-CH_2CH{=}CH_2$
Naltrexone	CH_3-	HO-	$-CH_2CH(CH_2)_2$

Fig. 4. Structures of 6-keto-opioids.

highly varied clinical uses of the 6-keto-opioids, there has been only limited GC–MS assay development work with these compounds. Cone and co-workers [102,103] developed a multi-purpose assay for opiates with SIM operating in the methane CI mode. The assay was developed for measurement of morphine, codeine, hydrocodone, hydromorphone and eight potential metabolites in biological fluids. A variety of organic solvents were tested for extraction efficiency; chloroform–isopropanol (9:1) provided the best overall recoveries for the twelve analytes. SIM assays also have been reported for oxycodone [104] and oxymorphone [105] in urine of different animal species. Methane CI was instrumental in these assays in providing high-molecular-mass fragment ions for monitoring. The limit of detection for oxymorphone and oxycodone was approximately 20 ng/ml. Recently, a highly sensitive SIM assay with negative ion CI was developed for naltrexone and its major metabolite, 6β-naltrexol [106]. Plasma and urine samples were extracted with an organic solvent and derivatized prior to analysis. The derivatization process was performed in two steps. The first process involved conversion of the 6-keto group of naltrexone to an N-oxime with methoxyamine; this step was followed by derivatization with pentafluoropropionic anhydride. The lower level of quantitation was 0.1 ng/ml with a precision of ±20%. Application of the method to the measurement of naltrexone and 6β-naltrexol in blood following a subcutaneous depot implant of naltrexone indicated that blood levels were consistently in the 0.1–1.0 ng/ml range for a period of up to 33 days after depot implantation.

4.4. Buprenorphine

Buprenorphine is a synthetic derivative of thebaine with potent mixed agonist-antagonist analgesic properties. It is readily absorbed and metabolized in humans to an active metabolite, norbuprenorphine, and to inactive conjugates (Fig. 5). Cone *et al.*, [107] analyzed urine and feces following subcutaneous, sublingual and oral buprenorphine administration by GC–CI-MS (positive- and negative-ion modes) and found a pre-

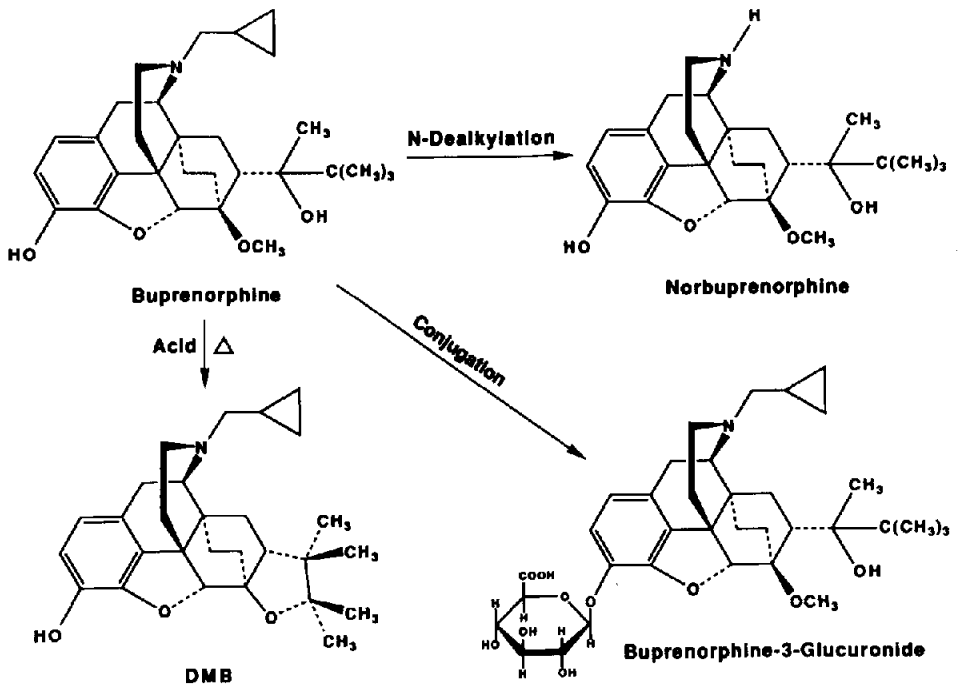


Fig. 5. Metabolism and rearrangement of buprenorphine.

ponderance of the norbuprenorphine metabolite over the parent drug in all biological specimens. Cone *et al.*, [108] also determined by GC–MS that buprenorphine and norbuprenorphine are unstable in the presence of acid and heat and undergo rearrangement with loss of a molecule of methanol to form new compounds. An example of the rearrangement for buprenorphine to DMB (demethoxybuprenorphine) is shown in Fig. 5. The new compounds are the result of formation of a tetrahydrofuran ring system via condensation of the two side-chain substituents. This rearrangement substantially alters and improves the chromatographic characteristics of buprenorphine. Blom *et al.* [109] took advantage of the improved chromatographic characteristics of the rearrangement products in the design of a sensitive SIM assay for buprenorphine and norbuprenorphine in plasma. Samples were extracted with an organic solvent, toluene 2-butanol, at pH 9.4 and back-extracted in dilute sulfuric acid. The acid solution was heated at 110°C yielding the rearrangement products. The sample then was extracted with an organic solvent, evaporated and derivatized with pentafluoropropionic anhydride. The lower limit of the SIM assay was 150 pg/ml. This assay was suitable for monitoring the disposition of buprenorphine and norbuprenorphine in plasma for 24 h and for more than seven days in urine. Recently, a SIM CI (isobutane) method was reported for measurement of buprenorphine and norbuprenorphine in plasma by Ohtani *et al.* [110]. The method consisted of extraction with an organic solvent followed by derivatization with pentafluoropropionic anhydride at room temperature, thus avoiding rearrangement. The lower limit of this assay was 200 pg/ml for both analytes. The assay had coefficients of variation for within- and between-run precision of 3–5.7%.

4.5. *Simultaneous opiate assays*

Although there can be substantial structural variation in opiates and metabolites found in biological specimens, many can be be isolated by simple extraction techniques. Generally, the use of liquid–liquid extraction requires modification of the polarity of the organic solvent with alcohol and adjustment of the aqueous phase to a basic pH range. Kintz *et al.* [111] reported that morphine, codeine, heroin, 3- and 6-acetylmorphine, nalorphine, naloxone, ethylmorphine and naltrexone could be extracted from plasma with chloroform–isopropanol–*n*-heptane (50:17:33) at pH 9.2, followed by back-extraction with dilute acid and re-extraction with chloroform. Recoveries were in the range 65.5–81.6%. Cone *et al.* [103] determined the effect of different concentrations of alcohol (isopropanol) in chloroform upon recovery of codeine, morphine and ten potential metabolites. Although chloroform without isopropanol provided highest recoveries for the codeine-related analogues (greater lipid solubility), the morphine-related compounds were optimally extracted with chloroform–isopropanol in ratios ranging from 95:5 to 4:1. In a separate study, Cone *et al.* [112] reported the optimal pH for recovery of eight opium alkaloids (morphine, codeine, normorphine, norcodeine, noscapine, thebaine, papaverine and oripavine) to be 9.5 when using methylene chloride–isopropanol as the extraction solvent.

The simultaneous extraction and analysis of multiple opiate analytes requires attention to recovery efficiencies and to chromatographic resolution. Cone *et al.* [103] analyzed codeine, morphine and ten additional analytes by SIM under methane CI conditions by means of a Silar-5CP packed column. However, in several cases, resolution was insufficient and an additional column phase had to be used to differentiate isomers. In a similar study of opium alkaloids and metabolites in urine of opium eaters, Cone *et al.* [112] measured eight opium alkaloids with an OV-210-packed column and SIM methane CI detection. Although resolution was not complete, the specificity of the detector was sufficient to overcome lack of resolution.

A rapid, simultaneous assay of morphine, codeine and hydromorphone in blood using a one-step extraction procedure was reported by Saady *et al.* [113]. The samples were extracted with toluene–hexane–isoamyl alcohol (78:20:2) at pH 9.9.

Recoveries were between 55 and 60% and limits of sensitivity were 20 ng/ml for morphine and codeine and 80 ng/ml for hydromorphone.

Procedures for the determination of 6-acetylmorphine, morphine, codeine and other opiate analytes have been reported by Bowie and Kirkpatrick [114] and Sticht *et al.* [115]. Both methods employed liquid–liquid extraction and analysis by fused-silica capillary column GC–MS. The methods were designed primarily for the detection of 6-acetylmorphine in urine, thus allowing differentiation of illicit heroin abuse from licit morphine or codeine use.

GC–MS also has been reported to be useful in the qualitative identification of opium components found in Asian folk remedies. Smith and Nelsen [116] used Bond-Elut Certify columns for opiate extraction and SIM for analysis. Significant amounts of 3-acetylmorphine, 6-acetylmorphine, 6-acetylcodeine and heroin were identified leading to the conclusion that these preparations consisted of mixtures of opium with herbal medicines. The authors speculated that partial morphine acetylation could have occurred as a result of heating opium in the presence of aspirin and acetaminophen during the preparation and/or use of these home-made remedies.

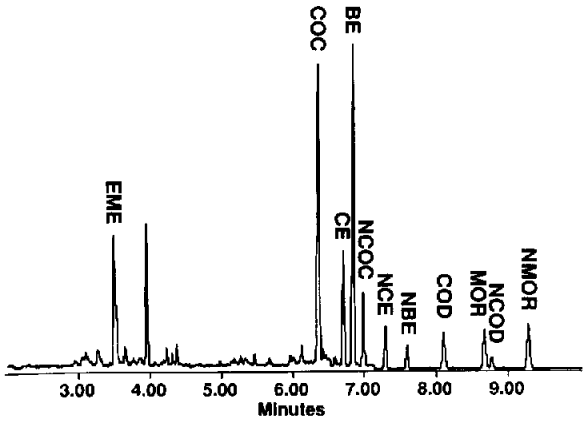


Fig. 6. Total ion current chromatogram (SIM mode) of cocaine, opiates and metabolites extracted from human hair. Abbreviations are as follows: EME = ecgonine methyl ester; COC = cocaine; CE = cocaethylene; BE = benzoylecgonine; NCOC = norcocaine; NCE = norcocaethylene; NBE = Benzoylnorecgonine; COD = codeine; MOR = morphine; NCOD = norcodeine; and NMOR = normorphine.

5. SIMULTANEOUS ASSAY OF COCAINE AND OPIATES

Very little work has been reported on the simultaneous determination of cocaine and opiates. Recently, Cone *et al.* [33] reported a method for the simultaneous determination of cocaine, benzoylecgonine, ecgonine methyl ester, norcocaine, cocaethylene, norcocaethylene and benzoylnorecgonine in hair samples. The extraction method utilized SPE cartridges packed with a copolymeric phase material. This cartridge also was found to be amenable for extraction of opiates under the same conditions utilized in the cocaine determinations. Subsequently, the method was extended to a simultaneous assay for cocaine, cocaine metabolites, morphine, codeine and opiate metabolites [117]. Fig. 6 illustrates the GC MS total ion current chromatogram of a hair extract which contained known amounts of cocaine, morphine, codeine and metabolites. Drug standards and deuterated internal standards were added to drug-free control hair in concentrations representing therapeutic levels. The sample was extracted with acid followed by neutralization and extraction by SPE. The extract was treated with a silyl-derivatizing reagent and analyzed by GC–MS in the SIM mode. Baseline resolution was obtained for all analytes. Detector response was linear across a broad concentration range. Fig. 7 illustrates SIM chromatograms of a hair extract from a drug user who had a documented history of simultaneous heroin and cocaine abuse. The sample was analyzed in the same manner as described for Fig. 6. Responses are clearly evident for cocaine, cocaethylene, benzoylecgonine, norcocaine, codeine, morphine and normorphine. The advantages of simultaneous analyses for drugs of abuse are exemplified in this hair analysis where there was only a small amount of biological material available for assay.

6. CONCLUSIONS

Opiate and cocaine abuse continues to represent significant health problems for much of modern society. As a result, analytical chemists and toxicologists are frequently called upon to

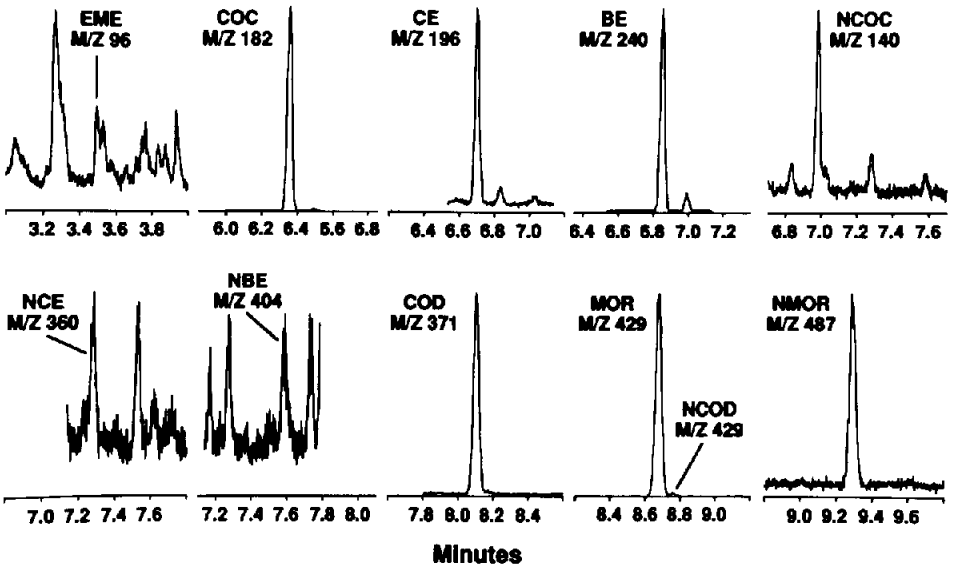


Fig. 7. Selected ion monitoring for cocaine and opiates in human hair of a documented heroin/cocaine abuser. Abbreviations are defined in Fig. 6.

analyze biological specimens and drug seizures for the presence of parent drug and breakdown products. The variety of metabolites originating from biotransformation and degradation processes make this a formidable problem. The use of GC–MS for the identification and measurement of drugs of abuse is currently believed to be crucial to acceptance of evidence in legal proceedings because of its sensitivity and specificity [3].

Presently, a variety of GC–MS assays are available for the determination of cocaine, opiates and metabolites, although many methods focus only upon detection of the administered drug and/or metabolite(s). However, the number of methods for the simultaneous determination of parent drug and metabolite is growing. This trend is facilitated by the development of multipurpose SPE columns that allow isolation and purification of multiple analytes of substantially different chemical structures. Improvements in extraction techniques are being coupled with improvements in chromatographic resolution gained through use of capillary column GC and with improvements in the sensitivity and specificity of MS detection. These combinations ultimately will result in the development of highly efficient assays for the sensitive determination of multiple cocaine and opiate analytes in biological specimens by GC–MS.

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