LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY IN FORENSIC TOXICOLOGY

Jan F. Van Bocxlaer,[1] Karine M. Clauwaert,[1] Willy E. Lambert,[1] Dieter L. Deforce,[2] Elfriede G. Van den Eeckhout,[2] and André P. De Leenheer[1]

[1]*Laboratorium voor Toxicologie, Universiteit Gent, Gent, Belgium*
[2]*Laboratorium voor Farmaceutische Biotechnologie, Universiteit Gent, Gent, Belgium*

Received 5 June 2000; revised 5 June 2000; accepted 7 June 2000

Liquid chromatography–mass spectrometry has evolved from a topic of mainly research interest into a routinely usable tool in various application fields. With the advent of new ionization approaches, especially atmospheric pressure, the technique has established itself firmly in many areas of research. Although many applications prove that LC–MS is a valuable complementary analytical tool to GC–MS and has the potential to largely extend the application field of mass spectrometry to hitherto "MS-phobic" molecules, we must recognize that the use of LC–MS in forensic toxicology remains relatively rare. This rarity is all the more surprising because forensic toxicologists find themselves often confronted with the daunting task of actually searching for evidence materials on a scientific basis without any indication of the direction in which to search. Through the years, mass spectrometry, mainly in the GC–MS form, has gained a leading role in the way such quandaries are tackled. The advent of robust, bioanalytically compatible combinations of liquid chromatographic separation with mass

Correspondence to: Prof. Dr. A.P. De Leenheer, Laboratorium voor Toxicologie, Harelbekestraat 72, B-9000 Gent, Belgium
E-mail: Andre.DeLeenheer@rug.ac.be

spectrometric detection really opens new perspectives in terms of mass spectrometric identification of difficult molecules (e.g., polar metabolites) or biopolymers with toxicological relevance, high throughput, and versatility. Of course, analytical toxicologists are generally mass spectrometry users rather than mass spectrometrists, and this difference certainly explains the slow start of LC–MS in this field. Nevertheless, some valuable applications have been published, and it seems that the introduction of the more universal atmospheric pressure ionization interfaces really has boosted interests. This review presents an overview of what has been realized in forensic toxicological LC–MS. After a short introduction into LC–MS interfacing operational characteristics (or limitations), it covers applications that range from illicit drugs to often abused prescription medicines and some natural poisons. As such, we hope it can act as an appetizer to those involved in forensic toxicology but still hesitating to invest in LC–MS.
 Mass Spec Rev 19: 165–214, 2000
Keywords: *LC–MS; forensic toxicology; drug analysis*

I. INTRODUCTION

A growing tendency exists, nationally as well as internationally, to use increasingly more scientific and technical evidence in legal matters. With respect to potentially toxic substances, their effects, and their involvement in various criminal cases, such answers can be provided by forensic toxicology (Yinon, 1991; Baillie, 1992; Foltz, 1992). Forensic science has been defined as the study and practice of applying science to the purpose of justice. The task of the forensic scientist can be summarized as the identification, interpretation and, more and more, searching for evidence materials. Indeed, the particular character of forensic toxicology implies that in the majority of the cases only faint indications are available that concern the direction in which the analytical search must be performed. The introduction of mass spectrometric techniques in this field has revolutionized the way forensic laboratories have approached many of the analytical problems that they are confronted with. On-line combination with a chromatographic technique, mainly gas chromatography (GC) until now (Maurer, 1992), in order to have a preliminary separation of the compounds of interest from interfering substances or matrix, has greatly enhanced the analytical potential. The mass spectrometer combines two apparent controversies, a universal detector, which at the same time affords high selectivity. Moreover, the resulting mass spectrum presents the investigator with an unequivocal identity proof of the studied substance or provides quasi-effortless valuable identification data for unknowns. Almost unnoticed sometimes, these advantages were accompanied by a substantially increased detection sensitivity and in many cases simplified sample treatment, thus greatly reducing analysis time. It is not surprising at all to see that gas chromatography–mass spectrometry (GC–MS) has evolved to the method of choice in the forensic laboratory.

Many of the advantages of mass spectrometric detection, common to gas chromatography, have long been envied by the liquid chromatography community. Liquid chromatography (LC or HPLC) has become increasingly important as a separation technique, especially for biomedical applications. In toxicology, too, the use of liquid chromatography has grown over the years, and it sometimes presents the investigator with some clear advantages over an alternative gas chromatographic approach (Lambert, Van Bocxlaer & De Leenheer, 1997). It is estimated that nearly 70% of everyday samples in the toxicological laboratory can be handled by LC (Hoja et al., 1997c). Indeed, this broad range of physico-chemically different compounds that can be separated with the technique makes it very attractive, especially to those confronted with continuously new and often mysterious analytical challenges. Moreover, the fact that, unlike GC, derivatization is virtually never necessary, certainly promotes LC as the better alternative for the analysis of polar and thermolabile compounds. A combination of liquid chromatography and mass spectrometry (LC–MS) provides a valuable analytical contribution in many areas of research that allows the power of MS to be applied to a much wider array of compounds. Consequently, much effort has been made over the past decades in developing a suitable interface between liquid chromatography and the mass spectrometer. With the advent of new ionization techniques, a breakthrough was finally realized and various, often complementary systems, are now available. Today, as evidenced by the growing number of LC–MS applications being published, the technique has left the experimental stage and has firmly established itself (Maurer, 1998). Systems are nowadays available that totally comply with all of the present requirements (sensitivity, robustness, flexibility) of a bioanalytical application, including many of the (forensic) toxicological issues. It is a fact that LC–MS has become complementary to GC–MS in the field of semivolatile compounds, and it excels for nonvolatile and/or high molecular weight molecules. This complementarity is very well illustrated by the importance that LC–MS has gained in the past few years in pharmaceutical research laboratories with respect to bioanalytical (e.g., pharmacokinetic) work (Robson, Draper & Tennant, 1994; Gelpì, 1995; Highton & Oxford, 1995). On the contrary, the use of LC–MS in forensic toxicology and toxicology in general remains comparatively scarce. LC–MS instruments are still substantially, at least twice, more expensive than their GC–MS counterparts. GC–MS has served forensic toxicologists faithfully for the majority of their analytical problems and often the

'trusted' throughput of at least two GC–MS instruments, which might become available against one LC–MS, is balanced against what is seen as the small percentage of alternative analyses dedicated to LC–MS. This factor, of course, disregards the contribution that LC–MS can make to their existing procedures (speed, no derivatization, etc.), helping in an accelerated pay-off of the initial capital investment.

The aim of this review is in part to demonstrate, through the work already performed, the great promise that LC–MS holds for measuring drug concentrations in body fluids, identifying the nature of evidence materials, and investigating the implication of toxins in suicide or suspect homicide cases. Convincing case reports are probably the best way to exemplify the extra potential LC–MS offers to toxicologists.

Following a summarizing introduction to the relevant mass spectrometric instrumentation and techniques, a comprehensive overview and discussion will be presented of the applications published in forensic LC–MS.

II. INSTRUMENTATION OVERVIEW

A. Interfacing

In combining the two analytical techniques, LC and MS, three major difficulties must be solved. First of all, the apparent flow-rate incompatibility is expressed in the need to introduce the effluent from the LC column (typically 1 mL/min) into the high vacuum of the mass spectrometer. A further problem constitutes the solvent composition as a result of the frequent use of non-volatile mobile phase additives by chromatographers; e.g., buffers or ion-pairing agents. Nonetheless, LC is capable of providing the separation of compounds that are unsuitable for GC analysis. Analysts have turned to HPLC to develop analyses for polar, ionic, nonvolatile, high molecular mass, and thermally labile analytes. The added value of LC is clearly lost if mass spectrometric detection is unable to cope with these compounds as well. In order to address some of these quandaries, a wide variety of technological approaches were developed: the LC–MS interfaces (Garcia & Barceló, 1993; Verpoorte & Niessen, 1994; Gelpì, 1995; Niwa, 1995; Higton & Oxford, 1995; Careri, Mangia & Musci, 1996). It is beyond the scope of this article to go into the details of any one of these methods used in LC coupling. A brief overview will be presented as a guideline for the following sections; more in-depth information can be found in many excellent, dedicated review articles (Bowers, 1989; Tomer & Parker, 1989; Fenn et al., 1990; Mellon, 1991; Clench, 1992; Burlingame, Boyd & Gaskell, 1994; Niessen & Tinke, 1995; Niessen, 1998) and books (Niessen, 1999).

Intrepid researchers have addressed the above-mentioned problems from a number of different angles. The problem of the vaporized solvent that enters the mass spectrometer vacuum system has been paramount for some, whereas others were challenged by the perspective of ionizing large molecular weight compounds such as biopolymers. As it happened, both approaches have sort of come together in the atmospheric pressure ionization techniques. Furthermore, the ionization of the molecules has evolved from an individual event such as electron impact ionization to an integral aspect of the process of solvent removal. For the problem of vapor load to the mass spectrometer vacuum system, a number of different approaches have been described, but only a few of them have really found widespread application. Generally speaking, one can distinguish among three approaches—as presented schematically in Fig. 1. Firstly, nebulization of the column effluent, removal of the mobile phase constituents, and vaporization of the analytes followed by ionization (moving belt [MB] and particle beam [PB] interfaces). Alternatively, direct ionization from a microliter effluent stream continuously deposited on a target (continuous-flow fast atom bombardment interface [CF–FAB]). Finally, nebulization of the effluent in either an atmospheric-pressure or a reduced-pressure region, desolvation of the droplets, followed by either gas-phase chemical ionization [CI] or ion evaporation (direct liquid introduction [DLI], thermospray [TSP], electrospray [ESI], and atmospheric-pressure chemical ionization [APCI]) (Verpoorte & Niessen, 1994).

1. *Moving Belt and Particle Beam Interfaces*

The principle of the MB interface is simple: the chromatographic eluate is mechanically transported from

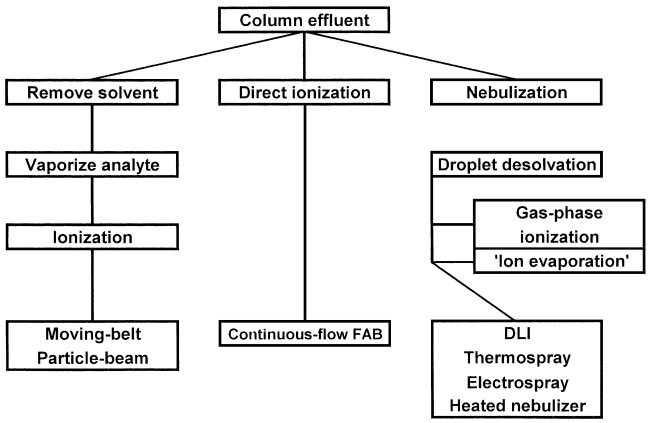


FIGURE 1. Basic principles of LC–MS interfaces. (Reproduced from Verpoorte and Niessen, 1994, with kind permission from John Wiley & Sons Limited, Copyright 1994).

the end of the column to the ion source, and the mobile phase is removed during this transport (Games et al., 1981). Excellent reviews on the moving belt technique can be found in the papers of Arpino and of Abian (Arpino, 1989; Abian, 1999). Nowadays, the belt interface principle has largely been abandoned in favor of more universal interface types.

The PB interface for LC–MS can produce classical, library-searchable EI and CI mass spectra of volatile or semivolatile compounds (Winkler et al., 1988; Behymer, Bellar & Budde, 1990; Creaser & Stygall, 1993; Cappiello & Famiglini, 1995). The major weakness of PB lies in the limited range of compounds that can be analyzed. More detailed information on this interface can be found in the Niessen LC–MS book (Niessen, 1999). Despite the advent of new, more universal interface approaches, over the years the PB has maintained a distinctive role in the analysis of small molecules (Cappiello, 1996).

2. *Continuous-Flow Fast Atom Bombardment*

CF–FAB, an adaptation of the conventional FAB ionization mode, can equally be used to introduce a continuously flowing LC eluate into the mass spectrometer (Barber et al., 1982; Caprioli, Fan, & Cottrell, 1986; Caprioli & Suter, 1992; Niwa, 1995, Niessen, 1999). This type of interface is capable of analyzing nonvolatile, thermally labile, and high molecular mass compounds. Its main limitation concerns the low flow rates that must be used. This limit imposes the use of drastic splitting for conventional columns, and, as such, results in an increased detection limit. In addition, the FAB technique suffers from high background-ion contributions of the mandatory matrix. In view of the later evolutions in interfacing LC with MS, on-line LC CF–FAB has largely been replaced.

3. *Direct Liquid Introduction*

DLI is probably the simplest LC–MS interface. Detailed descriptions have been published (Tomer & Parker, 1989; Vékey, Edwards & Zerilli, 1989; Gagné, Roussis & Bertrand, 1991; Garcia & Barceló, 1993). The DLI method has fallen out of favor because two main drawbacks of the interface are its lack of sensitivity when conventional HPLC columns are used and most of all, the frequent clogging problems of the orifice (which basically precludes the use of buffers).

4. *Thermospray*

The thermospray interface (Blakley & Vestal, 1983; Vestal, 1984) is also one of the interfaces that serve two purposes: it not only reduces the amount of solvent that enters the mass spectrometer vacuum but at the same time ionizes the analytes. The LC eluent (up to 2 mL/min) is sprayed through a heated capillary into a specially designed heated ion chamber. A supersonic jet of vapor is produced, in which the electrically charged droplets shrink as the solvent evaporates in the low-pressure region. When the droplet charge density becomes high enough, free ions are expelled from the droplet. Other ionization mechanisms, e.g., gas-phase ion-molecule reactions, take place simultaneously and all contribute to the generation of ions. These processes are detailed in various excellent papers (Vestal & Fergusson, 1985; Tomer & Parker, 1989; Arpino, 1990; Wolfender, Maillard & Hostettmann, 1994) and the Niessen's LC–MS book (Niessen, 1999). The main drawback of this technique is the difficulty to maintain a stable signal intensity because small changes in the instrumental parameters, such as temperature, produce large changes in the spectra. Instrumental parameters must be optimized for each individual compound studied and according to the specific LC working conditions (Garcia & Barceló, 1993). Nevertheless, the TSP interface was the first viable answer to two of the main demands in routine LC–MS coupling:conventional column flow rates with polar solvents and ionization of thermally labile and nonvolatile compounds. It has, as such, been the method of choice for most of the ''pre-API'' applications, as reflected in the number of publications available.

These different interfaces all have their specific advantages but, unfortunately, also a number of important disadvantages. The various interfaces generally also have their own particular application field, which in fact constitutes their most important limitation. This limitation, which they all have in common, is the lack of universality in their use. On the contrary, those interfaces that can be grouped under the common denominator of atmospheric pressure ionization (API) (Whitehouse et al., 1985) devices have been shown to alleviate this limitation to a great extent. These API devices largely owe their popularity (which shows from the current literature) to this universality, to their robustness, sensitivity, and of course the formidable advantage of multiple charge ionization, which electrospray ionization provides for the mass spectrometric analysis of high-molecular weight molecules such as peptides, proteins, and nucleic acids (Fenn et al., 1989; Kebarle & Tang, 1993). The ESI and APCI interfaces, which are based on this API principle, are generally supplied on the same modern instrument, and are nowadays becoming the mainstays of LC–MS (Niessen, 1999).

The strategies used in these interfaces to achieve the elimination of the solvent and ionization are in fact very similar to the earlier DLI and TSP approaches. The more recent API interfaces differ in the atmospheric pressure in the ion source and the direct ion desorption from charged

droplets (Fenn et al., 1990; Smith et al., 1990; Bruins, 1991). The two basic interfaces (besides some different commercial adaptations), which have emerged from the development of API, actually only differ in the nebulization principle, although having an important impact on the application range that they cover.

5. Atmospheric Pressure Chemical Ionization

In APCI, the eluent is pneumatically nebulized from a capillary (Henion, Thomson & Dawson, 1982). This approach enables desolvation of the nebulized drops and the formation of a vapor of analyte and solvent molecules. APCI is achieved in the same region, using a discharge electrode, to ionize solvent molecules, which after several ion-molecule reactions, transfer a charge to the analyte. Finally, the ions are sampled from the atmospheric-pressure region into the high vacuum through a system of nozzle and skimmers, sometimes with a counter-current drying gas (Huang et al., 1990; Zhao, Zhu & Lubman, 1992). The ionization mechanisms in APCI are very similar to those in conventional medium pressure chemical ionization (Covey, Lee & Henion, 1986). The technique has also many similarities to the TSP interface, but has the advantage of easy operation and a much less critical temperature control (Niessen & Tinke, 1995). The APCI interface can be used with aqueous liquid flow-rates of up to 2 mL/min, using volatile as well as small amounts of nonvolatile buffers (Bruins, 1994; Garcia, Huang & Stansbury, 1996). In terms of molecular mass and analyte polarity, APCI does not have the potential that the ESI interface has. APCI allows the very sensitive determination of analytes with moderate polarity and molecular mass (Niessen, 1999). Because the ionization is CI, it is a soft ionization technique and no informative fragmentation is achieved.

6. Electrospray

The technology of electrospray interfacing has been developed by Fenn et al. (1990). ESI differs from APCI in a number of technical and ionization process aspects. Here, the spray, consisting of charged droplets, is generated as a result of passage of the LC eluent through a metal capillary tube, which is maintained at several kV different from that of a surrounding counter electrode (Wachs et al., 1991; Kebarle & Tang, 1993; Reiser & Fogiel, 1994). Many designs pneumatically assist the nebulization (Hoja et al., 1997c). The ionization takes place in the liquid phase under atmospheric pressure, and is believed to consist of the direct emission of ions from microdroplets, which are generated in the spray through solvent evaporation. Besides ion evaporation of preformed ions, gas-phase ion-molecule reactions also play an important role (Voress, 1994; Hirabayashi et al., 1997). ESI offers the best sensitivity when used at low flow rates, which can only be attained with small bore columns or through post-column splitting. The latter, however, does not decrease sensitivity because the response has been proven to be concentration-sensitive and not mass-flow sensitive (Hopfgartner et al., 1993). In order to increase the structural information to be obtained with this equally soft ionization technique, some designs allow a voltage to be introduced in the intermediate vacuum region in order to induce in-source fragmentation. Of course, the impetus for the interest in ESI is (Fenn et al., 1989) the multiple charging of, e.g., proteins, which results in an ion envelope in the region *m/z* 500–1500 that allows protein determinations with mass spectrometry (Niessen & Van der Greef, 1992). ESI has the broadest application range (Gaskell, 1997). It enables the sensitive determination of high-molecular weight compounds as well as thermolabile or very high polarity molecules such as glucuronide conjugates or quaternary amines (Niessen, 1998). Certainly, in ESI the eluent is vital for the ionization process, and it should always contain at least small amounts of a volatile buffer, acid, or base.

B. Mass Analysis

Formed ions can subsequently be analyzed in a number of mass filters. The most frequently encountered filters are the quadrupole systems. Alternatively, some interfaces are linked to sector field instruments, allowing high mass resolution measurements, and lately an increasing number of applications use ion trap–based instruments. The relative merits of the various mass analyzers were discussed by Brunnée (1987). There is no such thing as an ideal mass analyzer, they are very difficult analytical utilities when it comes to such figures of merit as dynamic range or resolution. The choice largely depends on the application. Because most ionization principles used in LC–MS are soft ionization techniques, they basically produce only protonated molecular ions. In order to obtain more structural information, which is vital for the identification of unknowns in many forensic cases, fragmentation is induced through ion-molecule collisions (CID) in the ion source or by using tandem-MS. This coupling of mass filters in tandem-MS occurs in various combinations. The triple quadrupole geometry seems to be the most frequently used, but any combination, also hybrid, has been tried. In this respect, an interesting development has been introduced. This novelty combines a quadrupole mass analyzer, a quadrupole-type collision chamber for CID, and a time-of-flight (TOF) mass analyzer. The advantage of the TOF lies in its high mass resolution measurement (e.g., FWHM $>$ 5000 at *m/z* 1500 for the Micromass QTOF and $>$ 10,000 for its successor, the QTOF II), which can be achieved with high stability

over time (Weickhardt, Moritz & Grotemeyer, 1996). Such a high mass resolution has, of course, many advantages, also in the field of forensic toxicology, such as increased selectivity, identification power, and for biopolymers more accurate mass determination. The array detection principle also allows higher ''full scan'' sensitivity. Although as yet only limited data are available, it does seem that a quantitative relationship can be achieved (Muddiman et al., 1996; Purves, Gabryelski & Li, 1998; Clauwaert et al., 1999).

An MS–MS combination creates additional operational possibilities because both analyzers can be operated in either the scan or selected-ion mode, resulting in the four basic mass analysis modes when combined with CID. Product-ion scanning (PIS) has many advantages for the identification of unknowns, possibly metabolites of drugs or toxins, in the complex forensic–toxicological matrices. Selectivity is achieved by the LC separation aided by the first MS through the selection of one single ion. The second (scanning) mass analyzer provides the vital structural information. Precursor-ion scanning is the reverse approach, and offers a convenient way of identifying precursor-ions or fragmentation patterns. In constant neutral-loss scanning, the two mass filters are scanned with a fixed mass difference to monitor the loss of a neutral species from the precursor-ion. Finally, and very important in quantitative work regarding target-component analysis, selected ion monitoring in both analyzers results in its tandem-MS equivalent:selected reaction monitoring (SRM, also multiple reaction monitoring, MRM), giving the ultimate selectivity and sensitivity. Of course, ion-trap instruments have an adapted implementation of these mass analysis functions, and the Q-TOF inherently monitors all ions simultaneously in the second mass analyzer, the principle of which makes selection of some ions for sensitivity reasons obsolete (Hill, 1990; Hoja et al., 1997c).

III. LC–MS: WHAT TO INCLUDE?

In the present overview, the scope has been reduced to LC–MS in strictly forensic–toxicological applications. It is based on publications found through on-line searching of various analytical/bioanalytical/medical literature databases, as well as on a detailed scrutiny of the relevant literature. LC–MS procedures for the identification and/or quantitation of drugs, poisons, or toxins and their metabolites, relevant for forensic toxicology, are reviewed. The main focus is on the analysis of these compounds in biosamples (biofluids, tissues, hair, etc.). Clinical toxicology, drug metabolism, or pharmacokinetic studies were eliminated from the searches unless an obvious and present relationship in use or abuse potential could be established with forensic science. Some applications from the field of doping control complied with these criteria, and have been included, as well as very few cases that show a highly ingenious analytical approach with a potential to the field of forensic toxicology.

We did not attempt to limit our applications to certain interfaces, although it is clear that some of them show only a token presence in terms of practical application in toxicological problems. In concordance with the overwhelming presence of API-based applications, the majority of recent LC–MS papers deal with either ESI or APCI, the focal points will be mainly these approaches, which will inevitably dominate in LC–MS in the forthcoming years. As an illustration of this API dominance, Fig. 2 shows the chronological evolution of LC–MS interfaces used in the forensic toxicological publications on which this review is based.

Besides discussing the scientific contribution of each publication, key practical information is included when relevant. With respect to the mass spectrometric part, this inclusion was reasonably straightforward. Although particular instrumental settings are, of course, instrument-type specific, they most often bear a clear relationship with one another and can at least be used as a starting point for method development or adaptation to local circumstances. For the method validation data, especially when quantitative results are presented, the situation is much more difficult. We have experienced an inferior treatment, or at least reportage, of even the most basic validation aspects. Different definitions or criteria of such parameters as detection limits or linearity are omnipresent or no indication or supporting data are given on which approach is actually used. This limit clearly makes comparison, even intra-compound inter-application comparison, difficult to impossible at times. Future contributions to the field of forensic toxicological mass spectrometry should in our view, taking into consideration the importance of quantitative results for interpretation of the particular case, pay ample attention to proper validation and its unequivocal and complete presentation (e.g., for linearity, not only correlation coefficient but slope and intercept, their confidence limits or standard errors), according to established and agreed criteria.

IV. GENERAL PERFORMANCE AND APPLICATIONS

A. Drugs of Abuse

1. Opiates

The extensive use of morphine as a narcotic analgesic together with the widespread abuse of opiates (especially

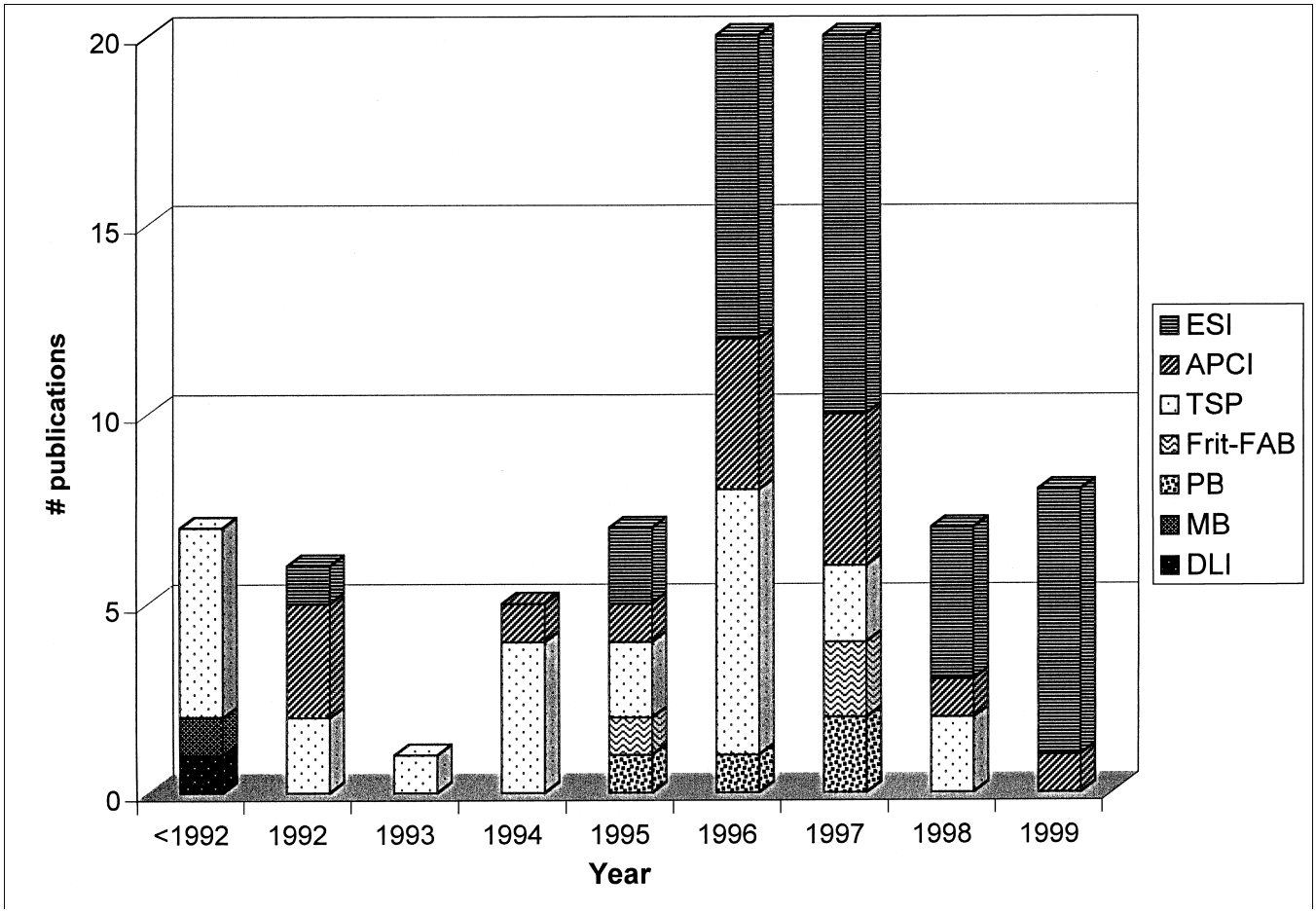


FIGURE 2. Chronological evolution of interfaces used in published forensic LC applications.

of heroin) has necessitated the development of techniques for the unequivocal detection of these compounds in biological samples. Heroin is quickly deacetylated to 6-monoacetylmorphine (6-MAM) and subsequently to morphine. Morphine, in turn, is conjugated into either morphine-3-glucuronide (M3G) or morphine-6-glucuronide (M6G); the latter is a metabolite with analgesic activity.

Joyce et al. (Joyce, Ardrey and Lewis 1985) have used thermospray LC–MS for the analysis of an illicit heroin sample. Because no samples of biological origin were analyzed, this method is not treated in detail. The article is valuable in that it clearly illustrated that, in this case, TSP ionization does not necessarily provide unequivocal molecular weight information. Indeed, in some cases no $[M+H]^+$ ion (protonated molecular ion) is present at all, but instead the $[M+NH_4]^+$ ion was exclusively formed, thus potentially complicating identification. Lurie, Cooper and Krull (1993) described the use of photoirradiation with thermospray mass spectrometric detection for the analysis of heroin. Photoirradiation can result in the formation of new photoproducts as well as a dramatically increased fragmentation, thus providing more informative spectra. They did not analyze any samples of biological origin; consequently, their method is not described in detail.

Tatsuno et al. (1996a), describes the use of thermospray LC–MS for the determination of morphine, 6-MAM, M3G, and M6G in human urine. Extraction was performed with solid phase extraction (SPE), and chromatographic separation was achieved on a reversed phase column, which is eluted with ammonium acetate and acetonitrile in the gradient elution mode. Experiments with the TSP vaporizer temperature showed that the highest sensitivities were obtained when the vaporizer temperature was linearly decreased from 170–150°C, concomitant with the 0–40% acetonitrile increase through the gradient elution. The resulting mass spectra were dominated by a single peak, which was used for quantitation and identification. This base peak was

actually the protonated molecular ion for morphine (*m/z* 286) and 6-MAM (*m/z* 328) but, on the contrary, for the aglycone of the morphine-glucuronides (*m/z* 286), which swiftly forms by the easy cleavage of the glucuronic acid moiety. The detection limits of this method were 2–40 ng/mL in the selected ion monitoring (SIM) and 50–400 ng/mL in the scan mode. We consider them relatively high in comparison with other methods, as can be seen from Table 1. The coefficients of variation and recoveries were also determined for the procedure; calibration was performed, using a calibration curve of peak area versus concentration, but, unfortunately, no internal standard is used. Table 1 lists some of the results.

The National Institute of Standards and Technology (NIST) has developed, together with a GC–MS and probe MS–MS method, a TSP–LC– MS protocol to certify the concentrations of morphine and codeine in an urinary standard reference material, using deuterated internal standards (Tai et al., 1994). Mixed-mode SPE columns were used to extract morphine and codeine from urine. A C_8 column with an isocratic mobile phase of trifluoroacetic acid and ammonium acetate in water–methanol, provided the separation. Selected ion monitoring of the protonated molecular ions at *m/z* 286, 289, 300, and 303 for morphine, morphine-d_3, codeine, and codeine-d_3, respectively, was performed. The limit of detection of the method was estimated to be approximately 2 ng/mL. Interestingly, it was concluded that the results obtained for the standard reference materials with all three methods, GC–MS, probe MS–MS, and LC–MS, were in good agreement.

Bogusz, Maier and Driessen (1997) determined morphine, its two glucuronides, and 6-MAM by means of LC–APCI–MS in body fluids (blood, urine, vitreous humor, and cerebrospinal fluid) of heroin victims, using SPE and morphine-d_3 as the internal standard. The compounds were separated on a reversed phase HPLC column with a mixture of aqueous ammonium formate and acetonitrile. The APCI LC–MS instrument was used in the positive ion mode, using an extractor voltage of 10 V for the analysis of morphine, M3G, and M6G (a voltage at which the glucuronides underwent only moderate in-source fragmentation) and 40 V for 6-MAM (voltage necessary for partial in-source fragmentation of 6-MAM). The ions that were monitored and used for quantitation were *m/z* 286 ($[M+H]^+$ ion of morphine and most distinct fragment of the glucuronides), *m/z* 328 ($[M+H]^+$ ion of 6-MAM) and *m/z* 289 ($[M+H]^+$ ion of the internal standard). The ions at *m/z* 462 (protonated molecular ions of M3G and M6G) and *m/z* 211 and 268 (fragments of the 6-MAM molecule) were monitored for identity confirmation. Quantitation was based on multipoint calibration graphs, and the method was extensively validated, including linearity, determination of recoveries, detection limits, total precision, and an analyte stability time-profile. In a second publication of the same group (Bogusz et al., 1997), the same instrumental configuration is used. That publication describes the determination of the above-mentioned compounds, now in combination with codeine and codeine-6-glucuronide (C6G), in the same biological fluids and using a similar SPE procedure. A mixture of six internal standards was used (morphine-d_3, M3G-d_3, M6G-d_3, codeine-d_6, C6G- d_3 and 6-MAM-d_6). Chromatographic separation was achieved, using a reversed phase column. The mobile phase consisted of an ammonium formate buffer and acetonitrile, used in the isocratic mode, but the flow rate was time-programmed from 0.6 to 1.1 mL/min. This flow-rate gradient approach had important advantages in view of reproducibility. Moreover, it was shown that this gradient does not exert a visible influence on the resulting mass spectra; an acetonitrile gradient did. As in the previous article, the positive ion APCI mode was used. Bogusz et al. devised an elaborate procedure for the SIM detection of the compounds in one single chromatographic separation, using different time windows (for details, see Table 1). For quantitation, the protonated molecular ions of the analytes and of their respective deuterated analogs were always used. The method was completely validated, including the determination of recoveries, detection limits, quantitation limits, total precision, and an analyte stability time-profile. Its application to the routine determination of opiates in blood, urine, and other body fluids in forensic cases proved the method's practical use as well as its robustness. It is unclear why APCI was chosen for this application. Other reports show that ESI works well for opiates, and that the APCI probe assembly temperature profile, unlike its ESI counterpart, always entails a certain risk of thermal degradation.

Zuccaro et al. (1997) describe LC–APCI–MS for the simultaneous determination of heroin, 6-MAM, codeine, and morphine and its glucuronides, and report on its application on serum samples from heroin-treated mice. In addition, they investigated the pharmacokinetics in this animal model of all the heroin metabolites. After an ethyl-phase SPE, with nalorphine as the internal standard, the compounds were chromatographed, using a normal phase column. The mobile phase consisted of methanol/acetonitrile/water/formic acid. Separation was achieved by isocratic elution with effluent splitting before introduction into the atmospheric pressure ionization source of the MS. For the analysis of M3G and M6G, they use an extractor voltage of 50 V (a fragment with *m/z* 286, due to the formation of an aglycone, is exceedingly produced with an increased voltage) and 70 V for morphine, heroin, codeine, and nalorphine (voltage necessary to partially produce in-source fragmentation). Zuccaro et al. obtained spectra that are in close accordance with the spectra

TABLE 1. Operational characteristics of LC-MS applications in the field of opiate analysis

Compound	Sample	Work-up + IS	Chromatography	Effluent treatment	Interface	Mass analysis type & detection mode	Monitored ions	Validation data	Ref
Morphine, M3G, M6G, 6-MAM	Urine	SPE (C_{18}), No IS[a]	L-column, ODS, 150 x 4 mm 100 mM ammonium acetate and acetonitrile, gradient elution from 0 to 40 % acetonitrile flow-rate: 1 mL/min.	/	+ TSP	Q, SIM + scan	*m/z* 286: morphine + M3G- and M6G-aglycone (q) *m/z* 328: 6-MAM (q) *m/z* 462: M3G and M6G (i)	LOD (SIM): 2-40 ng/mL (U.P.) Within-day prec.: 4.5-9.5 % (n=10) Linearity: - 2500 ng/mL	Tatsuno 1996a
Morphine, codeine	Urine	SPE (mixed-mode), Morphine-d_3, codeine-d_3	Zorbax RX-C_8 column, 250 x 4.6 mm, 5 µm p.s. 0.2 % trifluoroacetic acid, 0.1 M ammonium acetate in water-methanol (75/25) flow-rate: 1.25 mL/min.	/	+ TSP	Q, SIM	m/z 286: morphine (q), *m/z* 289: morphine-d_3 (q), *m/z* 300: codeine (q), *m/z* 303: codeine-d_3 (q)	LOD: 2 ng/mL (response factors)	Tai 1994
Morphine, M3G, M6G, 6-MAM	Blood Urine CSF VH	SPE (C_{18}), Morphine-d_3	Superspher RP select B, 125 x 3 mm, 4 µm p.s. 50 mM ammonium formate (pH 3) and acetonitrile (95/5 or 90/10 for 6-MAM flow-rate: 0.6 mL/min.	/	+ APCI	Q, SIM	*m/z* 211, 268: 6-MAM (i) m/z 286: morphine + M3G- and M6G-aglycone (q) *m/z* 328: 6-MAM (q) *m/z* 462: M3G and M6G (i)	LOD: 0.1- 1 ng/mL (s/n≥3) Total prec.: 6-9% (n=5) Linearity: 5-500 ng/mL	Bogusz 1997
Morphine, M3G, M6G, codeine, C6G, and 6-MAM	Blood Urine CSF VH	SPE (C_{18}), Deuterated analogs of 6 compounds	Superspher RP 18 125 x 3 mm, 4 µm p.s. 50 mM ammonium formate (pH 3) and acetonitrile (95/5) flow-rate gradient: 0.6 - 1.1 mL/min.	/	+ APCI	Q, SIM	*m/z* 286: morphine (q), and M3G-and M6G-aglycone (i), *m/z* 289: morphine-d_3 (q), and M3G-d_3 and M6G-d_3 aglycone (i), *m/z* 300: codeine (q), and C6G-aglycone (i), *m/z* 303: C6G-d_3-aglycone (q), *m/z* 306: codeine-d_6 (q), *m/z* 328: 6-MAM (q), *m/z* 334: 6-MAM-d_6 (q), *m/z* 462: M3G, M6G (q), *m/z* 465: M3G-d_3 and M6G-d_3 (q), *m/z* 476: C6G (q), *m/z* 479: C6G-d_3, (q)	LOD: 0.5- 100 ng/mL (s/n≥3) Total prec.: 3-10 % (n=3) Linearity: 5-500 ng/mL	Bogusz 1997
Morphine, M3G, M6G, codeine, heroin, and 6-MAM	Serum	SPE (C_2), Nalorphine	Supelcosil NP LC-Si, 250 x 2.1 mm, 5 µm p.s. methanol-acetonitrile-water-formic acid (59.8-5.2-34.65-0.35) flow-rate: 0.23 mL/min.	1/5 split	+ APCI	Q, SIM	*m/z* 286: morphine (q), *m/z* 300: codeine (q), *m/z* 312: nalorphine (q), *m/z* 328: 6-MAM (q), *m/z* 370: heroin (q), *m/z* 462: M3G and M6G (q)	LOD: 0.5-4.0 ng/mL (s/n≥3) Total prec.: 1.1-6.2 % (n=15) Linearity: 10-10000 ng/mL	Zuccaro 1997

TABLE 1. (*Continued*)

Morphine, M3G	Urine	SPE (C_{18}), No IS	L-column ODS, 150 mm, 4.6 mm 50 mM ammonium acetate and methanol (86-14) flow-rate: 1.0 mL/min.	/	+ APCI	Q, SIM	*m/z* 286: morphine (q), M3G-aglycone (i) *m/z* 462: M3G (q)	LOD (SIM): 1-3 ng/mL (s/n≥3) Within-day prec.: 4.8-3.2 %(n=11)	Nishi-kawa 1992
Morphine, M3G, M6G	Serum	SPE (C_2), Codeine, naltrexone	RP LC-ABZ, 250 x 4.6 mm, 5 µm p.s. convex gradient elution in 10 min. from 85-15 water-methanol to 40-60 flow-rate: 0.8 mL/min.	1/44 split	+ ESI	Q, SIM	*m/z* 286: morphine, M3G, M6G (q)	LOD: 10-100 ng/mL (s/n≥3) Total prec.: 6.5-7.2 % (n=6) Within-day prec.: 6.9-7.4 % (n=6) Linearity : 10-1000 ng/mL	Pacifici 1995
Morphine, M3G, M6G	Serum	SPE (C_{18}), No IS	RP YMC ODS-AL, 100 x 4.6 mm, linear gradient from water and acetonitrile 96/4 to 30/70 with 3 mmol/L formic acid flow-rate: 1 mL/min.	1/50	+ ESI	Q, SIM	*m/z* 286: morphine (q) *m/z* 462: M6G, M3G (q)	LOD: 240 pg/mL (s/n≥3) Total prec.: 3.9-9.0 % (n=30) Within-day prec.: 2.4-8.6 % (n=30) Linarity : 0.84 -500 ng/mL	Tyrefors 1996
Morphine, M3G, M6G, codeine, C6G, and 6-MAM, dihydro-morphine (DHM), dihydrocode ine (DHC))	Blood Urine CSF VH	SPE (C_{18}), Deuterated analogues (DHM and DHC, deut. morphine and codeine resp. used as IS)	Superspher RP 18 125 x 3 mm, 4 µm p.s. 50 mM ammonium formate (pH 3) and acetonitrile (90/10) flow-rate: 0.3 mL/min for M3G, M6G, DHM and morphine flow-rate: 0.6 mL/min. for C6G, 6-MAM, DHC and codeine	/	+ APCI	Q, SIM	cf row 4, *m/z* 288 (q) : DHM *m/z* 199, 245 (i), 302 (q) : DHC	LOD: 0.1 - 100 ng/mL (s/n≥3) Total prec.: 2-11% (n=3) Linearity: 5-500 ng/mL (morphine, M3G, M6G, codeine, C6G, 6-MAM) and 1-200 ng/mL (DHC, DHM)	Bogusz 1998
Morphine, codeine, norcodeine, 6-MAM, pholcodine and codethyline	Blood, plasma and urine	LLE, Nalorphine	Spherisorb 5 RP 8S 100 x 2.1 mm, 5 µm p.s. 0.1 % formic acid and 2 mM ammonium formate in water and acetonitrile (20/80) flow rate: 0.4 mL/min.	/	+ESI	QQQ, MRM	*m/z* 286 - 165: morphine *m/z* 300 - 165: codeine *m/z* 286 - 165: norcodeine *m/z* 328 - 165: 6-MAM *m/z* 399 - 114: pholcodine *m/z* 314 - 165: codethyline *m/z* 312 - 165: nalorphine	Total prec.: 5.5 - 17.8 % (n=6) Within-day prec.: 2.5 - 10.5 % (n=6) Linearity: 10 - 1000 ng/mL	Cailleux 1999
Morphine, M3G, M6G	Plasma	SPE (C_{18}), Deuterated analogues (M6G, deut. morphine)	YMC ODS-AQ 150 x 2 mm, 3 µm p.s. 0.1 % formic acid in water and acetonitrile (95/5) flow-rate: 0.2 mL/min.	/	+ESI	QQQ, MRM	*m/z* 286 - 286: morphine *m/z* 462 - 286: M3G *m/z* 462 - 286: M6G *m/z* 289 - 289: morphine-d_3 *m/z* 465 - 289: M3G-d_3	Total prec.: 1.7 - 4.8 % (n=9) Within-day prec.: 1.8 - 7.8 % (n=4) Linearity: 0.25- 10 ng/mL (M3G and M6G) and 0.5 - 10 ng/mL (morphine) using quadratic fitting	Slawson 1999

[a] Non-standard abbreviations: CSF, cerebrospinal fluid; VH, vitreous humor; IS, internal standard; q, quantitation; i, identification, p.s., particle size; Q ; quadrupole; U.P., undefined procedure.

reported by Bogusz et al. (1997) and Bogusz, Maier and Driessen (1997), although they more frequently report the presence of molecular ion adducts with sodium. It is our own experience that the latter very much depends on such conditions as source temperature, which indeed differs considerably (Bogusz et al., (1997) using 170 vs. 60°C in the latter application), although both figures are relatively low for APCI. Quantitation was performed, using the $[M+H]^+$ ions of the studied compounds (including the glucuronides) and nalorphine. Detection limits and total and within-day precision are reported (see Table 1). No heroin, M6G, or codeine was detected in mouse serum samples after a subcutaneous 20 mg/kg heroin dose.

Nishikawa et al. (1992) used LC–APCI–MS to determine morphine and morphine-3-glucuronide in human urine. The samples were purified with SPE. LC reversed phase separation was carried out with 50 mM ammonium acetate and methanol. Quantitation was performed, using the protonated molecular ions of morphine and of M3G. The limits of detection (using SIM and scan) and coefficients of variation were determined, and are reported in Table 1.

Pacifici (Pacifici et al., 1995) describes a serum assay of morphine and its 3- and 6-glucuronides, based on high performance liquid chromatography–ESI mass spectrometry. The analytes and internal standards, codeine and naltrexone, were subjected to SPE. A reversed phase column and gradient elution was used. In the SIM mode, the following ions ($[M+H]^+$) were monitored: *m/z* 286 for morphine, *m/z* 300 for codeine, *m/z* 342 for naltrexone, and *m/z* 462 for M3G and M6G. Linearity, apparently without using the internal standard, and total and within-day precision are determined (see Table 1). The method proved useful for the determination of serum levels of morphine, M3G, and M6G in pain patients, heroin addicts, and in morphine-treated mice. As in the heroin-treated mice (Zuccaro et al., 1997), no M6G could be detected in the samples of the morphine-treated mice.

Tyrefors et al. (1996) described a method for the determination of morphine and its glucuronides, using LC–MS with electrospray ionization. The compounds were extracted from serum with SPE, and separated on a C_{18} reversed phase column with a gradient of water/acetonitrile that contained formic acid as a modifier. The column eluate was subjected to a split, before it was delivered to the mass spectrometer. The compounds were SIM-detected by their protonated molecular ions at *m/z* 286 for morphine, and *m/z* 462 for M3G and M6G. Linearity and total and within-day precision were determined; data can be found in Table 1.

In a more recent publication, Bogusz et al. (1998) present a method for the determination of various opiate agonists and their metabolites (morphine, M3G, M6G, 6-MAM, codeine, C6G, dihydrocodeine, dihydromorphine, methadone, and buprenorphine) together with cocaine and metabolites and LSD in serum, blood, urine, and other biological matrices (cerebrospinal fluid, vitreous humor, and diluted bile). LC–APCI is used in the selected ion monitoring mode. Concerning the opiates, this report is very similar to the previous publication (Bogusz et al., 1997), except for the use of constant flow-rates instead of flow-rate gradients, and the dihydromorphine (DHM) and dihydrocodeine (DHC), which have been added (determined with morphine-d_3 and codeine-d_6, respectively, as internal standard). Concerning the synthetic opiates, cocaine, and LSD, they are addressed in later specific sections.

Cailleux et al. (1999) report a specific, sensitive, rapid (the chromatographic run takes only 6 min), and robust LC–ESI–MS/MS (triple quadrupole instrument) analysis of opiates (morphine, 6-MAM, codeine, norcodeine, codethyline, and a nalorphine internal standard). After a liquid–liquid extraction, the compounds are separated on a narrow bore reversed phase column (isocratic elution), using formic acid and ammonium acetate in water and acetonitrile. The MRM transitions used for detection and quantitation are all shown in Table 1. The method was validated, and linearity and total as well as within-day precision were determined. Quantification limits were also investigated, and established at 10 ng/mL, a concentration that can be determined with a CV% $\leq 20\%$.

A comparable HPLC–electrospray–MS/MS method, in this case capable of detecting subnanogram concentrations of morphine and its 3- and 6-glucuronide metabolites (M3G and M6G), was presented by Slawson et al. (1999). They use a narrow bore (obviously more or less becoming a standard) reversed phase column and isocratic elution with water, containing formic acid and acetonitrile, to analyze the compounds of interest in human plasma after an SPE extraction. For the glucuronides of morphine, they use the SRM transition of *m/z* 462 (protonated molecular ion) to *m/z* 286 (CID product ion, that corresponds to the loss of glucuronic acid). However, the analytical challenge they encountered in the development of their assay was the extensive CID of morphine and its deuterated internal standard. To overcome this unwanted fragmentation, which they always obtained despite the different conditions tested, and the corresponding loss of sensitivity for the analysis of morphine, they monitored the transition of *m/z* 286 (protonated molecular ion) to *m/z* 286, resulting, unfortunately, in more baseline noise and less specificity. The linearity of the method (Table 1) was determined by calculating product ion peak area ratios for compounds and their respective internal standards (morphine-d_3 for morphine and M6G, and M3G-d_3 for M3G). These peak area ratios were plotted against their corresponding concentrations, using a quadratic curve with 1/x weighting. This nonlinearity is rather unfortunate

because it is generally accepted that linearity should be actively pursued. It does not seem that any attempts were made to overcome the nonlinear character of the measurement, nor is any fundamental reason elaborated why these authors failed with linear regression, which is the standard regression model. One reason we think, and that becomes more and more evident from other researchers' experience, might be a nonlinearity in ionization efficiency due to suppression effects in the ESI source. Probably, quantitative LS–(ESI)–MS/MS users should pay more attention to the intricacies of the ionization process in the future because it obviously largely impacts on their final results. Table 1 again presents some operational characteristics and validation data such as within-day and total precision. An ion chromatogram obtained with this procedure from a patient (administered 0.14 mg/kg IV morphine, 12 h prior to sample collection) plasma sample extract is shown in Fig. 3. As low as 535 pg/mL of morphine was quantified.

Weinmann and Svoboda (1998) present an interesting and promising application for the determination of morphine and codeine as well as amphetamine and benzoylecgonine in serum and urine. A complete description of the method is not given, because they did not use LC–MS, but rather flow-injection ESI tandem mass spectrometry after SPE. However, their method is worth mentioning, because they obtain quantitative results that are comparable to GC–MS, over a broad concentration range (2–1000 ng/mL serum). The LOD ($s/n \geq 3$) of the method is 2 ng/mL for codeine and 4 ng/mL for morphine. Nevertheless, some seriously limiting restraints cannot be overlooked; e.g., no differentiation can be made between M3G and M6G because the masses of both protonated molecular ions and of their major MS/MS transition (collision-induced de-glucuronidation) are identical.

Essentially all of the mentioned manuscripts show that the use of LC for the determination of opiates is unarguably advantageous compared to GC applications because both M3G and M6G can be analyzed without any prior deglucuronidation. A major complication of the use of LC and single mass spectrometry, however, addressed in virtually all of the relevant publications, concerns the

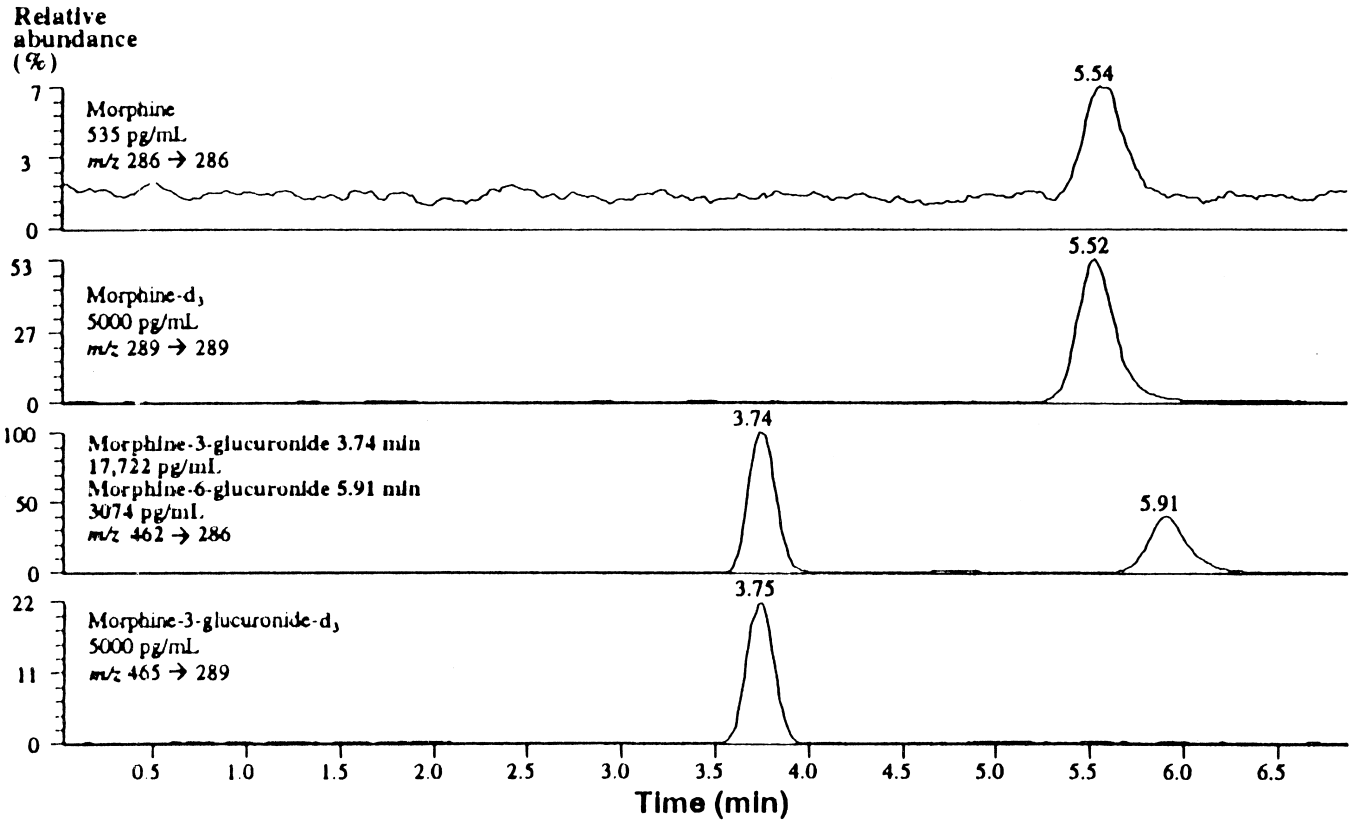


FIGURE 3. Ion chromatogram from an extracted patient plasma sample fortified with 5000 pg/mL internal standards Mor-d_3 (5.52 min) and M3G-d_3 (3.75 min). Mor (0.14 mg/kg IV) was administered 12 h prior to sample collection. Measured concentrations were 535 pg/mL (Mor, 5.54 min), 17,722 pg/mL (M3G, 3.74 min), and 3074 pg/mL (M6G, 5.91 min). (Reproduced from Slawson et al. with kind permission from Preston Publications, Copyright 1999).

fact that these glucuronides show an overwhelming tendency to fragment into glucuronic acid and the aglycone; the aglycone being morphine in this case. This unwanted in-source fragmentation can be remedied by adapting the ionization conditions in order to preserve more of the protonated molecule ion. Unfortunately, this change entails that the other important opiates no longer undergo any substantial informative in-source fragmentation. Some authors circumvent this problem by a time-programmed (after elution of the early glucuronides), e.g., extractor voltage increase (Zuccaro et al., 1997). This time-programming, of course, implies that the chromatographic stage of the opiate analysis must be well-designed and controlled, resulting in an adequate separation of at least morphine, M3G, and M6G. Furthermore, it shows that the use of gradient elution will be inevitable for the successful separation of all the heroin metabolites. However, it has been pointed out (Tatsuno et al., 1996a; Bogusz, Maier & Driessen, 1997) that a changing organic modifier percentage influences the ionization process. Bogusz et al. (1997) have indicated that the alternative to flow-programming could alleviate this unwanted influence. The use of single quadrupole instruments (used in many of the reviewed reports) is sufficient with respect to, e.g., pharmacokinetic studies.

In forensic cases and research into alternative matrices (hair, saliva), however, a single protonated molecular ion is, although resulting in a sensitive analysis, inadequate for unequivocal identification. CID, using LC–MS/MS, certainly proves to be the better approach in these cases and circumvents the problems of lack of in-source fragmentation of morphine under those conditions where M3G and M6G show an extensive tendency to fragment.

Unfortunately, some of the described publications, especially the older ones, report quantitative data, but lack information on the quality of their quantitative data; e.g., no linearity, no precision, and no information on the method used to obtain LOD's. This lack makes it difficult, to say the least, for a reader to evaluate a method or to adopt it.

2. *Cocaine and Metabolites*

Cocaine, the major alkaloid of the coca plant, is a potent drug with medical use and strong abuse potential. The growing public health danger of cocaine is a result of the advent of alternative drug administration routes such as crack smoking, in addition to snorting and intravenous administration. To investigate cocaine abuse, the detection of benzoylecgonine (BE) or ecgonine methyl ester (EME), two major (inactive) metabolites of cocaine with a relatively long half-life, is often required. These polar metabolites inevitably require derivatization prior to gas chromatography. Other important metabolites of cocaine are norcocaine, a psychoactive metabolite. norbenzoylecgonine, a metabolite that causes seizures. and cocaethylene (CE). The latter is a pharmacologically remarkable transesterification product of cocaine that is formed when cocaine and ethanol are taken together. CE appears together with its own metabolites norcocaethylene and ecgonine ethyl ester. The pyrolysis products of cocaine, anhydroecgonine and anhydroecgonine methyl ester, are formed when cocaine is heated, and are as such important markers to identify ''crack'' smokers. However, these thermal degradation products may also be formed during GC analysis (González et al., 1995).

Wang and Bartlett (1998) thoroughly explored the CID fragmentation mechanism of cocaine and 15 of its important metabolites via infusion of standards, including benzoylecgonine, the pyrolysis products anhydroecgonine and its methyl ester, as well as the thermally labile but highly toxic metabolite cocaine *N*-oxide. The CID mass spectra of three deuterium-labeled compounds and deuterium-exchanged cocaine (solution of cocaine in deuterium oxide) were also studied to confirm the identity of certain fragment ions and to aid in the fragmentation elucidation. The proposed general fragmentation pattern of protonated cocaine and its metabolites is shown in Fig. 4. This report of Wang and Bartlett is highly successful in offering a thorough understanding of the fragmentation of cocaine and its related compounds. The study shows that the transition to be monitored for an LC–MS/MS assay would, for the majority of these compounds, be from the protonated molecule ion to a fragment of *m/z* 182 for cocaine or *m/z* 196 for cocaethylene (ions marked c in Fig. 4). In the case of the interesting pyrolysis product anhydroecgonine methyl ester, a fragment ion (designated f) at *m/z* 122 was observed to be the most abundant at the selected collision energy.

In a previously reviewed procedure, Tatsuno et al. (1996a) used thermospray LC–MS to determine cocaine and BE, together with a number of opiates and some amphetamines in human urine. As was mentioned, extraction is performed with SPE and chromatographic separation is achieved on a reversed phase column with gradient elution. Cocaine and BE both produced the $[M+H]^+$ ion, at *m/z* 304 and 290, respectively, as the base peak in this TSP–MS experiment. As was already pointed out, a relatively insensitive detection was obtained (cf. Table 2).

Nishikawa et al. (1994a) developed a method for the simultaneous determination of cocaine and four of its metabolites (BE, norcocaine, EME, and ecgonine) in urine by LC–APCI–MS. An aqueous, steric exclusion chromatographic column was used with a mobile phase system that consisted of ammonium acetate/acetonitrile. An SPE procedure that consisted of two solid phase cartridges was developed to selectively extract the

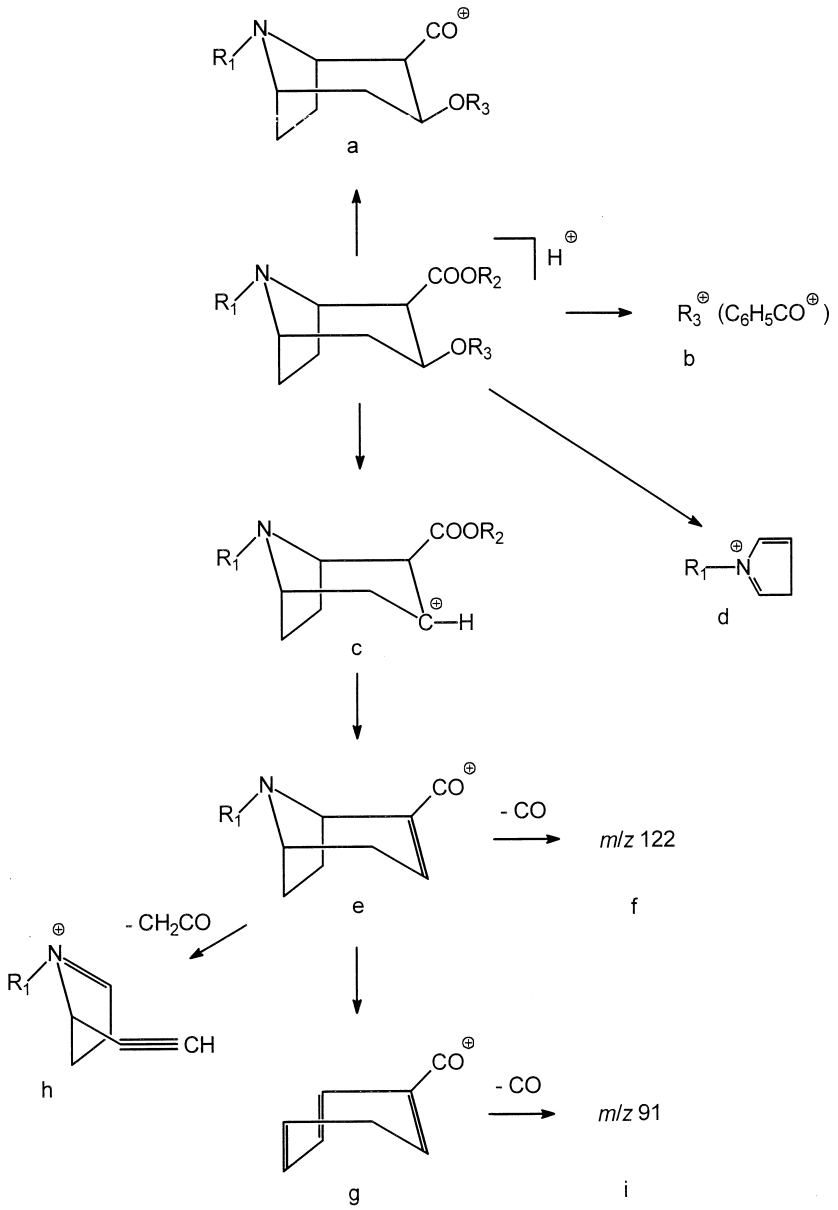


FIGURE 4. General fragmentation pathway of protonated cocaine and its metabolites. (Reproduced from Wang and Bartlett, 1998, with kind permission from John Wiley & Sons Limited, Copyright 1998).

compounds from urine. After extraction, both eluates were mixed and injected on the LC–APCI–MS. The base peaks in the resulting mass spectra were the protonated molecular ions at *m/z* 186 for ecgonine, *m/z* 200 for EME, *m/z* 290 for norcocaine and BE, and *m/z* 304 for cocaine. Other ions, corresponding to loss of a carboxyl, hydroxyl, or benzoyl group, were also observed. In this respect, Nishikawa found out that, contrary to their findings on morphine and its glucuronides (Nishikawa et al., 1992), the mass spectral patterns of cocaine and its metabolites hardly change with varying conditions of the mobile phase. Quantitative validation parameters such as detection limits and linearity data, together with some operational characteristics, can be found in Table 2.

Sosnoff et al. (1996) analyzed BE in blood spots (whole blood spotted onto filter paper) collected from newborn babies, using a sensitive liquid chromatographic–APCI tandem mass spectrometric method. MS detection was performed in the positive-ion APCI mode on a triple quadrupole mass spectrometer. Using APCI–MS/MS, Sosnoff obtained fragmentation patterns that are in perfect concordance with the fragmentation scheme

proposed by Wang and Bartlett (1998). MRM was used for quantitative purposes. The transition at *m/z* 290–168 of native BE compared to the corresponding transition for the internal standard (trideuterated BE) at *m/z* 293–171 was used. The BE transitions at *m/z* 290–105 and 290–82 were monitored for additional confirmation. Chromatographic analysis was performed on two serial C_{18} columns. Validation data can again be found in Table 2. The method developed was used for confirmatory analyses, after screening with radio immunoassay, in a large epidemiological study for the prevalence of cocaine use during late pregnancy, which is a problem that has become a public heath concern in the U.S. Actually, it is estimated that the use of cocaine (crack) during pregnancy is associated with a 200–300% increase in cost of delivery of these so-called ''crack babies''; that increase annually amounts up to a cost of approximately 1 billion dollars to the US health care system.

As mentioned earlier, Bogusz et al. (1998) described the determination of opiate agonists and LSD together with cocaine and two of its metabolites, BE and EME, in biological fluids. The biological fluids were extracted with a common solid-phase extraction method after addition of the appropriate deuterated internal standards, and separation was again achieved on a reversed phase column. These authors used a single quadrupole instrument equipped with an APCI source, in the positive ionization mode. Procedures were written for the SIM detection of the particular drugs. The reported validation achievements (Table 2) prove the usefulness of the method for multicomponent analysis, even though the use of two different injections (two different chromatographic conditions used for the determination of EME and cocaine/BE, respectively) after one common solid phase extraction unnecessarily complicates the method.

Singh et al. (1999) describe a validated stable isotope dilution LC–MS/MS assay, using APCI on a triple quadrupole instrument, for the trace analysis in plasma of cocaine and its major metabolites, benzoylecgonine, ecgonine methyl ester, and norcocaine. Their method requires no cleanup, apart from protein precipitation with acetonitrile, and they use deuterated internal standards for all four investigated compounds. Multiple reaction monitoring (transitions, cf. Table 2) was performed after chromatographic separation on a reversed phase column, using gradient elution with ammonium acetate/formic acid in water and acetonitrile. Besides the excellent linearity and precision results (Table 2), they proved that their method was sensitive enough to permit detailed study of the pharmacokinetics of cocaine and its metabolites after intravenous dosage to rats.

Clauwaert et al. (1998) used LC–ESI–MS/MS to investigate 12 positive hair samples. They applied LC with fluorescence detection to analyze hair samples for cocaine, BE, and cocaethylene, using two in-house synthesized internal standards, 2′-methylbenzoylecgonine and 2′-methylcocaine (molecular masses of 304 and 318, respectively). LC–ESI–MS/MS was used to provide complementary confirmation of cocaine or metabolite identity in the hair samples. Chromatographic separation was achieved on a C_{18} column and a gradient of aqueous ammonium acetate and methanol/acetonitrile. Experiments were conducted on a triple quadrupole system. The compounds were isolated from the hair matrix, using an SPE procedure with mixed-mode cartridges. Validated quantitative results were obtained with fluorescence detection; none was reported with MS detection. The LC–MS/MS method successfully corroborated the results obtained with LC/fluorescence.

Wang and Bartlett (1999) published a method, using LC–MS, to identify and quantitate cocaine-*N*-oxide, a thermally labile compound, in meconium, plasma, amniotic fluid, fetal tissue, and placental tissue. They found that cocaine-*N*-oxide converts to cocaine when heated to over 100°C; that conversion prevents, GC(–MS) analysis. After SPE, they inject their samples on a C_8 column, using gradient elution with ammonium acetate in water, methanol, and acetonitrile mixture. Their work was performed on a triple quadrupole MS with ESI (positive ion mode), however, only using single MS (SIM) following the ions *m/z* 304.3 (cocaine), 307.3 (codaine-d_3) and 320.2 (cocaine-*N*-oxide). Linearity and LOD data can be found in Table 2. The paper features full scan MS spectra for cocaine and cocaine-*N*-oxide. Unfortunately, like all ESI-generated (single) MS spectra, they are less informative because only the protonated molecular ion is generated, whereas, moreover, somewhat confusing in this case because of the many large background peaks. Having MS/MS capabilities, a CID MS/MS spectrum would probably have been more appropriate.

Cailleux et al. (1999) describe the use of a liquid chromatograph–ESI tandem mass spectrometer as a confirmatory tool for the presence of cocaine, benzoylecgonine, ecgonine methyl ester, cocaethylene, and anhydroecgonine methylester; together with the opiates, in urine, plasma, and blood. After addition of the internal standards, the samples are liquid–liquid extracted and separated on a narrow bore reversed phase column with isocratic elution, using formic acid and ammonium acetate in water and acetonitrile. The MRM transitions used for detection and quantitation as well as their validation results can be found in Table 2. Compared to other methods, also doing opiates and cocaine and metabolites, this paper uses two separate LC separations for both groups, together with separate MS conditions. The chromatographic separation times, on the other hand, were kept short (e.g., 6 min for the cocaine group compounds) to provide rudimentary separation, but

TABLE 2. Operational characteristics of LC-MS applications in the field of cocaine analysis.

Compound	Sample	Work-up + IS	Chromatography	Effluent treat-ment	Inter-face	Mass analysis type & detection mode	Monitored ions	Validation data	Ref
Cocaine, benzoylecgonine	Urine	SPE (C_{18}), No IS[a]	L-column, ODS,150 x 4 mm 100 mM ammonium acetate and aceto-nitrile, gradient elution from 0 % to 40 % acetonitrile flow-rate: 1 mL/min.	/	+ TSP	Q, scan and SIM	*m/z* 304: cocaine (q) *m/z* 290: benzoylecgonine (q)	LOD (SIM): 2-40 ng/mL (U.P.) Within-day prec.: 4.5-9.5 % (n=10) Linearity: - 500 ng/mL	Tatsuno 1996a
Cocaine, benzoylecgonine, ecgonine methyl ester, norcocaine, ecgonine	Urine	SPE (C_{18} + mixed mode), No IS	Asahipak GS-320H, 250 x 7.6 mm, (steric exclusion chromato-graphy column) 10 mM ammonium acetate and acetonitrile (90/10) 2 min followed by 30 min 20 mM ammonium acetate and acetonitrile(50/50) flow-rate: 1 mL/min.	/	+APCI	Q, SIM	*m/z* 304: cocaine (q) *m/z* 290: benzoylecgonine (q) *m/z* 200: ecgonine methyl ester (q) *m/z* 290: norcocaine (q) *m/z* 186: ecgonine (q)	LOD (s/n≥3):50 ng/mL (cocaine), 800 ng/mL (ecgonine) Linearity: 1-5 µg/mL	Nishikawa 1994a
Benzoylecgonine	Dried blood spots	Elution of spots, BE-d_3	2 serial C_{18} columns, 30 x 4.6 mm, 3 µm p.s. 25 mM ammonium acetate in water and methanol (50/50) flow-rate: 1 mL/min.	/	+APCI	QQQ, MRM	*m/z* 290 - 168 (q), *m/z* 290 - 105 (i), *m/z* 290 - 82 (i): benzoyl-ecgonine *m/z* 293 - 171 (q): benzoyl-ecgonine-d_3	LOD (s/n≥3): 2 ng/mL Linearity: 0-166 ng/mL	Sosnoff 1996
Cocaine, benzoylecgonine, ecgonine methyl ester	Urine blood serum	SPE, Deut. IS	Superspher RP C_{18}, 125 x 3 mm, 4 µm p.s. 50 mM ammonium formate buffer pH 3.0 and acetonitrile flow-rate: 0.2 - 1 mL/min.	/	+APCI	Q, SIM	*m/z* 304 (q), 182 (i): cocaine *m/z* 312 (q), 185 (i): cocaine-d_8 *m/z* 290 (q), 168 (i): BE *m/z* 298 (q), 171 (i): BE-d_8 *m/z* 200 (q), 182 (i): EME *m/z* 203 (q), 185 (i): EME-d_8	LOD (s/n≥3): 0.5 ng/mL (cocaine), 0.2 ng/mL (benzoylecgonine, EME) Total prec.: 5-10 % (n=3) Linearity: 1-200 ng/mL	Bogusz 1998

Cocaine, benzoylecgonine, ecgonine methyl ester and norcocaine	Plasma	Protein precipitation, Deut. IS	Reversed phase YMC basic column, 150 x 4.6 mm, 5 µm p.s. 5 mM ammonium acetate, 0.1 % formic acid in water/acetonitrile flow-rate: 0.8 mL/min.	/	+APCI	QQQ, MRM	*m/z* 304 -182: cocaine *m/z* 309-182: [$^2H_5$]cocaine *m/z* 290-168: benzoylecgonine *m/z* 295-168: N-C[$^2H_5$]-benzoyl-ecgonine *m/z* 200-182: ecgonine methyl ester *m/z* 203-185: N-C[$^2H_3$]-ecgonine methyl ester *m/z* 290-168: norcocaine *m/z* 295-168: [$^2H_5$]norcocaine	Total prec.: 0.8-4.1% (n=15) Linearity: 2-1000 ng/mL	Singh 1999
Cocaine, benzoylecgonine, cocaethylene	Hair	SPE, 2'-methyl-BE and 2'-methyl-cocaine	Reversed phase Hypersil BDS C_{18} 125 x 2.1 mm, 5 µm p.s. 0.045 M ammonium acetate in water/methanol/ acetonitrile flow-rate: 0.2 mL/min.	1/20	+ESI	QQQ	m/z 304: cocaine m/z 318: 2'-methylcocaine m/z 290: BE m/z 304: 2'-methylBE m/z 318: cocaethylene	/	Clauwaert 1998
Cocaine-N-oxide	Plasma	SPE, Deutera-ted cocaine	Eclipse XDB-C_8 column, 150 x 2.1 mm, 5 µm p.s. 20 mM ammonium acetate in water/methanol/ acetonitrile flow-rate: 0.3 mL/min.	/	+ESI	QQQ, SIM	*m/z* 304.3: cocaine *m/z* 307.3: codaine-d_3 *m/z* 320.2: cocaine-N-oxide	LOD: 1 ng/mL (U.P.) Linearity: 0.01 - 2 µg/mL	Wang 1999
Cocaine benzoylecgonine, ecgonine methyl ester, cocaethylene anhydroecgonine methylester	Urine, plasma blood	LLE, Deutera-ted IS	Spherisorb 5RP 8S, 100 x 2.1 mm, 5 µm p.s. 0.1 % formic acid, 2 mM ammonium formate in water/acetonitrile flow-rate: 0.4 mL/min.	/	+ESI	QQQ, MRM	*m/z* 304.2-182.2: cocaine *m/z* 290.2-168.2: benzoylecgonine *m/z* 220.2-182.2: ecgonine methyl ester *m/z* 318.2-196.2: cocaethylene *m/z* 182.2-122.2: anhydroecgonine methyl ester	Total prec.: 1.6 - 11.3% (n=6) Within-day prec.: 1.2 - 16.5 % (n=6) Linearity: 10 - 1000 ng/mL	Cailleux 1999
Cocaine, benzoylecgonine	Urine	Filtration, Coc.- d_3	Advantage Basic C_8 column, 50 mm x 2 mm, 5 µm p.s. Ammonium formate buffer (pH 4) and acetonitrile flow-rate: 0.6 mL/min.	/	+ESI	QQQ, MRM	*m/z* 304.2-182.0 (q), 304.2-105.0 (i), 304.2-82.1 (i): cocaine *m/z* 290.2-168.2 (q), 290.2-105.2 (i), 290.2-77.2 (i): benzoyl-ecgonine *m/z* 307.2-185.2 (q), 307.2-104.9 (i), 307.2-85.1 (i): cocaine-d_3	LOD (s/n≥3): 2.5 ng/mL Total prec.: < 6.9 % (n=5) Within-day prec.: < 2.5% (n=5) Linearity: 7.5 - 1000 ng/mL	Jeanville 2000

[a] Non-standard abbreviations: IS, internal standard; q, quantitation; i, identification; p.s., particle size; Q, quadrupole; U.P., undefined procedure.

invoked the superior selectivity of MS/MS. Despite a peculiar looking MS/MS spectrum illustrated for morphine, the validation parameters are excellent, indicating a robust method. Moreover, it seems that no ionization suppression, induced by the co-eluting peaks is evident, even at 1 μg/mL concentration levels.

Jeanville et al. (2000) also describe a rapid, sensitive, and selective method for the confirmation and quantitation of benzoylecgonine and cocaine in urine, this time by gradient liquid chromatography tandem mass spectrometry. These authors kept specimen preparation to a minimum, solely consisting, after addition of the internal standard (cocaine-d_3), of a filtration step. After a fast gradient elution of the compounds, using an ammonium formate buffer (pH 4) and acetonitrile, on a narrow bore C_8 column (analysis time 2.1 min), discrete MRM transitions (cf. Table 2) were monitored for quantitation and identification. The validation results are shown in Table 2. Apart from the quantitative assay presented, an interesting part of this paper deals with the mechanistics of the fragmentation of cocaine and related products, in which deuterated analogs and MS^n on an LCQ (ion trap) are utilized. The achievements of the method are impressive, certainly in terms of analysis speed. Nevertheless, certain considerations must be made. First, the short LC analysis times are relative to the, as stated by the authors, necessary adequate time needed to recondition the analytical system prior to the next injection (time not specified). Secondly, all assays have been performed on a spiked, pooled urine batch and no real time samples are analyzed. Pooling urine inevitably means averaging such factors like endogenous interferences, diurnal concentration variations, etc. We think the validation of a method is incomplete unless a critical mass of real samples are analyzed, in this case of drug abusers, which, in addition, frequently have a poly-drug abuse profile. Finally, these authors do encounter, and clearly describe, the limitations of scanning instruments when working with fast separations (narrow peaks). Obviously, such detectors as the TOF detector are a desirable alternative in this case.

From the above-reviewed manuscripts, we can conclude that LC–MS and especially LC–MS/MS can be successfully used to determine cocaine and/or its metabolites in challenging circumstances [e.g., complex matrices such as hair (Clauwaert et al., 1998), or low sample quantities; e.g., 12 μL blood (Sosnoff et al., 1996)]. Structural information on the basis of product-ion spectra allows for the mandatory unequivocal identity confirmation. Fundamentally, the use of LC–MS/MS has three important advantages compared to GC-based analytical approaches. LC separations require no derivatization of the polar metabolites, the thermally labile but highly toxic metabolite, cocaine *N*-oxide, can only be monitored with LC, and thermal degradation complications during injection as well as the artifactual formation of the pyrolysis products, anhydroecgonine and anhydroecgonine methyl ester, are obviously avoided. Irrespective of the high quality work on cocaine that has already been published, we think that the literature still lacks the method that toxicologists are most interested in. This ideal method is a single (LC–MS/MS) method, which determines cocaine and most of its metabolites, including the polar metabolites BE, ecgonine, and EME, the hydroxylated and methoxylated metabolites, cocaine-*N*-oxide, norcocaine, the pyrolysis products anhydroecgonine, and its methyl ester, in biological matrices such as urine or serum. The development of such a method will indeed be challenging. The presence of metabolites with the same molecular masses (e.g., hydroxylated isomers with molecular mass 319, cocaine and norcocaethylene with molecular mass 303, or benzoylecgonine and norcocaine with molecular mass 289) and common fragments (e.g., *m/z* 82, 105, and 182) makes a good chromatographic separation a necessity. A difficulty to overcome in the development of such a quantitative analysis will be the extraction procedure. It is hard to find an extraction method that can achieve high recoveries for compounds that have such a wide range of polarities such as cocaine and its metabolites. Unless, of course, the sample clean-up is eliminated all together. The inherent selectivity of mass spectrometry should allow the analyst to develop a rather rudimentary or nonspecific extraction procedure (consequently with high recoveries for all metabolites) with the possibility to distinguish cocaine and its metabolites from matrix compounds on the basis of their specific ions. Some of the latest works presented clearly go into that direction and achieve good quantitative results, despite some of the ventilated reservations with respect to the ionization process being influenced by co-eluting substances.

3. LSD

Lysergide (lysergic acid diethylamide, LSD) is one of the most potent hallucinogenic drugs known. Illicit LSD is generally found in tablet form or impregnated on small paper squares that contain 50–100 μg of the substance. LSD is extensively metabolized in man to give *N*-demethyl-LSD (nor-LSD, an important metabolite) and 13- and 14-hydroxy-metabolites. Approximately only 1% of the unchanged drug is excreted in urine. A normal LSD dose, sufficient to produce a ''trip'', results within a few hours after ingestion in plasma and urine concentrations at the sub-nanogram/milliliter level. Urine samples, screening positive for LSD, must be confirmed. However, in addition to the very low levels of LSD found in biological samples, LSD is photosensitive, relatively nonvolatile, thermally unstable at GC temperatures, and

readily adsorbed onto glass, and it exhibits, to a certain degree, irreversible adsorption onto GC columns. Therefore, HPLC is a very suitable separation technique for trace LSD samples. Fluorescence detection is capable of meeting the required detection limits. however, it is now generally recognized that some form of absolute identification is necessary. All of the above-mentioned factors make LC–MS(/MS) the method of choice for the analysis of LSD in biological samples.

Duffin, Wachs and Henion (1992) described the conversion of a commercial benchtop GC–MS (Hewlett-Packard Model 5970 Mass-selective detector) to a mass spectrometer applicable for LC–MS. The modified MS can sample gas-phase ions formed at atmospheric pressure either by pneumatically assisted electrospray (ion spray) or by APCI. The latter approach was used by Duffin et al. to analyze LSD standards. Table 3 compiles some of the operational characteristics for this report and the following reports. From an MS point of view, Duffin et al. chose to use in-source fragmentation through a potential difference between the ion-sampling capillary and the sample cone to achieve more informative fragmentation. The resulting mass spectra showed the protonated molecular ion at *m/z* 324 and fragment ions at *m/z* 281, 223, and 208. A SIM signal for as little as 250 pg LSD injected could be achieved. Nevertheless, a forensically valid detection limit (each of the four selected ions must be observed with its relative abundance ratio within $\pm 20\%$ of the normal observed abundance ratio) of 2.0 ng LSD/mL urine was reported.

Rule and Henion (1992) reported the use of on-line immunoaffinity chromatography–HPLC–ESI–MS. They used a commercially available protein G column, and primed it with an anti-lysergic acid diethylamide (anti-LSD) antiserum to capture the corresponding drug from human urine. Desorption and column-switching techniques afforded direct confirmation of the drugs' presence by LC–MS (see also Table 3). The detection limit of the method was 1 ng/mL LSD, although the authors claimed a detection limit of only 500 pg/mL LSD when monitoring only the protonated molecular ion at *m/z* 324. Of course, eliminating the two qualifier ions makes confirmation of identity less certain if not critical. Rule and Henion concluded that their method, although not being useful for quantification because of antibody saturation at high drug concentrations, could be very useful in those situations where confirming the presence of drugs in a rapid, inexpensive, and automated fashion is the primary objective. In a later publication of the same group (Cai & Henion, 1996a), an on-line immunoaffinity extraction-coupled column LC–MS/MS system for the determination of LSD and its five analogs (iso-LSD, nor-LSD, iso-nor-LSD, de-ethyl-LSD, and nor-allyl-LSD) is described. A comparable immunoaffinity and valve-switching system was used, although a packed capillary column was used as analytical column. The mass spectrometer used was a triple quadrupole system (both mass analyzers operated at unit mass resolution) with an electrospray interface. Cai and Henion experimentally found that by the MRM approach, using the transitions *m/z* 324–223 for LSD and iso-LSD, *m/z* 310–209 for nor- and iso-nor-LSD, *m/z* 296–208 for de-ethyl-LSD, and *m/z* 350–208 for nor-allyl-LSD, allowed the detection of 2.5 pg/mL of these compounds in spiked urine samples (albeit using 100 mL of urine). The developed IAE–LC–LC–MS/MS/MRM method is used to determine several positive human urine specimens. The quantitative aspect of the method (except for the sensitivity) has not been evaluated, and no solutions for the antibody saturation phenomenon were given. However, due to the extreme sensitivity, the method holds great promise for the qualitative determination of LSD and its metabolites in biological samples. On the basis of their practical experience and results achieved, the authors also concluded that their technique can be useful for the selective enrichment and characterization of drug metabolites with similar substructures as the parent drug (these compounds will be retained by the anticompound antibodies). Data for some oxylated LSD metabolites are illustrated in support of this conclusion.

Cai and Henion (1996b) also have investigated the in-vitro metabolism of LSD by human liver microsomes. The determination of LSD human liver in-vitro metabolites and of these metabolites in positive human urine samples was performed by HPLC and capillary electrophoresis, coupled with tandem MS. The mass spectrometer they used was a triple quadrupole system used with an electrospray interface. An example of an (infusion) CID spectrum of LSD together with the proposed fragmentation pattern as achieved with this instrumental configuration is shown in Fig. 5. This article also contains an interesting section on mass spectral characterization and proposed fragmentation patterns with CID. Ample MS data are provided, and the article is an excellent starting point for a novice in the LSD field. The manuscript also exhaustively reports on the application of MS/MS techniques in the detection of unknown metabolites (precursor-ion scanning, neutral loss analysis, . . .). It eloquently informs the reader about the state-of-the-art MS approach, and about the results achieved in the case of LSD. Indeed, two new in-vitro metabolites, lysergic acid ethylamide and 2-oxo-LSD, were positively identified. Several other potential in-vitro metabolites were detected, and experimental evidence suggested that they were mono- and trioxylated metabolites of LSD. In human urine specimens, several LSD-related compounds were detected: iso-LSD was present at the highest concentrations, followed by nor-LSD and iso-nor-LSD. Lysergic acid ethylamide, the major in-vitro metabolite of LSD by

TABLE 3. Operational characteristics of LC-MS applications in the field LSD analysis.

Compound	Sample	Work-up + IS	Chromatography	Effluent treatment	Interface	Mass analysis type & detection mode	Monitored ions	Validation data	Ref
LSD	Standards	/	C18 RP column, 100 x 1.0 mm, 5 µm p.s. acetonitrile/water (80/20) with 10 mM ammonium formate and 5 mM triethylamine flow-rate: 30 µL/min.	/	+ APCI	Q[a], scan and SIM	*m/z* 324 ($[M+H]^+$), 281, 223, 208	LOD (scan): 2 ng/mL (relative ion abundance ratio ± 20%)	Duffin 1992
LSD	Urine	IAE on-line, No IS	Regis ISRP C_{18} (10 x3.0 mm) trapping and Zorbax cyano analytical column, 150 x 4.6 mm, 5 µm p.s. acetic acid, pH 5.0/acetonitrile 50/50 containing 3 or 5 mM ammonium acetate flow-rate: 1.2 mL/min.	/	+ ESI (pneumatically assisted)	Q, SIM	*m/z* 324 ($[M+H]^+$), 223, 208	LOD (SIM): 0.5 ng/mL (s/n≥10)	Rule 1992
LSD, iso-LSD, nor-LSD, iso-nor-LSD, de-ethyl-LSD, nor-allyl-LSD	Urine	IAE on-line, No IS	C_{18} packed capillary column, 150 x 0.3 mm, 3 µm p.s. methanol 30% / acetonitrile 30% / 0.1% acetic acid / 5mM ammonium acetate flow-rate: 3.5 µL/min.	/	+ ESI	QQQ, MRM	*m/z* 324-223: LSD and iso-LSD *m/z* 310-209: nor- and iso-nor-LSD *m/z* 296-208: de-ethyl-LSD *m/z* 350-208: nor-allyl-LSD	LOD: 2.5 pg/mL (s/n≥10)	Cai 1996a
LSD and several metabolites or analogs	Liver, microsomes, and urine	LLE & SPE (Bond Elut Certify) for urine, No IS	Spherisorb ODS-2 100 x 1.0 mm, 5 µm p.s. 10/10 to 49.5/49.5 methanol/acetonitrile with 0.1% acetic acid and 5mM ammonium acetate gradient flow-rate: 60 µL/min.	/	+ ESI (pneumatically assisted)	QQQ, neutral loss and parent-ion scanning, MRM	Various ions or transitions depending on purpose (unknown metabolite identification or identity confirmation)	LOD: 50 pg/mL (U.P.)	Cai 1996b

LSD	Urine	SPE (C_{18}), LAMPA	Superspher RP 18 125 x 3 mm, 4 µm p.s. 50 mM ammonium formate (pH 3) and acetonitrile (75/25) flow-rate: 0.5 mL/min.	/	+ APCI	Q, SIM	*m/z* 324 ($[M+H]^+$), 281, 223: LSD *m/z* 324: LAMPA	LOD: 0.5 ng/mL (s/n≥3) Total prec: 11% (n=3) Linearity: 0.5 - 10 ng/mL	Bogusz 1998
LSD	Urine	SPE (Bond Elut Certify) or IAE, Methyser-gide	Hypersil C_{18}, 125 x 3.0 mm, 3 µm p.s. acetonitrile/0.1 M ammonium acetate buffer with 0.2 % triethylamine, (pH 8.0) (25/75) flow-rate: 0.5 ml/min.	/	+ ESI	Q, scan and SIM	*m/z* 324 ($[M+H]^+$), 281, 223: LSD *m/z* 354 ($[M+H]^+$): methysergide	LOD: 0.5 ng/ mL (U.P.) Linearity: 0.5 - 10 ng/mL	Webb 1996
LSD	Urine	SPE, Methyser-gide	Hypersil C_{18}, 125 x 3.0 mm, 3 µm p.s. 0.1 M acetate buffer (pH 8.0)/acetonitrile/triethyl-amine (75/25/0.25) flow-rate: 0.5 ml/min.	/	+ ESI	Q, SIM	*m/z* 324 ($[M+H]^+$), 281, 223: LSD *m/z* 324 ($[M+H]^+$), 281, 223: LAMPA: *m/z* 354 ($[M+H]^+$): methysergide	LOD (SIM): 0.3 ng/mL (s/n≥10) LOD (scan): 0.5 ng/mL (s/n of weakest monitored ion≥5) Total prec.: 4.9% (n=5) Linearity: 0.5 - 10 ng/mL	White 1997
LSD, nor-LSD	Urine	Extrelut, LSD-d_3	Nucleosil C_{18}, 150 x 1.0 mm 2 mM ammonium formate (pH 3)/ acetonitrile (70/30) flow-rate: 40 µL/min.	/	+ ESI	Q, SIM	*m/z* 324 ($[M+H]^+$), 223: LSD *m/z* 310 ($[M+H]^+$), 237, 209: nor-LSD *m/z* 327 ($[M+H]^+$): LSD-d_3	LOD: 0.1 and 0.2 ng/mL LSD, resp. nor-LSD: (relative abundance of confirmatory ions versus quantitation ions ± 20%) Total prec.: < 14% (n=6) Within-day prec.: < 9% (n=6) Linearity: 0.05-20 ng/mL	Hoja 1997b
LSD, nor-LSD	Urine blood, serum and plasma	SPE (Bond Elut Certify), LSD-d_3	Zorbax SB-phenyl, 750 x 4.6 mm, 3.5 mm p.s. 35% 0.2 M aqueous ammonium acetate (pH 4.0) in acetonitrile/*n*-propanol (954:46) flow-rate: 250 µL/min.	/	+ ESI	QQQ, MRM	*m/z* 324.2-197.1, 324.2-223.1 and 324.2-208.1: LSD *m/z* 310,2-209.1 and 310.2-237.1: nor-LSD *m/z* 327.2-200.1, 327.2-211.1 and 327.2-226.1: LSD-d_3	LOD: 0.025 ng/mL (U.P.) Total prec.: 4.4 to 9.1% Within-day prec.: 2.2 to 2.7% Linearity: 0.05 - 5 ng/mL (non-linear regression)	de Kanel 1998
LSD	Urine	SPE (Bond Elut Certify), LSD-d_3	Hypersil ODS 125 x 3.0 mm, 3 µm p.s. acetonitrile/0.1 M ammonium acetate with 0.25 % triethylamine, (pH 8.0) (25/75) flow-rate: 0.5 ml/min.	/	+ ESI	Q, SIM	*m/z* 324 ($[M+H]^+$), 223, 197: LSD *m/z* 327 ($[M+H]^+$): LSD-d_3	LOD (LSD): 0.5 ng/mL (s/n≥3 for weakest ion) Within-day prec.: 6.5 -11.6 % Linearity: 0.5 - 10 ng/mL	White 1999

[a]Non-standard abbreviations: IAE, immunoaffinity extraction; LLE, liquid-liquid extraction; Q, quadrupole; p.s., particle size; U.P., undefined procedure.

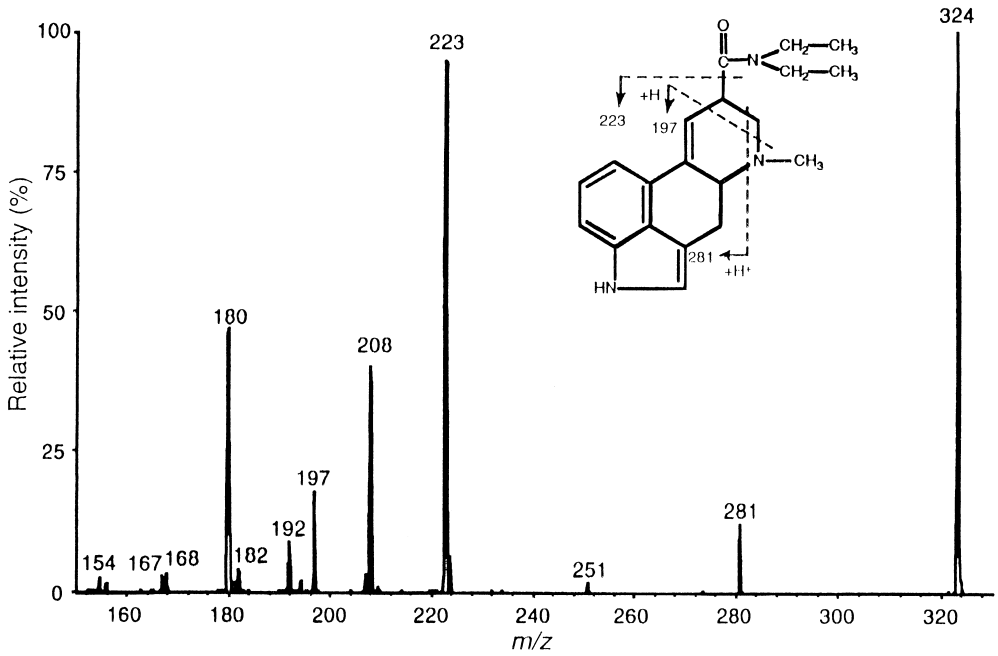


FIGURE 5. Infusion collision-induced dissociation product ion mass spectrum of LSD. Infusion of methanolic solution of LSD (5 ng/μL) at 2 μL/min. (Reproduced from Cai & Henion, 1996b, in Journal of Analytical Toxicology, with kind permission of the authors and Preston Publications, a division of Preston Industries, Inc.).

human microsomal preparations, was also present in the human urine.

Bogusz et al. (1998) determined opiate agonists, cocaine and metabolites, and lysergic acid diethylamide in serum, blood, urine, and other biological matrices, using LC–APCI–MS with lysergic acid *N*-methylpropyl amide (LAMPA) as an internal standard for LSD. A more extensive description of the instrumentation and conditions used has been given previously, and Table 3 provides the instrumental parameters and method characteristics as well as the relevant validation data of this LSD assay. This report contains a straightforward method in which maximum use is made of the power of LC–MS. As the authors stated, it provides a good example of the routine application of the new MS techniques in forensic casework.

Webb et al. (1996) published the use of HPLC in combination with ESI–MS for the confirmation and quantitation of LSD in urine samples after screening with a radio immunoassay, or a novel enzyme immunoassay procedure. The samples were extracted with a SPE procedure, using mixed mode solid phase columns or, alternatively, an immunoaffinity extraction procedure. The latter extraction approach, however, showed low recovery problems at high LSD concentrations, suggesting saturation of the affinity gel's LSD-binding capacity. That saturation phenomenon obviously is a treacherous characteristic of immunoaffinity extraction methods. Methysergide (at *m/z* 354, the $[M+H]^+$ ion) was used as the internal standard. It was chosen over LSD-d_3 because the latter has an important (qualifier) ion, *m/z* 281, in common with LSD. By applying a high enough voltage to the extractor, a characteristic in-source fragmentation pattern for LSD was obtained, containing two structurally significant fragment ions at *m/z* 223 and 281 together with the protonated molecule ion at *m/z* 324 (used for quantitation). The degree of fragmentation, however, was subject to change from day to day, and equally varied with different batches of mobile phase. Hence, the fragmentation voltage must be frequently checked and adjusted appropriately. An extensive interference study was conducted (104 compounds), and validation (see also Table 3) showed that the method displayed good linearity between 0.5 and 10 ng/mL (ensuring correct *m/z* 223 and 281 relative intensities to *m/z* 324 within a 10% error margin). In a second publication of the same group (White et al., 1997), identical LC–MS results were reported, without the immunoaffinity extraction.

A sensitive and highly specific method for the determination of LSD and *N*-demethyl-LSD or nor-LSD, using ESI–LC–MS and LSD-d_3 as an internal standard, has been developed by Hoja et al. (1997b). The main MS parameters were optimized for the protonated molecular ion of LSD (*m/z* 324) (details in Table 3). Practical

(forensic) detection limits (allowing unambiguous identification through relative abundances of qualifier ions versus quantitation ions within 20% to those of drug standards) were 0.1 ng/mL and 0.25 ng/mL for LSD and *N*-demethyl-LSD, respectively. This achievement means that the lower quantitative concentrations are considerably less than what is obtained with GC–EI–MS and comparable with GC–CI–MS. As such, the authors claimed that the method allows LSD detection in urine for at least 30–48 h post-dose. The presented analytical method combines sensitivity, specificity, speed, precision, and reliability and certainly relieves the analyst of some of the pitfalls of present GC-based methods without sacrificing performance.

de Kanel et al. (1998) have also developed a forensic procedure for the quantitative analysis of LSD and the qualitative confirmation of its metabolite, *N*-demethyl-LSD (nor-LSD) in blood, serum, plasma, and urine samples, using LSD-d_3. A Zymark RapidTrace was used to fully automate the solid-phase extractions of all specimen types. Analysis used HPLC, followed by positive electrospray ionization MS/MS. As usual, the preliminary optimization of MS(/MS) parameters, using infused compound, was needed to achieve optimum sensitivity. The MRM scheme comprised a total of eight transitions which are summarized in Table 3. The quantitative aspect of the method (for LSD only) is also thoroughly documented for all specimen types. Especially selectivity has been addressed in depth. A rigorous set of conditions (qualifier product ion ratios, relative retention time) was used to certify the identity of the measured compounds and a possible interference, LAMPA, was specifically investigated. Using an $y = a + b(1/x) + c(1/x^2)$ function allowed calibration between 0.05 and 5.0 ng/mL. However, the use of nonlinear regression compromises the method to some extent. The use of nonlinear regression should be avoided, or at least be justified. Finally, the stability of LSD and its metabolite was also evaluated. The obtained stability data again confirmed the relative instability of these molecules. This report illustrates the advantage of MS/MS over single MS. Of course, specificity is higher but, more subtle, the common fragment of LSD and LDS-d_3, complicating its use as an IS, is no longer a point in the MRM transitions.

Recently, White and co-workers have published an isotope dilution MS method for the determination of LSD, in which they specifically address the use of LSD-d_3, with its common fragments to LSD, versus methysergide as internal standard (White, Kidd & Webb, 1999). In essence, the procedural characteristics of this report are similar to the previous ones (discussed before in this section); the main difference was the choice of alternative SIM-scheme ions that concern the IS (now LSD-d_3). In this respect, the overriding consideration was that no compromise in detection limits could be made, nor was it forensically acceptable to monitor fewer ions, thus reducing reliability of identification. Examination of the in-source fragmentation spectrum of LSD revealed an *m/z* 197 ion (structure established) with 10–15% relative abundance, not present in the LSD-d_3 spectrum (which contains the corresponding *m/z* 200 ion). Albeit of minor abundance, it was shown experimentally that acceptable qualifier ratios could still be achieved at the LOD. It was shown that the use of LSD-d_3 instead of methysergide improved accuracy and reproducibility of measurement with a factor of 5. It proved, however, vital to optimize the interface and MS parameters at the time of analysis to achieve spectra that have the projected ion intensities within a narrow margin.

It is obvious that the quality and relative quantity of the reports on LC–MS analysis of LSD and eventually some of its metabolites promote it as the method of choice. Many analytical approaches have been put forward for the confirmation analysis of LSD in toxicology. Most methods fail to achieve substantial sensitivity; those methods that are successful make use of complex multistep extraction procedures, derivatization of analytes, or large sample volumes. Most, if not all, of these limitations are alleviated with LC–MS, and additionally, stability problems can be minimized. It is our opinion that very little can be added or improved to some of the analytical methods presented. It is fair to say that LSD analysis with LC–MS can be presented as a model application for this technique.

4. Amphetamines

Amphetamine (A), methamphetamine (MA), and certainly their analogs 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyethylamphetamine (MDEA) play an increasingly important part in the illicit drug markets all over the world. All of these compounds and their experimental analogs (''designer drugs'') synthesized by clandestine chemists in an effort to evade regulations and/or modify pharmacological properties, have potent sympathomimetic effects and abuse potential. Especially MA in the so-called ''ice'' form has spread rapidly in the US, whereas mainly MDMA and MDEA are found throughout Europe in the young people's rave and acid house cultures. Their popularity stems from a combinatory effect of stimulation and the claimed promotion of emotional harmony and reduced enmity. Consequently, these amphetamines have become increasingly important in forensic analytical research.

A previously discussed paper (Tatsuno et al., 1996a), presented a method for the simultaneous detection of a number of illicit drugs, including A, MA, ephedrine, and

TABLE 4. Operational characteristics and validation data of LC-MS applications in the field of amphetamine analysis.

Compound	Sample	Work-up + IS	Chromatography	Effluent treat-ment	Interface	Mass analysis type & detection mode	Monitored ions	Validation data	Ref
Amphetamine, methamphetam-ine, ephedrine, methylephedrine	Urine	SPE[a] (C_{18}), No IS	L-column, ODS, 150 x 4 mm 100 mM ammonium acetate and acetonitrile, gradient elution from 0 % to 40 % acetonitrile flow-rate: 1 mL/min.	/	+ TSP	Q, SIM + scan	m/z 136: amphetamine (q) *m/z* 150: methamphetamine (q) *m/z* 166: ephedrine (q) *m/z* 180: methylephedrine (q)	LOD (SIM): 2-40 ng/mL (U.P.) Within-day prec.: 4.5-9.5 % (n=10) Linearity: - 500 ng/mL	Tatsuno 1996a
Enantiomers of amphetamine, methamphetam-ine and *p*-hydro-xymetham-phetamine	Urine	SPE (cation exchan-ge), N-ethyl-aniline	β-cyclodextrin phenylcarbamate-bonded silica column, 150 x 6 mm, 5 µm p.s. 100 mM ammonium formate (pH 6), acetonitrile and methanol (60/10/30) flow-rate: 1 mL/min.	/	+ TSP	Q, SIM	*m/z* 136: amphetamine (D and L) *m/z* 150: methamphetamine (D and L) *m/z* 166: *p*-hydroxymethamphet-amine (D and L)	LOD: 0.5 - 1.0 ng/mL (U.P.)	Katagi 1996
MDA, MDMA, MDEA	/	/	Waters NovaPack C_{18}, 150 x 3.9 mm, 4 µm p.s. 100mM ammonium acetate and aceto-nitrile flow-rate: 0.4 mL/min. (TSP & ESI); 0.7 mL/min. (APCI)	+ 0.6 mL/min. 100mM ammoni um acetate (TSP only)	+ TSP + ESI + APCI	QQQ, MRM and full scan	TSP & APCI: *m/z* 180($[M+H]^+$) - 163: MDA *m/z* 194($[M+H]^+$) - 163: MDMA *m/z* 208($[M+H]^+$)- 163: MDEA ESI: *m/z* 221($[M+CH_3CN]^+$) -163: MDA *m/z* 235($[M+CH_3CN]^+$) - 163: MDMA *m/z* 249($[M+CH_3CN]^+$) - 163: MDEA	LOD: (on-column, s/n≥3) MRM: TSP: 10 - 30 pg ESI: 100 - 500 pg APCI: 1000 - 5000 pg Scan: TSP: 100 - 1000 pg ESI: 1000 pg APCI: 1000 - 2000 pg	Bogusz 2000
Amphetamine and methamphet-amine	Urine	/	β-cyclodextrin phenylcarbamate-bonded silica column, 150 x 6 mm, 5 µm p.s. 100 mM ammonium formate (pH 6), acetonitrile and methanol (60/10/30) flow-rate: 1 mL/min.	/	+ ESI	Q, SIM	*m/z* 136: amphetamine (D and L) *m/z* 150: methamphetamine (D and L)	LOD: 0.5 - 1.0 ng/mL (U.P.) Linearity: 1- 5000 ng/mL Within-day prec.: 1.41 - 2.07%	Katagi 1998

Amphetamine, methamphetamine, MDA, MDMA, MDEA	Serum, urine	LLE phenylisothiocyanate (PIT) deriv.; 4 deuterated analogs	Superspher Select B, 125 x 3 mm 50 mM ammonium formate (pH 3.0) and acetonitrile (55/45) flow-rate: 0.9 mL/min.	/	+ APCI	Q, SIM	*m/z* 271(q), 119 (q): Amphetamine *m/z* 285 (q), 150 (q), 119 (i): Methamphetamine *m/z* 315 (q), 163 (q) MDA *m/z* 329 (q), 194 (q), 163 (i): MDMA *m/z* 343 (q), 208 (q): MDEA (all PIT derivatives)	LOD: 5 ng/mL (amphetamine, MDA), 1 ng/mL (methamphetamine, MDA, MDEA) (s/n ≥3) Total prec.: 5 -7 % (n=7) Linearity: 5 - 1000 ng/mL	Bogusz 1997
MDA, MDMA	Blood, post-mortem hepatic tissue, fly larvae, chitinized insect tissues	LLE, MDMA -d_5	Hamilton PRP, 150 x 1.2 mm 10 mM ammonium hydroxide and acetonitrile (25/75) flow-rate: 0.3 mL/min.	/	+ ESI	Q, SIM	*m/z* 221 ($[M+CH_3CN]^+$) (q), 180 ($[M+H]^+$)(i), 163 (i): MDA *m/z* 235 ($[M+CH_3CN]^+$) (q), 194 ($[M+H]^+$)(i), 163 (i): MDMA *m/z* 240 ($[M+CH_3CN]^+$)(q), 199 ($[M+H]^+$)(i), 165 (i): MDMA-d_5	/	Goff 1997
Amphetamine, methamphetamine, MDA, MDMA, MDEA and 11 related compounds	Serum	SPE, Amphetamine-d_{11}, methamphetamine-d_{10}, MDEA-d_5, MDMA -d_7	Superspher 100 RP 18, 125 x 3 mm, 4 µm p.s. 50 mM ammonium formate (pH 3.0) and acetonitrile (75/25) flow-rate: 0.3 mL/min.	/	+ APCI	Q, SIM	*m/z* 136 ($[M+H]^+$) (q), 119 (q): Amphetamine *m/z* 150 ($[M+H]^+$) (q), 119 (q): Methamphetamine *m/z* 180 ($[M+H]^+$) (q), 163 (q): MDA *m/z* 194 ($[M+H]^+$)(q), 163 (q): MDMA *m/z* 208 ($[M+H]^+$) (q), 163 (q): MDEA	LOD: 1 - 2 ng/mL (s/n≥3) Total prec.: 4 - 12 % (n=3) Linearity: 5 - 500 ng/mL	Bogusz 2000
MDA, MDMA	Whole blood, serum, vitreous humour	LLE, MDEA	Hypersil BDS C_{18}, 100 x 2.1mm, 3 µm p.s. 0.1 M ammonium acetate in H_2O, methanol and acetonitrile (8/1/1) flow-rate: 0.2 mL/min.	/	+ ESI	Q-TOF, full spectrum, high resolution	*m/z* 180.1 ($[M+H]^+$) - 133.2 + 135.2 + 163.1[$]: MDA *m/z* 194.1 ($[M+H]^+$) - 133.2 + 135.2 + 163.1[$]:MDMA *m/z* 208.1 ($[M+H]^+$) - 163.1[$]:MDEA [$]extracted ion chromatogram	LOD: 250 pg/mL (MDMA, s/n ≥3) Linearity: 1 - 1000 ng/mL	Clauwaert 1999

[a]Non-standard abbreviations: SPE, solid phase extraction; IS, internal standard; LLE, liquid-liquid extraction; Q, quadrupole; p.s., particle size; U.P., undefined procedure.

methylephedrine in urine. They used thermospray LC–MS with a linear decrease in vaporizer temperature in accordance with the mobile phase composition. Using gradient elution, the acetonitrile content in the mobile phase increased from 0 to 40%, thus reducing sensitivity under constant temperature conditions. Under optimized conditions, the four amphetamines produced simple mass spectra dominated by the protonated molecular ion (see Table 4 for details). For quantitative purposes, these intense ions were chosen in a SIM experiment. This experiment resulted in linear calibration curves for each compound, although no internal standard was used. The validation parameters of the analysis (Table 4) were, unfortunately, not presented in detail, so that specific detection limits for the individual amphetamines are unknown, but are in the low ng/mL range, using the SIM mode. Extraction and chromatography have been addressed previously in the opiates section.

Most amphetamines are chiral molecules, with the individual enantiomers differing in their pharmacokinetic and pharmacodynamic properties. Consequently, chiral separations and quantification of individual enantiomers are important. Moreover, medication that contains a single enantiomer is perfectly legal in some countries. In forensic toxicological investigations, stereospecific differentiation combined with unequivocal identification can be vital. Katagi et al. (1996) have chosen a chiral-differentiating stationary phase with an acetonitrile–methanol–ammonium acetate mobile phase. This mobile phase was used in the identity-verification part of their work with TSP LC–MS as opposed to the quantitative measurements, which used UV-detection. Urinary chiral analysis is performed for MA and its metabolites A and *p*-hydroxymethamphetamine. Sample preparation consisted of SPE extraction of 10-mL urine samples, and *N*-ethylaniline was used as internal standard, although only added after the extraction. The MS was operated in the positive ion mode with filament off. The resulting spectra only show the protonated molecular ions (see Table 4), which were consequently monitored in the SIM experiments. Method performance parameters are also compiled in Table 4.

Verweij and Lipman (1996) have used some amphetamines (MDA, MDMA, MDEA) to compare TSP, ESI, and APCI ionization in view of their obtainable detection limits for low-molecular weight compounds. They used the classical aqueous ammonium acetate–acetonitrile mixture of various compositions as LC eluent on a C_{18} column, with extra post-column ammonium acetate addition in the case of TSP. A tandem quadrupole MS was used, and MS/MS experiments were conducted. MRM was preferred to maximize sensitivity. They selectively optimized the various MS parameters in detail, according to the ionization method used. It was shown that, whereas relatively large amounts of (volatile) salts were needed in TSP ionization, ESI and APCI required a substantial reduction in salt content for optimum ionization efficiency (although some acetate had to be present) as well as a high-as-possible acetonitrile content in the mobile phase. TSP proved most sensitive, especially with MRM, resulting in low picogram range detection limits for amphetamines. Quantitatively variable adduct formation between protonated molecular ion and acetonitrile was observed, especially in ESI and APCI, probably accounting for some of the observed lower sensitivity (2–20 times).

Semi-microcolumn HPLC with a column-switching system was combined with ESI–MS for the analysis of MA and A (Katagi et al., 1998) in urine. Samples were directly introduced into the system. After removal of other components of the sample matrix by the column-switching process, MA and A were both successfully analyzed with enantiomeric resolution. Linear calibration curves were obtained for all the analytes throughout the concentration range from 1 ng/mL to 5 μg/mL. Precision data (four enantiomer peaks) and detection limits have been included in Table 4. The HPLC–ESI–MS system studied here appeared highly sensitive and useful towards forensic applications.

A manuscript by Bogusz, Kala and Maier, (1997) presents a bioanalytical method for A, MA, MDA, MDMA, and MDEA as well as for eight related, licit, or illicit compounds (although the latter eight are nonquantitative), using HPLC and either APCI mass spectrometry or diode array UV-detection. A liquid–liquid extraction with ether was chosen for clean-up of serum and urine samples, followed by phenylisothiocyanate derivatization (UV-detectable, stable derivatives). Although, of course, primarily interesting for UV detection, the same derivatives are used in these authors' MS work (although this approach is abandoned in a later, related publication (Bogusz, Krüger & Maier, 2000)). Separation was performed on a reversed phase column, using aqueous ammonium formate–acetonitrile. APCI–MS was performed in the positive ionization mode. Based on a fragmentation study, a selective scan procedure was developed for the detection of the various A-related compounds, including alternate switching of the extractor voltage between 15 V (no fragmentation) and 40 V (in-source fragmentation present). As such, protonated molecular ions could be monitored as well as, alternatively some of the most specific fragments, for identification purposes. The MS procedure was limited to A, MA, 3,4-methylenedioxyamphetamine (MDA), MDMA, MDEA, and deuterated internal standards. The authors conclude that APCI–MS with in-source fragmentation results in a more differentiated fragmentation pattern compared to either GC–EI–MS or GC-CI-MS. Such an

enhanced fragmentation pattern results in a substantial advantage for unequivocal differentiation between A and congeners from commonly used sympathomimetic amines. Quantitative results are shown in Table 4. The limits of detection were in the 0.001 to 0.005 ng/mL range, making APCI–MS at least as sensitive as GC–MS .

Goff et al. (1997) used ESI–MS to determine MDMA and its metabolite MDA in blood, post-mortem hepatic tissue, fly larvae, and chitinized insect tissues (puparial cases). Their aim was to evaluate the effects of MDMA and MDA in decomposing tissues on specific larvae species developmental rates, which are frequently used in estimating the post-mortem interval. Liquid–liquid extraction of the various samples was performed, if necessary after homogenization, and the extracts were analyzed on a reversed phase column. The separation was achieved with acetonitrile and ammonium hydroxide. A triple quadrupole MS with ESI interface was used to monitor three ions for each compound (see Table 4) and the deuterated internal standard (MDMA-d_5), using the most abundant ion current for quantification. All the analyzed samples, from rabbits treated with MDMA and colonies fed on tissues from these animals, showed the presence of MDMA, and the concentrations were in all samples (also entomological samples) directly related to the dosage administered. No validation data were presented, but concentrations found were in the high pg/mg to low ng/mg range (Table 4).

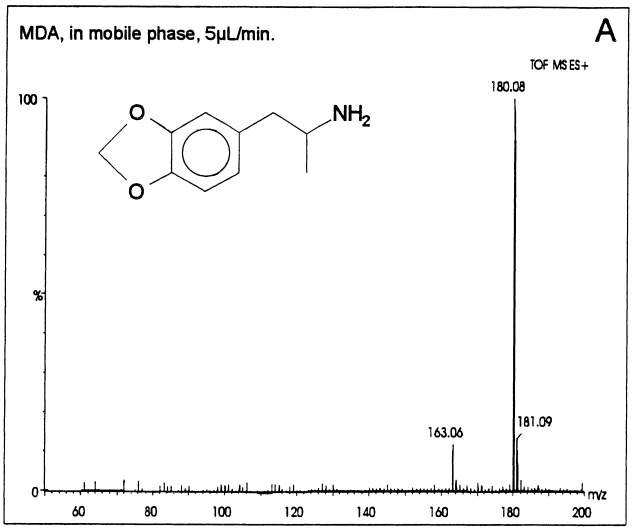
Bogusz, Krüger and Maier (2000) describe the analysis of A, MA, illicit designer drugs (MDA, MDMA, and MDEA), and other phenethylamines (e.g., the novel MBDB and BDMPEA, although the other phenethylamines without validation) in serum after solid phase extraction. The extracts are examined with HPLC–APCI–MS. The drugs are separated on a C_{18} column with acetonitrile and an ammonium formate buffer as a mobile phase. The method is applied for routine determination of the amphetamines and related drugs in serum. For quantitation, SIM procedures (*m/z* values can be found in Table 4) were applied, using the protonated molecular ions, although selected characteristic fragments, through in-source fragmentation, were also followed to allow unequivocal differentiation (especially necessary because some drugs, e.g. MDEA and MBDB, have the same nominal molecular mass and the 8-min chromatographic separation provides no complete resolution between certain peak pairs). The method was partially (only some compounds) validated, and from these results the authors conclude that their single quadrupole approach, using in-source fragmentation, is sufficient for the positive identification of the examined drugs, and as such is a real alternative to the much more expensive triple quadrupole instruments to serve their goal, a rapid and sensitive investigation, e.g., to be used for road-side testing (confirmation), for the most important amphetamines, illicit designer drugs, and other phenethylamines.

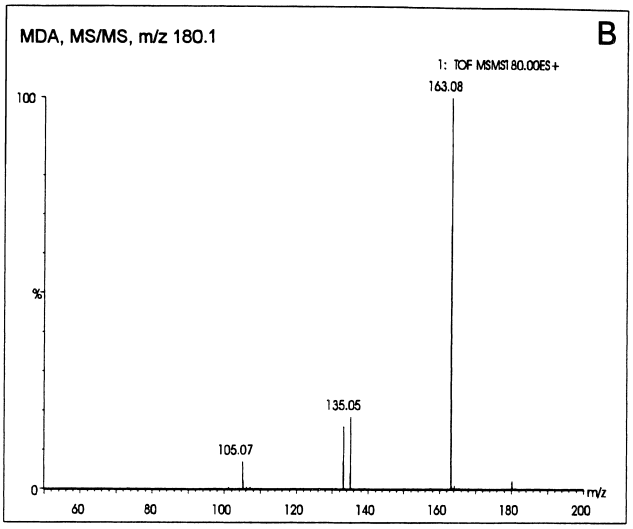
Clauwaert et al. (1999), on the other hand, describe the investigation of the quantitative potential of the Q-TOF MS with electrospray ionization, using MDMA. They compared the quantitative data obtained by liquid chromatography with fluorescence detection and LC–Q-TOF MS, for MDMA, using MDEA as internal standard, in aqueous standards and biological extracts (blood, serum and vitreous humor, which were all liquid–liquid extracted) and found perfect superimposability. They concluded that the Q-TOF achieves a linear dynamic range for quantitative LC–MS/MS work (3 decades), exceeding that of fluorescence detection, at a much better absolute sensitivity. Their work proves that the Q-TOF can be an attractive alternative for triple quadrupole MS systems, the commonly used LC–MS/MS instruments in forensic toxicology, because of its ability to obtain informative full-scan MS/MS spectra at levels that would be impossible using a conventional triple quadrupole MS. An illustration of the spectra of MDA, MDMA, and MDEA, recorded by this technique, is given in Fig. 6. This advantage is clearly not degraded by the (formerly questioned) quantitative possibilities of such an instrument.

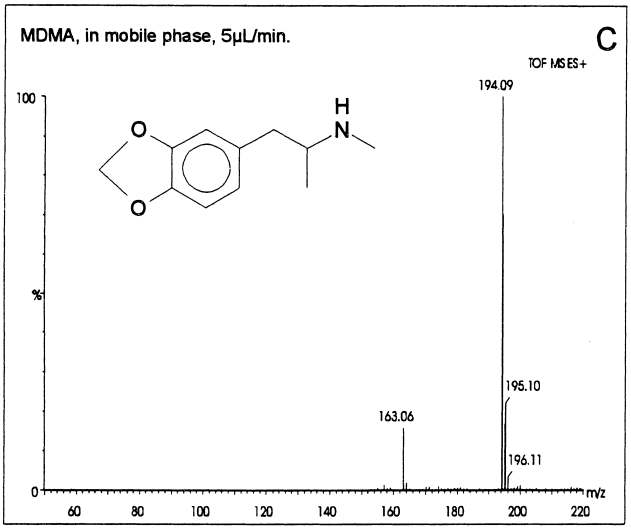
It is clear that LC–MS provides a valuable alternative to GC–MS for the analysis of amphetamines and related compounds. Certainly, the fact that a more differentiated fragmentation pattern is achieved is of forensic importance. Although one report indicates that thermospray ionization results in better sensitivity, the majority of the recent publications use ESI or some APCI; that ionization choice probably reflects availability more than the reasonability of choice. Apart from the enantiomeric separations, we think that the approaches presented all share a common feature. It is our impression that MS is still too much used as a powerful detection technique for an essentially chromatographic analysis. Only the most recent contributions have started exploiting the full capabilities of MS(/MS) in an effort to develop high throughput analysis on, e.g., short LC columns. Although improving through time, validation is still the Cinderella of quantitative LC–MS(/MS), albeit its vital role in proving a method's practical use and reliability in applications, which forensic toxicology is all about.

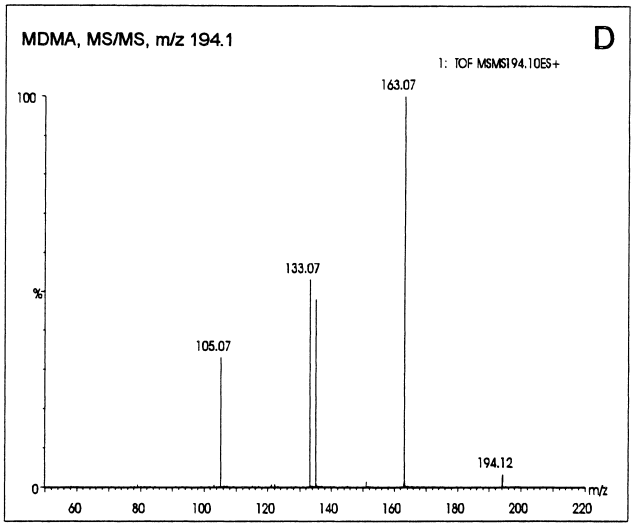
5. *Cannabinoids*

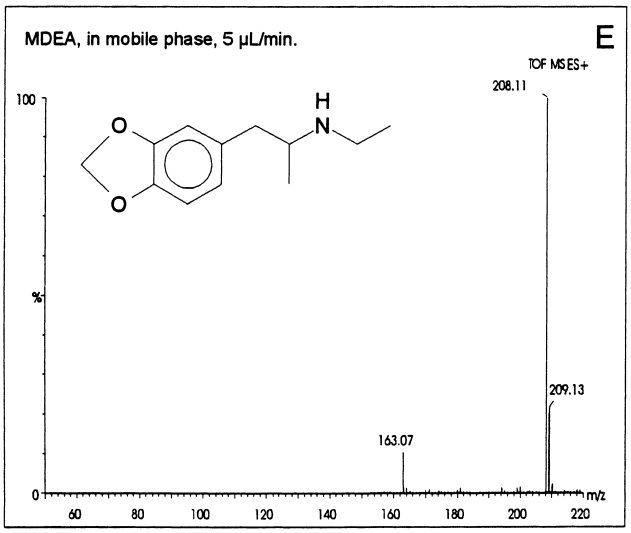
Cannabis sativa or Indian hemp in all of its different forms is the most widely used illicit drug. The resin which the plant secretes is especially rich in psychoactive compounds. The cannabinoids are a large group of different constituents with characteristic psychoactive properties. The most important psychoactive compound

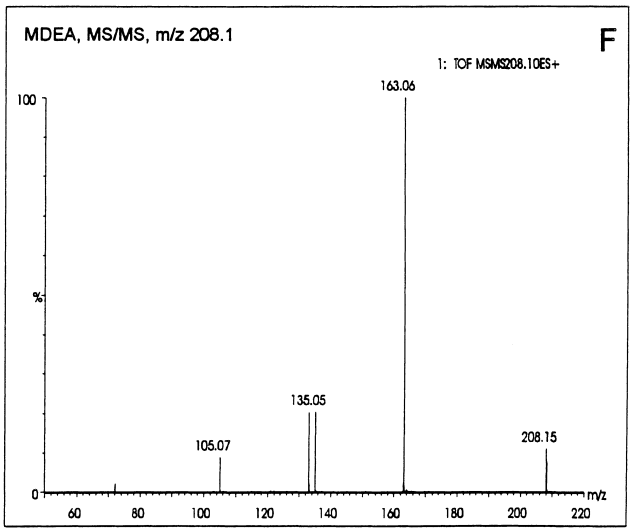

MDA, in mobile phase, 5µL/min.
A
TOF MS ES+
180.08
163.06
181.09
NH2
O
O
100
%
0
60
80
100
120
140
160
180
200
m/z


MDA, MS/MS, m/z 180.1
B
1: TOF MSMS180.00ES+
163.08
135.05
105.07
100
%
0
60
80
100
120
140
160
180
200
m/z


MDMA, in mobile phase, 5µL/min.
C
TOF MS ES+
194.09
195.10
163.06
196.11
H
N
O
O
100
%
0
60
80
100
120
140
160
180
200
220
m/z


MDMA, MS/MS, m/z 194.1
D
1: TOF MSMS194.10ES+
163.07
133.07
105.07
194.12
100
%
0
60
80
100
120
140
160
180
200
220
m/z


MDEA, in mobile phase, 5 µL/min.
E
TOF MS ES+
208.11
209.13
163.07
H
N
O
O
100
%
0
60
80
100
120
140
160
180
200
220
m/z


MDEA, MS/MS, m/z 208.1
F
1: TOF MSMS208.10ES+
163.06
135.05
105.07
208.15
100
%
0
60
80
100
120
140
160
180
200
220
m/z

is the Δ^9-tetrahydrocannabinol (THC). Cannabinol and cannabidiol are other constituents that have been extensively investigated for pharmacological activity. In the body, the main THC metabolite is 11-hydroxy-THC; however, the major urinary metabolite is the 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC–COOH), which is excreted primarily as its glucuronic acid conjugate.

It is surprising to find so little LC–MS research in the cannabinoid field, taking into consideration its abuse frequency, the relatively low concentrations to be detected when assaying biological samples (about 1 ng THC/mL whole blood is related to cannabis use 3–4 h before blood collection), and the thermal instability of some of the molecules. In fact, we only found two reports that use LC–MS. One deals with the determination of THC–COOH in urine, and the other one with the simultaneous separation and identification of major neutral and acidic cannabinoids in hashish samples. Rustichelli et al. (1996) used LC–particle beam-MS to investigate the great differences between the relative amounts and content patterns of cannabinoids in drug samples. These variations reflect variations in age, storage conditions, and heterogeneity of plant starting material, and can furnish information on, e.g., geographical origin. The samples were powdered in liquid nitrogen and extracted with petroleum ether. A 125×4.0 mm (5 µm particle size) reversed phase (C_{18}) analytical column was used, with a mobile phase that consisted of methanol/water (isocratic elution) and a flow rate of 0.5 mL/min., compatible with the MS interface. The MS was a single stage quadrupole instrument equipped with a Particle Beam Interface. Full-scan spectra were acquired every second under electron impact over the *m/z* mass range of 65–400. It was, as expected, possible to perform a successful library search on the resulting EI mass spectra. For quantitative purposes, UV detection was used in an identical instrumental configuration. Interestingly, the authors found that several of the acidic cannabinoids, as identified through retention time in the UV chromatogram, produced spectra that were identical to their neutral counterparts. It was concluded that the thermal lability of these acidic cannabinoids was so high as to result in their decarboxylation in the ionization chamber of the particle beam interface. Unfortunately, no indications were given on the sensitivity of this LC–MS setup.

Urinary THC–COOH has almost always been assayed with GC–MS . In that respect, preliminary hydrolysis of the drug or metabolite conjugates in urine is mandatory. Breindahl and Andreasen (1999) have used LC–MS instead of GC–MS, but have kept the hydrolysis step. As mentioned in the opiates section, LC–MS allows intact analysis of conjugates such as in this case of THC–COOH. Consequently, deconjugation was an option to these authors. Although, quantitative data on conjugates of THC–COOH in urine are of interest, the aim of this analytical method was confirmation of immunoassay-based drug screening in, e.g., workplace testing. In that respect, cut-off levels have been agreed to for THC–COOH as such and deconjugation is, of course, a necessity for these authors from an interpretation rather than a technical point of view. Moreover, the authors also state that conjugation of THC–COOH with glucuronic acid is variable. The elimination of a derivatization step for the thermolabile THC–COOH was the major impetus for these authors to switch from GC–MS to LC–MS. Their isotope dilution (THC–COOH-d_3) standardized method uses SPE extraction and separation on a C_8 analytical column (gradient elution using water–acetonitrile–formic acid). The MS detector was used in the ESI + mode. Positive and negative ion mode both were explored, and found to be suitable. Although it would seem logical to use negative ions, considering the easily ionized carboxylic acid group of THC–COOH, the positive ion mode was chosen for reasons of compatibility with other methods. This result is remarkable because negative ions would most probably benefit from sensitivity, and the switching from negative to positive modes between applications is easy on most instruments. SIM is used at *m/z* 345 (protonated molecular ion of THC–COOH) and 348 (trideuterated IS). Some experiments were performed with in-source fragmentation in order to achieve unequivocal identity information. In SIM, the often-used qualifier fragment ion intensity ratio criterion of ± 20 could be achieved in sample analyses, but at the expense of a LOD (S/N ≥ 3 criterion) increasing from 2 to 15 ng/mL. The method was fully and thoroughly validated. Linearity (seven-point calibration curve in urine), within-run precision (2.2–4.3% CV in a 2.5–125 ng/mL concentration range), and precision (0.7–2.6% deviation) were all very good. We consider this method a very nice quantitative contribution, clearly illustrating the potential

FIGURE 6. MS and MS/MS spectra of MDA, MDMA, and MDEA with respective molecular structures. A. MDA MS spectrum; B. MDA MS/MS spectrum (*m/z* 180.1 precursor mass); C. MDMA MS spectrum; D. MDMA MS/MS spectrum (*m/z* 194.1 precursor mass); E. MDEA MS spectrum. F. MDEA MS/MS spectrum (*m/z* 208.1 precursor mass). (Reproduced from Clauwaert et al., 1999, with kind permission from John Wiley & Sons Limited, Copyright 1999).

LC–MS, in its simplest form, has for forensic toxicology, and emphasizing an analytical application rather than a mass spectrometric innovation.

6. *Miscellaneous*

In a report in the Journal of Analytical Toxicology, Tai et al. (1996) describe the use of an isotope-dilution TSP–LC–MS for the certification of phencyclidine (1-(1-phenylcyclohexyl)-piperidine; PCP) concentrations for two reference materials in freeze-dried human urine. PCP has been around in the illegal drug circuit for a long time. Its persistence in street drugs is probably due to its cheap and easy synthesis. The drug abuse program of the NIDA includes PCP as a targeted drug, and for testing of Federal employees, the cut-off concentration of PCP was set at 25 ng/mL. The reference materials investigated here all have concentrations at or around this cut-off level. PCP-d_5 was used as an internal standard at a concentration that approximated the expected level of the analyte. Sample preparation was performed with mixed-mode SPE cartridges with an absolute recovery that averaged 73%. As is the custom in reference methodology, calibrators were made in water to give weight ratios of unlabeled-to-labeled compound that ranged between 0.8 and 1.3; i.e., around the expected PCP concentration. Aliquots of 50–100 µL were separated on a C_{18} column with an isocratic mobile phase that consisted of heptanesulfonic acid, ammonium acetate, and glacial acetic acid in methanol–water at a flow rate of 1 mL/min. A thermospray source interfaced to a quadrupole mass spectrometer was used to monitor the $[M+H]^+$ ions at *m/z* 244 and 249 for PCP and PCP- d_5, respectively. TSP ionization was used (i.e., discharge and electron ionization turned off). Through optimization of the TSP control settings for stability and sensitivity, a limit of detection was achieved for PCP estimated to 0.5 ng/mL. Good precision was achieved, and the CV of single measurements within a set of samples ranged from 3.5 to 4.6 %. The authors have simultaneously used a GC–MS method for the certification process, and found good agreement between both methods (relative difference of 3.9–8.4%, depending on the reference material batch used). Overall, the LC–MS results tended to be slightly lower than the GC–MS results for reasons the authors failed to explain.

γ-Hydroxybutyrate (GHB) is a suppressant of the central nervous system that is used in some European countries as an anesthetic adjunct. GHB became popular among athletes because of its perceived anabolic benefits. It was found that numerous side effects exist, and the FDA has banned the product. Recent reports indicate that illicit GHB use is on the rise. it has been implicated in an increasing number of sexual assault cases, and some deadly incidents have equally been reported. GHB and its precursor, the γ-butyrolactone (GBL) are difficult to analyze because of the small, polar nature of the molecules, their interconvertibility, and the lack of a chromophore, which makes UV-detection difficult at best. Mesmer and Satzger (1998) reported on the quantitative HPLC–UV analysis of GHB and GBL, using LC–TSP–MS as a qualitative confirmation method. The method was used to confirm the presence of these products in illegal preparations. The TSP discharge-on ionization mode was preferred, and the mass range *m/z* 80–220 amu was scanned. Sample preparation was essentially dissolution or dilution in methanol. A 300 × 3.9 mm C_{18} column was used with a binary mobile phase that consisted of ammonium or triethylammoniumacetate (pH 4.0) and methanol at a flow rate of 0.75 mL/min. (especially for the MS experiments as the UV approach used an incompatible phosphate buffer system). Unfortunately, the sensitivity that could be achieved was rather poor. A standard chromatogram with low signal-to-noise ratio was shown for 14 and 20 µg GHB and GBL, respectively (probably amount injected, although not specified). Interesting are the resulting spectra. In the GHB spectrum, the base peak (*m/z* 122, $[M+NH_4]^+$) was accompanied by the $[M+NH_4]^+$ peak (*m/z* 104) of the lactone, which indicated the conversion of GHB in the heated ion source. Contrary to GC(-MS) methods, which intentionally transform GHB to GBL for chomatographic purposes, LC–MS offers the possibility of distinguishing between the two molecules. Nevertheless, this analysis clearly is a case in which LC–MS offers only limited advantages over GC(-MS).

B. Forensic Toxicologically Relevant Compounds

In this section, various compounds are addressed, which can have forensic implications, and which the forensic toxicologist encounters regularly. Nevertheless, it is in our view incorrect to denote them as drugs of abuse. Two important groups are treated in detail. All others for which an LC–MS approach was found, have been compiled in a table (Table 6) with respect to their operational characteristics and method highlights.

1. *Benzodiazepines*

Drugs of the benzodiazepine group have anxiolytic, hypnotic, tranquillizing, and anti-convulsive properties. They were originally developed in the sixties, and members of this group of drugs are used extensively in today's society. This success has boosted efforts of many pharmaceutical companies to develop and market new molecules. Their efforts have resulted in a large number of different benzodiazepines (BZ) being encountered in the daily toxicological work. Their relatively low toxicity

makes them drugs of choice in comparison to older, more dangerous drugs used for similar purposes. Besides the problem of dependence, which results from overprescribing and/or inappropriate prescribing, benzodiazepine abuse on the street is the primary concern of forensic toxicologists. In the latter case, the doses used are abnormally high compared to therapeutic doses, and injection, frequently of oral preparations, is preferred. Rapid injection gives a rush, but it also seems that concurrent use of benzodiazepines and heroin increases the intensity and duration of the effect of the opiate. Finally, benzodiazepines are frequently encountered, in very low concentrations, in clinical and forensic samples that involve road traffic offenses.

For the analysis of benzodiazepines in biological matrices, a variety of spectroscopic, chromatographic, and immunological methods are available. GC–MS has become the method of choice, but unfortunately not for all BZ. Some of them show thermal instability, and rearrangement and/or degradation reactions result in several reaction products, making accurate quantification difficult. Verweij, Lipman and Zweipfennig (1992) have used LC–TSP–MS to circumvent this problem, and were able to separate ten different BZ, using 0.4 mL/min methanol–50 mM aqueous ammonium acetate on a C_{18} (150 × 3.9 mm) column. Post- column, another 0.8 mL/min. of 50 mM aqueous ammonium acetate was added to enhance ionization. A triple quadrupole MS system with a TSP interface was used. All operating conditions of the interface (temperatures, voltages) were optimized, as well as the ionic strength of the eluent, and were kept constant for all ten BZ. MS/MS detection (MRM, positive ions) was used with argon as collision gas, and the collision energy was optimized for each single ion transition. brotizolam *m/z* 395–314; clonazepam *m/z* 316–151; desmethylflunitrazepam *m/z* 300–198; diazepam *m/z* 285–165; flunitrazepam *m/z* 313–268; ketazolam *m/z* 285–185; loprazolam *m/z* 465–408; lormetazepam *m/z* 335–289; nitrazepam *m/z* 282–152; triazolam *m/z* 343–308). Extraction from blood was performed, using Extrelut-packed extraction columns. Standardization was in blood but only for flunitrazepam, desmethylflunitrazepam, and lormetazepam (10, 50, and 250 ng/mL). The sensitivity achieved was good. full-scan LC–MS of standards resulted in a sensitivity range of 25–250 ng/mL (S/N≅3), depending on the BZ (nitro group substitution reduced sensitivity). It also showed that a higher vaporizer temperature was beneficial for the S/N ratio, and changes especially affected the signal rather than the noise level. Collision energy voltages were extremely critical for sensitivity, but once optimized, MRM experiments resulted in sensitivities in the range 1–10 ng/mL (again for standard solutions). Ketazolam posed a problem. It was completely converted into diazepam, apparently at the tip of the heated vaporizer. Probably, TSP ionization was, due to the heating in the interface, not the ideal solution for thermolabile BZ.

An interface where the involvement of heating is insignificant is the FAB interface. Sato and co-workers have used this approach in two consecutive papers on the identification of chlordiazepoxide and metabolites (desmethylchlordiazepoxide, demoxepam, desmethyldiazepam, and oxazepam. all pharmacologically active and reported as thermolabile) and of triazolam in serum (Sato et al., 1992a,b). Triazolam, for example, is a short-acting BZ with therapeutic doses as low as 0.125–0.25 mg, resulting in a reported maximum therapeutic blood concentration of 10 ng/mL. In both cases a capillary LC system was used (150 × 0.3 mm i.d., packed with 5 μm ODS packing material), with a mobile phase that consisted of methanol–water–acetic acid–glycerol at a flow rate of 4 μL/min. On-line analysis was performed, using a column-switching device to inject as much as 500 μL of sample, concentrate it on a precolumn, and separate it on the capillary column. A capillary column allows introduction of the entire effluent through a porous stainless steel frit interface into the FAB–MS. The latter is a double-focusing MS, with a xenon fast atom beam, used in the scanning mode (*m/z* 50–800). Extraction of the BZ from serum was performed with C_{18} extraction cartridges, resulting in an extraction recovery of 95% for triazolam, and in excess of 90% for chlordiazepoxide, desmethyldiazepam, and oxazepam. The presented procedure resulted in detection limits of 2–5 ng/mL serum for triazolam (1–2.5 ng on column), and 4–10, 1–2, 2–4 ng/mL for chlordiazepoxide, desmethyldiazepam, and oxazepam, respectively. In the positive ion FAB spectrum of triazolam, the protonated molecular ion showed up as the base peak at *m/z* 343, together with the chlorine isotope peaks. Desmethyldiazepam and oxazepam also only showed the protonated molecular ion (*m/z* 271 and 287, respectively); chlordiazepoxide, desmethylchlordiazepoxide, and demoxepam, on the other hand, displayed the $[MH-O]^+$ ion as the base peak (*m/z* 284, 270, and 271, respectively), in addition to the $[M+H]^+$ ion (*m/z* 300, 286, and 287, respectively) and respective chlorine isotope peaks. Figure 7 shows an example of the spectra obtained with this technique. Otherwise, very little fragmentation is produced. Unfortunately, in the *m/z* 286 mass fragmentogram at the retention time of desmethylchlordiazepoxide, an interference was present from chlordiazepoxide. One of the disadvantages the authors agreed upon is the generally qualitative, or at best semiquantitative, results that FAB–MS produce. This disadvantage means that accurate quantitation is impossible unless a stable-isotopic standard is used; the authors failed to use those standards here. Nevertheless, the detection limits achieved were outstanding even in the less

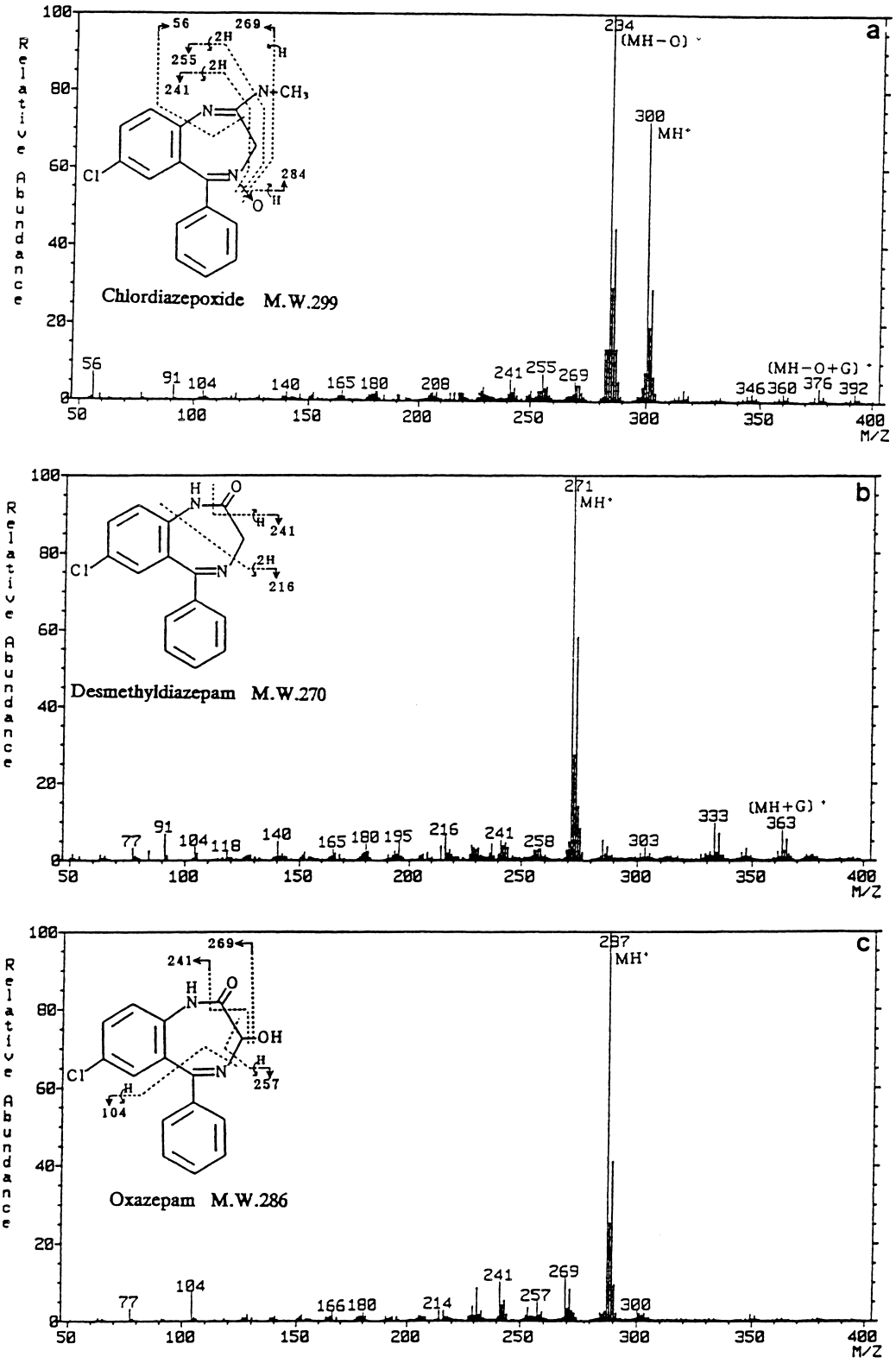


FIGURE 7. FAB mass spectra of authentic chlordiazepoxide (a), desmethyldiazepam (b), and oxazepam (c). The amount of each drug was 10 ng on column. (Reproduced from Sato et al., 1992, with kind permission of the Japanese Association of Forensic Toxicology).

sensitive scan mode. The major disadvantage was, however, the FAB interface, certainly when compared to the more recent API interfaces and certainly for low molecular mass compounds; it is not really the state-of-the-art anymore.

Kleinschnitz, Herderich and Schreier (1996) reported the use of an ultrasensitive method for the quantification of five 1,4-benzodiazepines (diazepam, desmethyldiazepam, nitrazepam, flunitrazepam, and medazepam) from human serum and urine, using LC–ESI–MS/MS. Extraction of serum and urine used either C_{18} or Narc-2 solid phase extraction columns. Diazepam-d_5 and *N*-desmethyldiazepam-d_5 were both used for internal standardization. Separations were performed on a reversed phase HPLC column (100×2.0 mm I.D., 5 μm) with 100 μL/min methanol–water–acetonitrile. The triple quadrupole MS was operated in the MRM mode with argon as collision gas. An extractor voltage of 5 V efficiently destroyed solvent clusters. The selected ion transitions for MRM were *m/z* 285–257 (diazepam), *m/z* 271–140 (desmethyldiazepam), *m/z* 271–242 (medazepam), *m/z* 282–236 (nitrazepam), *m/z* 314–268 (flunitrazepam), *m/z* 290–262 (diazepam-d_5), and *m/z* 276–213 (desmethyldiazepam-d_5), representing the $[M+H]^+$ ion and the most abundant product ion. Calibration graphs (seven points) were achieved with a dynamic range between 1 and 1000 ng/mL, using nonweighted linear regression ($r > 0.999$; no other calibration graph statistics were presented). The routine quantification limit was set at 2 ng/mL for serum and urine ($S/N \geq 10$). With respect to validation, accuracy (% recovery, between 90 and 110%), and reproducibility (all within 10%, although the used procedure remains undefined), data were presented for control samples in serum and urine. An interesting finding was also that no hydrogen/deuterium exchange could be observed for the internal standards. According to the authors, the main advantage of this approach was, logically, the high specificity of the instrumental part of the analysis as well as the lack of a derivatization step. Obviously, it is clear that the presented method closely approaches the best, nowadays available, analytical methodology for analyzing BZ.

Practically the same methodology is used by Crouch et al. (1999) for the quantitation of alprazolam (AL) and α-hydroxyalprazolam (OH-AL) in human plasma. Liquid/liquid extraction and narrow bore reversed phase (C_{18}) HPLC separation (isocratic, 250 μL/min. using a methanol–water–0.1% formic acid mixture) is followed by MS/MS detection in the positive ion MRM mode (triple quadrupole system). Pentadeuterated analogs (AL-d_5 and OH-AL-d_5) were used as respective internal standards. The following MRM ion transitions were monitored:AL *m/z* 309–205, OH-AL *m/z* 325–216, AL-d_5 *m/z* 314–210, and OH-AL-d_5 *m/z* 330–221. A very complete, validated evaluation into the quantitative nature of the method was performed. Linear calibration curves were achieved for both compounds between 0.05 and 50 ng/mL ($r > 0.990$, ten points and an extra blank). Weighted ($1/X$ or $1/X^2$) regression tended to improve precision at the lower concentrations; however, at the expense of the precision at the higher levels. Sensitivity (LOQ) for OH-AL was found to be 5- to 10-times better than what previously had been reported. It was now possible to quantitate OH-AL following a single dose, which was previously reported as undetectable. Accuracy and precision were evaluated at three different concentration levels (2.0, 5.0, and 20.0 ng/mL) and within-day precision figures (CV%) for AL were all <5.6, for OH-AL all <8.4 ($n = 5$). Total precision ($n = 7$) was $<11.8\%$ for AL and $<9.6\%$ for OH-AL. Accuracy was within 6.6% of the target value. Clearly, these are extremely good results, taking into consideration the minute concentrations detected. This report illustrates quite well the advantages of LC–MS over, e.g., GC–MS, especially in terms of sample preparation (simple liquid–liquid extraction, no derivatization step), sensitivity, and speed of analysis; the described LC procedure has only a total analysis time of <5 min per sample. There can be no doubt that the ESI LC–MS/MS approach is emerging as the method of choice within LC–MS, of BZ in this case. The presented method demonstrates quite unequivocally that top level routine quantitative performance can be achieved with an instrumental approach (ESI) that is at present comparable in complexity and ease-of-use to many non-MS techniques.

One other approach is the APCI interface, which presents itself as a viable alternative to ESI in the case of small molecules. It should be stressed that heat is applied and that heat eventually may compromise the analysis of some thermolabile molecules. Senda et al. (1995) report the use of LC–APCI–MS for the quantitative analysis of triazolam and its major metabolites (α-hydroxytriazolam and 4-hydroxytriazolam) in plasma and urine, down to the pg/mL level. An ODS (150×4.6 mm) column was used, and up to 200-μL volumes were injected. The exact volume is unclear, as is the description of the extraction, which seems to be a simple solvent extraction. In any event, much more interesting is the investigation into the selection of a mobile phase that is most appropriate for the APCI interface-mediated ionization, in order to achieve maximum sensitivity (the mobile phase acts as a proton donor or acceptor). Ammonium acetate (50 mM) solutions of different pH (3.5–10) were used in combination with either methanol or acetonitrile in a linear solvent gradient from initially 50–100% organic phase (within 15 min). The relation to peak height (i.e., sensitivity or ionization efficiency) was evaluated. Data showed that the lower the pH of the aqueous phase, the higher the sensitivities for all compounds concerned, irrespective of the organic com-

ponent used in the solvent composition. In fact, a 100-fold increase in sensitivity was observed at pH 4.0 compared to pH 10. For the quantitative determination, selected ions were monitored, in each case the protonated molecular ion, $[M+H]^+$ (*m/z* 343, 359, 359, 285 for, respectively, triazolam, α-hydroxytriazolam, 4-hydroxytriazolam, and diazepam, which was used as internal standard). Linear calibration curves were prepared in the range 0–10 (plasma) or 0–300 ng/mL (urine) with correlation coefficients of 0.999. The quantification limits achieved (S/N ≥ 3) were 20 pg/mL, although it is unclear whether this limit was for standards or biological extracts. Reproducibilities varied from ca. 5–2.5% CV. Whether this value was within-day or total precision was again unclear. What is important with respect to this paper is the proof that APCI is a viable approach for BZ, and that the high sensitivity achieved. Sensitivity is a vital aspect for BZ analysis because some of the newer derivatives are very potent and their serum concentrations are so low that even the routine enzyme-immunologic assay procedures fail to detect them, although they can be deadly when combined with, e.g., alcohol or other kinds of tranquillizers.

The success of the BZ and the great number of different representatives is reflected in the number of analytical applications being published, also in the field of LC–MS. Because not all of them are equally interesting for forensic applications, but because the operational characteristics can be an interesting starting point, we have chosen to summarize them in Table 5.

As has already been mentioned in the section concerning opiates (Section IV.A.1.), the analysis of intact glucuronide conjugates is a valuable option when using LC–MS. This deduction is equally valid for BZ, which also show this type of conjugation, although the formation of pharmacodynamically active conjugates is less important compared to the opiates.

2. *Synthetic Opioids*

This group comprises those compounds that have been synthesized either as a structural analog of, e.g., morphine or later on, without any chemical relationship to the natural opiates but having similar activity. In fact, many of these compounds were synthesized in an effort to obtain a narcotic analgesic effect as potent as, e.g., morphine, but without the addictive, euphoric effects, which have made drugs of abuse of the opiates. The advent of partial agonists such as buprenorphine has initially spurred hope of having created such a compound with low dependency potential. Perhaps predictably, the anticipated inferior abuse potential did not turn out to be the case, and buprenorphine is now abused just as all the other representatives such as dextropropoxyphene, bezitramide, pethidine, or tilidine. Of course, methadone is also part of this group of compounds. It has gained widespread importance as a useful substitution product for heroin in the treatment of opiate addicts.

Verweij and co-workers have been among the first to use TSP mass spectrometry for the quantitative analysis of methadone (MT), dextropropoxyphene (DP), and dextromoramide (DM) in whole blood, together with a number of other compounds. Their results appear in two different publications, which unfortunately contain virtually the same data (Verweij, Hordijk & Lipman, 1995, 1996). They preferred LC–MS/MS over more classical GC–MS because these compounds often have long GC retention times, which sometimes lead to substantial absorption onto the analytical column, or are thermolabile like dextropropoxyphene. Bond Elut certify SPE was used after diluting the whole blood with buffer. An acidic as well as a neutral/basic fraction was eluted. The latter fraction was used, but the acidic fraction can eventually be used for other molecules. A C_{18} cartridge column (150×3.9 mm, 4-μm particle size) was isocratically eluted with a mixture of methanol and 50 mM ammonium acetate in water at a flow-rate of 0.6 mL/min. To enhance ionization in the TSP source, an extra 0.5 mL/min 50 mM ammonium acetate in water was added post-column. The MS was a tandem quadrupole mass spectrometer equipped with a TSP interface (filament-on mode). Different collision energies were used in Q2: MT, 10 V; DP, 6 V; and DM–20 V. For optimum selectivity, MRM was applied to monitor the transition of the $[M+H]^+$ protonated molecular ion to the most intense fragment ion in the MS/MS spectrum (MT *m/z* 310–265; DP *m/z* 340–266; DM *m/z* 393–306). In the MS optimization experiments, it was found that low TSP repeller voltages could be used, but that a rather high vaporizer temperature was beneficial in terms of signal-to-noise ratio. Also, and quite logically, the collision energy was critical. Such an optimized experiment results in detection limits (S/N ≥ 3) on-column of 50 pg, equivalent to 50 pg/mL blood and better than in comparable GC–MS experiments. No thermal influence on the compounds could be observed. From the quantitative point of view, only DM was investigated. A calibration graph (five concentrations) made in whole blood and ranging from 0.5 to 12.5 ng/mL was linear ($r = 0.99$). No internal standard was used. The authors concluded that their method was more sensitive and much faster than comparable GC–MS methods with chromatographic analysis times shortened. Clearly, the TSP approach presented here achieves valuable results but, as indicated before, has become virtually obsolete with the advent of API-based LC–MS. Those pioneers in toxicological LC–MS analysis, at the time limited by availability of solely this, (although then promising) thermospray interface, have now largely reinvested in ESI-based instruments.

In 1997, Kintz et al. developed an enantioselective separation for methadone and its main metabolite in human hair, using ESI–MS. Methadone used for substitution therapy is a racemic mixture of the biologically active R-form and the almost inactive S-form. The authors' main aim was to study the methadone and metabolite (2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolium, EDDP) distribution into hair. A major limitation of blood and urine analysis is the relatively short retrospective time period for drug abuse demonstration. Hair analysis expands the time window for detection because it reveals an exposure to illicit drugs from weeks to months prior to the test. Hair was enzymatically hydrolyzed, and extracted using C_{18} based extraction columns. Trideuterated analogs of MT and EDDP were used as internal standards. The extracts were injected on a chiral-AGP column (100 by 4.0 mm, alpha-1 acid glycoprotein phase). A binary mobile phase, consisting of isopropanol and 2 mM ammonium formate, pH 5.8, was used to elute the compounds under gradient conditions. A 1:9 post-column split reduced the column flow to 50 μL/min towards the API source. The MS was a single quadrupole system, equipped with a proprietary ion spray source. The instrument was operated in the positive ionization mode. MS data were collected either in scan or in SIM, focusing on the following ions: *m/z* 265 and 310 (MT), 268 and 313 (MT-d_3), 278 (EDDP), and 281 (EDDP-d_3). Good separation was obtained between the optical isomers, and a linear correlation in the range 0.5–20 (MT) or 0.2–10.0 ng/mg (EDDP) was achieved. Total precision (10-day period) was calculated for the various enantiomers at a 5.0 (MT) and 1.0 (EDDP) ng/mg level, and resulted in CV's of 13.6% (EDDP) and 17.1% (MT). The detection limits (S/N $\geq$ 3) were 0.2 and 0.1 ng/mg hair (MT and EDDP, respectively), which appear slightly better than a reported GC–MS procedure. On using the method, the enantiomers of MT and EDDP were all found in the hair samples of addicts enrolled in an opiate detoxification scheme. The results, although not conclusive, suggested a predominance of the R-enantiomer of methadone in human hair. This publication represents a nice example of applied LC–MS. The MS information given is limited to what is essential for the application and excellent clinical-toxicological results, generated with the technique, are the main point.

Methadone, buprenorphine (BP), and various opiates in different biological matrices were studied in a report already discussed in the opiates section (Bogusz et al., 1998). Ample attention was paid to the mass spectrometric fragmentations, which revealed that the APCI spectrum for buprenorphine showed extensive fragmentation whereas this fragmentation was not observed in an LC–ESI–MS experiment published in another study (Tracqui, Kintz & Mangin, 1997a). Clearly, ESI produces ionization conditions that are milder compared to APCI, in which elevated temperature will play an important role. For MT, on the other hand, comparable spectra were achieved with both ionization techniques. Good linearity, precision (4% CV%), and detection limits (0.5 and 0.2 for BP and MT, respectively) were achieved.

Buprenorphine is, in fact, a semisynthetic opioid, closely related to morphine, although 25–40 times more potent. It exhibits partial agonist activity at the μ-opiate receptor and antagonist activity at the κ-opiate receptor, resulting in a potent analgesic with poor respiratory depressant activity and a lack of significant withdrawal symptoms, albeit having addictive potential. Determination is difficult because of the low therapeutic plasma levels (steady-state concentrations of 0.1 to 8 ng/mL) and a major active metabolite norbuprenorphine (norBP). Tracqui, Kintz and Mangin, (1997a) have tried to cope with these problems by using LC–ESI–MS for the analysis of BP and norBP in biological fluids and hair samples. In an earlier publication (Kintz et al., 1996), the same approach was presented for the analysis of BP in a sweat patch aimed for the long-term continuous monitoring of substance abusers. Hair samples were pretreated as usual; the resulting extract was processed as all other biological samples (blood, plasma, urine). This treatment meant adding BP-d_4 as internal standard and performing a liquid–liquid extraction. The sweat patch was eluted with methanol. A C_{18} reversed phase column (150 × 2.0 mm) was used, and was isocratically eluted at 0.2 mL/min with acetonitrile/2 mM ammonium acetate buffer pH 3.0. A post-column split of 1/3 was applied to be compatible with the ESI source (although pneumatically assisted, so essentially able to cope with the entire 0.2 mL/min column effluent). This source was mounted onto a single-stage quadrupole MS, which was operated either in the scan mode (*m/z* 260–475) or SIM mode at *m/z* 414 (norBP), *m/z* 468 (BP), and *m/z* 472 (BP-d_4). As usual in ESI, simple MS spectra were obtained that contained one unique peak that corresponded to the protonated moleculer ion, which was monitored in the SIM experiments. Under these conditions, good precision data (within-day 5, respectively, 13% CV at concentration levels of 10, respectively, 1 ng/mL (BP as well as norBP), and total 8.1% CV, only for BP at 10 ng/mL; as well as excellent accuracy and detection limits, S/N $>$ 3 (0.1 and 0.05 ng/mL blood or 24 pg/mg hair, BP and norBP) were achieved. The authors concluded that their approach is the method of choice for the thermo-instable BP and norBP because it is simple, rapid (no tedious derivatization step required), highly specific, and sensitive; moreover, it produced unequivocal MS data, which are vital for forensic work. The latter, of course, only holds when full-scan spectra are obtained; full-scanning will affect quantitative results.

TABLE 5. Operational characteristics of some benzodiazepine LC-MS applications.

Compound	Sample	Work-up + IS	Chromatography	Effluent treatment	Interface	Mass analysis type & detection mode	Monitored ions	Validation data	Ref
Bromazepam	Serum	Not specified, No IS[a]	Nucleosil C_{18}, 300 x 4.0 mm methanol/0.2 % ammonia solution (75/25) flow-rate: 0.6 mL/min.	/	MB + CI	Double focusing sector instrument, SIM (high resolution)	*m/z* 316 ($[M+H]^+$): bromazepam (^{79}Br isotope) *m/z* 318 ($[M+H]^+$): bromazepam (^{81}Br isotope)	Linearity: 200-1000 ng/mL	Tas 1986
Glucuronide of N-1-hydroxy-ethyl flurazepam	Urine	Amberlite XAD-2, No IS	Hypersil ODS, 100 x 4.6 mm, 5 µm p.s. methanol/water (70/30) flow-rate: 0.6 mL/min.	/	DLI - and + CI	Q, scan	- CI: *m/z* 508 (M^-), *m/z* 332 ($[M-Glu+H]^-$) + CI: *m/z* 509 ($[M+H]^+$); *m/z* 333 ($[M-Glu+2H]^+$)	/	Dragna 1989
Brotizolam (1), clonazepam (2), desmethylflunitrazepam (3), diazepam (4), flunitrazepam (5), ketazolam(6), loprazolam (7), lormetazepam (8), nitrazepam (9), and triazolam (10)	Whole blood	SPE (Bond Elut Certify), No IS	Waters Novapak C_{18}, 150 x 3.9 mm, 4 µm p.s. methanol/50 mM aq. ammonium acetate (60/40) flow-rate: 0.4 mL/min.	+ 0.8 mL/min. 50 mM ammonium acetate post-column	+ TSP	QQQ, MRM	*m/z*: 395-314 (1); *m/z*: 316-151 (2); *m/z*: 300-198 (3); *m/z*: 285-165 (4); *m/z*: 313-268 (5); *m/z*: 285-165 (6); *m/z*: 465-408 (7); *m/z*: 335-287 (8); *m/z*: 282-152 (9); *m/z*: 343-308 (10)	LOD: 0.05 - 1 ng/mL (s/n≥3) Linearity: 10-250 ng/mL	Verweij 1996
Twenty different benzodiazepines	Standards, no biological samples	/	Partisil ODS-3 column, 110 x 4.7 mm, 5 µm p.s. acetonitrile/0.1 M ammonium acetate (60/40) flow-rate: 1.3 mL/min.	/	+ TSP	Q, scan or SIM	monitored ions: see manuscript 77	/	Lurie 1992

Triazolam and metabolites (1-hydroxymethyl-triazolam and 4-hydroxytriazolam)	Urine	SPE (C_{18}), Temaze-pam	TSKgel Octyl-80 Ts, 150 x 4.6 mm methanol/100 mm ammoniumacetate (50/50) flow-rate: 1.0 mL/min.	/	+ TSP	Q, scan or SIM	*m/z* 343 ($[M+H]^+$): triazolam *m/z* 359 ($[M+H]^+$): 1-hydroxymethyltriazolam *m/z* 359 ($[M+H]^+$): 4-hydroxytriazolam:	LOD: 5-10 ng/mL (U.P.) Linearity: 10-500 ng/mL	Nishi-kawa 1995 and Nishi-kawa 1998
Triazolam, nimetazepam, lormetazepam, and flurazepam	Stand-ards, no biolo-gical samples	/	CLC-ODS, 150 x 4.6 mm acetonitrile/water (40/60) containing 0.1 M ammonium acetate flow-rate: 1.0 mL/min.	/	+ TSP (either filament on or off)	Q, SIM	*m/z* 343 ($[M+H]^+$): triazolam *m/z* 296 ($[M+H]^+$): nimetazepam *m/z* 335 lormetazepam *m/z* 388 ($[M+H]^+$): flurazepam	/	Yoshida 1997
31 different benzodiazepines and metabolites	Stand-ards	/	TSKgel octyl-80 Ts, 150 x 4.6 mm gradient elution, methanol/ammoni um acetate flow-rate: 1.0 mL/min.	/	+ TSP & + APCI	Q, scan and SIM	TSP: only $[M+H]^+$ ion for most compounds APCI: $[M+H]^+$ ions and some fragment ions	/	Tatsuno 1996b
Oxazepam glucuronide	Urine	SPE (C_{18}), No IS	L-column (C18) 150 x 4.6 mm methanol/50 mM ammonium acetate (70/30) flow-rate: 1.0 mL/min.	/	+ APCI	Q, SIM	*m/z* 287 ($[M+H]^+$ ion of oxazepam aglycone)	LOD: 50 ng/mL (U.P.) Linearity: 0.25 - 1.0 µg/mL	Tatsuno 1992

[a] Non-standard abbreviations: IS, internal standard; Q, quadrupole; p.s., particle size; U.P., undefined procedure.

A comparable approach was published by Hoja et al. (1997a) for BP and norBP in whole blood and in urine or serum (Hoja et al., 1996). Their extraction procedure was much more complicated, but this complication was probably due to the fact that it was especially meant for postmortem and hemolyzed blood samples. Strangely, these authors also included an enzymatic (β-glucuronidase-arylsulfatase) hydrolysis step for their blood samples. SIM analysis was performed, using conditions very much comparable to those in the report discussed previously. The authors stressed the importance of controlling the in-source fragmentation (extractor voltage). BP yields only very few fragment ions when optimizing this voltage; one dealkylation fragment (*m/z* 414) and one resulting from dehydratation of the tertiary alcohol moiety (*m/z* 396). The method described has been thoroughly validated with good overall results. Precision evaluation for both compounds at various concentrations (down to 0.1 ng/mL) results in $<4\%$ CV within-day and <10 total precision). The limit of quantitation was 0.1 ng/mL for both analytes, and weighted-regression analysis (1/x) revealed linearity between 0.1 and 100 ng/mL ($r > 0.9999$). It was concluded that especially the sensitivity that could be achieved promoted LC–MS to become the method of choice for BP, and norBP, and that the robustness of the overall analytical approach allowed the routine use of this method for toxicological purposes.

Bezitramide (BZ) or burgodin is a potent, long-acting, orally active narcotic analgesic. Its use in a clinical setting has decreased over the past years, but it is nowadays abused by drug addicts for its euphoric effect; death of overdose, generally in combination with other drugs, has been reported. Bezitramide is considered a prodrug; rapid hydrolysis of a propionyl group leads to an active metabolite to which the main action is attributed. De Baere et al. (1999) have developed an LC–ESI–MS/MS method for the identification and quantitation of despropionyl-bezitramide (D-BZ) in postmortem specimens (blood, urine, stomach contents, bile, liver, and kidney). *N*-methyldespropionylbezitramide was used as an internal standard, and all samples (or tissue homogenates) were liquid–liquid (back) extracted. Separation was performed isocratically on a classical ODS reversed phase column, using ammonium acetate (1 mM), methanol, and acetonitrile. A 1/20 flow split was inserted between the column and ESI source. A triple quadrupole MS was used for detection; in the MRM mode for the quantitative aspects and selected CID, product-ion spectra were recorded for identity confirmation purposes considered vital in their forensic work. Ion transitions of *m/z* 437.2–111.2 (BZ) and 451.2–111.2 (D-BZ), being the $[M+H]^+$ fragmentation to a common fragment, were monitored. Calibration curves were constructed in blood and urine only, over a range of 0 to 150 ng/mL (eight concentration levels). Weighted regression analysis gave good linearity ($r > 0.9994$). A validation was performed: precision ($n = 5$, CV%) within-day 6.4 (10 ng/mL) and 1.6 (50 ng/mL); total 10.2 (10 ng/mL) and 7.4 (50 ng/mL), accuracy ($n = 5$) percent of target within 12%. LOD was not determined formally, but was inferred from the LOQ established at the lowest calibrator (1 ng/mL). All validation experiments were performed only on blood. Using this method, various postmortem samples of three fatalities due to combined drug overdose, including bezitramide, were analyzed. D-BZ was positively identified in all cases, using the low-energy CID product ion scan experiments. The highest concentrations of D-BZ were found in stomach contents (logically, for its oral administration) and liver tissue, clearly exceeding those of blood and certainly urine. Because the procedure is a further development of an LC–fluorescence method, the same samples and calibrators were assayed with fluorescence detection. Good quantitative correlations were found between both methods, although the linear dynamic range of the MS detector was far better than that of the fluorescence detector, except for the tissue samples. For the latter, much lower concentrations were found with the LC–Fl approach; that difference was attributed to the dirty character of these extracts that literally quenched the fluorescence response. We have found very few reports on the forensic investigation of tissues that use LC–MS. The dirty sample ''quenching'' problem put forward by these authors is also interesting from an MS point of view. It remains to be seen whether such dirty matrices will not have the same impact on MS detection; in this case, not on the detection or mass analysis part (inherently selective, e.g., through MRM), but on the ionization process. Indeed, we ourselves have experimental data that suggest an outspoken matrix suppression effect in ESI ionization when sample clean-up is only rudimentary. The phenomenon is untraceable when limiting applications to qualitative investigations but detrimental when progressing to quantitative LC–MS work.

It is clear that in the field of these synthetic opioids, even more than for the opiates, LC–MS emerges as the first choice. Especially, the high sensitivity, needed for these generally very potent molecules, appears advantageous and discriminates the LC–MS approach from all the previously developed GC–MS- or LC-based analytical methods. Also, this breakthrough has only been established with the advent of the versatile API interfaces. Up until then, TSP had been tried, but were clearly proven not routine enough to develop an analytical effort into a viable quantitative method that is routinely applicable. It is our opinion that in this field LC–MS, using atmospheric pressure ionization, has taken the lead and will keep it for the foreseeable future.

TABLE 6. Miscellaneous forensically relevant LC-MS applications.

Class of drugs + compounds analyzed	Sample	Work-up + IS	Chromatography	Effluent treat-ment	Inter-face	Mass analysis type & detection mode	Monitored ions	Validation data	Ref
Barbiturates Heptabarbital metabolite (5-ethyl-5-(1'-3'-or 6'-cycloheptadienyl) barbituric acid	Plasma, Urine	LLE[a] + HPLC (fraction collec-tion), No IS	Nucleosil C_{18}, 150 x 3.0 mm, 5 µm p.s. 1) 50 mM ammonium acetate in methanol/ water (50/50) 2) methanol/water (50/50) flow-rate: 1.2 mL/min.	/	+ TSP	QQQ, parent and daughter ion scan	Several ions monitored	/	Heeremans 1991
Antidepressants Amitriptyline, nortriptyline	Plasma	LLE, Imipra-mine	Hypersil silica, 150 x 2.1 mm acetonitrile/0.1 M ammonium acetate (94/6) flow-rate: 0.4 mL/min.	/	PB/+CI (methane)	Q, SIM	$[M+H]^+$ at *m/z* 278: amitriptyline *m/z* 264: nortriptyline *m/z* 281: imipramine	LOD: 2 (ami.) and 5 (nor.) ng/mL (s/n ≥ 3) Within-day prec.: 1.2 (ami.) and 11.7 % (nor.) (n=5) Linearity: 10-1000 ng/mL	Kudo 1997
Antidepressants Doxepin, desipramine, imipramine, amitryptiline, trimipramine	Plasma	LLE, Imipramine-d_3	SB-C18 Mac Mod, 15 x 2.1 mm acetonitrile/0.003 M ammonium acetate (pH 3.3) (34/66) flow-rate: 1.4 mL/min.	/	+ ESI (pneuma-tically assisted)	TOF, full spectrum, high resolution	Extracted ion chromatogr.: *m/z* 280.2: doxepine *m/z* 267.2: desipramine *m/z* 281.2: imipramine *m/z* 278.2: amitryptiline *m/z* 295.2: trimipramine *m/z* 280.2: doxepine *m/z* 284.2: imipramine-d_3	Total prec.: 1.0 - 17.7% (n=3) Within-day prec.: 2.9 - 12.8% (n=6) Linearity: 2 - 100 (desi.); 1 - 50 (all others) ng/mL Qualitative accurate mass measurements to < 10 ppm	Zhang 2000
Neuroleptics Iloperidone and metabolite	Plasma	SPE (mixed mode), Iloperi-done methyl derivative	Hypersil C_{18}, 100 x 4.6 mm, 5 µm p.s. acetonitrile/2.5 mM ammonium formate pH 3.5 (70/30) flow-rate: 0.55 mL/min.	Splitting 1/5	+ ESI (pneuma-tically assisted)	QQQ, SIM	$[M+H]^+$ at *m/z* 427: iloperidone *m/z* 429: metabolite *m/z* 441: iloperidone methyl derivative	LOD : 100 pg/mL (s/n ≥ 3) Total prec.: 0-9% (ilo.) and 0.9-12.5% (met.) (n=6) Within-day prec.: < 10.5% (n=6) Linearity: 0.250 - 20 ng/mL	Mutlib 1995

TABLE 6. *(Continued)*

Class of drugs + compounds analyzed	Sample	Work-up + IS	Chromatography	Effluent treatment	Interface	Mass analysis type & detection mode	Monitored ions	Validation data	Ref
Neuroleptics Chlorprotixene, flupenthixol, thiothixene and zuclopenthixol	Whole blood	SPE (certify), No IS	Asahipak ODP-50, 125 x 4.0 mm acetonitrile/50 mM ammonium acetate (85/15) flow-rate: 0.6 mL/min.	+ 0.6 mL/min 50 mM ammonium acetate post-column	+ TSP	QQQ, MRM	*m/z* 316-271: chlorprotixene *m/z* 435-265: flupenthixol *m/z* 444-335: thiothixene *m/z* 401-128: zuclopenthixol	LOD: 0.5 - 10 ng/mL (s/n ≥ 3) Chlorprotixene only : Within-day prec.:7.5 %(n=6) Linearity: 1 - 200 ng/mL	Verweij 1994
Neuroleptica Sultopride	Plasma	LLE, Tiapride	Hypersil silica, 150 x 2.1 mm acetonitrile/0.1 M ammonium acetate (94/6) flow-rate: 0.4 mL/min.	/	PB/-CI (methane)	Q, SIM	$[M-CH_3]^-$ at *m/z* 339: sultopride *m/z* 313: tiapride	Within-day prec.:7.8% (n=5) Linearity: 10-1000 ng/mL	Jitsufuchi 1997
Neuroleptics Haloperidol, reduced haloperidol metabolite	Plasma	LLE, Chloro-haloperi-dol	Nucleosil C_{18}, 150 x 1.0 mm acetonitrile/2 mM ammonium formate pH 3.0 (45/55) flow-rate: 50 µL/min.	/	+ ESI	Q, SIM	*m/z* 123.1; 165.0; 376.2; 378.2 ($[M + H]^+$): haloperidol *m/z* 109.1; 360.2; 378.2; 380.2 ($[M + H]^+$): metabolite *m/z* 392.2: chlorohaloperidol	LOD: 0.075 ng/mL (haloperidol); 0.100 ng/mL (metabolite) (U.P.) Total prec.: 9.4 and 11.2 % (n=6) Within-day prec.: 11.1 and 8.5% (n=6) Linearity: 0.1-50 and 0.25-50 ng/mL	Hoja 1997d
Hypnotics Bromisovalum	Bone marrow of skeleto-nized human remains	LLE, Aryliso-propyl-acetyl-urea	Chemcobond 5-ODS-H, 150 x 4.6 mm methanol/0.1 M ammonium acetate (50/50) flow-rate: 1 mL/min.	/	+ TSP (filament on)	Q, SIM	*m/z* 223 ($[M+H]^+$): bromisovalum *m/z* 185: arylisopropyl-acetylurea	LOD: 2 - 3 µg/mL (U.P.) Linearity: up to 100 µg/mL	Maeda 1997
Hypnotics Bromisovalum	Plasma and whole blood	SPE (C_{18}), 2-Bromo-hexanoyl-urea	Hypersil ODS, 100 x 2.1 mm methanol/water (1/1) flow-rate: 0.2 mL/min.	/	PB/+EI	Q, SIM	*m/z* 182: bromisovalum + 2-bromohexanoylurea	LOD: 100 ng/g (U.P.) Linearity: 500 - 5000 ng/g	Nagata 1995

Cardiac glycosides Digoxin, digitoxin, lanatoside C, and acetyldigitoxin	Plasma	LLE, Oleandrin	Novapak C_{18}, 150 x 2.0 mm, 4 µm p.s. acetonitrile/2 mM ammonium formate pH 3.0 (gradient) flow-rate: 0.2 mL/min.	Splitting 1/3	+ ESI (pneumatically assisted)	Q, SIM	$[M+NH_4]^+$ at *m/z* 799: digoxin *m/z* 783: digitoxin *m/z* 1003: lanatoside C *m/z* 825: acetyldigitoxin *m/z* 894: oleandrin	LOD: 0.15 - 0.60 ng/mL (s/n ≥ 2) Total prec.:12.7 - 18.9% (n=10) Within-day prec.: 5.7 - 13% (n=6) Linearity: 0 - 100 ng/mL	Traqui 1996
Quaternary ammonium compounds 12 compounds including suxamethonium, pancuronium, neostigmine	Urine	LLE, No IS	Asahipak GS320H acetonitrile/100 mM ammonium acetate (30/70) flow-rate: 1 mL/min.	/	+ TSP	Q, SIM	generally the $[M+H]^+$ ion except for suxamethonium and pancuronium	LOD: 0.5 - 80 ng/mL (s/n ≥ 3) Within-day prec.: 3.4 - 7.5% (n=5)	Nishikawa 1994b
Quaternary ammonium compounds Suxamethonium	Rat Tissue	SPE (CBA), Hexamethonium	Develosil ODS-UG-5, 150 x 0.3 mm methanol/0.1% trifluoroacetic acid, both + 0.4% glycerol (gradient) flow-rate: 5 µL/min.	/	Frit-FAB	SIM	*m/z* 403: $[M+TFA]^+$	LOD: 5 ng/g (s/n ≥ 3) Within-day prec.: 8.8% (n=5) Linearity: 30 - 1000 ng/g	Tsuchihashi 1997
Anticoagulants Warfarin and its oxidative metabolites	Microsomal incubations and urine	SPE (C_{18}), Deuterated 6-hydroxywarfarin	Novapak C_{18}, 150 x 4.6 mm 4 µm p.s., acetonitrile/0.25 M ammonium acetate	/	+ TSP (filament off)	Q, SIM	Base peak ($[M+H]^+$) and at least one extra ion, generally $[M+H-H_2O]^+$ *m/z* 309, 291: warfarin	LOD : 10-50 ng/mL (U.P.) Within-day prec.: 6 % (n=3) Linearity: 0 - 200 ng/mL	Spink 1989
Anticoagulants Phenprocoumon	Plasma, urine	LLE, *p*-chlorophenprocoumon	Nucleosil C_{18}, 125 x 4 mm, 5 µm p.s., acetonitrile/0.1 M ammonium acetate (1/1), flow-rate 1 mL/min	/	+ TSP	Q, SIM	*m/z* 281: phenprocoumon *m/z* 315: *p*-chlorophenprocoumon	/	De Vries 1991
Nicotine Nicotine and cotinine	Plasma	LLE, Deuterated. analogues	Hypersil BDS C_{18}, 100 x 3.0 mm, 3 µm p.s., acetonitrile/methanol/10 mM ammonium acetate (53/32/15), flow-rate 1.4 mL/min	/	+ APCI	QQQ, MRM	*m/z* 163.2 - 84.0: nicotine *m/z* 166.2 - 87.0: nicotine-d_3 *m/z* 177.2 - 98.0: cotinine *m/z* 180.2 - 101.0: cotinine-d_3	nicotine/cotinine: LOD: 30 pg/mL/300 pg/mL (s/n ≥ 3) Total prec.: < 6.42% (n=30) Within-day prec.: < 6.34% (n=5) Linearity: 1 - 50 ng/mL/10 - 500 ng/mL	Xu 1996

TABLE 6. *(Continued)*

Class of drugs + compounds analyzed	Sample	Work-up + IS	Chromatography	Effluent treatment	Interface	Mass analysis type & detection mode	Monitored ions	Validation data	Ref
Nicotine Nicotine and 17 metabolites (of which 5 quantitatively)	Urine	Centrifugation and filtration, Cotinine-d_3	Polystyrene-divinylbenzene, 50 x 4.6 mm, 5 µm p.s., methanol/water (gradient), flow-rate 0.5 mL/min	+ 0.9 mL/min. 0.05 M ammonium acetate post-column	+ TSP (filament off)	Q, SIM	$[M+H]^+$ at *m/z* 163: nicotine and demethylcotinine *m/z* 177: cotinine *m/z* 179: nicotine-1'-*N*-oxide *m/z* 180: cotinine-d_3 *m/z* 193: trans-3'-hydroxy-cotinine	LOD: 20 -90 ng/mL (U.P.) Total prec.: 6% (n=5) Within-day prec.: 5 - 7% (n=6) Linearity: 20-8000 ng/mL	McManus 1990
Nicotine Nicotine and 8 metabolites	Urine	Centrifugation and filtration, Cotinine-d_3	Polystyrene-divinylbenzene, 50 x 4.6 mm, 5 µm p.s., methanol/water (gradient), flow-rate 0.5 mL/min	+ 0.9 mL/min. 0.05 M ammonium acetate post-column	+ TSP (filament off)	Q, SIM	$[M+H]^+$ at *m/z* 163: nicotine and demethylcotinine *m/z* 177: cotinine *m/z* 179: nicotine-1'-*N*-oxide *m/z* 180: cotinine-d_3 *m/z* 193: cotinine-N-oxide and trans-3'-hydroxy-cotinine	/	Byrd 1992
Nicotine Cotinine-*N*-glucuronide	Urine	Centrifugation and filtration, Cotinine-*N*-glucuronide-d_3	LC-SCX, 250 x 4.6 mm, isopropanol/50 mM ammonium formate pH 3.4 (45/55), flow-rate 1 mL/min	+ 0.4 mL/min. 0.1 M ammonium acetate post-column	+ TSP (filament off)	Q, SIM	*m/z* 177: cotinine-*N*-glucuronide *m/z* 180: cotinine-*N*-glucuronide-d_3 (protonated cotinine aglycons)	LOD: 246 ng/mL (curve stats.) Linearity: 0 - 10500 ng/mL	Byrd 1994
Xanthins Caffeine, theophylline, and theobromine	Plasma, urine	Extrelut and in-line preconcentration, 7-Ethyl-theophylline	Develosil ODS-HG-5, 150 x 0.3 mm, 17 M acetic acid/glycerol/methanol/water (gradient), flow-rate 4 µL/min	/	frit-FAB (positive ion mode)	double focusing sector, scan	all ions, in scanning mode	Theobr./theofyll./caffeine: LOD: 100/20/20 ng/mL (s/n ≥ 5) Total prec.: 2.7-9.9% (n=5) Within-day prec.: 2.4-9.6% (n=5)	Hieda 1995
Natural poisons Amanitins	Urine	SPE (C_{18}), No IS	Kromasil RP-18, 125 x 2.0 mm, 5 µm p.s., methanol/ammonium acetate (0.02 M, pH 5) (22/78), flow-rate 75 µL/min	/	+ ESI	Q, SIM	$[M+H]^+$ at *m/z* 919: α-amanitin *m/z* 920: β-amanitin	LOD: 10 ng/mL (s/n ≥ 3)	Maurer 1997

Natural poisons Oleandrin	Blood, urine	LLE, Digoxin	Novapak C_{18}, 150 x 2.0 mm, acetonitrile/2 mM ammonium formate pH 3 (gradient)	/	+ ESI (pneumatically assisted)	Q, SIM	*m/z* 594: oleandrin *m/z* 799: digoxin	Within-day prec.: 12.7% (n=6)	Tracqui 1998
Natural poisons Colchicine	Whole blood, plasma, urine	LLE, Tofisopam	C_{18}, 250 x 1.0 mm, 5 µm p.s., acetonitrile/2 mM ammonium formate pH 3 (75/25), flow-rate 50 µL/min	/	+ ESI (pneumatically assisted)	Q, scan and SIM	$[M+H]^+$ at *m/z* 400: colchicin *m/z* 383: tofisopam	LOD: 0.6 ng/mL (s/n ≥ 3) Total prec.: 12.6% (n=10) Within-day prec.: 9.1-10.8% (n=10) Linearity: 5 - 200 ng/mL	Tracqui 1996
Natural poisons L-hyoscyamine	Plasma	LLE, Scopolamine	BDS C_{18}, 50 x 3.0 mm, 3 µm p.s. acetonitrile/methanol/10 mM ammonium acetate (625/375/150) flow-rate: 0.5 mL/min.	/	+ APCI	QQQ, MRM	*m/z* 290.2 ($[M+H]^+$) - 124.0: L-hyoscyamine *m/z* 304.2 ($[M+H]^+$) - 138.0: scopolamine	LOD: 3 pg/mL (s/n ≥ 3) Total precision : < 6.7% (n=3) Within-day prec.: < 3.5% (n=5) Linearity: 0.020 - 0.5 ng/mL	Xu 1995
Natural poisons Reserpine	Plasma	LLE or SPE (MP-1 discs), Rescinnamine	Betasil C_{18} 100 x 1.0 mm, 5 µm p.s. acetonitrille/5 mM ammonium acetate (80/20) flow-rate: 50 µL/min.	/	+ ESI (pneumatically assisted)	QQQ, MRM	*m/z* 609 ($[M+H]^+$) - 195: reserpine *m/z* 635 ($[M+H]^+$) - 221: rescinnamine	LLE: LOD: 10 pg/mL (s/n ≥ 3) Within-day prec.: 5.2 % (n=5) Linearity: 0.01 - 5 ng/mL SPE: LOD: 100 pg/mL (s/n = 7) Linearity: 0.1 - 5 ng/mL	Anderson 1997
Natural poisons Aconitine, mesaconitine, hypaconitine, jesaconitine	Blood, urine	SPE (C_{18} and Sep-pak PS-1), No IS	Inertsil ODS 150 x 4.6 mm tetrahydrofuran/0.3 % trifluoroacetic acid /glycerin (19/81/0.3) flow-rate: 1 mL/min.	Splitting 1/1000	Frit-FAB (positive ion mode)	Double focusing sector, SIM	$[M+H]^+$ at *m/z* 646: aconitine *m/z* 632: mesaconitine *m/z* 616: hypaconitine *m/z* 676: jesaconitine	LOD: 0.5 ng/mL blood Within-day prec.: 10.7 - 14.2 % (n=7)	Ohta 1997

[a]Non-standard abbreviations: LLE, liquid-liquid extraction; Q, quadrupole; p.s., particle size; TOF, time-of-flight; U.P., undefined procedure.

3. Miscellaneous

A large number of prescription drugs, natural products, etc., have or will, sooner or later, find their way into the field of forensic toxicology. For a number of those compounds, certainly those in which the pharmaceutical industries have interests, an LC–MS analytical method has been reported. They are, nevertheless, at present less important from a general forensic viewpoint. Rather than describing them in detail, we have compiled them in Table 6, selecting only those that are really relevant to the review subject.

V. CONCLUSIONS AND OUTLOOK

It is clear that LC–MS has found its way into the field of forensic toxicology and toxicology in general. The variety of application fields that have been addressed in this literature review show that, due to its versatility, LC–MS has proven itself successful in virtually any analytical challenge. In fact, it is our impression that LC–MS and forensic toxicology have been brought together from three different angles. In the early beginning, a number of experimental mass spectrometrists have used forensically relevant compounds such as drugs of abuse to validate their, mainly instrumental, research. Although valuable to show the different LC–MS capabilities and provide operational examples (certainly the earlier interfaces were sometimes complicated and in constant need of fine tuning), the results are mainly qualitative and far from proven in a routine user environment. On the other hand, there are the LC toxicology people who finally see the detector that they always have dreamed of. Many applications have this sort of background, and although of high analytical quality (validated reliability), we do think they do not give LC–MS all the credit that it deserves. Indeed, the mass spectrometer is much too often thought of as a powerful (and expensive) detector for, essentially, a chromatographic separation. Certainly, the high initial investment of an LC–MS system makes high throughput an important issue. The generally long chromatographic separation times de facto prevent high throughput; therefore, short LC columns and rudimentary separations, combined with the high resolving power of MS/MS should certainly be a future issue, albeit keeping in mind the pitfalls of matrix ionization suppression effects. Recent work by Jeanville et al. (2000) and especially Zhang, Heining and Henion (2000) demonstrates such high throughput possibilities; the latter with an 18 sec sample analysis time for tricyclic antidepressants in serum, using the fast acquisition rates of a TOF mass spectrometer. At last, there are, of course, those who are now used to the comfort of GC–MS find themselves limited by either the difficulties of a GC separation (thermal instability, complex derivatization procedures) or confronted with new analytical problems, which GC simply cannot cope with. Many high quality applications, favorably illustrating the power of LC–MS, are the result. They range from polar metabolite investigations such as the measurement of intact glucuronides, e.g., of opiates, to the analysis of complex high molecular mass natural poisons and ionized quaternary amine xenobiotics.

Obviously, LC–MS has reached a level of maturity that makes it a robust and routinely applicable alternative. It is fair to say that powerful analytical methods have been developed that can challenge any other analytical approach such as the much-acclaimed GC–MS. Some nice examples can be found in the section on cocaine and metabolites. The analysis of, e.g., LSD on the other hand, reveals the superiority of LC–MS in some specific cases. It should, however, be clear that basically all of these applications are target component analysis applications. The much-used profiling analysis for unknowns in forensic toxicology is probably less-suited to LC–MS.

Although a slow starter in the field of forensic toxicology, we think LC–MS is quickly making new ground and will catch up in the near future. It is not surprising to see that the advent of the atmospheric pressure ionization techniques has played an important role in the acceptance of LC–MS. Most, if not all, of the recent work uses either ESI or APCI, which are quickly becoming a standard. The fact of a standard emerging will certainly have the effect of persuading less-experienced users in getting involved. They will find the choice much more straightforward, one interface combination serving virtually all potential problems, but above all, assess their investment (which is still substantial) as most likely lasting, with the LC–MS analytical approach at cruising speed.

ACKNOWLEDGMENTS

The authors thank the various colleagues who have provided input to this review, as well as all those co-workers who assisted them with the literature.

REFERENCES

Abian J. 1999. The coupling of gas and liquid chromatography with mass spectrometry. J Mass Spectrom 34:157–168.

Anderson MA, Wachs T, Henion JD. 1997. Quantitative ionspray liquid chromatographic/tandem mass spectrometric determination of reserpine in equine plasma. J Mass Spectrom 32:152–158.

Arpino P. 1989. Combined liquid chromatography mass spectrometry. Part I. Coupling by means of a moving belt interface. Mass Spectrom Rev 8:35–55.

Arpino P. 1990. Combined liquid chromatography mass spectrometry. Part II. Techniques and mechanisms of thermospray. Mass Spectrom Rev 9:631–669.

Baillie TA. 1992. Advances in the application of mass spectrometry to studies of drug metabolism, pharmacokinetics and toxicology. Int J Mass Spectrom Ion Proc 118/119:289–314.

Barber M, Bordoli RS, Elliot GJ, Sedgwick RD, Tyler AM. 1982. Fast-atom bombardment mass spectrometry. Anal Chem 54:645A–657A.

Behymer TD, Bellar TA, Budde WL. 1990. Liquid chromatography/particle beam/mass spectrometry of polar compounds of environmental interest. Anal Chem 62: 1686–1690.

Blakley CR, Vestal ML. 1983. Thermospray interface for liquid chromatography–mass spectrometry. Anal Chem 55: 750–754.

Bogusz MJ, Kala M, Maier R-D. 1997. Determination of phenylisothiocyanate derivatives of amphetamine and its analogues in biological fluids by HPLC–APCI–MS or DAD. J Anal Toxicol 21:59–69.

Bogusz MJ, Maier R-D, Driessen S. 1997. Morphine, morphine-3-glucuronide, morphine-6-glucuronide, and 6-monoacetylmorphine determined by means of atmospheric pressure chemical ionization-mass spectrometry–liquid chromatography in body fluids of heroin victims. J Anal Toxicol 21:346–355.

Bogusz MJ, Krüger K-D, Maier R-D. 2000. Analysis of underivatized amphetamines and related phenethylamines with high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. J Anal Toxicol 24:77–84.

Bogusz MJ, Maier R-D, Erkens M, Driessen S. 1997. Determination of morphine and its 3- and 6-glucuronides, codeine, codeine-glucuronide and 6-monoacetylmorphine in body fluids by liquid chromatography atmospheric pressure chemical ionization mass spectrometry. J Chromatogr 703:115–127.

Bogusz MJ, Maier R-D, Krüger K-D, Kohls U. 1998. Determination of common drugs of abuse in body fluids using one isolation procedure and liquid chromatography-atmospheric pressure chemical-ionization mass spectrometry. J Anal Toxicol 22:549–558.

Bowers LD. 1989. High-performance liquid chromatography/mass spectrometry: state of the art for the drug analysis laboratory. Clin Chem 35:1282–1287.

Breindahl T, Andreasen K. 1999. Determination of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid in urine using high-performance liquid chromatography and electrospray ionization mass spectrometry. J Chromatogr B 732: 155–164.

Bruins AP. 1991. Mass spectrometry with ion sources operation at atmospheric pressure. Mass Spectrom Rev 10:53–77.

Bruins AP. 1994. Atmospheric-pressure-ionization mass spectrometry. II. Applications in pharmacy, biochemistry and general chemistry. Trends Anal Chem 13:81–90.

Brunnée C. 1987. The ideal mass analyser: fact or fiction. Int J Mass Spectrom Ion Proc 76:125– 237.

Burlingame AL, Boyd RK, Gaskell SJ. 1994. Mass spectrometry. Anal Chem 66:634R– 683R.

Byrd GD, Chang K-M, Greene JM. 1992. Evidence for urinary excretion of glucuronide conjugates of nicotine, cotinine, and trans-3′-hydroxycotine in smokers. Drug Metab Dispos 20:192–197.

Byrd GD, Uhrig MS, deBethizy JD, Caldwell WS, Crooks PA, Ravard A, Riggs RM. 1994. Direct determination of cotinine-*N*-glucuronide in urine using thermospray liquid chromatography/mass spectrometry. Biol Mass Spectrom 23:103–107.

Cai J, Henion J. 1996a. On-line immunoaffinity extraction-coupled column capillary liquid chromatography/tandem mass spectrometry: trace analysis of LSD analogs and metabolites in human urine. Anal Chem 68:72–78.

Cai J, Henion J. 1996b. Elucidation of LSD in vitro metabolism by liquid chromatography and capillary electrophoresis with tandem mass spectrometry. J Anal Toxicol 20:27–37.

Cailleux A, Le Bouil A, Bonsergent G, Turcant A, Allain P. 1999. Determination of opiates and cocaine and its metabolites in biological fluids by high-performance liquid chromatoraphy with electrospray tandem mass spectrometry. J Anal Toxicol 23:620–624.

Cappiello A. 1996. Is particle beam an up-to-date LC–MS interface? State of the art and perspectives. Mass Spectrom Rev 15:283–296.

Cappiello A, Famiglini G. 1995. Analysis of thermally unstable compounds by a liquid chromatography/mass spectrometry particle beam interface with a modified ion source. Anal Chem 67:412–419.

Caprioli RM, Fan T, Cottrell JS. 1986. Continuous-flow sample probe for fast-atom-bombardment mass spectrometry. Anal Chem 58:2949–2959.

Caprioli RM, Suter MJ-F. 1992. Continuous-flow fast-atom bombardment: recent advances and applications. Int J Mass Spectrom Ion Processes 118–119:449–476.

Careri M, Mangia A, Musci M. 1996. Applications of liquid chromatography–mass spectrometry interfacing systems in food analysis:pesticide, drug and toxic substance residues. J Chromatogr A 727:153–184.

Clauwaert KM, Van Bocxlaer JF, Lambert WE, Van den Eeckhout EG, Lemière F, Esmans EL, De Leenheer AP. 1998. Narrow-Bore HPLC in combination with fluorescence and electrospray mass spectrometric detection for the analysis of cocaine and metabolites in human hair. Anal Chem 70:2336–2344.

Clauwaert KM, Van Bocxlaer JF, Major HJ, Claereboudt JA, Lambert WE, Van den Eeckhout EG, Van Peteghem CH, De Leenheer AP. 1999. Investigation of the quantitative properties of the quadrupole orthogonal acceleration time-of-flight mass spectrometer with electrospray ionisation

using 3,4-methylenedioxymethamphetamine. Rapid Comm Mass Spectrom 13:1540–1545.

Clench MR. 1992. A comparison of thermospray, plasmaspray, electrospray and dynamic FAB. VG Monographs 3:1–22.

Covey TR, Lee ED, Henion JD. 1986. High-speed liquid chromatography/tandem mass spectrometry for the determination of drugs in biological samples. Anal Chem 58:2453–2460.

Creaser CS, Stygall, JW. 1993. Particle beam liquid chromatography-mass spectrometry: instrumentation and applications: a review. Analyst 118:1467–1480.

Crouch DJ, Rollins DE, Canfield DV, Andrenyak DM, Schulties JE. 1999. Quantitation of alprazolam and α-hydroxyalprazolam in human plasma using liquid chromatography electrospray ionization MS–MS. J Anal Toxicol 23:479–485.

De Baere SM, Lambert WE, Esmans EL, De Leenheer AP. 1999. Identification and quantitation of despropionylbezitramide in postmortem samples by liquid chromatography coupled to electrospray ionization tandem mass spectrometry. Anal Chem 71:2908–2914.

de Kanel J, Vickery WE, Waldner B, Monahan RM, Diamond FX. 1998. Automated extraction of lysergic acid diethylamide (LSD) and *N*-demethyl-LSD from blood, serum, plasma and urine samples using the Zymark RapidTrace™ with LC/MS/MS confirmation. J Forensic Sci 43:622–625.

De Vries JX, Kymber KA. 1991. Thermospray and particle beam liquid chromatographic–mass spectrometric analysis of coumarin anticoagulants. J Chromatogr 562:31–38.

Dragna S, Aubert C, Cano JP. 1989. Direct liquid inlet liquid chromatographic/mass spectrometric identification and high-performance liquid chromatographic analysis of a benzodiazepine glucuronide. Biomed Environ Mass Spectrom 18:359–362.

Duffin KL, Wachs T, Henion JD. 1992. Atmospheric pressure ion-sampling system for liquid chromatography/mass spectrometry analyses on a benchtop mass spectrometer. Anal Chem 64:61–68.

Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. 1989. Electrospray ionization for mass spectrometry of large biomolecules. Science 24:664–671.

Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. 1990. Electrospray ionisation-principles and practice. Mass Spectrom Rev 9:37–70.

Foltz RL. 1992. Recent applications of mass spectrometry in forensic toxicology. Int J Mass Spectrom Ion Proc 118/119:237–263.

Gagné J-P, Roussis SG, Bertrand MJ. 1991. Simple direct liquid introduction system usable as an interface for liquid chromatography-mass spectrometry on quadrupole and magnetic-sector mass spectrometers. J Chromatogr 554:239–304.

Games DE, Hirter P, Kuhnz W, Lewis E, Weerasinghe NCA, Westwood SA. 1981. Studies of combined liquid chromatography–mass spectrometry with a moving-belt interface. J Chromatogr 203:131–138.

Garcia JF, Barceló D. 1993. An overview of LC–MS interfacing systems with selected applications. J High Res Chromatogr 16:633–641.

Garcia DM, Huang SK, Stansbury WF. 1996. Optimization of the atmospheric pressure chemical ionization liquid chromatography mass spectrometry interface. J Am Soc Mass Spectrom 7:59–65.

Gaskell SJ. 1995. Electrospray:principles and practice. J Mass Spectrom. 2:677–688.

Gelpì E. Biomedical and biochemical applications of liquid chromatography–mass spectrometry. J Chromatogr A 703:59–80.

Goff ML, Miller ML, Paulson JD, Lord WD, Richards E, Omori AI. 1997. Effects of 3,4-methylenedioxymethamphetamine in decomposing tissues on the development of Parasarcophaga ruficornis (Diptera: Sarcophagidae) and detection of the drug in postmortem blood, liver tissue, larvae, and puparia. J Forensic Sci 42:276–280.

González ML, Carnicero M, de la Torre R, Ortuño J, Segura J. 1995. Influence of the injection technique on the thermal degradation of cocaine and its metabolites in gas chromatograpy. J Chromatogr B 664:317–327.

Heeremans CEM, Stijen AM, Van der Hoeven RAM, Niessen WMA, Danhof M, Van der Greef J. 1991. Liquid chromatography-thermospray mass spectrometry for identification of a heptabarbital metabolite and sample work-up artifacts. J Chromatogr 554:205–214.

Henion JD, Thomson BA, Dawson PH. 1982. Determination of sulfa drugs in biological fluids by liquid chromatography/mass spectrometry/mass spectrometry. Anal Chem 54:451–456.

Hieda Y, Kashimura S, Hara K, Kageura M. 1995. Higly sensitive and rapid determination of theophylline, theobromine and caffeine in human plasma and urine by gradient capillary high-performance liquid chromatography-frit-fast atom bombardment mass spectrometry. J Chromatogr 667:241–246.

Higton DM, Oxford JM. 1995. Mass spectrometry: pharmaceutical sciences. Appl Spectroscopy Rev 30:81–118.

Hill RE. 1990. The widening horizons of bioanalytical mass spectrometry. Clin Chim Acta 194:1–18.

Hirabayashi A, Sakairi M, Takada Y, Koizumi H. 1997. Recent progress in atmospheric pressure ionization mass spectrometry. Trends Anal Chem 16:45–52.

Hoja H, Marquet P, Verneuil B, Lotfi H, Dupuy J-L, Dreyfuss MF, Lachâtre G. 1996. Dosage de buprénorphine et de norbuprénorphine dans l'urine et le sérum par chromatographie liquide couplée à la spectrométrie de masse avec ionisation de type electrospray (LC–ES/MS). Analusis 24:104–107.

Hoja H, Marquet P, Verneuil B, Lotfi H, Dupuy J-L, Lachâtre G. 1997a. Determination of buprenorphine and norbuprenorphine in whole blood by liquid chromatography–mass spectrometry. J Anal Toxicol 21:160–165.

Hoja H, Marquet P, Verneuil B, Lotfi H, Dupuy J-L, Lachâtre G. 1997b. Determination of LSD and *N*-demethyl-LSD in

urine by liquid chromatography coupled to electrospray ionization mass spectrometry. J Chromatogr B 692: 329–335.

Hoja H, Marquet P, Verneuil B, Lotfi H, Pénicaut B, Lachâtre G. 1997c. Applications of liquid chromatography–mass spectrometry in analytical toxicology: a review. J Anal Toxicol 21:116–126.

Hoja H, Marquet P, Verneuil B, Lotfi H, Dupuy JL, Pénicaut B, Lachâtre G. 1997d. Determination of haloperidol and its reduced metabolite in human plasma by liquid chromatography–mass spectrometry with electrospray ionization. J Chromatogr 688:275–280.

Hopfgartner G, Bean K, Henion J, Henry R. 1993. Ion spray mass spectrometric detection for liquid chromatography: a concentration- or mass-flow sensitive device? J Chromatogr 647:51–61.

Huang EC, Wachs T, Conboy JJ, Henion JD. 1990. Atmospheric pressure ionization mass spectrometry. Detection for the separation sciences. Anal Chem 62:713A–725A.

Jitsufuchi N, Kudo K, Tokunaga H, Imamura T. 1997. Selective determination of sultopride in human plasma using high-performance liquid chromatography with ultraviolet detection and particle beam mass spectrometry. J. Chromatogr. 690, 153–159.

Jeanville PM, Estapé ES, Needham SR, Cole MJ. 2000. Rapid confirmation/quantitation of cocaine and benzoylecgonine in urine utilizing high performance liquid chromatography and tandem mass spectrometry. J Am Soc Mass Spectrom 11:257–263.

Joyce JR, Ardrey RE, Lewis AS. 1985. Thermospray LC/MS applied to the analysis of complex mixtures of non-polar materials. Biomed Mass Spectrom 12:588–592.

Katagi M, Nishikawa M, Tatsuno M, Miyazawa T, Tsuchihashi H, Suzuki A, Shirota O. 1998. Direct analysis of methamphetamine and amphetamine enantiomers in human urine by semi-microcolumn HPLC electrospray ionization mass spectrometry. Jpn J Toxicol Environ Health 44:107–115.

Katagi M, Nishioka H, Nakajima K, Tsuchihashi H, Fujima H, Wada H, Nakamura C, Makino K. 1996. Direct high-performance liquid chromatographic and high-performance liquid chromatographic-thermospray-mass spectrometric determination of enantiomers of methamphetamine and its main metabolites amphetamine and p-hydroxymethamphetamine in human urine. J Chromatogr B 676:35–43.

Kebarle P, Tang L. 1993. From ions in solution to ions in the gas phase. The mechanisms of electrospray mass spectrometry. Anal Chem 65:972A–986A.

Kintz P, Eser HP, Tracqui A, Moeller M, Cirimele V, Mangin P. 1997. Enantioselective separation of methadone and its main metabolite in human hair by liquid chromatography/ion spray-mass spectrometry. J Forensic Sci 42:291–295.

Kintz P, Tracqui A, Mangin P, Eser Y. 1996. Sweat testing in opioid users with a sweat patch. J Anal Toxicol 20: 393–397.

Kleinschnitz M, Herderich M, Schreier P. 1996. Determination of 1,4-benzodiazepines by high-performance liquid chromatography–electrospray tandem mass spectrometry. J Chromatogr B 676:61-67.

Kudo K, Jitsufuchi N, Imamura T. 1997. Selective determination of amitriptyline and nortriptyline in human plasma by HPLC with ultraviolet and particle beam mass spectrometry. J Anal Toxicol 21:185–189.

Lambert WE, Van Bocxlaer JF, De Leenheer AP. 1997. Potential of high performance liquid chromatography with diode array detection in forensic toxicology. J Chromatogr B 689:45–53.

Ligon WV Jr, Dorn SB. 1990. Particle beam interface for liquid chromatography/mass spectrometry. Anal Chem 62:2573–2580.

Lurie IS, Cooper DA, Klein RFX. 1992. High-performance liquid chromatographic analysis of benzodiazepines using diode array, electrochemical and thermospray mass spectrometric detection. J Chromatogr 598:59–66.

Lurie IS, Cooper DA, Krull IS. 1993. High-performance liquid chromatography using continuous on-line post-elution photoirradiation with subsequent diode-array UV or thermospray mass spectrometry detection. J Chromatogr 629:143–151.

Maeda H, Oritani S, Nagai K, Tanaka T, Tanaka N. 1997. Detection of bromisovalum from the bone marrow of skeletonized human remains: a case report with a comparison between gas chromatography/mass spectrometry (GC/MS) and high-performance liquid chromatography/mass spectrometry (LC/MS). Med Sci Law 37:248–253.

Maurer HH. 1992. Systematic toxicological analysis of drugs and their metabolites by gas chromatography–mass spectrometry. J Chromatogr B 580:3–41.

Maurer HH. 1998. Liquid chromatography–mass spectrometry in forensic and clinical toxicology. J Chromatogr 713: 3–25.

Maurer HH, Kraemer T, Ledvinka O, Schmitt CJ, Weber AA. 1997. Gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–mass spectrometry (LC–MS) in toxicological analysis. Studies on the detection of clobenzorex and its metabolites within a systematic toxicological analysis procedure by GC–MS and by immunoassay and studies on the detection of α- and β-amanitin in urine by atmospheric pressure ionization electrospray LC–MS. J Chromatogr 689:81–89.

McManus KT, deBethizy JD, Garteiz DA, Keyerematen GA, Vesell ES. 1990. A new quantitative thermospray LC–MS method for nicotine and its metabolites in biological fluids. J Chromatogr Sci 28:510–516.

Mellon FA. 1991. Liquid chromatography/Mass spectrometry. VG Monographs 2:1–19.

Mesmer MZ, Satzger RD. 1998. Determination of gamma-hydroxybutyrate (GHB) and Gamma-butyrolactone (GBL) by HPLC/UV-VIS spectrophotometry and HPLC/Thermospray Mass Spectrometry. J Forensic Sci 43:489–492.

Muddiman DC, Gusev AI, Martin LB, Hercules DM. 1996. Direct quantification of cocaine in urine by time-of-flight mass spectrometry. Fres J Anal Chem 354: 103–110.

Mutlib AE, Strupczewski JT. 1995. Picogram determination of iloperidone in human plasma by solid-phase extraction and by high-performance liquid-chromatography-selected-ion monitoring electrospray mass spectrometry. J Chromatogr B 669:237–246.

Nagata T, Kudo K, Imamura T, Jitsufuchi N. 1995. Sensitive and selective determination of bromisovalum by high-performance liquid chromatography/particle beam mass spectrometry. Forensic Sci Int 74:205–211.

Niessen WMA. 1998. Advances in instrumentation in liquid chromatography–mass spectrometry and related liquid-introduction techniques. J Chromatogr A 794:407–435.

Niessen WMA. 1999. Liquid chromatography–mass spectrometry, 2nd edition. New York: Marcel Dekker.

Niessen WMA, van der Greef J. 1992. Liquid chromatography–mass spectrometry. New York: Marcel Dekker.

Niessen WMA, Tinke AP. 1995. Liquid chromatography–mass spectrometry. General principles and instrumentation. J Chromatogr A 703:37–57.

Nishikawa M, Nakajima K, Igarashi K, Kasuya F, Fukui M, Tsuchihashi H. 1992. Determination of morphine-3-glucuronide in human urine by LC/APCI–MS. Jpn J Toxicol Environ Health 38:121–126.

Nishikawa M, Nakajima K, Tatsuno M, Kasuya F, Igarashi K, Fukui M, Tsuchihashi H. 1994a. The analysis of cocaine and its metabolites by liquid chromatography/atmospheric pressure chemical ionization-mass spectrometry. Forensic Sci Int 66:149–158.

Nishikawa M, Tatsuno M, Tsuchihashi H, Igarashi K, Kasuya F, Fukui M. 1994b. The analysis of quaternary ammonium compounds in human urine by thermospray liquid chromatography–mass spectrometry. Jpn J Toxicol Environ Health 40:534–541.

Nishikawa M, Tatsuno M, Katagi M, Tsuchihashi H. 1995. Determination of triazolam and its metabolites in human urine by EMIT, gas chromatography–mass spectrometry and thermospray liquid chromatography–mass spectrometry. Jpn J Toxicol Environ Health 41:59–66.

Nishikawa M, Tsuchihashi H. 1998. Applications of LC/MS in forensic chemistry. J Toxicol Toxin Rev 17:13–26.

Niwa T. 1995. Clinical mass spectrometry. Clin Chim Acta 241–242:1–414.

Ohta H, Sato Y, Tsundo N. 1997. Determination of Aconitum alkaloids in blood and urine samples. 1. High-performance liquid chromatographic separation, solid-phase extraction and mass spectrometric confirmation. J Chromatogr 691:351–356.

Pacifici R, Pichini S, Altieri I, Caronna A, Passa AR, Zuccaro P. 1995. High-performance liquid chromatographic–electrospray mass spectrometric determination of morphine and its 3- and 6-glucuronides: application to pharmacokinetic studies. J Chromatogr B 664:329–334.

Purves RW, Gabryelski W, Li L. 1998. Investigation of the quantitative capabilities of an electrospray ionization ion trap/linear time-of-flight mass spectrometer. Rapid Commun Mass Spectrom 12:695–700.

Reiser RW, Fogiel AJ. 1994. Liquid chromatography/mass spectrometry analyses of small molecules using electrospray and fast-atom bombardment ionization. Rapid Commun Mass Spectrom 8:252–257.

Robson JN, Draper S, Tennant K. 1994. Atmospheric pressure ionization liquid chromatography–mass spectrometry in the quantification of drugs in biological fluids. Anal Proc 31:159–161.

Rule GS, Henion JD. 1992. Determination of drugs from urine by on-line immunoaffinity chromatography-high-performance liquid chromatography–mass spectrometry. J Chromatogr B 582:103–112.

Rustichelli C, Ferioli V, Vezzalini F, Rossi MC, Gamberini G. 1996. Simultaneous separation and identification of hashish constituents by coupled liquid chromatography–mass spectrometry (HPLC–MS). Chromatographia 43: 129–134.

Sato K, Moore CM, Mizuno Y, Hattori H, Katsumata Y. 1992a. Analyses of drugs by capillary high performance liquid chromatography/fast atom bombardment-mass spectrometry, Part 1. Identification of triazolam in human serum after its oral administration of only a 0.25 mg tablet. Jpn J Forensic Toxicol 10:9–15.

Sato K, Moore CM, Kobayashi K, Mizuno Y, Katsumata Y. 1992b. Analyses of drugs by capillary high performance liquid chromatography/fast atom bombardment-mass spectrometry, Part 2 Identification of chlordiazepoxide and its metabolites in human serum. Jpn J Forensic Toxicol 10:23–30.

Senda N, Kohta K, Takahashi T, Shizukuishi K, Mimura T, Fujita T, Nakayama M. 1995. A highly sensitive method to quantify triazolam and its metabolites with liquid chromatography–mass spectrometry. Biomed Chromatogr 9:48–51.

Singh G, Arora V, Fenn PT, Mets B, Blair I. 1999. A validated stable isotope dilution liquid chromatography tandem mass spectrometry assay for the trace analysis of cocaine and its major metabolites in plasma. Anal Chem 71: 2021–2027.

Slawson MH, Crouch DJ, Andrenyak DM, Rollins DE, Lu JK, Bailey PL. 1999. Determination of morphine, morphine-3-glucuronide, and morphine-6-glucuronide in plasma after intravenous and intrathecal morphine administration using HPLC with electrospray ionization and tandem mass spectrometry. J Anal Toxicol 23:468–473.

Smith RD, Loo JA, Edmonds CG, Barinaga CJ, Udseth HR. 1990. New developments in biochemical mass spectrometry: electrospray ionization. Anal Chem 62:882–899.

Sosnoff CS, Ann Q, Bernert JT Jr, Powell MK, Miller BB, Henderson LO, Hannon WH, Fernhoff P, Sampson EJ. 1996. Analysis of benzoylecgonine in dried blood spots by liquid chromatography-atmospheric pressure chemical

ionzation tandem mass spectrometry. J Anal Toxicol 20:179–184.

Spink DC, Aldous KM, Kaminsky LS. 1989. Analysis of oxidative warfarin metabolites by thermospray high-performance liquid chromatography/mass spectrometry. Anal Biochem 177:307–313.

Stroh JG, Carter Cook J, Milberg RM, Brayton L, Kihara T, Huang Z, Rinehart KL. 1985. On-line liquid chromatography/fast atom bombardment mass spectrometry. Anal Chem 57:985–991.

Tai SS-C, Christensen RG, Coakley K, Ellerbe P, Long T, Welch MJ. 1996. Certification of phencyclidine in lyophilized human urine reference materials. J Anal Toxicol 20:43–49.

Tai SS-C, Christensen RG, Paule RC, Sander LC, Welch MJ. 1994. The certification of morphine and codeine in a human standard reference material. J Anal Toxicol 18: 7–12.

Tas AC, van der Greef J, ten Noever de Brauw MC, Plomp TA, Maes RAA, Hohn M, Rapp U. 1986. LC/MS determination of bromazepam, clopenthixol, and reserpine in serum of a non-fatal case of intoxication. J Anal Toxicol 10:46–48.

Tatsuno M, Nishikawa M, Katagi M, Tsuchihashi H, Igarashi K, Kasuya F, Fukui M. 1992. Determination of oxazepam glucuronide in human urine by liquid chromatography/APCI-mass spectrometry. Jpn J Toxicol Environ Health 38:162–167.

Tatsuno M, Nishikawa M, Katagi M, Tsuchihashi H. 1996a. Simultaneous determination of illicit drugs in human urine by liquid chromatography–mass spectrometry. J Anal Toxicol 20:281–286.

Tatsuno M, Nishikawa M, Tsuchihashi H, Igarashi K, Kasuya F, Fukui M. 1996b. Thermospray and atmospheric pressure chemical ionization liquid chromatographic–mass spectrometric analysis of benzodiazepines and their metabolites. Jpn J Toxicol Environ Health 42:248–256.

Tomer KB, Parker CE. 1989. Biochemical applications of liquid chromatography–mass spectrometry. J Chromatogr 492:189–221.

Tracqui A, Kintz P, Ludes B, Rougé C, Douibi H, Mangin P. 1996. High-performance liquid chromatography coupled to ion spray mass spectrometry for the determination of colchicine at ppb levels in human biofluids. J Chromatogr 675:235–242.

Tracqui A, Kintz P, Mangin P. 1997a. HPLC/MS determination of buprenorphine and norbuprenorphine in biological fluids and hair samples. J Forensic Sci 42:111–114.

Tracqui A, Kintz P, Ludes B, Mangin P. 1997b. High-performance liquid chromatography–ionspray mass spectrometry for the specific determination of digoxin and some related cardiac glycosides in human plasma. J Chromatogr 692:101–109.

Tracqui A, Kintz P, Branche F, Ludes B. 1998. Confirmation of oleander poisoning by HPLC/MS. Int J Legal Med 111:32–34.

Tsuchihashi H, Nishikawa M, Tatsuno M. 1997. The analytical procedure by Frit-FAB LC–MS and detectable period of suxamethonium in rats tissues. Jpn J Toxicol Environ Health 43:108–113.

Tyrefors N, Hyllbrant B, Ekman L, Johansson M, Långström B. 1996. Determination of morphine, morphine-3-glucuronide and morphine-6-glucuronide in human serum by solid-phase extraction and liquid-chromatography with electrospray ionization. J Chromatogr A 729:279–285.

Vékey K, Edwards DMF, Zerilli LF. 1989. Liquid chromatographic–mass spectrometric studies on rifamycin antibiotics. J Chromatogr 474:317–327.

Verpoorte R, Niessen WMA. 1994. Liquid chromatography coupled with mass spectrometry in the analysis of alkaloids. Phytochem Anal 5:217–232.

Verweij AMA, Hordijk ML, Lipman PJI. 1994. Quantitative liquid chromatography, thermospray/tandem mass spectrometric (LC/TSP/MS/MS) analysis of some tranquillizers of the thioxantene group in whole-blood. J Liq Chromatogr 17:4099–4110.

Verweij MA, Hordijk ML, Lipman PJL. 1995. Quantitative liquid chromatograpic thermospray-tandem mass spectrometric analysis of some analgesics and tranquillizers of the methadone, butyrophenone, or diphenylbutylpiperidine groups in whole blood. J Anal Toxicol 19:65–68.

Verweij AMA, Hordijk ML, Lipman PJL. 1996. Liquid chromatographic-thermospray tandem mass spectrometric quantitative analysis of some drugs with hypnotic, sedative and tranquillising properties in whole blood. J Chromatogr B 686:27–34.

Verweij AMA, Lipman PJL, Zweipfenning PGM. 1992. Quantitative liquid chromatography, thermospray/tandem mass spectrometry (LC/TSP/MS/MS) analysis of some thermolabile benzodiazepines in whole blood. Forensic Sci Int 54:67–74.

Verweij AMA, Lipman PJL. 1996. Comparison of mass spectrometric ionization techniques for the analysis of phenethylamines. J Chromatogr Sci 34:379–382.

Vestal ML. 1984. High-performance liquid chromatography–mass spectrometry. Science 226:275–281.

Vestal ML, Fergusson GJ. 1985. Thermospray liquid chromatograph/mass spectrometer interface with direct electrical heating of the capillary. Anal Chem 57:2373–2378.

Voress L. 1994. Electrospray MS. Anal Chem 66:481A–486A.

Wachs T, Conboy JC, Garcia F, Henion J. 1991. Liquid chromatography–mass spectrometry and related techniques via atmospheric pressure ionization. J Chromatogr Sci 29:357–366.

Wang P, Bartlett MG. 1998. Collision-induced dissociation mass spectra of cocaine, and its metabolites and pyrolysis products. J Mass Spectrom 33:961–967.

Wang P, Bartlett MG. 1999. Identification and quantitation of cocaine *N*-oxide: a thermally labile metabolite of cocaine. J Anal Toxicol 23:62–66.

Webb KS, Baker PB, Cassells NP, Francis JM, Johnston DE, Lancaster SL, Minty PS, Reed GD, White SA. 1996. The

analysis of lysergide (LSD): the development of novel enzyme immunoassay and immunoaffinity extraction procedures together with an HPLC–MS confirmation procedure. J Forensic Sci 41:938–946.

Weickhardt C, Moritz F, Grotemeyer J. 1996. Time-of-flight mass spectrometry: state-of-the art in chemical analysis and molecular science. Mass Spectrom Rev 15:139–162.

Weinmann W, Svoboda M. 1998. Fast screening for drugs of abuse by solid-phase extraction combined with flow-injection ionspray-tandem mass spectrometry. J Anal Toxicol 22:319–328.

White SA, Catterick T, Harrison ME, Johnston DE, Reed GD, Webb KS. 1997. Determination of lysergide in urine by high performance liquid chromatography combined with electrospray ionisation mass spectrometry. J Chromatogr B 689:335–340.

White SA, Kidd AS, Webb KS. 1999. The determination of lysergide (LSD) in urine by high performance liquid chromatography-isotope dilution mass spectrometry (IDMS). J Forensic Sci 44:375–379.

Whitehouse CM, Dreyer RN, Yamashita M, Fenn JB. 1985. Electrospray interface for liquid chromatographs and mass spectrometers. Anal Chem 57:675–679.

Winkler PC, Perkins DD, Williams WK, Browner RF. 1988. Performance of an improved monodisperse aerosol generation interface for liquid chromatography/mass spectrometry. Anal Chem 60:489–493.

Wolfender J-L, Maillard M, Hostettmann K. 1994. Thermospray liquid chromatography-mass spectrometry in phytochemical analysis. Phytochem Anal 5:153–182.

Xu A, Havel J, Linderholm K, Hulse J. 1995. Development and validation of an LC/MS/MS method for the determination of L-hyoscyamine in human plasma. J Pharm Biomed Anal 14:33–42.

Xu AS, Peng LL, Havel JA, Petersen ME, Fiene JA, Hulse JD. 1996. Determination of nicotine and cotinine in human plasma by liquid chromatography-tandem mass spectrometry with atmospheric-pressure chemical ionization interface. J Chromatogr B 682:249–257.

Yinon J. 1991. Forensic applications of mass spectrometry. Mass Spectrom Rev 10:179–224.

Yoshida M, Akane A, Yoshimura S, Okii Y, Tokiyasu T, Watabiki T. 1997. Determination of benzodiazepines, triazolam, nimetazepam, lormetazepam and flunitrazepam by thermospray liquid chromatography-mass spectrometry. Res Prac Forensic Med 40:103–107.

Zhang H, Heining K, Henion J. 2000. Atmospheric pressure ionization time-of-flight mass spectrometry coupled with fast liquid chromatography for quantitation and accurate mass measurement of five pharmaceutical drugs in human plasma. J Mass spectrom 35:423–431.

Zhao J, Zhu J, Lubman DM. 1992. Liquid sample injection using an atmospheric pressure direct current glow discharge ionization source. Anal Chem 64:1426–1433.

Zuccaro P, Ricciarello R, Pichini S, Pacifici R, Altieri I, Pellegrini M, D'Ascenzo G. 1997. Simultaneous determination of heroin, 6-monoacetylmorphine, morphine, and its glucuronides by liquid chromatography–atmospheric pressure ionspray-mass spectrometry. J Anal Toxicol 21: 268–277.