# II
# New Trends in Alkaloid Isolation and Structure Elucidation

*Modern Alkaloids: Structure, Isolation, Synthesis and Biology.* Edited by E. Fattorusso and O. Taglialatela-Scafati

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# 12
# Analysis of Tropane Alkaloids in Biological Matrices

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## 12.1
## Introduction

Tropane alkaloids are an important class of structurally related natural products, having in common the azabicyclo[3.2.1]octane skeleton. It is now well established that the tropane ring system derives its pyrrolidine ring from ornithine and/or arginine [1] and that *N*-methyl-$\Delta^1$-pyrrolinium salt is the common intermediate (Figure 12.1). The majority of these alkaloids are mono-, di-, and tri-esters of hydroxytropanes with various organic acids, such as tropic, atropic, cinnamic, angelic, tiglic, senecioic, isovaleric, and truxillic acids. Hyoscyamine and scopolamine (hyoscine) are important representatives of this class of alkaloids. They are ester derivatives of tropane-3α-ol and its 6–7 epoxide with tropic acid, respectively (Figure 12.1).

Tropane alkaloids are commonly found in genera belonging to three families: Solanaceae, Erythroxylaceae, and Convolvulaceae, but they occur also sporadically in a number of other families including Proteaceae and Rhizophoraceae [2]. To date, more than 200 tropane alkaloids have been isolated from biological material [1]. The natural form of hyoscyamine is the (−)-form. (−)-Hyoscyamine is easily racemized, yielding atropine. Dimeric (e.g. schizanthines) and trimeric (grahamine) tropane alkaloids have also been found [3]. There is considerable structural diversity within this class of compounds. Another important tropane alkaloid, cocaine, occurs in the genus *Erythroxylum*. It is an ester of ecgonine, a derivative of tropane-3β-ol carrying a carboxyl goup at C-2 (Figure 12.1). Recently, the calystegines, a new type of *nor*tropane alkaloids, have been structurally characterized. A typical feature of calystegines is a hydroxyl group on the bridgehead carbon 1 (Figure 12.1). According to the number of hydroxyl groups on the *nor*tropane skeleton (3, 4, or 5), they belong to the series A, B, or C, respectively. To date, 15 calystegines have been identified and, in contrast to most other tropane alkaloids, they are not esterified. They were first identified in *Calystegia sepium*, Convolvulaceae [4], and later detected in numerous other Convolvulaceae [5], Solanaceae, Moraceae [6], Erythroxylaceae [7], and Brassicaceae [8]. Particularly high concentrations are found in potato skins and eggplants.

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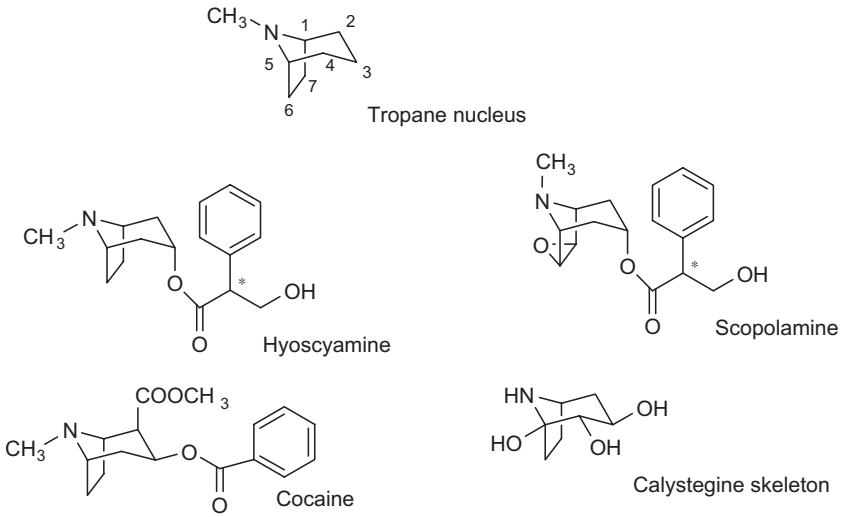


**Fig. 12.1** Tropane alkaloids of pharmaceutical interest.

From a pharmacological point of view, tropane alkaloids show antimuscarinic activity (parasympathetic inhibition), which at the peripheral level translates into antispasmodic effects on the gastrointestinal and genitourinary systems. Atropinic drugs dilate the pupil (mydriasis) and result in a loss of accommodation (cycloplegia). In terms of its effects on the central nervous system, atropine can prevent vagal reflexes induced by the manipulation of visceral organs. Scopolamine is used to prevent nausea and vomiting caused by motion sickness. Cocaine is the parent compound of local anesthetic; however, its use is limited because cocaine has stimulatory effects on the central nervous system. To achieve the latter effect, leaves can be chewed or pure cocaine sniffed or injected.

Semisynthetic derivatives have also been commercialized. These compounds are quaternary derivatives that have no side effects on the central nervous system. They are used against spasm of the bladder or of the intestine and to treat bronchospasm associated with chronic obstructive pulmonary disease.

Owing to their structural similarity with monosaccharides, calystegines exhibit potent inhibitory activities against glycosidases. Thus, they are of considerable interest as potential antiviral, anticancer, and antidiabetic agents. The pharmacological properties of these compounds have been comprehensively reviewed [9,6].

Among the main toxic plants responsible for human deaths throughout the world, those producing alkaloids and, in particular, containing tropane alkaloids (e.g. *Atropa belladonna, Datura* sp., and *Hyoscyamus niger*) are of significant importance [10]. Therefore, it remains critical to have rapid and accurate analytical methods for the identification and quantification of tropane alkaloids in plants or in biological fluids.

In this review, plant material and numerous biological fluids, mainly blood plasma and serum, urine, and saliva, as well as some alternative matrices such as bile, amniotic fluid, cord blood, meconium, and maternal hair among others will be

considered. The tropane alkaloids of interest reported in this review are cocaine and its metabolites, hyoscyamine, as well as its racemate atropine, scopolamine and their derivatives. Calystegines and other polyhydroxylated compounds will not be further discussed in this chapter.

## 12.2 Extraction

### 12.2.1 Plant Material

The classical method for the extraction of tropane alkaloids is based on the Stas–Otto procedure. In this protocol, the alkaloids are transferred to either aqueous or organic layers simply by changing the pH of the two-phase system: the salts are soluble in water whereas the free bases are soluble in organic solvents. Chloroform is frequently used in tropane alkaloid extraction and analysis. El Jaber-Vazdekis *et al.* [11] have demonstrated that dichloromethane, a solvent less hazardous than chloroform, can advantageously replace the latter for the extraction of tropane alkaloids with a similar eluotropic value. Classically, extraction of tropane alkaloids is carried out using percolation, maceration, digestion, decoction, and extraction using a Soxhlet apparatus [12]. These techniques have been used for many decades. However, they are very time-consuming and require relatively large quantities of polluting solvents. Furthermore, some problems may arise using some of these procedures or solvents. Prolonged treatment with strong alkali leads to hydrolysis or racemization of hyoscyamine to atropine. Diethyl ether should be avoided not only because of its volatility and flammability but also because of its tendency to form alkaloid *N*-oxides in the presence of peroxides [13].

Another major drawback of classical extractions is that additional clean-up procedures are frequently required before chromatographic analyses. Solid phase extraction (SPE) avoids the emulsion problems often encountered in liquid–liquid extraction. A wide range of adsorbents are commercially available and may be divided into three classes: polar, ion-exchange, and nonpolar adsorbents. Solid-supported liquid-liquid extraction on Extrelut columns is frequently reported for efficient clean-up of crude tropane alkaloid mixtures. Basified aqueous solutions of alkaloids may be transferred to Extrelut columns and the bases recovered in dichloromethane–isopropanol mixture [13].

### 12.2.2 Supercritical Fluid Extraction

Supercritical fluid extraction (SFE) has become a method of choice for the extraction of plant material [14]. It represents an interesting alternative technique compared to conventional liquid–solid extraction, with lower solvent consumption and working temperature. The free bases of hyoscyamine and scopolamine are extractable with

pure supercritical $CO_2$, but the addition of alkaline modifiers such as methanol basified with diethylamine is necessary to extract the salts of hyoscyamine and scopolamine from plant material [15]. Chemometry was used to optimize supercritical fluid extraction of hyoscyamine and scopolamine from hairy root culture of *Datura candida* × *D. aurea* [16]. A polar modifier was required in order to extract hyoscyamine and scopolamine quantitatively. The optimal conditions were 20 % methanol in $CO_2$ at a pressure of 15 MPa and 85 °C. Extracted amounts were in good agreement with those obtained by traditional liquid–solid extraction.

Cocaine has been extracted from coca leaves and the optimization procedure was investigated by means of a central composite design [17]. Pressure, temperature, nature, and percentage of polar modifier were studied. A rate of 2 mL/min $CO_2$ modified by the addition of 29 % water in methanol at 20 MPa for 10 min allowed the quantitative extraction of cocaine. The robustness of the method was evaluated by drawing response surfaces. The same compound has also been extracted by SFE from hair samples [18–20].

### 12.2.3 Microwave-assisted Extraction

The use of microwave energy as a heating source in analytical laboratories started in the 1970s and was applied to acid digestions [21]. Later, the use of microwave-assisted extraction was reported by Ganzler [22,23]. The fundamental principles of microwave energy for digestion, extraction, and desorption have been reviewed by Zlotorzynski [24]. With the wide availability of microwaves ovens, this type of heating system has been introduced into many analytical laboratories [25]. Microwave heating depends on the presence of polar molecules or ionic species. Furthermore, the chosen solvent should absorb the microwaves without leading to strong heating so as to avoid degradation of the compounds of interest. Disruption of hydrogen bonds, resulting from dipole rotation of molecules, and migration of dissolved ions facilitate the penetration of solvent molecules into the matrix and allow the solvation of extracted components [26]. Heating with microwaves is instantaneous and occurs in the center of the sample, leading to homogenous, very fast extractions. Two technologies are available: either closed vessels, under controlled pressure and temperature, also called pressurized microwave-assisted extraction (PMAE) or open vessels, also called focused microwave-assisted extraction (FMAE) at atmospheric pressure [27]. Most of the papers dealing with microwave energy have been dedicated to the extraction of pesticides and organometallic compounds from environmental matrices, and very few applications have been published which concern the extraction of alkaloids. In particular, very little is reported on the microwave-assisted extraction of tropane alkaloids. Cocaine and benzoylecgonine have been extracted from coca leaves by FMAE [28]. Several parameters including the nature of the extracting solvent, the particle size distribution, the sample moisture, the applied microwave power, and the radiation time were studied by means of a central composite design. Bieri *et al.* [29] analyzed cocaine distribution in 51 wild *Erythroxylum* species. Extraction was performed using FMAE on 100 mg of hydrated plant material with 5 mL methanol

for 30 s. After filtration, the samples were analyzed by GC-MS without further purification.

### 12.2.4 Pressurized Solvent Extraction

Pressurized solvent extraction (PSE), also called pressurized fluid extraction (PFE), accelerated solvent extraction (ASE®), pressurized liquid extraction (PLE), or enhanced solvent extraction (ESE), is a solid–liquid extraction that has been developed as an alternative to conventional extractions such as Soxhlet, maceration, percolation, or reflux. It uses organic solvents at high pressure and temperature to increase the efficiency of the extraction process. Increased temperature decreases the viscosity of the liquid solvent, enhances its diffusivity, and accelerates the extraction kinetics. High pressure keeps the solvent in its liquid state and thus facilitates its penetration into the matrix, resulting in increase extraction speed [30]. PSE was applied to the rapid extraction of cocaine and benzoylecgonine from coca leaves [31]. Several parameters including the nature of the extracting solvent, the pressure, temperature, extraction, addition of alkaline substances, and sample granulometry were investigated. Critical parameters were pressure, temperature, and extraction time. They were optimized by means of a central composite design. It was demonstrated that an extraction time of 10 min was sufficient to extract cocaine quantitatively at 80 °C and 20 MPa.

### 12.2.5 Solid-phase Microextraction

Solid-phase microextraction (SPME) was introduced by Arthur and Pawliszyn in 1990 [32]. It is a simple, solvent-free preparation method. It can be conducted as a direct extraction in which the coated fiber is immersed in the liquid sample or in a headspace configuration for sampling the volatiles from the headspace above the liquid sample placed in a vial. The SPME process consists of two steps: (a) the sorbent is exposed to the sample for a specified period of time; (b) the sorbent is transferred to a device that interfaces with an analytical instrument for thermal desorption using GC or for solvent desorption with HPLC. SPME has become a widely used technique in many areas of analytical chemistry, such as food analysis, environmental sampling, and biological analysis. The state-of-the-art of SPME including recent developments and future challenges has been comprehensively reviewed by O'Reilly *et al.* [33]. Reports on alkaloid analysis by SPME are still scarce and mainly concern cocaine in biological fluids [34,35] and human hairs [36]. SPME has been evaluated as an alternative injection technique in combination with fast GC to carry out a quantitative determination of cocaine first extracted from coca leaves by FMAE [37]. A 7 μm PDMS fiber allowed an extraction time of 2 min and a very short desorption time of 12 s for the compounds of interest (cocaine and cocaethylene as internal standard).

An effective combination of focused microwave-assisted extraction with solid-phase microextraction (FMAE-SPME) was carried out for the extraction of cocaine

from coca leaves prior to GC analysis [38]. SPME was performed in the direct immersion mode with a 100 μm polydimethylsiloxane coated fiber. A significant gain in selectivity was obtained with the incorporation of SPME in the extraction procedure. Therefore, the analysis time was reduced to 6 min compared to 35 min with conventional GC. A comparison of extraction methods, namely ultrasonic bath, hot solvent under reflux, and pressurized liquid extraction applied to the extraction of hyoscyamine, scopolamine, and other related alkaloids has been published [39]. A mixed-mode reversed phase cation-exchange SPE was optimized for simultaneous recovery of (−)-hyoscyamine, scopolamine, and scopolamine *N*-oxide from various *Datura* species. The alkaloids were qualitatively and quantitatively analyzed by HPTLC-densitometry without derivatization and compared with reversed phase high-performance liquid chromatography with diode array detection (RP-HPLC-DAD). Another densitometric method for the analysis of hyoscyamine and scopolamine in different solanaceous plants and hairy roots has been reported by Berkov and Pavlov [40].

### 12.2.6 Biological Matrices

The analytical process can be divided into four major steps: sample preparation, separation, detection, and data treatment. For the analysis of drugs and metabolites in biological matrices, sample preparation remains the most challenging task since the compounds of interest are often present at trace level in a complex matrix containing a large number of biomolecules (e.g. proteins) and other substances, such as salts.

Different procedures can be used for the preparation of biological matrices prior to separation and detection techniques as a function of the selectivity and sensitivity of the latter as well as of the matrix complexity. However, for liquid samples, conventional methods are generally carried out: simple dilution/filtration and injection (dilute and shoot), protein precipitation (PP), liquid–liquid extraction (LLE), and solid phase extraction (SPE). These techniques present advantages and drawbacks; the final choice depending on several criteria, among them: analyte concentration, selected analytical method, nature of the matrix, number of samples, time delivery, ease of automation, and so on. They can be performed manually as well as automatically in conventional and high-throughput modes. Comprehensive textbooks have been published on this topic in bioanalysis [41,42]. For solid biological matrices such as tissues or hair, a preliminary extraction with an organic solvent is performed followed by a purification step (e.g. LLE and SPE), if needed.

Tropane alkaloids are basic compounds, generally water soluble at acidic pH, while their unionized form (basic pH) is more soluble in apolar organic solvents. Therefore, LLE has been largely used to extract these compounds from biological fluids after addition of sodium carbonate, sodium borate, or ammonia to attain a pH $\geq$ 10 [43,44]. SPE has also been used for extracting tropane alkaloids from biological matrices. Some advantages exist for SPE methods versus LLE, including the absence of emulsion, fewer tedious tasks, lower solvent consumption, and possible automation in both off-line and on-line modes. For this purpose, conventional cartridges

packed with reversed phase materials (e.g. C8, C18, and other polymeric phases) have been extensively employed [45]. Other supports such as the hydrophilic–lipophilic water-wettable reversed phase sorbent Oasis HLB (Waters, Milford, MA, USA), commercialized in 1996, has gained considerable interest for the extraction of basic drugs, for example cocaine and metabolites. This kind of support presents a high retention capacity of polar analytes due to its ''polar-hook.'' In order to enhance selectivity and sensitivity for basic compounds, a different water-wettable polymeric sorbent (Oasis MCX, Waters) was introduced in 1999. It provides a dual mode of retention with strong cation-exchange and reversed phase mechanisms. These sorbents are commercialized under different formats (syringe barrel cartridges, 96-well extraction plates, microelution plates, and on-line columns) permitting the selection of the method of choice as a function of the number of samples to be analyzed, the sample volume available, the sensitivity required, and so on. A great number of generic procedures have been developed for the analysis of cocaine and its metabolites in urine, serum, plasma, whole blood, among others, since cocaine abuse has increased dramatically during the last decades. Scopolamine, and its internal standard atropine, were also extracted from human serum with a simple and automated SPE on Oasis HLB before liquid chromatography tandem mass spectrometry analysis [46].

Another possibility for increasing selectivity during the extraction process is the use of immuno techniques based on molecular recognition. An excellent review was published on this subject in 2003 by Hennion and Pichon [47] and the reader is invited to study this comprehensive review, and references therein, for more information. Immunoaffinity extraction sorbents, also called immunosorbents (ISs), contain antibodies that can retain an antigen with high affinity and selectivity. Several ISs have been used for extracting drugs, hormones, peptides, and other large biomolecules in environmental and biological matrices. However, the major drawbacks of ISs are their long development time and their cost. Therefore, synthetic antibodies or plastic antibodies, called molecularly imprinted polymers (MIPs), have been described and their use as solid phase extraction materials reviewed [48]. In this case, the molecular imprinting is the synthesis of highly crossed-linked resins in the presence of a given molecule of interest. After washing, the molecule is eliminated and the polymer contains cavities with the appropriate size and shape. Thus, the MIP can be applied to selectively bind the molecule and analogs in different matrices. Using this strategy, Nakamura *et al.* [49] developed a uniformly sized MIP for atropine. This selective sorbent was applied for determining atropine and scopolamine in pharmaceutical preparations containing *Scopolia* extracts. A column-switching procedure was used with the MIP as a precolumn and a conventional cation exchanger as the analytical column. The automated method was validated and showed good performances in terms of linearity, recoveries, and precision.

Microdialysis has also been used as a sampling method for measuring the concentration of drugs in human subcutaneous tissues for pharmacokinetic studies [50]. The microdialysates are simpler than other biological fluids and do not contain proteins, permitting their direct injection for analysis by liquid chromatography.

However, the presence of nonvolatile salts in the microdialysate can cause some contamination (after several injections) of the mass spectrometer, for instance. Thus, the ionization source needs frequent cleaning. Microdialysis combined with LC-MS/MS quantitation has been reported for scopolamine [46] for concentrations ranging from 50 pg/mL to 10 ng/mL. Free cocaine and benzoylecgonine were also investigated in rat brain with *in vivo* microdialysis [51] with off-line LC-MS analysis. Monitoring cocaine and its metabolites is of prime importance for understanding its effects in the brain. Recently, the same kinds of methods were used to determine unbound cocaine in blood, brain, and bile of rats with LC-UV and LC-MS/MS [52].

As previously mentioned, besides conventional matrices such as urine and blood, alternative matrices have become of great interest in toxicology. Different reviews describe the analysis of drugs of abuse in saliva, sweat, and hair [53–56]. For conventional matrices, LLE and SPE are usually the methods of choice. However, for hair analysis, a more drastic extraction step is necessary initially, followed by a purification step [57].

## 12.3 Analysis of Plant Material and Biological Matrices

Current methods for tropane alkaloids analysis have been well covered in the literature. An excellent comprehensive review written by B. Dräger [45] appeared in 2002, describing the analysis of tropane and related alkaloids in plant material. Sample preparation procedures were reviewed, as well as the analytical methods used for performing the separation and detection of tropane alkaloids, such as gas chromatography (GC), liquid chromatography (LC), and capillary electrophoresis (CE). Therefore, this chapter will not describe in detail these well-known analytical methods but discuss some recently developed applications for the analysis of tropane alkaloids in plant material and biological matrices.

### 12.3.1 Gas Chromatography

The isolation of atropine, scopolamine, and cocaine occurred long before the development of modern analytical techniques. Gas chromatography was the first instrumental technique available in the field of separation science and thus it is not surprising that these alkaloids were firstly analyzed by GC despite their low volatility. With the advent of capillary columns and the proliferation of various sample introduction and detection methods, GC has evolved as the dominant analytical technique for screening, identification, and quantitation of tropane alkaloids of plant origin as well as in biological fluids. The state-of-the-art of GC analysis of tropane alkaloids has been the subject of two comprehensive reviews [45,58]. We shall therefore mainly focus on publications which have appeared since 2002.

Currently, rapid and unambiguous identification of tropane alkaloids is routinely accomplished by GC-MS by ''fingerprint matching,'' comparing the MS spectra with

those available for authentic compounds. The fragmentation of tropane alkaloids is very well known and may help in the tentative identification of unknown derivatives. A fundamental parameter for identification in GC is the reproducibility of retention data. Supporting this is the basic concept of the retention index system, introduced by Kováts for isothermal conditions and by Van den Dool and Kratz for linear programmed temperatures. Incorporation of retention indices in peak identification criteria strongly assists and complements MS matches or alternative confirmatory detection techniques such as Fourier transform infrared spectroscopy (FTIR) or element-specific detectors. Today, with more reproducible column manufacturing technologies and the development of electronic gas flow controllers, instead of relative retention-based identification, a new software called retention time locking (RTL) developed by Agilent Technologies allows reliable identification from absolute retention time values. This software enables the chromatographic system to be adjusted so that the retention times of analytes remain constant even after routine maintenance procedures, column replacement, column shortening, and method transference from instrument to instrument. The use of RTL has been evaluated by Savchuk *et al.* [59] during the development of a unified procedure for the detection of drugs (including cocaine) in biological fluids. The high precision obtained with RTL has also been pointed out by Rasanen *et al.* [60], who prefer to use absolute retention time instead of the more laborious retention index system during toxicological screening for drugs.

Finally, the development of fast GC [61–63] and comprehensive two-dimensional GC (GC × GC) [64–66] address the continuous demand for increased speed and separation power in routine analysis. The former technique allows a dramatic reduction in analysis time without sacrificing resolution, while the latter offers a markedly increased separation power without altering the analysis time. A fast GC method for the analysis of cocaine and other drugs of forensic relevance has been published by Williams *et al.* [67]. They used a GC instrument in which the column was resistively heated at rates of up to 30°/s which allowed separation of 19 compounds within 1.5 min. A GC × GC time-of-flight mass spectrometry (TOF-MS) method has been proposed by Song *et al.* [68] for the analysis of a mixture of 78 drugs of interest, including cocaine and benztropine.

As part of a comparative study, roots, leaves, and seeds of three varieties of *Datura stramonium* L., namely var. *stramonium*, *tatula*, and *godronii* were investigated by GC-MS for their tropane alkaloid pattern [69]. In total, 25 alkaloids were directly detected in these varieties. The identification was based upon electron impact ionization MS data from commercially available libraries or from literature. Hyoscyamine and scopolamine were detected in all plant organs of the three *Datura stramonium* varieties grown in Bulgaria but not in the roots, seeds or leaves from the Egyptian variety *stramonium*. Among the chromatographically separated tropane alkaloids, some showed superimposable mass spectra and were accordingly determined as isomeric series. The difficulty of unambiguously distinguishing tiglioyl from its isomeric angeloyl or senecioyl derivatives based upon mass spectral information only is worth emphasizing. Thus, identification by GC-MS of such tropane derivatives without referring to authentic compounds should be considered with care and regarded as tentative only.

Similarly, screening of 12 different species and their varieties belonging to the tribe Datureae has been reported [70]. GC-MS investigation permitted on-line identification of 66 tropane alkaloids from crude leaf and root extracts using no more than 300 mg of plant material. Many of the alkaloids were described for the first time in the corresponding species, and their identification was based on their fragmentation pathways. One new tropane alkaloid was reported as well; however, its stereochemistry could obviously not be assigned.

Kartal *et al.* have discussed the quantitative analysis of hyoscyamine in *Hyoscyamus reticulatus* L., a plant growing in east Anatolia [71].

GC-MS analysis of the tropane content of shoots from *in vitro* regenerated plantlets from *Schizanthus hookeri* [72] allowed the detection of ten alkaloids ranging from simple pyrrolidine derivatives to tropane esters derived from angelic, tiglic, senecioic, or methylmesaconic acids. One of them, 3α-methylmesaconyloxytropane, is a new alkaloid. Its structure was deduced by comparing mass spectral data and retention indices with those of a synthetic reference compound. To date, the fastest GC analysis of tropane alkaloids dealt with the separation of isomeric secondary metabolites from the stem-bark of *Schizanthus grahamii* [73]. This study presents a systematic investigation of very fast GC applied for the baseline separation of a series of four hydroxytropane esters. Theoretical and practical relationships were used in the optimization steps, including selection of stationary phase, temperature, internal column diameter, and optimal practical gas velocity. This work provided a challenging application for isothermal analysis in conjunction with very short, narrow-bore columns. The investigated approach allowed a baseline separation of the alkaloids of interest in less than 9 s (Figure 12.2).

Even though sample preparation is usually the most time-consuming step in natural product research, this particular case study demonstrated that phytochemical investigations can positively benefit from fast GC methods to reduce the overall analysis time.

The usefulness of GC-MS analysis for biosynthetic studies was demonstrated by Patterson and O'Hagan [74] in their investigation of the conversion of littorine to hyoscyamine after feeding transformed root cultures of *Datura stramonium* with deuterium-labeled phenyllactic acids. This study complements previous investigations on the biosynthesis of the tropate ester moiety of hyoscyamine and scopolamine [75], where GC-MS played a key role. It also has general relevance in the biosynthetic pathway of tropane alkaloids in the entire plant kingdom [76].

Gas chromatography of cocaine of plant origin has mainly involved the analysis of the coca plant [77–79]. Identification and quantitation GC methods of minor naturally occurring tropane alkaloids in illicit cocaine samples have also been reviewed [80]. Moore *et al.* presented an in-depth methodology for the analysis of the coca plant by GC-FID, GC-ECD, and GC-MS for the identification of alkaloids of unknown structure [81]. Recently, Casale *et al.* [82] have analyzed the seeds from *Erythroxylum coca* for their alkaloidal content. Several tropane alkaloids were detected and characterized and it appeared that methylecgonidine (MEG) was the primary constituent and not an analytical artifact.

Thus far, the genus *Erythroxylum* and, more particularly, the coca plant, represented by the cultivated species *Erythroxylum coca* and *Erythroxylum novogranatense,*

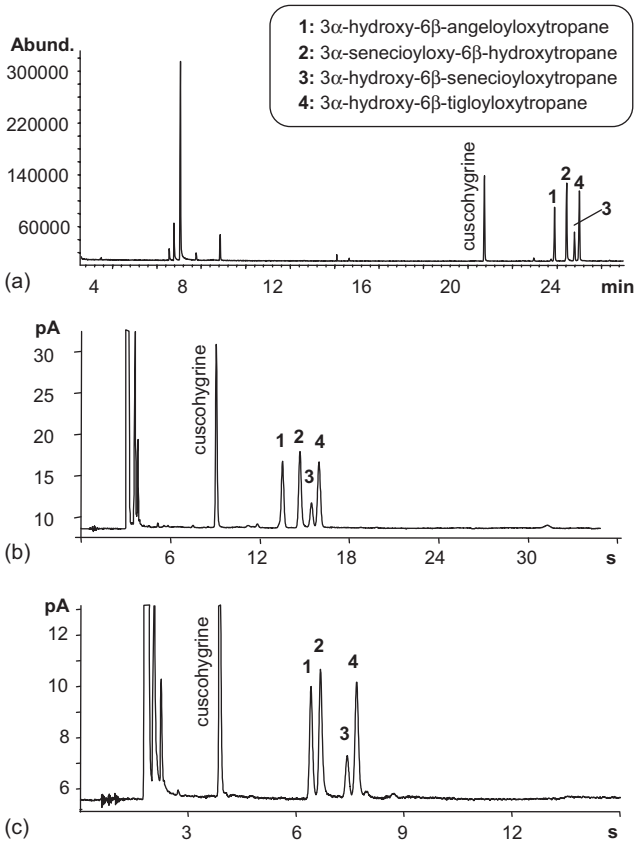


**Fig. 12.2** Separation of four isomeric tropane alkaloids by GC: (a) Analysis in linear programmed temperature GC-MS using a conventional 30 m × 0.25 mm i.d. × 0.25 μm film thickness HP5-MS column. (b) Fast GC separation on a 3 m × 0.1 mm i.d. × 0.1 μm DB5 column. (c) 1.5 m × 0.05 mm i.d. × 0.05 μm microbore BGB-1701 column operating at an average linear gas velocity ($H_2$) of 150 cm/s.

is the only natural source of cocaine. However, little attention has been paid to noncultivated *Erythroxylum* species for the possible presence of cocaine.

During a screening of tropane alkaloids in *Erythroxylum* species from Southern Brazil, Zuanazzi *et al.* [83] identified a new alkaloid as 3β,6β-ditigloyloxynortropane. The five investigated species were also screened for MEG, tropacocaine, and cocaine. Tropacocaine and MEG were present in two plants but no cocaine was detected in any species.

Leaf samples of various *Erythroxylum* species have been investigated for their cocaine content by a fast GC-MS method [29]. Amongst the 51 analyzed species, 28 had not been examined previously and cocaine was detected in 23 wild species. Cocaine content was less than 0.001 % for all wild species, except for *Erythroxylum laetevirens* in which a 10-fold higher concentration was determined. The qualitative

chromatographic profile of the latter species was very similar to that of cultivated coca species. Moreover, GC profiles and quantitative results showed that the so-called ''Mate de coca,'' was mainly composed of unadulterated coca leaves. This result agrees with previous GC-MS investigations on the cocaine content in coca tea [84,85].

An effective combination of FMAE and SPME to enhance selectivity for the quantitative GC analysis of cocaine in leaves of *E. coca* has been proposed by Bieri *et al.* [38]. The dual extraction step greatly improved the selectivity, thus allowing much faster GC-FID analysis. Finally, by optimizing the desorption step after SPME sampling and by using a fast GC method, Ilias *et al.* [37] were able to complete a quantitative cocaine analysis from coca leaves (i.e. sample preparation, extraction, and chromatography) in less than 1 h (Figure 12.3).

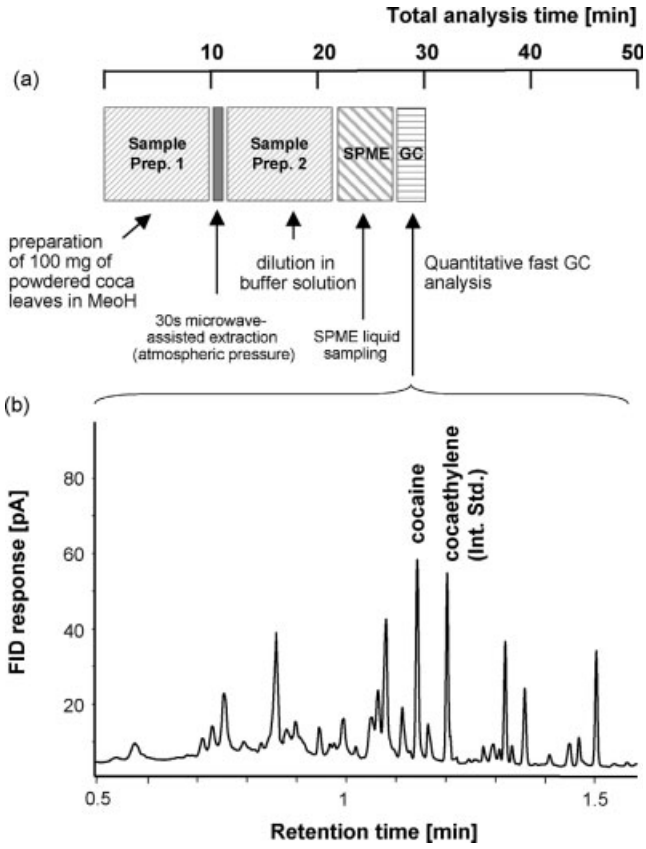


**Fig. 12.3** Fast cocaine determination in coca leaves by GC according to Ilias *et al.* (adapted from [37]). (a) Schematic of the total analysis time. (b) Fast GC-FID chromatogram using a short 100 μm i.d. column and a fast oven temperature programming.

Gas chromatography is an established separation method of major importance in forensic sciences. In particular, GC-MS is one of the most frequently used techniques in toxicology. The increasing incidence and variety of ''drugs of abuse'' have resulted in a growing demand for rapid and universal screening methods for the analysis of biological matrices. In particular, identification and quantification of cocaine in biological fluids and tissues are of great importance. A large range of GC methods have been developed for the analysis of cocaine and its main metabolites in biological fluids. A critical review of chromatographic procedures for testing hair sample for drugs has been published by Sachs and Kintz [86]. An exhaustive review concerning GC-MS analysis of body fluids for drugs of abuse has been written by Cody and Foltz [87]. Owing to the limited sensitivity of the flame ionization detector (FID), some reports pointed out the effectiveness of the element-sensitive nitrogen–phosphorus detector (NPD) [88,89] or electron capture detector (ECD) [90].

A GC-MS method was developed for the determination of hyoscyamine and scopolamine in blood serum [91,92]. Extraction was carried out using aqueous basic solution followed by a purification step on an Extrelut column. Derivatization was done with *N,O*-bis(trimethylsilyl)trifluoroacetamide/trimethylchlorosilane (99 : 1). GC-MS was performed on a HP-5 MS column (30 m $\times$ 0.25 mm i.d. with a 0.25 $\mu$m film thickness). The linearity was good between 10 and 5000 ng/mL. The limit of detection (LOD) was 5 ng/mL for each compound.

SPE and LLE are considered the methods of choice for preparing biological samples before a GC analysis of cocaine and metabolites. Farina *et al.* [43] have developed a simple, rapid, and sensitive method for determining cocaine in urine with a single-step LLE and using GC with NPD. A mean extraction recovery of 74 % was reported and the limits of detection and quantitation were 5 and 20 ng/mL, respectively.

Stir-bar sorptive extraction (SBSE) [93] with polydimethylsiloxane sorbent followed by thermal desorption–capillary gas chromatography–mass spectrometry has been used for the detection of drugs of abuse in biological fluids [94]. The following biological fluids were investigated: urine, blood, bile, and stomach content. The method was developed for 34 drugs, including cocaine. The GC-MS data were plotted in a contour plot with locked retention time on the *x*-axis and ion traces on the *y*-axis. Target solutes were identified by a spot in specific positions in the plot and the color of the spots was related to peak abundances. Semiquantitative information was readily obtained from the contour plots while precise quantification required calibration procedures.

A rapid, automated procedure for single-step and simultaneous extraction of cocaine and 11 related compounds was developed by Lewis *et al.* [95]. Fluid and tissue specimens were extracted using an automated SPE system (Zymark Rapid-Trace) with a Bond Elute-Certify I cartridge. Samples were derivatized with pentafluoropropionic anhydride/2,2,3,3,3-pentafluoro-1-propanol prior to GC-MS analysis. The method allowed differentiation between smoking crack and intranasal/intravenous cocaine use and was able to elucidate whether ethanol and cocaine were used simultaneously. Another way to determine cocaine in biological matrices is to measure its metabolites and/or degradation products. MEG is produced when

cocaine base is smoked and ecgonidine (EC) is a hydrolytic metabolite of MEG that has been identified in the urine of crack smokers. These compounds can be used as biomarkers to differentiate smoking from other routes of administration. A GC-MS method was developed by Scheidweiler *et al.* [96] to analyze MEG and EC in blood samples. The two compounds were extracted from plasma by SPE after methanol precipitation of proteins. The method was linear between 20 and 2500 μg/L for MEG and between 30 and 3000 μg/L for EC. The limit of detection was 10 μg/L.

Saliva is used increasingly as a matrix of choice for the detection of illicit drugs. The advantages of saliva over traditional fluids are that collection is almost noninvasive, easy to perform and can be achieved under close supervision to prevent adulteration or substitution of the samples. Saliva can be extracted and analyzed in the same manner as other biological fluids. In general, there is less interference from endogenous compounds than with blood or urine. The cocaine concentration in saliva, stored in a plastic container without the addition of citric acid or other stabilizers, remains unaltered at −4 °C for 1 week. Generally, cocaine and its metabolites can be extracted from saliva using a simple SPE procedure in acetate buffer at pH 4.0 [56]. Similarly, Campora *et al.* [97] developed an analytical method for the simultaneous determination of cocaine and its major metabolites, ecgonine methyl ester, and benzoylecgonine in the saliva of chronic cocaine users. The method involved LLE, derivatization with (99 : 1) BSTFA/TMCS, and GC-CIMS in positive ion mode using a single quadrupole detector with the appropriate deuterated standards. Chromatographic elution performed using a 12 m × 0.20 mm i.d. column, 0.33 μm film thickness of 5 % phenyl-methylsiloxane, and temperature programming. Selected ions were monitored, in SIM mode, for each compound studied. Samyn *et al.* [98] also used some alternative matrices, such as saliva and sweat for the detection of drugs of abuse in drivers. Cocaine was determined by GC-MS and the positive predictive values of saliva and sweat wipe were 92 % and 90 %, respectively.

Teske *et al.* [99] evaluated a programmed-temperature vaporizing injection for GC-MS determination of different drugs, among them cocaine, in biological fluids (blood, saliva, etc.). This method reduced the sample consumption (50 μL) and possessed good sensitivity.

Hair is also frequently a useful matrix for drug testing because drugs can be detected for a longer period than in blood or urine. A critical review of chromatographic procedures for drug testing of hair samples has been published by Sachs and Kintz [86]. Gruszecki *et al.* [100] reported the detection of cocaine and cocaethylene in postmortem biological specimens. Hair samples were washed successively at 37 °C with methanol and with phosphate buffer at pH 6.0 and dried. The powdered material was then refluxed in methanol. After filtration, the extract was evaporated to dryness, reconstituted in chloroform and analyzed by GC-MS on an HP I capillary column (50 m × 0.2 mm i.d., 0.1 μm film thickness). The MSD was operated in the SIM mode with a limit of detection for both cocaine and cocaethylene of 5 ng/mL.

It is also interesting to note that cocaine can be determined in amniotic fluid, cord blood, infant urine, meconium, and maternal hair to detect prenatal cocaine use [101]. GC-MS was performed after an appropriate extraction procedure and 51 of 115

subjects were positive for cocaine metabolites. Urine was most frequently positive in identified users, followed by hair.

### 12.3.2 High-performance Liquid Chromatography

The analysis of tropane alkaloids by liquid chromatography has been performed for over 30 years. The first high-performance liquid chromatography (HPLC) analysis of tropane alkaloids was published in 1973 and concerned the separation of atropine and scopolamine as well as homatropine and apoatropine [102]; the first analysis of these compounds in plant material appeared 12 years later [103].

Numerous HPLC methods for analyzing tropane alkaloids in plant material have been published and reversed phase (RP) columns appear to be the stationary phase of choice [45,58]. RP-18 is generally the preferred stationary phase, while others (RP-8, RP-6, and RP-cyano) are more rarely used. HPLC with UV detection is often employed for tropane alkaloids with UV-absorbing moieties. Gradient elution with a mixture of water–acetonitrile or water–methanol at acidic pH is generally used. A database of retention indices of 383 toxicologically relevant compounds, including some tropane alkaloids was published in 1994 [104] using gradient elution with a mixture of acetonitrile and phosphate buffer. The column was packed with a C18 stationary phase and the detection performed by a UV-diode array detector, permitting the on-line collection of UV spectra.

New generation columns made of high-purity silica with the absence or low content of metals can be endcapped to ensure low silanol activity [105] and thus prevent tailing of the alkaloids. Such columns (Luna 5 μm C-18, Phenomenex) coupled to a SPE unit (SupelClean LC-18) have been used for the simultaneous analysis of hyoscyamine, scopolamine, 6β-hydroxyhyoscyamine, and apoatropine in hairy roots of *Atropa belladonna* and of *Datura innoxia* [106]. The column was eluted with a mixture of acetonitrile, methanol, and 0.1 % TFA. Absolute LOD values were 0.6 and 0.8 ng for hyoscyamine and scopolamine, respectively. Another HPLC separation was developed to investigate the tropane alkaloid content in genetically transformed root cultures of *Atropa belladonna* [107]. After extraction with a mixture of chloroform–methanol and 25 % ammonia, the sample purification was carried out on a self-packed Extrelut column. Chromatography was performed using a Luna 5 μm C8 with an isocratic mixture of acetonitrile–phosphate buffer (pH 6.2)–methanol as eluent. The absolute LODs were 3 ng for hyoscyamine and 2.3 ng for scopolamine, with a corresponding signal-to-noise ratio of 3. A RP-HPLC-DAD method was developed and optimized for the analysis of hyoscyamine and scopolamine in 14 different leaf and seed samples of *Datura* sp. [39]. The separation was performed on an XTerra RP-18 column with gradient of acetonitrile in 15 mM ammonia solution. LODs were 0.25 and 0.29 ng/μL for hyoscyamine and scopolamine, respectively, with limits of quantification (LOQ) of 0.82 and 0.97 ng/μL. Kirchhoff *et al.* [108] developed an HPLC method without ion-pairing reagents to separate the degradation products and by-products of atropine. The separation was performed on a hydrophilic embedded RP-18 column (Thermo Hypersil Aquasil) characterized by hydrophilic endcapping. Acidic gradient elution with 20 mM phosphate

buffer–acetonitrile mixture was used. The method was applied to the atropine assay for eye drops. The short retention time of atropine of about 2 min and the baseline separation of the main degradation products are very convenient for routine analysis.

Since 1990, with the emergence of high-throughput analyses, the approach in natural products discovery has changed considerably to direct analysis of crude plant extracts with minimal sample manipulation. This approach led to the dereplication method, avoiding the tedious isolation of known or undesirable compounds [109]. Conventional detection systems used with HPLC, such as UV or fluorescence spectroscopy, provide only limited information on the molecular structure of the separated compounds. The coupling of HPLC with mass spectrometry (LC-MS, LC-$MS^n$) resulted in a powerful analytical tool for qualitative and quantitative determination of drugs and their metabolites. In particular, LC-ion-trap multiple-stage mass spectrometry (LC-IT-$MS^n$) and LC-time-of-flight mass spectrometry (LC-TOF-MS) are used to study fragmentation patterns and determine elemental formulae of analyzed compounds. However, mass spectrometry cannot provide unequivocal structural determination, particularly when no reference material is available or in the case of isomeric compounds. The complementary use of LC-NMR [110] has therefore become extremely attractive as it offers unparalleled structure elucidation capabilities. In this respect, chemical screening strategies have been developed using hyphenated techniques LC-DAD-UV, LC-MS, and LC-NMR [111] for on-line identification of natural products in crude plant extracts. Using LC-DAD-UV, LC-APCI-$MS^n$, LC-APCI-TOF-MS, and LC-NMR experiments, Zanolari *et al.* [112] identified 24 tropane alkaloids from the bark of *Erythroxylum vacciniifolium*. Among them, six new compounds were characterized. Bieri *et al.* [113] have published two fully automated LC-NMR approaches, namely loop storage and trapping, using a LC-UV-MS/SPE-NMR set-up to deal with the limited NMR sensitivity and overcome the short acquisition times during on-flow measurements. Both approaches were applied to the separation of four isomeric tropane alkaloids isolated from the stem-bark of an endemic plant from Chile, *Schizanthus grahamii* (Solanaceae). The chromatographic separation was carried out on a Hypercarb porous graphitic carbon column ($125 \times 4.6$ mm i.d., 5 μm particle size). In the loop storage approach, each peak was stored into a loop by means of a switching valve and after the separation of the individual compound, the content of each loop was transferred one at a time into the NMR flow cell and subjected to NMR spectroscopy without undesirable peak broadening (Figure 12.4a). In the second approach, LC was combined with parallel ion-trap MS detection for peak selection, and NMR spectroscopy using an SPE cartridge for postcolumn analyte trapping (Figure 12.4b). Nondeuterated solvents were used, reducing the cost and avoiding the interference in MS with the alcoholic exchangeable protons. After the trapping step, the cartridges were dried and analytes were sequentially flushed into the cryogenically cooled NMR cell with a deuterated solvent for measurements.

Analysis of tropane alkaloids in biological fluids has been developed mostly for cocaine and metabolites. Indeed, it is well recognized that cocaine remains one of the most widely consumed drugs of abuse worldwide. Generally, reversed phase liquid chromatography coupled with UV-VIS detection is employed for these analyses.

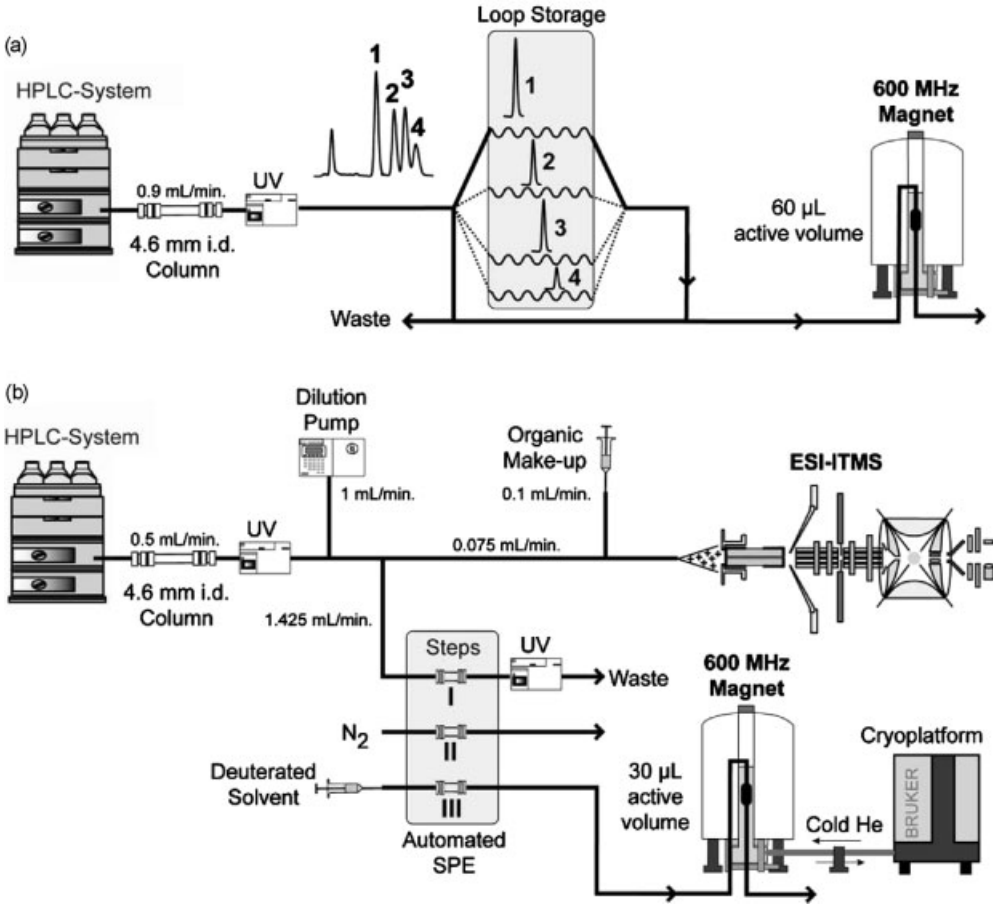


**Fig. 12.4** Instrumental set-up for the HPLC-NMR hyphenated approaches. (a) Peak sampling unit using storage loops. (b) Peak trapping onto SPE cartridges with parallel MS and cryogenically cooled NMR detection [113]. *Source:* Reproduced with permission of Wiley.

In the case of cocaine, its persistence in the blood stream is short owing to hydrolysis of the ester linkage [114]. Therefore, sensitive methods are needed for measuring cocaine in biological fluids. With UV detection, Chen *et al.* [52] investigated the disposition of unbound cocaine in rat blood, brain, and bile after microdialysis. A C18 column was used with a mobile phase containing 25 % acetonitrile (with triethylamine) and 75 % of 10 mM phosphate buffer (pH 4.0). The UV detector was set at 235 nm and the LOQ was 0.05 µg/mL. The *in vivo* recoveries were lower than 50 % owing to different factors affecting the dialysis efficiency. Thus, the method was not sensitive enough to determine cocaine in the bile dialysate and a LC-tandem mass spectrometry assay was developed instead. Raje *et al.* [44] developed a sensitive LC-UV

method for determining the benztropine analog AHN-1055 in rat plasma and brain. This compound, a novel cocaine abuse therapeutic, was first extracted by LLE and analyzed on a C18 column with a gradient profile. The mobile phase was a mixture of methanol and phosphate buffer set at pH 3.0 and detection was achieved at 220 nm. The sensitivity was sufficient (25 and 50 ppb in plasma and brain, respectively) for performance of a pharmacokinetic study. Harrison *et al.* [115] have developed a LC-UV method for determining the rapid hydrolysis of atropine by atropinesterase in plasma of dogs, goats, guinea pigs, humans, pigs, rabbits, and rhesus monkeys. The activity of this enzyme in rabbits and some plants is well described, while its presence in other animal species is very controversial. In this study, atropine and tropic acid were separated by reversed phase chromatography with a mixture of acetonitrile and phosphate buffer (pH 3.1) and detection was performed at 205 nm.

As mentioned above, UV detection is often not sensitive enough to determine low amounts of tropane alkaloids in biological fluids. Therefore, different LC methods have been published using MS and tandem MS. For this purpose an electrospray ionization (ESI) source is used, since tropane alkaloids are basic substances that can be easily protonated. Analysis of free cocaine and benzoylecgonine was performed by Fuh *et al.* [51] using a C18 column in the gradient mode with a mobile phase containing acetonitrile and acetic acid. With a single quadrupole, the authors investigated in-source collision-induced dissociation (CID) to promote fragmentation, with the skimmer voltage set at 125 V to obtain two major ions for each tested substance. Cocaine had characteristic ions at $m/z$ 304 and $m/z$ 182 and corresponding benzoylecgonine ions were seen at $m/z$ 290 and $m/z$ 168. The method was validated and applied to determine the free form of cocaine and its metabolite in rat brain following microdialysis. The same strategy was applied by Chen *et al.* [52] for the analysis of unbound cocaine in the bile fluid with a triple quadrupole MS. With the same ions (precursor at $m/z$ 304 and product ion at $m/z$ 182), the sensitivity was sufficient to determine that cocaine is excreted through the hepatobiliary system. Scopolamine and atropine, selected as internal standard, were also determined by LC-MS/MS in serum samples of volunteers after a simple and rapid SPE procedure and in microdialysates [46]. The sensitivity was excellent, with a limit of quantification of 20 pg/mL for scopolamine in serum, allowing the application of this method for conducting pharmacokinetic studies. Other authors used LC-MS/MS to analyze cocaine and its metabolite ecgonine methyl ester in human urine [116]. In this study, a nonconventional hydrophobic pentafluorophenylpropyl (PFPP) bonded silica stationary phase was used to strongly retain cocaine and its metabolite with good efficiency. A mobile phase with 90 % acetonitrile was then necessary to elute the compounds of interest, thus inducing a large enhancement of the ESI-MS signal. It is noteworthy that the MS signal of cocaine was 12 times greater with the PFPP column than with a conventional C18. Therefore, the urine was simply diluted (1 : 10) and injected directly in the LC-MS, saving time and money.

As already reported by Dräger in 2002 [45], the low UV absorption of tropane alkaloids means that other detection modes have been investigated. Besides mass spectrometry, electrochemical detection was tested by different authors, but sensitivity was not significantly improved since tropane alkaloids are only moderately

responsive compounds. Fluorescence detection can also be used to improve selectivity and sensitivity. However, tropane alkaloids are not natively fluorescent and require a prederivatization step. With 1-anthroylnitrile (1-AN) used as reagent, atropine was determined by LC-fluorescence at a concentration of 10 ng/mL [117]. Jamdar *et al.* [118] modified an analytical procedure to achieve a rapid and sensitive quantitation of cocaine and its major metabolites in plasma and urine with two analytical columns packed in series with a C8 and a cyanopropyl phase. For determining ecgonine methylester (EME) and rendering this compound UV-detectable, a derivatization was performed with 4-fluorobenzoyl chloride. Under optimized conditions, quantitation limits were 25 ng/mL for cocaine, benzoylecgonine, and norcocaine and 50 ng/mL for EME. Therefore, this method was sensitive enough to perform pharmacokinetic studies.

### 12.3.3 Capillary Electrophoresis

Capillary electrophoresis (CE) coupled with UV or MS detection has been widely used for the determination of pharmaceutical compounds because of its efficiency, accuracy, and high resolution. This separation method appears particularly interesting for the analysis of alkaloids because these compounds are easily protonated if an appropriate acidic buffer is chosen. In 1998, Stöckigt *et al.* [119] published a review in which they summarized the use of CE for the analysis of various alkaloids.

Surprisingly, in comparison with HPLC or GC methods, CE was not frequently applied to the analysis of tropane alkaloids. A capillary zone electrophoresis (CZE) method in combination with an on-column diode array detection was developed and validated for the simultaneous determination of atropine, scopolamine, and derivatives in pharmaceutical preparations [120,121] or in plant material [122]. Enantioseparation of atropine using sulfated β-cyclodextrin has been optimized by means of a central composite design. The method has been developed for the enantiomeric purity evaluation of (−)-hyoscyamine as well as for the separation of littorine (a positional isomer of hyoscyamine) from atropine enantiomers in genetically transformed root cultures of *Hyoscyamus albus* [123,124]. In plant material, hyoscyamine and scopolamine are generally present together with other tropane alkaloids with similar structures and mass-to-charge ratios. Therefore, micellar electrokinetic capillary chromatography (MEKC) is frequently found more appropriate for analysis of the alkaloids in plant extracts. Using the same set-up as CZE, MEKC only requires the addition of a surfactant at a concentration above its critical micelle concentration to the running buffer. In particular, MEKC is able to separate neutral and ionic analytes in the same run. This method was applied to the simultaneous analysis of six tropane alkaloids, including hyoscyamine and scopolamine [125]. The optimized conditions have been applied to the analysis of tropane alkaloids found in hairy roots of *Datura candida* × *D. aurea*. MEKC was selected for the quantitative determination of hyoscyamine in *Belladonna* extract [126] and for the simultaneous quantitative determination of hyoscyamine, scopolamine, and littorine in different hairy root clones of *Hyoscyamus muticus* [127] and other solanaceous plant extracts [128].

The use of nonaqueous media has gained considerable importance in the analysis of pharmaceuticals by CE. In particular, very high efficiency and resolution, short analysis time, and the possibility of increasing the analyte solubility have been demonstrated. In addition, nonaqueous media are suitable for on-line coupling to mass spectrometry. Nonaqueous capillary electrophoresis (NACE) has been developed for the separation of eight tropane alkaloids [129]. The optimized method was applied to the analysis of hyoscyamine and scopolamine in genetically transformed roots of *Datura candida* × *D. aurea* and results have been compared with those obtained by MEKC. A simple NACE method has been described for the separation of several atropine- and scopolamine-related drugs. The robustness has been verified using a full factorial design and, after validation, the method was applied for the determination of *N*-butylscopolamine in different pharmaceutical preparations [130].

The on-line coupling of CE with electrospray ionization mass spectrometry (CE-ESI-MS) allows high separation efficiency together with high sensitivity and selectivity as well as molecular structural information. A CE-UV-ESI-MS method was developed for the analysis of hoscyamine, scopolamine, and other tropane derivatives [131]. The differentiation of hyoscyamine from littorine, commonly encountered in plant material, was demonstrated using in-source collision-induced dissociation. The developed method was applied to the analysis of these alkaloids in *Belladonna* leaf extract and in *Datura candida* × *D. aurea* hairy root extract. Recently, CE coupled with electrochemiluminescence detection has been used for the determination of atropine and scopolamine in *Flos daturae* [132].

Even if capillary electrophoresis is a very powerful technique and has gained considerable interest in pharmaceutical and biomedical analysis [133], its use in the determination of tropane alkaloids in biological matrices remains very restricted. Several reasons could explain this lack of interest in CE. First, sensitivity with UV detection, even at low wavelength, is often not sufficient to determine drugs and metabolites at sub-part-per-million levels. This lack of sensitivity is mainly due to the short optical path length afforded by the small internal diameter of the capillary and to the small injected volume. Second, the injection is matrix-dependent; therefore, a sample preparation is generally required, such as a liquid–liquid extraction or a solid phase extraction, for reducing the complexity of the biological matrix. Third, the coupling of MS with CE is not yet recognized as a completely routine technique. However, several strategies can be used to overcome these drawbacks and a large number of publications have appeared since the late 1990s dealing with the quantification of drugs and metabolites in blood, urine, and other biological matrices by CE [134–138]. Presently, tropane alkaloids are only rarely analyzed by CE. In 1998, Plaut and Staub developed a micellar electrokinetic chromatography (MEKC) procedure for the determination of atropine in the presence of strychnine and tetracaine in blood and gastric contents [139]. After sample preparation by LLE, atropine was detected at the part-per-million level by UV detection with scopolamine as internal standard. Tagliaro *et al.* [140–142] used capillary electrophoresis for analyzing illicit substances, such as cocaine, in hair samples. The sample preparation was conventional, with a washing procedure followed by extraction in acidic medium overnight and purification in ready-to-use Toxi-Tubes A. The analysis was conducted at pH 9.2,

with UV detection. A field-amplified sample stacking technique was performed during the injection for improving sensitivity. The detection of concentrations of cocaine as low as 0.5 ng/mg have been reported.

CE also suffers from limited reproducibilities of compound migration times. The major reason is the variability of the electroosmotic flow (EOF). In order to overcome this drawback, capillaries can be permanently or dynamically coated. The latter is more advantageous, since the coating can be replaced and regenerated after each run. CElixir, also called CEofix, has been developed as a commercially available dynamic coating to stabilize the EOF [143] and Boone *et al*. [144] used this procedure for a systematic toxicological analysis. A set of 73 compounds was analyzed, among them atropine and cocaine. It was clearly demonstrated that a coated capillary gave better results for the identification of basic drugs in terms of higher reproducibility, identification power, and shorter analysis time than conventional CE. More recently, Alnajjar *et al*. [145] used CE with native fluorescence and laser-induced fluorescence for the separation and detection of multiple drugs of abuse in biological fluids. Cocaine was analyzed in urine after solid phase extraction and derivatization using fluorescein isothiocyanate isomer I. Before derivatization, cocaine was subjected to an N-demethylation reaction involving the use of 1-chloroethyl chloroformate. The sensitivity of the method was excellent (approximately 100 pg/mL) with excitation and emission wavelengths of 488 and 522 nm, respectively.

### 12.3.4
### Desorption Electrospray Ionization Mass Spectrometry

Since 2000, the development of ambient desorption electrospray ionization (DESI) as an ion source for mass spectrometry has emerged as an interesting alternative in cases where the analyte is otherwise destroyed by sample preparation, as a simple preliminary screening test in emergency toxicology, or in high-throughput applications [146,147]. It allows for the rapid analysis of samples under ambient conditions and without any sample preparation. Typically, DESI is carried out by directing electrosprayed droplets and ions of solvent onto the surface of a complex sample of interest. The instrumentation, mechanisms, and applications of DESI in forensics, chemistry, and biology have been reviewed [148]. Another report presented the analysis of 21 commercial drugs as well as some illicit Ecstasy tablets [149]. DESI-MS has also been used for the screening of cannabis samples, resulting in the rapid detection of the major cannabinoids [150]. *In situ* detection of tropane alkaloids in *Datura stramonium* and *Atropa belladonna* has been investigated [151]. The effects on analytical performance of operating parameters, including the electrospray high voltage, heated capillary temperature, the solvent infusion rate, and the carrier gas pressure, were evaluated. Fifteen out of nineteen known alkaloids for *D. stramonium* and the principal alkaloids of *A. belladonna* were identified using DESI in combination with tandem mass spectrometry with methanol : water (1 : 1) as the spray solvent. Total analysis time was reduced to a minimum, as there is no sample preparation and no separation.

## 12.4 Conclusions

Tropane alkaloids are an important class of natural products possessing different and interesting pharmacological activities. Hyoscyamine (atropine in the racemate form), scopolamine, and cocaine are the major representatives of this class. They are commonly found in plant materials, mainly in genera belonging to three families: Solanaceae, Erythroxylaceae, and Convolvulaceae. The importance of these compounds requires that there are accurate analytical methods for their determination in plants and in biological matrices. This chapter describes the state-of-the-art of analytical procedures (extraction and analysis) for analyzing tropane alkaloids.

Extraction procedures of plant materials: classical percolation, maceration, digestion, decoction, and so on, as well as supercritical fluid extraction, microwave-assisted extraction, pressurized solvent extraction, and solid-phase microextraction are described. For biological matrices, liquid–liquid, and solid phase extractions are mainly used for different samples such as blood, urine, microdialysates, and saliva, among others.

Analyses of tropane alkaloids are mainly carried out by GC and HPLC and to a lesser extent by CE. This review describes recent applications developed for the analysis of this class of compounds in plant materials and biological matrices. Of course, mass spectrometry is generally used as the detection technique because of its high sensitivity and selectivity, but other techniques such as UV, fluorescence, flame ionization detection, nuclear magnetic resonance, among others have also been investigated. Finally, desorption electrospray ionization mass spectrometry is reported as a new interesting detection technique for the rapid analysis of samples without any sample preparation.

## References


1 Lounasmaa, M. and Tamminen, T. (1993) The tropane alkaloids, In Cordell, G. A. (Ed.) *The Alkaloids*, Vol. 44, Academic Press, San Diego, CA.

2 Christen, P. (2000) Tropane alkaloids: old drugs used in modern medicine, In Atta-ur-Rahman (Ed.) *Studies in Natural Products Chemistry*, Vol. 22, Elsevier, Amsterdam, NL.

3 Griffin, W. J. and Lin, G. D. (2000) *Phytochemistry*, **53**, 623–637.

4 Goldmann, A., Milet, M. L., Ducrot, P. H., Lallemand, J. Y., Maille, M., Lepingle, A., Charpin, I., Tepfer, D. (1990) *Phytochemistry*, **29**, 2125–2128.

5 Schimming, T., Tofern, B., Mann, P., Richter, A., Jenett-Siems, K., Dräger, B., Asana, N., Gupta, M. P., Correa, M. D., Eich, E. (1998) *Phytochemistry*, **49**, 1989–1995.

6 Dräger, B. (2004) *Natural Products Report*, **21**, 211–223.

7 Brock, A., Bieri, S., Christen, P., Dräger, B. (2005) *Phytochemistry*, **66**, 1231–1240.

8 Brock, A., Herzfeld, T., Paschke, R., Koch, M., Dräger, B. (2006) *Phytochemistry*, **67**, 2050–2057.

9 Asano, N., Nash, R. J., Molyneux, R. J., Fleet, G. W. J. (2000) *Tetrahedron: Asymmetry*, **11**, 1645–1680.

10 Gaillard, Y. and Pepin, G. (1999) *Journal of Chromatography, B*, **733**, 181–229.

11 El Jaber-Vazdekis, N., Gutierrez-Nicolás, F., Ravelo, A. G., Zárate, R. (2006) *Phytochemical Analysis*, **17**, 107–113.

**12** Cannell, R. J. P. (1998) Natural products isolation, *Methods in Biotechnology*, Vol. 4, Humana Press, Totowa, NJ.

**13** Woolley, J. G. (1993) Tropane alkaloids, In Watermann, P. G. (Ed.) *Methods in Plant Biochemistry*, Vol. 8, Academic Press, London.

**14** Castioni, P., Christen, P., Veuthey, J. -L. (1995) *Analusis*, **23**, 95–106.

**15** Choi, Y. H., Chin, Y. -W., Kim, J., Jeon, S. H., Yoo, K. -P. (1999) *Journal of Chromatography, A*, **863**, 47–55.

**16** Brachet, A., Mateus, L., Cherkaoui, S., Christen, P., Gauvrit, J. -Y., Lantéri, P., Veuthey, J. -L. (1999) *Analusis*, **27**, 772–778.

**17** Brachet, A., Christen, P., Gauvrit, J. -Y., Longeray, R., Lantéri, P., Veuthey, J. -L. (2000) *Journal of Biochemical and Biophysical Methods*, **43**, 353–366.

**18** Later, D. W., Richter, B. E., Knowles, D. E., Anderson, M. R. (1986) *Journal of Chromatographic Science*, **24**, 249–253.

**19** Morrison, J. F., Chesler, S. N., Yoo, W. J., Selavka, C. M. (1998) *Analytical Chemistry*, **70**, 163–172.

**20** Veuthey, J. -L., Edder, P., Staub, C. (1995) *Analusis*, **23**, 258–265.

**21** Abu Samra, A., Morris, J. S., Koirtyohann, S. R. (1975) *Analytical Chemistry*, **47**, 1475–1477.

**22** Ganzler, K., Salgò, A., Valko, K. (1986) *Journal of Chromatography*, **371**, 299–306.

**23** Ganzler, K. and Salgò, A. (1987) *Zeitschrift fuer Lebensmittel-Untersuchung und -Forschung*, **184**, 274–276.

**24** Zlotorzynski, A. (1995) *Critical Reviews in Analytical Chemistry*, **25**, 43–76.

**25** Neas, E. D. and Collins, M. J. (1988) Microwave heating: theoretical concepts and equipment design, In Kingston, H. M. and Jassie, L. B. (Eds.) *Introduction to Microwave Sample Preparation. Theory and Practice*, American Chemical Society, Washington DC.

**26** Ganzler, K., Szinai, I., Salgò, A. (1990) *Journal of Chromatography*, **520**, 257–262.

**27** Camel, V. (2001) *Analyst*, **126**, 1182–1193.

**28** Brachet, A., Christen, P., Veuthey, J. -L. (2002) *Phytochemical Analysis*, **13**, 162–169.

**29** Bieri, S., Brachet, A., Veuthey, J. -L., Christen, P. (2006) *Journal of Ethnopharmacology*, **103**, 439–447.

**30** Kaufmann, B. and Christen, P. (2002) *Phytochemical Analysis*, **13**, 105–113.

**31** Brachet, A., Rudaz, S., Mateus, L., Christen, P., Veuthey, J. -L. (2001) *Journal of Separation Science*, **24**, 865–873.

**32** Arthur, C. L. and Pawliszyn, J. (1990) *Analytical Chemistry*, **62**, 2145–2148.

**33** O'Reilly, J., Wang, Q., Setkova, L., Hutchinson, J. P., Chen, Y., Lord, H. L., Linton, C. M., Pawliszyn, J. (2005) *Journal of Separation Science*, **28**, 2010–2022.

**34** Gentili, S., Cornetta, M., Macchia, T. (2004) *Journal of Chromatography, B*, **801**, 289–296.

**35** Follador, M. J. D., Yonamine, M., de Moraes Moreau, R. L., Silva, O. A. (2004) *Journal of Chromatography, B*, **811**, 37–40.

**36** Bermejo, A. M., López, P., Álvarez, I., Tabernero, M. J., Fernandez, P. (2006) *Forensic Science International*, **156**, 2–8.

**37** Ilias, Y., Bieri, S., Christen, P., Veuthey, J. -L. (2006) *Journal of Chromatographic Science*, **44**, 394–398.

**38** Bieri, S., Ilias, Y., Bicchi, C., Veuthey, J. -L., Christen, P. (2006) *Journal of Chromatography, A*, **1112**, 127–132.

**39** Mroczek, T., Glowniak, K., Kowalska, J. (2006) *Journal of Chromatography, A*, **1107**, 9–18.

**40** Berkov, S. and Pavlov, A. (2004) *Phytochemical Analysis*, **15**, 141–145.

**41** Wells, D. A. (2003) High throughput bioanalytical sample preparation, *Methods and Automation Strategies. Progress in Pharmaceutical and Biomedical Analysis*, Vol. 5, Elsevier, Amsterdam, NL.

**42** Van Hout, M. W. J., Niederländer, H. A. G., de Zeeuw, R. A., de Jong, G. J. (2003) New developments in

integrated sample preparation for bioanalysis, In Smith, R. M. and Wilson, I. A. (Eds.) *Handbook of Analytical Separations: Bioanalytical Separations*, Vol. 4, Elsevier, Amsterdam, NL.

**43** Farina, M., Yonamine, M., Silva, A. A. (2002) *Forensic Science International*, **127**, 204–207.

**44** Raje, S., Dowling, T. C., Eddington, N. D. (2002) *Journal of Chromatography, B*, **768**, 305–313.

**45** Dräger, B. (2002) *Journal of Chromatography, A*, **978**, 1–35.

**46** Oertel, R. (2001) *Journal of Chromatography, B*, **750**, 121–128.

**47** Hennion, M. -C. and Pichon, V. (2003) *Journal of Chromatography, A*, **1000**, 29–52.

**48** Sellergren, B. (2001) Molecularly imprinted polymers in solid-phase extractions, In Lanza, F. (Ed.) *Molecularly Imprinted Polymers-Man-made Mimics of Antibodies and Their Applications in Analytical Chemistry: Techniques and Instrumentation in Analytical Chemistry*, Vol. 23, Elsevier, Amsterdam, NL.

**49** Nakamura, M., Ono, M., Nakajima, T., Ito, Y., Aketo, T., Haginaka, J. (2005) *Journal of Pharmaceutical and Biomedical Analysis*, **37**, 231–237.

**50** Müller, M., Schmid, R., Georgopoulos, A., Buxbaum, A., Wasicek, C., Eichler, H. -G. (1995) *Clinical Pharmacology and Therapeutics*, **57**, 371–380.

**51** Fuh, M. -R., Tai, Y. -L., Pan, W. H. T. (2001) *Journal of Chromatography, B*, **752**, 107–114.

**52** Chen, Y. -F., Chang, C. -H., Wang, S. -C., Tsai, T. -H. (2005) *Biomedical Chromatography*, **19**, 402–408.

**53** Kidwell, D. A., Holland, J. C., Athanaselis, S. (1998) *Journal of Chromatography, B*, **713**, 111–136.

**54** Samyn, N., Verstraete, A., van Haeren, C., Kintz, P. (1999) *Forensic Science Review*, **11**, 1–19.

**55** Gaillard, Y. and Pépin, G. (1999) *Journal of Chromatography, B*, **733**, 231–246.

**56** Kintz, P. and Samyn, N. (2002) *Therapeutic Drug Monitoring*, **24**, 239–246.

**57** Kintz, P. (1996) *Drug Testing in Hair*, CRC Press, New York, USA.

**58** Baerheim-Svendsen, A. and Verpoorte, R. (1984) Chromatography of alkaloids, *Part b: Gas–Liquid Chromatography, and High-Performance Liquid Chromatography*, Elsevier, Amsterdam, NL.

**59** Savchuk, S. A., Simonov, E. A., Sorokin, V. I., Dorogokupets, O. B., Vedenin, A. N. (2004) *Journal of Analytical Chemistry*, **59**, 954–964.

**60** Rasanen, I., Kontinen, I., Nokua, J., Ojanpera, I., Vuori, E. (2003) *Journal of Chromatography, B*, **788**, 243–250.

**61** Cramers, C. A., Janssen, H. -G., van Deursen, M., Leclercq, P. A. (1999) *Journal of Chromatography, A*, **856**, 315–329.

**62** Korytar, P., Janssen, H. -G., Matisova, E., Brinkman, U. A. T. (2002) *Trends in Analytical Chemistry*, **21**, 558–572.

**63** Klee, M. S. and Blumberg, L. M. (2002) *Journal of Chromatographic Science*, **40**, 234–247.

**64** Bertsch, W. (2000) *Journal of High Resolution Chromatography*, **23**, 167–181.

**65** Marriott, P. and Shellie, R. (2002) *Trends in Analytical Chemistry*, **21**, 573–583.

**66** Dalluge, J., Beens, J., Brinkman, U. A. T. (2003) *Journal of Chromatography, A*, **1000**, 69–108.

**67** Williams, T. A., Riddle, M., Morgan, S. L., Brewer, W. E. (1999) *Journal of Chromatographic Science*, **37**, 210–214.

**68** Song, S. M., Marriott, P., Kotsos, A., Drummer, O. H., Wynne, P. (2004) *Forensic Science International*, **143**, 87–101.

**69** Berkov, S., Zayed, R., Doncheva, T. (2006) *Fitoterapia*, **77**, 179–182.

**70** Doncheva, T., Berkov, S., Philipov, S. (2006) *Biochemical Systematics and Ecology*, **34**, 478–488.

**71** Kartal, M., Kurucu, S., Altun, L., Ceyhan, T., Sayar, E., Cevheroglu, S., Yetkin, Y. (2003) *Turkish Journal Of Chemistry*, **27**, 565–569.

**72** Jordan, M., Humam, M., Bieri, S., Christen, P., Poblete, E., Muñoz, O. (2006) *Phytochemistry*, **67**, 570–578.

**73** Bieri, S., Muñoz, O., Veuthey, J. -L., Christen, P. (2006) *Journal of Separation Science*, **29**, 96–102.

**74** Patterson, S. and O'Hagan, D. (2002) *Phytochemistry*, **61**, 323–329.

**75** O'Hagan, D. and Robins, R. J. (1998) *Chemical Society Reviews*, **27**, 207–212.

**76** Humphrey, A. J. and O'Hagan, D. (2001) *Natural Products Report*, **18**, 494–502.

**77** Casale, J. F. and Moore, J. M. (1996) *Journal of Chromatography, A*, **749**, 173–180.

**78** Casale, J. F. and Moore, J. M. (1996) *Journal of Chromatography, A*, **756**, 185–192.

**79** Moore, J. M. and Casale, J. F. (1997) *Journal of Forensic Sciences*, **42**, 246–255.

**80** Moore, J. M. and Casale, J. F. (1994) *Journal of Chromatography, A*, **674**, 165–205.

**81** Moore, J. M., Casale, J. F., Klein, R. F., Cooper, D. A., Lydon, J. (1994) *Journal of Chromatography, A*, **659**, 163–175.

**82** Casale, J. F., Toske, S. G., Colley, V. L. (2005) *Journal Of Forensic Sciences*, **50**, 1402–1406.

**83** Zuanazzi, L. A. S., Tremea, V., Limberger, R. P., Sobral, M., Henriques, A. T. (2001) *A. Biochemical Systematics and Ecology*, **29**, 819–825.

**84** Engelke, B. F. and Gentner, W. A. (1991) *Journal of Pharmaceutical Sciences*, **80**, 96.

**85** Jenkins, A. J., Llosa, T., Montoya, I., Cone, E. J. (1996) *Forensic Science International*, **77**, 179–189.

**86** Sachs, H. and Kintz, P. (1998) *Journal of Chromatography*, **713**, 147–161.

**87** Cody, J. T. and Foltz, R. L. (1995) *Forensic Applications of Mass Spectrometry*, 1–59.

**88** Hime, G. W., Hearn, W. L., Rose, S., Cofino, J. (1991) *Journal of Analytical Toxicology*, **15**, 241–245.

**89** Chasin, A. A. M., De Lima, I. V., De Carvalho, D. G., Midio, A. F. (1997) *Acta Toxicológica Argentina*, **5**, 77–80.

**90** Gunnar, T., Mykkanen, S., Ariniemi, K., Lillsunde, P. (2004) *Journal of Chromatography, B*, **806**, 205–219.

**91** Namera, A., Yashiki, M., Hirose, Y., Yamaji, S., Tani, T., Kojima, T. (2002) *Forensic Science International*, **130**, 34–43.

**92** Namera, A. (2005) Tropane alkaloids, In Suzuki, O. and Watanabe, K. (Eds.) *Drugs and Poisons in Human: A handbook of Practical Analysis*, Springer, Berlin.

**93** Baltussen, E., Cramers, C. A., Sandra, P. J. F. (2002) *Analytical and Bioanalytical Chemistry*, **373**, 3–22.

**94** Tienpont, B., David, F., Stopforth, A., Sandra, P. (2003) *LC-GC Europe*, **16** (12a), 5–13.

**95** Lewis, R. J., Johnson, R. D., Angier, M. K., Ritter, R. M. (2004) *Journal of Chromatography, B*, **806**, 141–150.

**96** Scheidweiler, K. B., Plessinger, M. A., Shojaie, J., Wood, R. W., Kwong, T. C. (2003) *Journal of Pharmacology and Experimental Therapeutics*, **307**, 1179–1187.

**97** Campora, P., Bermejo, A. M., Tabernero, M. J., Fernandez, P. (2003) *Journal of Analytical Toxicology*, **27**, 270–274.

**98** Samyn, N., DeBoeck, G., Verstraete, A. G. (2002) *Journal of Forensic Sciences*, **47**, 1380–1387.

**99** Teske, J., Putzbach, K., Engewald, W., Kleemann, W. J., Müller, R. K. (2003) *Chromatographia*, **57** (Suppl.), S/271–S/273.

**100** Gruszecki, A. C., Robinson, C. A., Jr., Embry, J. H., Davis, G. G. (2000) *American Journal of Forensic Medicine and Pathology*, **21**, 166–171.

**101** Eyler, F. D., Behnke, M., Wobie, K., Garvan, C. W., Tebbett, I. (2005) *Neurotoxicology and Teratology*, **27**, 677–687.

**102** Stutz, M. H. and Sass, S. (1973) *Analytical Chemistry*, **45**, 2134–2136.

**103** Pekic, B., Slavica, B., Lepojevic, Z., Gorunovic, M. (1985) *Die Pharmazie*, **40**, 422–423.

**104** Bogusz, M. and Erkens, M. (1994) *Journal of Chromatography, A*, **674**, 97–126.

**105** Herrero-Martinez, J. M., Mendez, A., Bosch, E., Roses, M. (2004) *Journal of Chromatography, A*, **1060**, 135–145.

**106** Kurzinski, L., Hank, H., László, I., Szöke, E. (2005) *Journal of Chromatography, A*, **1091**, 32–39.

**107** Hank, H., Szöke, E., Tóth, K., László, I., Kursinszki, L. (2004) *Chromatographia*, **60**, S55–S59.

**108** Kirchhoff, C., Bitar, Y., Ebel, S., Holzgrabe, U. (2004) *Journal of Chromatography, A*, **1046**, 115–120.

**109** Wolfender, J. -L., Rodriguez, S., Hostettmann, K. (1998) *Journal of Chromatography, A*, **794**, 299–316.

**110** Albert, K. (2002) *On-Line LC-NMR and Related Techniques*, John Wiley & Sons, Chichester, UK.

**111** Wolfender, J. -L., Queiroz, E. F., Hostettmann, K. (2005) *Magnetic Resonance in Chemistry*, **43**, 697–709.

**112** Zanolari, B., Wolfender, J. -L., Guilet, D., Marston, A., Queiroz, E. F., Paulo, M. Q., Hostettmann, K. (2003) *Journal of Chromatography, A*, **1020**, 75–89.

**113** Bieri, S., Varesio, E., Veuthey, J. -L., Munoz, O., Tseng, L. -H., Braumann, U., Spraul, M., Christen, P. (2006) *Phytochemical Analysis*, **17**, 78–86.

**114** Bowman, B. P., Vaughan, S. R., Walker, Q. D., Davis, S. L., Little, P. J., Scheffler, N. M., Thomas, B. F., Kuhn, C. M. (1999) *Journal of Pharmacology and Experimental Therapeutics*, **290**, 1316–1323.

**115** Harrison, P. K., Tattersall, J. E. H., Gosden, E. (2006) *Naunyn Schmiedebergs Archives of Pharmacology*, **373**, 230–236.

**116** Needham, S. R., Jeanville, P. M., Brown, P. R., Estape, E. S. (2000) *Journal of Chromatography, B*, **748**, 77–87.

**117** Takahashi, M., Nagashima, M., Shigeoka, S., Nishijima, M., Kamata, K. (1997) *Journal of Chromatography, A*, **775**, 137–141.

**118** Jamdar, S. C., Pantuck, C. B., Diaz, J., Mets, B. (2000) *Journal of Analytical Toxicology*, **24**, 438–441.

**119** Stöckigt, J., Unger, M., Stöckigt, D., Belder, D. (1998) Analysis of alkaloids by capillary electrophoresis and capillary electrophoresis-electrospray mass spectrometry, In Pelletier, S. W. (Ed.) *Alkaloids: Chemical and Biological Perspectives*, Vol. 12, Elsevier, Oxford, UK.

**120** Cherkaoui, S., Mateus, L., Christen, P., Veuthey, J. -L. (1997) *Journal of Chromatography, B*, **696**, 283–290.

**121** Cherkaoui, S., Mateus, L., Christen, P., Veuthey, J. -L. (1998) *Journal of Pharmaceutical and Biomedical Analysis*, **17**, 1167–1176.

**122** Eeva, M., Salo, J. -P., Oksman-Caldentey, K. -M. (1998) *Journal of Pharmaceutical and Biomedical Analysis*, **16**, 717–722.

**123** Mateus, L., Cherkaoui, S., Christen, P., Veuthey, J. -L. (2000) *Journal of Chromatography, A*, **868**, 285–294.

**124** Ye, N., Zhu, R., Gu, X., Zou, H. (2001) *Biomedical Chromatography*, **15**, 509–512.

**125** Cherkaoui, S., Mateus, L., Christen, P., Veuthey, J. -L. (1997) *Chromatographia*, **46**, 351–357.

**126** Mateus, L., Cherkaoui, S., Christen, P., Veuthey, J. -L. (1998) *Journal of Chromatography, A*, **829**, 317–325.

**127** Mateus, L., Cherkaoui, S., Christen, P., Oksman-Caldentey, K. -M. (2000) *Phytochemistry*, **54**, 517–523.

**128** Mateus, L., Cherkaoui, S., Christen, P., Veuthey, J. -L. (1999) *Current Topics in Phytochemistry*, **2**, 175–182.

**129** Cherkaoui, S., Mateus, L., Christen, P., Veuthey, J. -L. (1999) *Chromatographia*, **49**, 54–60.

**130** Cherkaoui, S., Mateus, L., Christen, P., Veuthey, J. -L. (1999) *Journal of Pharmaceutical and Biomedical Analysis*, **21**, 165–174.

**131** Mateus, L., Cherkaoui, S., Christen, P., Veuthey, J. -L. (1999) *Electrophoresis*, **20**, 3402–3409.

**132** Gao, Y., Tian, Y., Wang, E. (2005) *Analytica Chimica Acta*, **545**, 137–141.

**133** Veuthey, J. -L. (2005) *Analytical and Bioanalytical Chemistry*, **381**, 93–95.

**134** Boone, C. M., Waterval, J. C. M., Lingeman, H., Ensing, K., Underberg, W. J. M. (1999) *Journal of Pharmaceutical and Biomedical Analysis*, **20**, 831–863.

**135** Petersen, J. R. and Mohammad, A. A. (2001) *Clinical and Forensic Applications of Capillary Electrophoresis*, Humana Press, Totowa, NJ.

**136** Petersen, J. R., Okorodudu, A. O., Mohammad, A. A., Payne, D. A. (2003) *Clinica Chimica Acta*, **330**, 1–30.
**137** Thormann, W. (2003) *Therapeutic Drug Monitoring*, **24**, 222–231.
**138** Plaut, O. and Staub, C. (2002) *Chimia*, **56**, 96–100.
**139** Plaut, O. and Staub, C. (1998) *Electrophoresis*, **19**, 3003–3007.
**140** Tagliaro, F., Poiesi, C., Aiello, R., Dorizzi, R., Ghielmi, S., Marigo, M. (1993) *Journal of Chromatography*, **638**, 303–309.
**141** Tagliaro, F., Manetto, G., Crivellente, F., Scarcella, D., Marigo, M. (1998) *Forensic Science International*, **92**, 259–268.
**142** Tagliaro, F., Valentini, R., Manetto, G., Crivellente, F., Carli, G., Marigo, M. (2000) *Forensic Science International*, **107**, 121–128.
**143** Chevigné, R. and Louis, P. (1997) US patent 5 611 903.
**144** Boone, C. M., Jonkers, E. Z., Franke, J. P., de Zeeuw, R. A., Ensing, K. (2001) *Journal of Chromatography, A*, **927**, 203–210.
**145** Alnajjar, A., Butcher, J. A., McCord, B. (2004) *Electrophoresis*, **25**, 1592–1600.
**146** Takáts, Z., Wiseman, J. M., Gologan, B., Cooks, R. G. (2004) *Science*, **306**, 471–473.
**147** Takáts, Z., Cotte-Rodriguez, J., Talaty, N., Chen, H., Cooks, R. G. (2005) *Chemical Communications*, 1950–1952.
**148** Takáts, Z., Wiseman, J. M., Cooks, R. G. (2005) *Journal of Mass Spectrometry*, **40**, 1261–1275.
**149** Leuthold, L. A., Mandscheff, J. -F., Fathi, M., Giroud, C., Augsburger, M., Varesio, E., Hopfgartner, G. (2006) *Rapid Communications in Mass Spectrometry*, **20**, 103–110.
**150** Rodriguez-Cruz, S. E. (2006) *Rapid Communications in Mass Spectrometry*, **20**, 53–60.
**151** Talaty, N., Takáts, Z., Cooks, R. G. (2005) *Analyst*, **130**, 1624–1633.