Progress of Liquid Chromatography–Mass Spectrometry in Clinical and Forensic Toxicology

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Summary: The use of liquid chromatography–mass spectrometry (LC-MS) has recently exploded in various analytic fields, including toxicology and therapeutic drug monitoring (although still far behind pharmacokinetics). There is no doubt that LC-MS is currently competing with gas chromatography (GC)–MS for the status of the reference analytic technique in toxicology. This review presents, for the nonspecialist reader, the principles, advantages, and drawbacks of LC-MS systems using atmospheric pressure interfaces. It also gives an overview of the analytic methods for xenobiotics that could be set up with these instruments for clinical or forensic toxicology. In particular, as far as quantitative techniques are concerned, this review tries to underline the large number and variety of drugs or classes of drugs (drugs of abuse, therapeutic drugs) or toxic compounds (e.g., pesticides) that can be readily determined using such instruments, the respective merits of the different ionization sources, and the improvements brought about by tandem MS. It also discusses new applications of LC-MS in the field of toxicology, such as “general unknown” screening procedures and mass spectral libraries using LC–atmospheric pressure ionization (API)–MS or MS-MS, presenting the different solutions proposed to overcome the naturally low fragmentation power of API sources. Finally, the opportunities afforded by the most recent or proposed instrument designs are addressed. Key Words: Liquid chromatography–mass spectrometry—Electrospray—Atmospheric pressure chemical ionization—Therapeutic drug monitoring—General unknown screening.

Writing a review article is never easy, but it is particularly difficult when at least five such articles have been written in the past 4 years (1–5). The application of liquid chromatography–mass spectrometry (LC-MS) to toxicology has been a hot topic in recent years because the technology is now mature and validated and has helped to resolve some acute analytic problems in the field of toxicology, while a substantial number of other analytic methods, some very exciting, were developed as alternatives to gas chromatography (GC)–MS or high-performance liquid chromatography (HPLC) techniques.

The history of LC-MS coupling, as well as the principles, advantages, and drawbacks of the various and successive interfaces/ionization sources used, were detailed in most of the reviews cited above. In this paper, I will focus on the electrospray (ES) and atmospheric pressure chemical ionization (APCI) sources that equip the majority of the instruments sold recently and represent a large majority of the applications reported.

However, the main purpose of this new review, rather than listing and detailing all the analytic methods developed in toxicology, is to summarize what the reader who is not yet involved in this technique would be able to do, or not do, after acquiring such an instrument. For more detailed comparison of the different methods reported for the same application, or the same classes of toxic compounds, the reader is invited to refer to the reviews cited above and then, for a complete description of the methods most suited to his or her needs, to refer to the original experimental articles.
Finally, a new topic dealt with only briefly in a previous review article from my group (4)—general unknown screening procedures using LC-MS—will be discussed here in more detail.

**PRINCIPLES, ADVANTAGES, AND DRAWBACKS OF LC-MS COUPLING VIA ATMOSPHERIC PRESSURE INTERFACES**

The first description of atmospheric pressure ionization (API) sources for MS was made in the late 1950s, but the first systems were commercialized only in the 1970s. Nevertheless, it was not until the late 1980s that these coupling techniques really began to spread to the various fields of science, while the first applications in toxicology appeared in the early 1990s. API interfaces mainly comprise different versions of ES and APCI interfaces/sources. The common features of these interfaces can be described as a combination of a liquid introduction device, an API source, an ion sampling aperture, an interface between atmospheric pressure and high vacuum, and an ionic optical system, all of which meet different specifications, depending on the different makers (6). API interfaces and the related ionization sources described below were used for a large majority of the applications in forensic and clinical toxicology published in the past 3 years, and probably equip an even larger proportion of the instruments sold in toxicology laboratories.

**ES Interfaces**

In genuine ES sources, the liquid flow was nebulized exclusively by means of an intense electric field between the tip of the capillary inlet and the aperture, which was compatible only with very low flow rates (1–10 μL/min) (7). In 1987, Bruins et al (8) described a pneumatically assisted ES interface, which they called ionspray, that accepted flow rates up to 200 μL/min with no loss in sensitivity, contrary to most of the other high-flow rate solutions proposed previously, which resulted in higher detection limits (9). Pneumatically assisted ES sources combine the principles of ion evaporation (10) and ES, which are close ionization mechanisms (Fig. 1). Since the advent of IonSpray™ (named after Sciex LC-MS instruments) (Applied-Biosystems/MDS-Sciex; Concord, Ontario, Canada), similar solutions have been proposed by different manufacturers under various names (e.g., high-flow or megaflow ES). Further steps in accommodating high flow rates have consisted of an additional drying of the spray by an orthogonal, heated nitrogen beam, used in the Turbo-IonSpray™ interface (Sciex); or an orthogonal positioning of the ES with respect to the ion sampling orifice, using different technical solutions (Agilent Technologies orthogonal ES system (Agilent Technologies; Palo Alto, CA) or Micromass Z-spray™ ES source (Micromass; Manchester, UK)).

In the spray, the droplet size decreases rapidly, owing to an initial desolvation in the atmospheric pressure chamber, assisted in some instruments by a countercurrent of pure nitrogen. Therefore, the electrical field at the droplet surface increases until the droplets explode, giving smaller droplets that will undergo the same fate. The ions are extracted from the spray, which is frequently off-axis with respect to the orifice, by an electrical field directed toward the intermediate- or low-pressure chamber, where the residual clusters are broken down, owing to their acceleration through the electrical field. If this acceleration is increased, dissociation of ions can occur through collision with the residual solvent and gas molecules, and the fragments produced by this collision-induced dissociation (CID), called “in-source CID,” can be used as confirmation ions for quantitation methods in the selected ion monitoring (SIM) mode, or for structure elucidation. This interface allows the analysis of moderately nonpolar to highly polar compounds, even those that are thermally labile or those with a high molecular weight. In particular, it gives multicharged ions from peptides and proteins, making it possible to analyze them using single quadrupole instruments, as the m/z ratios resulting from molecules up to 30 to 200 kDa generally fall into their usual m/z range. Electrospray is surpassed in this application only by the more expensive matrix-assisted laser desorption ionization (MALDI) source. Other advantages of ES are that it is compatible with

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**FIG. 1.** A pneumatically assisted electrospray (ES) ionization source and triple-quadrupole mass spectrometer (MS). API, atmospheric pressure ionization.
Chromatographic gradients and can be coupled with capillary electrophoresis through a different introduction device. Its main drawbacks are a significant chemical noise in the low mass range (although lower than with thermospray ionization sources); “ion suppression” problems when different compounds enter the source at the same time and compete for ionization; low fragmentation (which can be enhanced afterwards by CID); and a limited admissible flow rate except, maybe, with some of the most recent types of sources. Concerning this last point, most users choose to use chromatographic columns with a 2.1-mm I.D. and a mobile phase flow rate of about 200 μL/min, with or without postcolumn splitting. Nevertheless, as previously noticed when reviewing the methods published for the same compound or class of compounds (4), the lower the column I.D. and mobile phase flow rate, the lower the limits of detection (LOD) or quantitation (LOQ) obtained: narrow-bore columns of about 1 mm I.D. with flow rates of 40 to 50 μL/min generally give the best results, inasmuch as the sensitivity of ES-type sources seems to be related to the concentration of the compounds of interest in the chromatographic effluent rather than to the amount per unit time (mass flow rate) admitted in the source. Indeed, this is attributed to a higher evaporation rate of mobile phase for low flow rates and, thus, to a better transmission of the ions formed toward the mass spectrometer (6).

APCI Interfaces

Horning et al (11) developed the APCI source in the early 1970s. Since then, different technical solutions and designs have been proposed (9), some of which are commercially available. Schematically, the chromatographic effluent flows through a heated nebulizer, either pneumatically assisted and housed in a quartz tube in which flows a make-up gas (Fig. 2), or assisted by a piezoelectric ultrasonic or a sonic spray device. Alternately, heat is not applied to the three concentric tubes (chromatographic effluent, nebulizing and make-up gases) but in a “vaporization zone” in the atmospheric pressure interface. However, the mixture of hot liquid and vapors expands into the atmospheric pressure interface, where it is ionized by a corona discharge. The ions formed from the solvent molecules transfer a charge to the analytes. As in the ES source, droplets can be further desolvated and ion clusters broken down when crossing a countercurrent stream of gas. The pumping stages, intermediate-pressure chamber, ion optic, and in-source CID possibilities are the same as those of ES, as both are generally available on the same instruments. However, although it can admit higher liquid flow rates, fewer applications were reported with this type of source than with ES, at least in the fields of clinical and forensic toxicology. The reasons for this are probably its more limited polarity range, partly overlapping that of GC-MS; its relative incompatibility with thermally labile compounds; and the higher background noise produced compared with ES. Its main advantage is that it extends the polarity range of compounds amenable to LC-API-MS toward less polar molecules. APCI sources can be either more or less sensitive than ES sources, depending on the type of source or manufacturer (more sensitive on LC-MSD from Agilent Technologies and less sensitive than TurboIonSpray™ sources from Applied Biosystem-Sciex).

APPLICATION OF LC-API-MS TO TOXICOLOGY

Quantitative Methods

The extensive use of LC-MS and LC-MS-MS by the pharmaceutical industry in the past few years and the huge volume of related literature have shown the potential value of these techniques for the analysis of small molecules, typically therapeutic drugs and metabolites,
whether qualitatively (metabolic studies) or quantitatively (pharmacokinetic studies). The same applies to toxicology, which shares a large number of compounds of interests with pharmacology. At the beginning of the expansion of LC-API-MS into toxicology laboratories, the most frequently analyzed compounds were those not amenable to GC-MS (e.g., LSD and glucuronides) or for which no sufficiently sensitive analytic technique was available so far (e.g., colchicine, cardiac glycosides). LC-API-MS and even LC-API-MS-MS are now applied in toxicology to compounds previously analyzed by less specific HPLC techniques or even by GC-MS owing to the simpler sample preparation generally needed. It would appear now that virtually all xenobiotics are amenable to LC-MS. Investigating this hypothesis is one of the aims of this review.

Quantitative Methods for Drugs of Abuse

Opiates and Opioids. The detection and quantitation of opiates in biologic fluids by LC-MS has been the object of many papers since 1994, most of which have already been analyzed in recent review papers (3–5). An ES source was used in at least eight methods (12–19) and an APCI source in at least three others (20–22). One method elicited the direct determination of heroin in mouse serum by LC-APCI-MS with a limit of detection of 0.5 ng/mL (14). Most others concerned heroin metabolites 6-monoacetylmorphine (6-MAM) and morphine, as well as codeine and the metabolites of morphine, namely glucuro-3- and glucuro-6-morphine (M3G and M6G, respectively). Overall results for 6-MAM showed LOQs equal to 4 ng/mL using ES and 0.5 to 2 ng/mL using APCI. For morphine they were 0.84 to 10 ng/mL with ES and 0.1 to 30 ng/mL with APCI; for codeine they were 5 and 4 ng/mL, respectively. Finally, the LOQs of M3G were 1 to 100 ng/mL and 1 to 30 ng/mL, respectively; those of M6G were 2 to 50 ng/mL and 2 to 5 ng/mL. Morphine glucuronides can be directly and accurately determined using LC-MS, in contrast to GC-MS, where they have to be hydrolyzed (with variable and often incomplete recovery) and their global concentration deduced from the difference between total and free morphine concentrations. The conclusion that can be drawn from these studies is that heroin, morphine, codeine, and their metabolites can be determined by LC-API-MS with a sensitivity at least equal to that of GC-MS (and maybe even better with APCI, although only a few methods were reported with such sources). In general, simpler sample preparation (no hydrolysis, one-step extraction, no derivatization) was used. For both these criteria of sensitivity and simplicity, as well as for its increased selectivity, tandem MS is undoubtedly a further improvement, as shown by four recent papers using ES ionization. In the first, morphine and codeine, as well as other drugs of abuse, were screened for and quantitated in serum and urine in a concentration range of 10 to 1,000 ng/mL using flow-injection analysis (FIA)-ES-MS-MS operated in the multiple reaction monitoring (MRM) mode (19). The second paper reported the fully validated determination of 6-MAM, morphine, codeine, norcodeine, pholcodine, and codehylthine in whole blood, plasma, and urine in the concentration range of 10 to 1,000 ng/mL, using nalmorine as internal standard and reversed-phase chromatography (RP-HPLC)-ES-MS-MS in the MRM mode (16). Third, Slawson et al (18) reported a validated method for the determination of morphine (0.25–10 ng/mL) and M3G and M6G (0.5–10 ng/mL) in plasma using morphine-d₃ and M3G-d₃ as internal standards and RP-HPLC and acquisition in the MRM mode. Finally, Naidong et al (17) reported a fully validated method for the determination of morphine (concentration range 0.5–50 ng/mL) and its two glucuronides (M6G, 1–100 ng/mL; M3G, 10–100 ng/mL) in human plasma using the respective deuterated internal standard, ion-pairing solid-phase extraction (SPE), chromatography on a nongrafted silica column using a water-acetonitrile mobile phase, and the MRM mode. For a definitive identification of the compounds detected, although MS-MS in the MRM mode is relatively specific, full mass spectra should be obtained in the product-ion scan mode, in which all the fragments generated by the dissociation in the collision cell of each given pseudo-molecular ion selected in the first quadrupole are recorded. However, whether in the MRM or product-ion scan mode, good chromatographic separation between morphine and its glucuronides seems an important point, because the glucuronides are often partly fragmented to morphine in the ionization source, even at low fragmentation voltage. However, a fully validated method including all the above-mentioned opiates and metabolites and all the respective deuterated standards is needed, to be proposed as an alternative gold standard to GC-MS.

LC-MS was also shown to be a convenient technique for the sensitive and specific determination of synthetic or semisynthetic opioids: dihydromorphine and dihydrocodeine (23); buprenorphine and norbuprenorphine, for which it is now considered the gold standard (24,25); methadone in hair (26) and different biofluids (23); and despropionyl-bezitramide, the active metabolite of the prodrug bezitramide (27). Methods using only old types of ionization sources/interfaces were reported for dextromoramide and dextropropoxyphene (thermospray [TSP]
ionization) and dextromethorphan (continuous-flow fast-atom bombardment). GC-MS was often used for the phenylperidines fentanyl, alfentanil, malfentanil, sufentanil, or remifentanil (28–30), whereas, except for the LC-TSP-MS determination of mrfentanol in plasma (with a 0.4–100-ng/mL quantitation range) (31), no paper concerning the LC-MS analysis of these drugs has been published so far. Nevertheless, LC-MS emerges as the first choice for several synthetic opioids, even more than for opiates (5).

**Cocaine and Metabolites.** At least a dozen papers, most of which were reviewed recently (5), reported LC-API-MS analytic methods for cocaine and its metabolites, mainly using tandem MS. Using an ES ionization source, Weinmann et al (19) presented the FIA-MS-MS analysis of benzoylecgonine in addition to that of morphine, codeine, and amphetamine in serum and urine after mixed-mode SPE. This previously mentioned study yielded a LOD of 2 ng/mL for benzoylecgonine and was linear in the concentration range of 10 to 1,000 ng/mL (19); possible matrix effects (ion suppression) were taken in account by the use of deuterated analogs of all the molecules of interest. However, benzoylecgonine has the same molecular mass as norcocaine (289 u), an active metabolite of cocaine (5) with which it also shares the same most intense fragment at m/z 168; therefore, the m/z 290 → 168 transition used by these authors should have been avoided (even if norcocaine concentrations in plasma or urine are generally much lower than benzoylecgonine concentrations) because no chromatographic separation was used in this method. Several other studies used LC-ES-MS-MS for the determination of cocaine and various metabolites. Clauwaert et al (32) designed a confirmation method for the detection of cocaine, benzoylecgonine, and cocaethylene in hair, for which unfortunately they did not report a LOD. In urine, Jeanville et al (33) obtained an LOQ of 7.5 ng/mL for cocaine and benzoylecgonine, using a simple filtration as sample preparation. Needham et al (34) obtained an LOQ of 1.0 ng/mL for the same molecules after a simple 1:10 dilution of urine samples and using a pentfluorophenylpropyl (PFPP) bonded silica chromatographic column to increase the retention of ecgonine methyl ester (EME) and to be able to use a high enough concentration of organic solvent in the mobile phase, thereby increasing the ES-MS signal because of more efficient desolvation. Cailleux et al (16) obtained LOQs of 10 ng/mL for cocaine, benzoylecgonine, EME, cocaethylene and anhydroecgonine methylester after liquid-liquid extraction. These latter authors obtained the same LOQs in plasma and whole blood and validated their technique in both these matrices, whereas Srinivasan et al (35) designed a validated method for the determination of cocaine and 12 metabolites in blood, amniotic fluid, and placental and fetal tissues with LOQs of 10 ng/mL, using SPE on underivatized silica columns. Wang and Bartlett (36) reported a single quadrupole method for the determination in plasma of cocaine-N-oxide, a thermally labile metabolite that converts to cocaine when heated, with an LOQ of 10 ng/mL. Finally, Jeanville et al (37) reported the first use of a quadrupole orthogonal acceleration time-of-flight (Q-TOF) instrument operated in the MS-MS mode for the determination of cocaine and EME in rat plasma. The procedure had a high-throughput, total turn-over time of 3.2 minutes owing to on-line SPE and rapid chromatography using a cyanopropyl-based SPE and rapid chromatography using a cyanopropyl-based column with retention characteristics similar to the PFPP stationary phase cited above (i.e., high retention for the extremely polar EME). Relying on the resolving ability of TOF instruments (high discrimination of mass differences and collection of a full-scan mass spectrum every 10–100 μs) to provide specificity, simple extraction and separation conditions could be used. Using the postacquisition, pseudo-MRM mode (TOF detectors can be operated only in the full scan mode), the LODs were 0.5 ng/mL for cocaine (m/z 304.2 → m/z 182.2 transition) and 0.05 ng/mL for EME (m/z 200.2 → m/z 182.2 transition). Moreover, despite the inherent limited dynamic range of TOF instruments (typically two orders of magnitude), modifications of the detection system and the use of correction algorithms improved the procedure so that it was linear over a large range (5.0–10,000 ng/mL for cocaine, 0.5–10,000 ng/mL for EME) and showed good intra- and interday precision and accuracy, using trideuterated cocaine as internal standard and three replicate injections of each sample (which tends to reduce the gain in analytic time of this technique).

Besides these methods, using ES-type sources, several groups proposed using APCI sources for the determination of cocaine and/or metabolites. The first combined double SPE, a wide-bore steric-exclusion chromatographic column, and SIM mode on a single-quadrupole instrument for the determination of cocaine, benzoylecgonine, EME, norcocaine, and ecgonine in urine, yielding LODs of 50 to 800 ng/mL (20). More recently, Bogusz et al (23) reported another SPE, single quadrupole, SIM method for the determination of cocaine, benzoylecgonine, and EME (in addition to opiates and LSD) in urine, blood, and serum using deuterated analogs of the compounds as internal standard, that yielded much better LODs of 0.2 ng/mL for benzoylecgonine and EME and 0.5 ng/mL for cocaine. The method was linear in the range 1 to 200 ng/mL but used two different chromatographic conditions, one for cocaine and benzoylecgonine...
and another for EME, confirming the particular chromatographic behavior of this last compound. A method was reported for the determination of benzoylecgonine in dried blood spots collected from newborns, using benzoyllecgonine-d₃ as internal standard, two serial C18 wide-bore analytic columns, and MS/MS in the MRM mode, with an LOD of 2 ng/mL (despite typical sample volumes of 12 μL) and linearity up to 166 ng/mL (38). Finally, Singh et al (39) designed a LC-APCI-MS-MS method for the determination of cocaine, benzoylecgonine, EME, and norcocaine in plasma using simple protein precipitation, gradient elution-reversed-phase chromatography on a wide-bore column, deuterated analogs as internal standard, and acquisition in the MRM mode. This method was linear in the range of 2 to 1,000 ng/mL for all four compounds. From all these studies, it is obvious that cocaine and its main metabolites can be easily determined using LC-MS or better LC-MS/MS, simple sample preparation (in particular, no derivatization required), and either ES or APCI sources. The other two conclusions that can be drawn from these papers are that the sensitivity of the detection of the most polar metabolites of cocaine, such as EME, is related to the proportion of organic solvent used to elute them, thus necessitating adapted chromatographic stationary phases; and that although providing more specificity and selectivity (higher signal-to-noise ratios [S/N]), MS-MS does not necessarily yield lower LODs than single-quadrupole MS detectors.

**Amphetamines and Related Compounds.** At least 10 papers have reported the LC-API-MS analysis of amphetamines and related compounds, some of which were already analyzed in previous review papers (2,5). Amphetamines are volatile but polar compounds that need to be derivatized before GC-MS analysis, which is probably why several teams developed LC-MS techniques. Unlike cocaine and its metabolites, many of these methods used single-quadrupole MS instruments, possibly due to the low molecular weight of these compounds, meaning that the daughter ions generated by LC-MS-MS are in an even lower m/z range, at which high background noise is unavoidable. All these techniques used the positive-ion detection mode. The first published method using an E-type source, although not validated, yielded LODs in the high pg/mg to low ng/mg range for methylenedioxy-methamphetamine (MDMA) and its metabolite methylenedioxyamphetamine (MDA) in rabbit blood and postmortem hepatic tissue, as well as in fly larvae and chitinized insect tissues, using a triple-quadrupole MS operated in the single-quadrupole SIM mode (three ions selected per compound). Sample preparation was by liquid-liquid extraction and MDMA-d₃ was used as internal standard (40). Katagi et al (41) developed a direct-injection, column-switching LC-ES-MS method for the determination of methamphetamine and amphetamine enantiomers in urine, using a β-cyclodextrin phenylcarbamate bonded silica column. Differentiating the enantiomers of amphetamines can be useful because D and L enantiomers have different pharmacologic properties, and in certain countries D-enantiomers are used in legal medications. The LOD was 0.5 to 1 ng/mL and the linearity range was 1 to 5,000 ng/mL for all compounds. The same team proposed an LC-ES-MS method for the determination of dimethylamphetamine (DMA) and its metabolites dimethylamphetamine-N-oxide (DMAO), amphetamine, and methamphetamine in urine (42). This study was initiated because DMA use can sometimes be difficult to differentiate from methamphetamine use in urine if DMA is totally metabolized. With that aim, the detection of DMAO, a thermally labile compound, is of primary importance, justifying the use of LC-MS. Indeed, GC-MS analysis of DMAO requires its prior reduction to DMA followed by a derivatization step, or alternately direct derivatization to trifluorooacetyl-methamphetamine, both pretreatments that render the simultaneous determination of the three compounds impossible. SPE and chromatographic separation were performed using a strong cation-exchanger phase to obtain sufficient retention for the more polar compounds. The quadrupole MS was operated in the positive-ion full-scan mode between m/z 100 and 500 for drug identification (LOD = 50 ng/mL for DMA and DMAO, 100 ng/mL for methamphetamine, 500 ng/mL for amphetamine) and in the SIM mode using the protonated molecular ion of each compound for drug quantitation (LODs 10 times less, respectively). The method was linear from twice the LODs in the SIM mode up to 5,000 ng/mL and was applied to the urine elimination study of DMA, showing that DMAO was detectable up to 3 to 5 days after intake. Kataoka et al (43) also used LC-ES-MS in the SIM mode for the determination of amphetamine, methamphetamine, MDA, MDMA, and MDEA in urine. After high dilution of urine in water and automated in-tube solid-phase microextraction (SPME) using a relatively polar capillary, they obtained LODs of 0.38 to 0.82 ng/mL (seemingly using standard solutions) owing to concentration of the amphetamines in the capillary during several draw/eject cycles, and LOQs of 32 to 79 ng/mL in urine, although the criteria used were unclear. In their previously cited study of FIA-ES-MS-MS analysis of drugs of abuse, Weinmann and Svoboda (19) reported the determination of amphetamine in urine with an S/N ratio of 4.4 at 2 ng/mL, following the m/z 136 → 91.
transitional. Finally, Clauwaert et al (44) reported a LC-ES-Q-TOF method in the pseudo-MRM mode (one parent ion and three extracted daughter ions) for the determination of MDMA in whole blood, serum, and vitreous humor, using MDEA as internal standard and liquid-liquid extraction. The quantitative potential of Q-TOF MS was evaluated by comparison with HPLC with fluorescence detection, showing a very low LOD of 0.25 ng/mL and a three-decade dynamic range (1–1000 ng/mL) for the Q-TOF, superseding that of fluorescence detection. However, the choice of MDEA as internal standard was probably not the best one, because a significant percentage of tablets sold as Ecstasy in Europe contain MDEA in addition to (or instead of) MDMA.

Using an APCI ionization source and single-quadrupole MS detection in the SIM mode (following two or three ions per molecule), Bogusz et al (45) designed a quantitative method for amphetamine, methamphetamine, MDA, MDMA, and MDEA in serum and urine that involved a phenylisothiocyanate derivatization of the compounds, mostly justified by the HPLC-diode-array UV method used as a comparison with LC-APCI-MS. Highly deuterated analogs of amphetamine, methamphetamine, MDEA, and MDMA were used as internal standard and LODs between 1 ng/mL (methamphetamine, MDMA, MDEA) and 5 ng/mL (amphetamine, MDA) were reached. Later, the same team reported an alternative LC-APCI-MS method for the determination of the same molecules using the same internal standard as well as 11 related compounds for which no validation data were provided (46). Moreover, although not clearly stated, it appears that different chromatographic conditions were applied to analyze some of these additional molecules. The main differences with the previous method were SPE instead of LLE and the absence of derivatization, resulting in quasi-molecular ions and fragments of lower masses. However, the LODs reached were similar or slightly better (1–2 ng/mL for the five principal analytes), whereas the linearity range was seemingly limited at its upper end (5–500 ng/mL instead of 1–1000 ng/mL). Ramos et al (47) reported an LC-APCI-MS-MS method for the determination of methylphenidate, an amphetamine-related stimulant drug used in the treatment of attention deficit hyperactivity disorder and narcolepsy. This method used a high-throughput sample preparation procedure based on semi-automated liquid-liquid extraction in a 96-well plate format, a very short chromatographic elution (T_R # 1.6 min) using a wide-bore column and detection in the MRM mode, using one transition. The LOQ was 50 pg/mL and the method was linear and validated in the range of 0.05 to 100 ng/mL.

Despite this apparent diversity of analytic methods for amphetamines, ES ionization sources and, as already mentioned, single-quadrupole MS were used by most teams. Moreover, although the low molecular weights of underivatized amphetamines are not optimal for LC-MS detection, LOQs better than those reported for GC-MS methods (classically, in the range of 10–50 ng/mL) could be attained while using simpler sample preparation procedures. However, the formal identification of the compounds detected is difficult using such types of instruments. In this respect, triple-quadrupole or even better Q-TOF mass spectrometers provide more selectivity and specificity and have proved to be more sensitive than quadrupoles.

Cannabinoids. It was not until 1999 that the first report of an LC-API-MS method for the determination of cannabinoids in biologic fluids appeared (48), to be followed a year later by a second paper (49). In the first, the authors developed a LC-ES-MS method for the urinary determination of 11-Nor-Δ⁹-tetrahydrocannabinol-9-carboxylic acid (THC-COOH), the main urinary metabolite of 11-Nor-Δ⁹-tetrahydrocannabinol (THC), the major psychoactive component of Cannabis sativa. Because THC-COOH is excreted mainly as its glucuronide in urine, the authors used basic hydrolysis of conjugates in urine samples before SPE (48). After gradient elution chromatography, THC-COOH and THC-COOH-d₄ (internal standard) were detected in the positive-ion SIM mode following, respectively, three and two m/z ratios. The LOD was 2 ng/mL for the quantitation ion of THC-COOH, 15 ng/mL when taking into account its qualifying ions and their relative intensity with respect to that of the quantitation ion (accepting ± 20% variability). Considering only the quantifying m/z ratio, the method was linear in the concentration range of 2.5 to 125 ng/mL, much lower than the concentrations found in many positive urine samples. However, such sensitive techniques are required to confirm or disprove the positive results obtained with immunologic techniques involving antibodies that partly cross-react with other cannabinoids or cannabinoid metabolites, in addition to THC-COOH and its glucuronide. The two questions raised by this report were already discussed in Van Bocxlaer et al’s review article (5): the relevance of THC-COOH-glucuronide hydrolysis when this molecule is theoretically directly amenable to LC-ES-MS (as demonstrated later on) and the questionable use of the positive ionization mode when THC-COOH is under anionic form in solution. Answers to the first question were provided both by the authors themselves (48), who advocated a variable conjugation of THC-COOH, and by the above-mentioned review article (5), which underlined...
the need for correct interpretation of the concentration measured with respect to regulatory cutoff levels. As for the second question, this previous review paper regretted that negative ionization was not used, owing to a supposed increase of sensitivity, which in my experience is seldom achieved. Indeed, although the S/N ratio is lower, the chromatographic signal is often of much lower intensity in the negative-ion mode than in the positive mode, because the chromatographic mobile phases are generally acidic. Therefore, it is not uncommon to use the positive-ion mode for those acidic compounds that can be easily protonated. As a confirmation, in the second paper dealing with cannabinoids, both THC-COOH and THC-COOH-glucuronide were analyzed in urine by LC-ES-MS-MS in the positive-ion mode (49). However, because no reference compound was available for the latter, only the former could be quantitated (using THC-COOH-d3 as internal standard), urine samples from cannabis users being used for defining the analytic conditions for the glucuronide. After liquid-liquid extraction, the compounds of interest were separated using gradient elution reversed-phase chromatography on a 2-mm I.D. column and detected in the MRM mode by selection of the protonated molecules in the first quadrupole and of one major fragment in the third quadrupole (and two of the THC-COOH-glucuronides). Indeed, because the major fragment of THC-COOH-glucuronide is the THC-COOH moiety, obtained in the first quadrupole by in-source CID even at low fragmentation voltage, the transition selected for THC-COOH also allowed the confirmation of THC-COOH glucuronide at its respective retention time. The LOD of THC-COOH was estimated to be less than 10 ng/mL, whereas no validated LOQ was reported. Linearity was assessed between 10 and 10,000 ng/mL. Interestingly, the authors compared THC-COOH concentrations in urine samples before and after complete enzymatic hydrolysis (as could be verified by the disappearance of the glucuronide mass signal). From this experiment it was possible to deduce the concentration of the glucuronide and to relate it to the area of its chromatographic peak in the nonhydrolyzed sample, or to its peak area ratio with the internal standard.

Another improvement would be a LC-API-MS method for the determination of THC, 11-hydroxy-THC (the main blood metabolite of THC), THC-COOH, and eventually THC-COOH-glucuronide in blood, with low enough LODs and without a derivatization step, to be competitive with GC-MS.

_LSD, Metabolites, and Epimers._ All the methods using LC-API-MS for the determination of LSD and its metabolites and epimers were previously reviewed (4,5), except one (50). Most of these methods used ES-type sources and single-quadrupole MS and most were limited to LSD in urine, with LODs ranging from 0.3 (51) to 0.5 ng/mL (52–54). Still using LC-ES-MS, my group designed validated methods for the determination of LSD and N-demethyl-LSD (or nor-LSD) in urine with LODs of 0.025 and 0.035 ng/mL and LOQs of 0.05 and 0.10 ng/mL, respectively (55). For the determination of LSD, nor-LSD, iso-LSD, and isonor-LSD in serum and blood, our LODs were 0.02 to 0.05 ng/mL and LOQs 0.05 to 0.10 ng/mL (56). Using LC-ES-MS-MS in the MRM mode, De Kanel et al (57) designed an analytic method for LSD and nor-LSD in urine, blood, serum, and plasma, using two or three transitions per compound and yielding LODs of 25 pg/mL for both compounds. This method was validated in these three matrices for LSD, with an LOQ of 50 pg/mL and a calibration range of 0.05 to 5 ng/mL, with good within-run and between-run precision and accuracy. The method was suitable only for qualitative identification of nor-LSD due to higher between-run variability. Cai and Henion (58,59) designed an LC-ES-MS-MS technique for LSD, nor-LSD, iso-LSD, nor-iso-LSD, and methysergide in liver microsomes and urine, using SPE, with an LOD of 50 pg/mL, as well as a second technique for LSD, nor-LSD, iso-LSD, iso-nor-LSD, de-ethyl-LSD, and nor-allyl-LSD in urine using immunoaffinity extraction, reaching a very impressive LOD of 2.5 pg/mL. However, no validation data were reported in these two papers.

Only three papers have reported analytic methods for LSD and/or metabolites using APCI sources. The first was a study by Henion’s team in 1992 (60) concerning the feasibility of LSD analysis by LC-MS in the scan and SIM modes using an ancestor of APCI interfaces. Using standard solutions of LSD, an LOD of 2 ng/mL was obtained in the scan mode. In their multielement LC-APCI-MS method for drugs of abuse in urine, Bogusz et al (23) obtained an LOD of 0.5 ng/mL and a linearity range of 0.5 to 10 ng/mL for LSD. More recently, LC-APCI-iontrap-MS-MS was used for the determination of nor-LSD and, principally, 2-oxo-3-hydroxy-LSD (O-H-LSD), the main urinary metabolite of LSD, whereas LSD and iso-LSD were determined by GC-MS (50). Although somewhat clumsy (e.g., an m/z 338 → 338 transition was used for O-H-LSD and quantitation was made by single-point calibration), this method was partially validated for O-H-LSD, using 2-oxo-3-hydroxy-LAMPA as internal standard. It showed an LOQ of 0.4 ng/mL and a linearity range up to 200 ng/mL. More importantly, this method was applied to the confirmation analysis of 74 positive urine specimens, showing that the O-H-LSD urine concentration was much higher than that of the other
compounds, with a mean O-H-LSD/LSD concentration ratio of 42.9 (range 1–778), emphasizing the importance of this metabolite for the detection window of LSD abuse.

From this review, it is clear that LC-MS is the method of choice for LSD analysis, eliciting the simultaneous determination of different metabolites and epimers in biofluids with high sensitivity and specificity, using either ES or APCI, MS or MS-MS. As already emphasized, tandem MS does not seem to yield lower LODs than single-quadrupole MS, but it is, nevertheless, an improvement in terms of specificity and S/N ratio. However, here again a complete and fully validated technique able to determine all of these compounds (or at least all those of clinical interest) in one run is still needed.

Summary. If virtually all drugs of abuse can be sensitively and specifically determined using LC-API-MS techniques, the status of gold standard in this field is still the prerogative of GC-MS, due to wide acceptance, identity of the analytic conditions required for the different families (e.g., no change of chromatographic column or mobile phase required), and reliability and affordability of instruments.

Quantitative Methods for Other Drugs and Toxic Compounds

A large volume of literature has been published on the determination of therapeutic drugs in biologic fluids (mainly by the pharmaceutical industry but also in the field of therapeutic drug monitoring) (6), as well as for the detection and quantitation of pesticides and industrial or environmental pollutants, all of which are of clinical or forensic relevance. It is beyond the scope of a single paper to review all these studies, and I will limit the task to discussing some particularly interesting classes of drugs.

Central Nervous System Drugs. Several LC-MS techniques have been reported for benzodiazepines with excellent sensitivity, and have been the subject of previous review articles (2,4,5), but few of these involved an API interface. Using LC-APCI-MS, LODs of 2 ng/mL were obtained for midazolam in pig plasma (61), whereas using LC-ES-MS, LODs of 0.2 ng/mL for midazolam and 0.5 ng/mL for its metabolite 1-hydroxymidazolam could be reached in human serum, with LOQs of 0.5 ng/mL for both (validated method) (62). LC-APCI-MS was used for the determination of triazolam in plasma and urine with an LOD of 0.02 ng/mL (63), and of oxazepam glucuronide in urine (LOD = 50 ng/mL) (64). Bogusz et al (65) described the determination of flunitrazepam and its metabolites 7-aminoflunitrazepam (7-AF), N-desmethylflunitrazepam (N-DT), and 3-hydroxyflunitrazepam (3-HF) in urine, serum, and blood using macrobore HPLC coupled to either ES-MS or APCI-MS (65). The comparison of ES and APCI in the positive-ion SIM mode, following a single ion per compound, showed that APCI was 7 times more sensitive for flunitrazepam, about 20 times more for N-DT, and 40 times more for 3-OHF compared with ES, but that there was no difference for 7-AF. The LODs were 0.2 ng/mL for flunitrazepam and 7-AF and 1 ng/mL for N-DT and 3-OHF, and the method was linear over a range of 1 to 500 ng/mL with the APCI source. This method is highly sensitive, but the use of a single ion for the identification of each compound limits its specificity. Despite the limited number of papers, LC-API-MS appears to be at least as sensitive as, but easier to handle than, GC-NICI-MS for the determination of benzodiazepines.

Four papers have reported analytic techniques for antidepressants in plasma. The first concerned the analysis of nortriptyline and its metabolite 10-hydroxynortriptyline in plasma using LC-APCI-MS-MS. LODs of 0.2 ng/mL were achieved, and the assay was linear in the range of 0.8 to 32 ng/mL (66). The second reported the determination of nefazodone and its metabolites in plasma by LC-ES-MS, achieving LODs of 4 and 2 ng/mL, respectively (67). Third, doxepin and desmethyldoxepin were determined in plasma by LC-ES-MS-MS, with respective LOQs of 0.320 and 0.178 ng/mL (68). Finally, Zhang et al (69) reported a LC-ES-TOF technique for doxepin, desipramine, imipramine, amitriptyline, and trimipramine, using imipramine-d3 as internal standard, that showed, in the extracted-ion mode, LOQs of 2 ng/mL for desipramine and 1 ng/mL for the other drugs. The method was linear in rather restricted concentration ranges (respectively, 2–100 and 1–50 ng/mL).

Several papers have reported LC-API-MS techniques for the determination of commercialized antipsychotic (or neuroleptic) drugs, whereas others have reported analytic techniques for candidate drugs under evaluation (e.g., sertindole, pramipexole, iloperidone). The butyrophenone haloperidol and its reduced metabolite were determined in serum by LC-ES-MS with LODs of 0.075 and 0.1 ng/mL and LOQs of 0.1 and 0.25 ng/mL, respectively (70). The thienobenzodiazepine olanzapine was determined in human plasma and serum using LC-APCI-MS-MS in the positive-ion MRM mode, with an LOQ of 0.25 ng/mL and linearity up to 50 ng/mL (71). A LC-ES-MS-MS technique was designed for the pharmacokinetic study and therapeutic drug monitoring of risperidone, a benzisoxazole derivative. Risperidone and its
metabolite 9-hydroxy risperidone were determined in plasma with an LOQ of 0.1 ng/mL and linearity up to 100 ng/mL (72). The phenothiazine trimeprazine and its main metabolites were determined in rat serum using LC-ES-MS with LODs of 0.4 ng/mL, and linearity was verified in the range 0.5 to 40 ng/mL for all compounds (73). In a recent paper, McClean et al (74) described an LC-ES-iontrap-MS-MS method for chlorpromazine, tri-fluoperazine, flupenthixol, and risperidone in a hair sample from a patient under clinical treatment for schizophrenia. Calibration and validation data were obtained from blank hair samples spiked with standard solutions of the drugs of interest, showing LODs in the range of 10^{-9} to 10^{-8} mol/L (i.e., roughly 0.3–3 ng/mL) and linearity in the range 5 \times 10^{-7} and 1 \times 10^{-5} mol/L for all compounds except risperidone, for which the range was 4.9 \times 10^{-7} to 9.6 \times 10^{-6} mol/L (mol/L is a rather surprising unit for a solid matrix such as hair). Finally, Kumazawa et al (75) reported a method for the determination of 11 phenothiazine antipsychotics in human whole blood and urine using LC-ES-MS-MS. The authors compared the single-quadrupole SIM and triple-quadrupole MRM modes, showing much lower LODs with the latter, due to much better S/N ratios (and not, as misinterpreted by the authors, higher sensitivity, because signal intensity was actually decreased). Using SPME and detection in the MRM mode, the LODs obtained were between 0.2 (flupentixol) and 200 ng/mL (clozapine) in whole blood and between 4 (thioridazine) and 22 pg/mL (proporciazine, clozapine) in urine; the method was linear and validated over wide concentration ranges.

Again, owing to the high sensitivity and specificity of the methods published, it is probable that LC-API-MS is going to be a technique of choice for antipsychotic drugs in toxicology.

**Cardiac Drugs.** At least two papers reported the determination of cardiac glycosides in human plasma or serum, both using LC-ES-MS and both applied to clinical and forensic toxicology. The first concerned digoxin, digitoxin, lanatoside C, and acetyldigoxin with LODs between 0.15 ng/mL (acetyldigoxin) and 0.60 ng/mL (lanatoside C) and linearity within the range 1 to 100 ng/mL (76). The second paper concerned 17 cardiac glycosides and metabolites and showed LODs between 1 ng/mL (deslanoside, digoxin, methyldigoxin) and 10 ng/mL (acetyldigoxin, gitaloxin) and linearity up to 100 ng/mL for all compounds (77). For the first time, these techniques allowed the MS confirmation of intoxication with cardiac glycosides, whereas it was previously possible to confirm the results obtained only using immunologic techniques, mainly radioimmunoassay.

Only one recent paper concerned the LC-API-MS analysis of beta-blocking drugs, but only in pharmaceutical preparations (78). However, the concentration range studied using pure solutions (100–300 ng/mL) suggests that such a technique could be applied to biologic fluids with sufficient sensitivity, provided a convenient extraction/concentration procedure was developed.

Several reports concerned the determination of calcium antagonists by LC-API-MS, mainly applied to pharmacokinetic studies. LOD and LOQ were respectively 0.25 and 0.5 ng/mL for both nifedipine enantiomers by LC-ES-MS/MS (79) and 0.08 and 0.24 ng/mL for nifedipine and dehydronifedipine by LC-APCI-MS/MS (80). An LOQ of 0.03 ng/mL was achieved for barnidipine using LC-ES-MS/MS (81). Obviously, although there are still no published techniques allowing the simultaneous detection and quantitation of all, or most, of the calcium antagonists, these data suggest that LC-API-MS could be a convenient analytic technique for these drugs too.

**Doping Agents.** The detection and identification of endogenous and exogenous anabolic steroids and their metabolites are of primary importance in sports doping control. The sulfate and glucuronide conjugates of testosterone and epitestosterone were determined in human urine using LC-ES-MS in the MRM mode. LODs “in the low nanomolar range” were achieved for these steroid conjugates, except that of testosterone sulfate, in urine from a normal man. This method was claimed to be convenient for the direct and precise determination of the testosterone/epitestosterone metabolite ratio in doping control, as an alternative to the classic testosterone/epitestosterone ratio obtained by GC-MS after enzymatic hydrolysis of glucuroconjugated metabolites (82). Even better, an LOD of 25 pg for epitestosterone glucuronide could be obtained using a 300 μm I.D. packed capillary HPLC column and similar MS conditions (83). Recently, Kuaranne et al (84) reported a study of the best ionization conditions of eight anabolic steroid glucuronides (of testosterone, epitestosterone, nandrolone, androsterone, and four new compounds synthesized in their laboratory), using ES or APCI in the positive or negative mode and triple-quadrupole MS. Using direct injections of standard compounds into the source, they concluded that the ES source and the positive-ion mode were the most promising for further development of LC-MS methods for anabolic steroid compounds (which confirms that the positive-ion mode is often more sensitive than the negative ion mode, even for acidic compounds).

Beta-agonists are increasingly used by athletes, presumably because of their bronchodilator as well as the
anabotic properties of some of them. Several papers, most of which have already been reviewed (2,85), reported analytic techniques for β₂-agonists, mostly applied to matrices from animals but easily applicable to samples from humans. Their LODs were 2.5 ng/mL for clenbuterol, fenoterol, metaproterenol, salbutamol, and terbutaline in plasma using LC-APCI-MS or (for salbutamol) -MS-MS (86); 0.05 ng/mL for cimaterol, clenbuterol, mabuterol, salbutamol, and terbutaline in cattle urine using LC-APCI-MS-MS (87); 0.05 ng/mL for clenbuterol, mabuterol, mapenterol, methylcnenbuterol, and toluoberol in cattle urine using LC-APCI-MS-MS (88); and 0.1 μg/kg for clenbuterol in cattle liver using LC-ES-iontrap-MS-MS (89). Hence, the determination of this class of drugs in various biologic fluids or tissues at the low or sub-ppb level is straightforward, with seemingly a preference for APCI interfaces. Pesticides. A historic paper compared APCI, ES, thermospray, and particle beam interfaces/sources for the analysis of carbamate pesticides in pepper, showing that APCI gave the best results, followed by ES and thermospray, whereas particle beam was not sufficiently sensitive (90). Another LC-APCI-MS method was developed for eight carbamates in serum, yielding LODs between 12 and 60 ng/mL in the SIM mode (91). The same team conducted a preliminary study of the LC-APCI-MS detection of 21 pesticides in the positive and negative ion mode, showing that some were detected in one mode whereas others were detected in both. These optimized MS conditions were applied to the determination of propoxur in a single spiked serum sample (92). Another team reported the LC-APCI-MS-MS determination of the chloroacetanilides alachlor mercapturate and metolachlor mercapturate in human urine. They obtained LOQs of 1 and 10 ng/mL, respectively, in the positive-ion MRM mode (93,94). A column-switching LC-LC-ES-MS-MS method was reported for the determination of chlorpyrifos and its main metabolite in human urine and serum. Chlorpyrifos was detected in the positive-ion mode and its metabolite in the negative ion mode, with similar LODs of 1.5 ng/mL in serum and 0.5 ng/mL in urine. The method was validated up to 100 ng/mL (95). Turcant et al (96) reported a nonfatal case of self-poisoning with metobromuron, a urea derivative, in which HPLC-DAD was used for the study of metobromuron toxicokinetics, and LC-ES-MS and LC-ES-MS-MS were used to elucidate the structure of four metobromuron metabolites in plasma and urine. Finally, in parallel with a GC-MS method for the determination of 47 organophosphate, organochloride, and other pesticides, Lacassie et al (97) reported an LC-ES-MS multiresidue technique for the determination of 11 carbamate and 3 benzimidazole pesticides in human urine, plasma, serum, and whole blood, with LODs in the SIM mode in the range of 2.5 to 50 ng/mL in serum, depending on the molecules. Clinical cases of intoxication with carbofuran and aldicarb were described in which the pesticides could be readily identified and quantitated.

However, much work remains to be done in the field of pesticide analysis by LC-MS in human biologic fluids or tissues, whether for forensic, occupational, or environmental toxicologic applications. In particular, owing to the very large polarity range of pesticides, the respective roles of GC-MS and LC-MS are still to be defined.

Summary. The wide range of chemical and pharmacologic or toxic properties, as well as circumstances of use, of the molecules that can be readily analyzed by LC-APCI-MS suggest it is a technique with wide applicability. Therapeutic drugs and, above all, the most recently commercialized ones were most often analyzed by LC-MS, which is not always the case for antidoping agents or pesticides. The capital cost of the instrument probably contributes to this situation.

“General Unknown” Screening Methods

The a priori identification of unknown xenobiotics in biologic fluids generally involves a panel of automated immunoassays for the most common drugs, and of chromatographic techniques, ideally coupled to specific detectors (MS or UV-DAD). Nevertheless, failures are not uncommon, particularly when polar or thermally labile compounds with no or little UV absorbency are involved. Moreover, MS is more specific and reliable than DAD and should always be preferred. In recent decades, GC-MS screening procedures have been universally applied for general unknown screening, owing to their specificity and sensitivity and to the availability of very large libraries of standardized spectra for chemicals of any kind; some of these libraries specialize in drugs, toxic compounds, and metabolites (98). However, because GC is limited to volatile and thermally stable compounds, and because some polar compounds need specific derivatization procedures not compatible with a general unknown approach, the coupling of MS with HPLC has long been considered a potential means of increasing the range of compounds amenable to MS.

Because electron ionization (EI) produces universally reproducible mass spectra, this ionization mode is generally regarded as the gold standard for the specificity of MS detection. In the past, only moving belt and particle beam interfaces were compatible with EI sources (4), but both suffered from an unavoidable volatilization step by heating and thus were not suited to polar or thermally
labile compounds. Nevertheless, one group recently proposed an application combining a double liquid-liquid extraction (for acidic and neutral compounds on the one hand and for alkaline compounds on the other) and a gradient chromatographic elution on a C18 column (chromatographic time 70 minutes), the first detection step using a DAD, followed by a second using MS (99). The authors used a particle beam interface and electron ionization. Mass spectra were acquired in the range of 50 to 400 u, then three different libraries were searched: a customized in-house library, the Pfleger-Maurer-Weber library, and the Wiley 138K library.

With this procedure, the researchers analyzed about 150 compounds, mainly drugs, with a LOD varying from 20 to more than 1,000 ng/mL in whole blood, corresponding to extraction recovery values between a few percent to 99%. Interestingly, many compounds with the highest LODs were polar compounds, such as morphine, benzoylcegonine, or ibuprofen, although it was attributed to low extraction recovery for the first two, amphoteric compounds. However, the main drawback of particle beam interfaces is that they are limited to rather nonpolar compounds that can be desolvated and transferred in the gas phase to the mass spectrometer. Moreover, virtually no manufacturer still commercializes particle beam interfaces. This procedure has been used to solve forensic cases with multidrug intoxication.

Though ES and APCI have superseded all the other types of interfaces/ionization sources for LC-MS, they are not compatible with EI. On the contrary, they involve a soft ionization process. This limitation can be bypassed by using CID, which provides thorough fragmentation of the compounds. CID consists of accelerating the ions generated and making them collide with molecules of a neutral gas, either in a specialized collision cell or in the intermediate-pressure part of the MS, between the atmospheric pressure source and the high vacuum of the mass analyzer (in-source CID). The first solution, necessitating ion-trap or tandem MS of any sort, supposes that a limited number of parent ions are selected in the first MS stage and submitted to fragmentation in the collision cell and that the resulting fragments are analyzed in the second MS stage. It can be used easily, with or without chromatographic separation, to confirm the identity of suspected compounds as long as fragmentation energy is standardized (in terms of nature and pressure of collision gas, and ion kinetic energy) and libraries of mass spectra of compounds of interest are built for each different type of MS. Weinmann et al (100) developed an MS-MS library of more than 500 therapeutic or illicit drugs using LC-ES-QqQ (API 365, Sciex) in the product-ion scan mode. Four different positive product-ion spectra were recorded in the library for each compound, corresponding to four different collision energies (101). The library searching algorithm used was that included in the Multiview 1.3 software (Sciex). The authors showed that these product-ion spectra were also helpful for identifying unknown metabolites of known drugs because many of them share common fragments with the parent drug (102). Using the same type of instrument equipped with a Turbo-IonSpray™ source, Gergov et al (103) built a library of MS-MS spectra from about 400 therapeutic and illegal drugs. The spectra were generally obtained using a collision energy of 35 eV, except that additional spectra were acquired at 20 or 50 eV for compounds giving no informative spectrum at 35 eV. For the detection of the compounds of interest, a first injection in the single-quadrupole SIM mode was performed and, if any of the [M+H]+ ions of these compounds gave a chromatographic peak at their expected retention time, an automatic procedure created new experimental conditions for the next injection of the extract in which these ions were selected as parent ions for product-ion scanning and library searching. However, it seems a rather complicated process when direct product-ion scanning of the 17 β-blocking drugs studied could probably have been applied, eventually using different time windows to limit the number of parent ions selected at any time. Moreover, in no way could this be described as general unknown screening, because only a limited number of selected compounds were screened for. Baumann et al (104) built an MS-MS library of 517 spectra using an ion-trap MS (LCQ, Thermoquest (Thermo Finnegan; San Jose, CA)) and either an ES or an APCI source operated in the positive-ion mode. Helium was used as collision gas in the trap. To obtain rich product ion spectra, wide-band excitation was used (i.e., resonance excitation at 20 u below the parent ion selected) to further dissociate the [M+H-H2O]+ ions generally produced by the rather soft fragmentation process involved in ion traps. Moreover, as fragmentation energy decreases linearly when mass increases, a mass-dependent correction was automatically applied to the collision energy (“normalized collision energy”). Owing to both these improvements, sufficiently specific MS-MS spectra of different drugs, as well as endogenous compounds, could be recorded. Library searching was possible owing to the NIST algorithm implemented in the Xcalibur data system (Thermo Finnegan) used. The examples given include the perfect distinction between the diastereoisomeric corticosteroids dexamethasone and betamethasone, injected as pure solutions; the identification of morphine-glucuronides in a urine sample of a drug addict, owing to a rather complicated MS(3) process involving the successive selection...
of the parent ion of the glucuronides, then of morphine, then the full-scan monitoring of the fragments further generated from the morphine moiety (m/z 462 → m/z 286 → full scan). Although theoretically interesting, these applications are obviously of little use in forensic or clinical toxicology, where simple multiple-ion monitoring (MRM) experiments would be sufficient to identify the suspected compounds (using relative retention time and one or two specific ion transitions) and to quantify them, provided calibration samples are prepared in parallel.

To be able to use MS-MS spectra library searching for general unknown screening, it is necessary to use an automatic process, called data-dependent acquisition or information-dependent acquisition, to select the parent ions of interest, totally unexpected by definition, and to dissociate them and monitor their fragments. This approach was used by Decaestecker et al (105), who used a Q-TOF instrument equipped with a Z-spray source, operated in the positive ion mode using a single set of voltage conditions. During the chromatographic run, the quadrupole initially transmitted all masses in the range of 50 to 450 u to the TOF detector (pass-band mode) until one or more ions reached a predefined threshold. Instantly, the quadrupole selectively transmitted these high-intensity ions (maximum of four ions) to the collision cell, and the resulting fragments were analyzed by the TOF detector. Then, the instrument switched back to the initial conditions after 4 seconds, except that a 2-minute refractory period was applied to the last selected ions. Seventeen common drugs were used to optimize the whole procedure, in particular fragmentation energy. This procedure was applied to the analysis of urine samples from toxicology cases, in which all the compounds previously detected by enzyme-multiplied immunoassay technique (EMIT) and HPLC-DAD were also identified by LC-MS. However, no details were given about the mass spectra library used (or the conditions in which such a library was built), nor about the library searching facilities available. Moreover, the very small number of compounds studied makes this report only preliminary. Data-dependent acquisition was also used by Fitzgerald et al (106), who used a particular instrumental setting, coupling the sample preparation/chromatographic separation parts of the REMEDI HS instrument from Biorad Diagnostic (Bio-Rad Laboratories; Hercules, CA) to a Finnigan (Thermo Finnegan) ion-trap mass spectrometer via an ES source. A postcolumn splitting system was used, directing 5% of the mobile phase to the MS and 95% to waste. After direct injection of urine samples in the column switching system, the purified and separated analytes were ionized using a single set of voltage conditions and analyzed in the full-scan mode between 50 and 500 u. When an ion exceeded a preset threshold, this ion was selected and fragmented by CID in the ion trap, and the resulting fragments were recorded in the product-ion scan mode. Finally, the MS reverted back to the full-scan mode. This procedure was tested on urine samples spiked with only 17 drugs again, resulting in only a very preliminary study, inasmuch as no precise CID conditions were provided, nor any fit values of CID spectra obtained from these spiked samples and from pure standards.

The major advantage of such techniques is their high specificity and selectivity, as the spectra recorded come from a single parent ion. However, their main drawback is that the setting of a given intensity threshold is hardly compatible with the intense and, above all, highly variable background noise produced by extracts of real samples (in particular forensic samples), or by gradient chromatographic elution, except if this threshold is given a high value (which would result in very poor sensitivity).

Finally, both these papers reported quantitative data for test compounds, the principle of which is questionable because the reliability of quantitation depends on extraction recovery, which is low for many compounds when using a standard extraction procedure. In my opinion, general unknown screening procedures should never be used as a quantitation tool, whatever the analytic technique used, except maybe in an emergency when no other method is available, and provided that the quantitative results are delivered with caution. Here again, if one is equipped with tandem MS, quantitation should better be performed in the MRM mode, using a dedicated method.

An alternative to the use of MS-MS spectra for general unknown screening is single MS spectra, provided enough fragment ions can be produced. Although ES and APCI generate very few fragments, fragmentation can be obtained by in-source CID, whose principle is similar to that used in collision cells. The molecular or pseudomolecular ions produced by the ionization process are accelerated through an ambient of neutral gas with the molecules of which they will collide. In-source CID involves an acceleration potential, the name of which varies depending on the manufacturer (orifice voltage for Sciex (Applied Biosystems/MDS-Sciex), cone voltage for Waters-Micromass (Waters; Milford, MA), octapole offset voltage for Finnigan, and fragmentor voltage” for Agilent Technologies). Nitrogen and residual vapors from the mobile phase are as the ambient gas, in a zone corresponding roughly to the transition between the atmospheric to intermediate-pressure parts of the
instrument. The fragments produced by in-source CID are generally the same as those produced by conventional CID in the collision cell of an MS-MS instrument, but not necessarily with the same intensity. As shown by most papers analyzed in the previous section, in-source CID is routinely used to generate confirmation ions for quantitative methods using single-quadrupole instruments in the selected ion monitoring mode. My group reported the first application of in-source CID for general unknown screening using an ionspray interface (107). Acquisition was performed in the full-scan mode, from 100 to 1,100 u, with a step of 0.2 u. In-source CID was performed at four, continuously alternated orifice voltages, both in the positive- and negative-ion modes: positive-ion mode with low CID energy (20 eV); positive-ion mode with high CID energy (80 eV); negative-ion mode with low CID energy (−20 eV); and negative-ion mode with high CID energy (−80 eV). The resulting recordings were automatically separated into four different chromatograms with respect to polarity and orifice voltage value. To obtain both fragment-ions and the protonated molecule (in the positive mode) or molecular ion (in the negative mode) for the majority of the compounds tested, weakly and highly fragmented spectra of the same polarity were added within each acquisition time. The result was a pair of full mass reconstructed spectra used as library entries, a positive and a negative one, obtained by adding spectra at +20 and +80 V on one hand and spectra at −20 and −80 V on the other. The total turnover time of 5.8 seconds for looping these four acquisition conditions still corresponded to a convenient time resolution with respect to HPLC peak width, even with the narrow-bore column used. Indeed, reversed-phase chromatographic separation of extracted chemicals was performed on a Nucleosil C18, 5 mmol/L, pH 3 ammonium formate as mobile phase (flow rate: 40 μL/min). A narrow-bore column and a rather long chromatographic separation with gradient elution were chosen because unlike MS-MS, where a precursor ion is selected before fragmentation, in-source CID needs to be preceded by an efficient chromatographic separation procedure for good selectivity (no interference), good sensitivity (no ion suppression), and reproducible fragmentation (fragmentation efficiency being dependent on the ion density in the transition zone (9)).

We showed that in-source CID fragmentation was dependent on the distance the accelerated ions have to cross before entering the high-vacuum region (108). This is partly dependent on the geometry of the instrument (ionization source as well as transition zone between pumping stages), but it can be adjusted by means of the distance between the ionization needle and the MS entrance orifice, which is variable in certain types of instrument (109). In our experiments, this distance was finely tuned between about 5.8 and 6.2 mm to obtain a standard fragmentation pattern for glafenine (test compound). We and others (109,110) showed that such a tuning procedure ensured reproducible in-source CID spectra, whether using a single or different instruments, even with different configurations (e.g., ionization sources, spray mode, pumping stages).

The reconstructed CID-MS spectra thus obtained compared favorably with LC-MS-MS spectra in terms of number and intensity of ions in the spectrum, due to molecular or pseudomolecular ions of higher intensity and sometimes to the presence of high-mass adducts (109). They were even as rich as EI spectra. However, when using ES ionization in the positive mode, pseudomolecular ions and fragments are often protonated, or even form adducts with sodium or potassium, whereas when using EI, molecular ions and fragments are obtained rather in the M+ form. The mixture of soft and harder fragmentation conditions used in the present procedure generally enables the simultaneous detection of molecular or pseudomolecular ions as well as fragments, which is not always the case with EI (because of excessively strong fragmentation conditions) or with MS-MS (because no single fragmentation energy would provide both molecular or pseudomolecular ions and fragments for most molecules). Moreover, the use of both positive and negative ionization in the same run provided significantly more information than either of them alone for those compounds giving both positive and negative ions, and allowed the detection of compounds giving only negative ions. Less than half the molecules were ionized in the negative mode, but most acidic compounds, hardly amenable to GC-MS without convenient derivatization, were detected using this polarity.

Under these chromatographic and mass spectral conditions, one library containing about 1,100 reconstructed mass spectra was built in the positive ionization mode, and another library containing about 500 reconstructed spectra was built in the negative ionization mode. These libraries include spectra from about 1,300 therapeutic drugs, drugs of abuse, pesticides, plants, and industrial and domestic toxicants; they are updated regularly.

In addition, software was developed in cooperation with the manufacturer to automatically reconstruct such spectra and compare each pair of positive and negative spectra, together with their retention time (in optional, standardized chromatographic conditions) to those in the libraries (Fig. 3) (107).
The intra- and interassay variability of reconstructed mass spectra was studied for nine compounds. Intra-assay precision coefficients of variation (n = 6) of the relative intensity of a significant ion with respect to the most intense ion in the spectrum was less than 25% for all nine compounds, whether in the positive or the negative mode, and was less than 10% for most of them. Interassay precision coefficients of variation over 6 consecutive days were between 5.8% and 22.0% in the positive-ion mode and between 11.9% and 33.3% in the negative mode, and were generally high for the low ion ratios. This variability, including that of the chromatographic background noise, was found acceptable for correct identification of compounds, inasmuch as a low weight was attributed to this intensity ratio in the library search procedure. Moreover, visual inspection of the spectra recorded showed a satisfactory reproducibility of the spectrum pattern, including m/z ratios of low intensity. Even Bogusz et al (111), who reported a poor interlaboratory reproducibility of in-source CID mass spectra with a different type of instrument and ES and APCI sources (no standardization of fragmentation using a test compound was applied), found a satisfactory short-term and long-term intra-laboratory reproducibility when using ES sources. These authors (111) as well as others (110,112) confirmed that the mobile-phase composition in terms of ionic strength, pH, or organic solvent content had little or no influence on fragmentation. The optimal formate concentration for the intensity of the MS signal was found to be 2 (109) to 2.5 mmol/L (112). Further, it was verified that the reconstructed mass spectra were not dependent on concentration in a wide range (e.g., 10 ng/mL to 10 μg/mL (108)), except when saturation of the channel electron multiplier occurred (110). Finally, the last problem to solve with this procedure was to detect even small signals against a high background noise. After testing different solutions, such as background subtraction, examination of reconstructed mass chromatograms in small pass-bands (m/z ranges), contour mapping (or “eagle’s view”) of the three-dimensional traces (time, m/z, and intensity), it was found that the best results by far were obtained using an algorithm present in the standard software provided with the instrument, the so-called enhance procedure. This signal processing algorithm is similar to that developed by Visentini et al (113) and called TICfilt. It eliminates the contributions of solvent and buffer ions to the acquired mass spectra, recognizes and removes noise spikes caused by experimental variations, and extracts weak eluent peaks containing significant ions from the overall total ion chromatogram (TIC) trace, based on the premise that the occurrence of background ions is more frequent than that of ions due to analytes. An example of the efficiency of this algorithm is presented in Figure 4, in which the “enhanced” mass chromatogram is superimposed over the raw mass chromatogram. Chromatographic peaks undetectable on the latter trace can be detected easily (and even resolved, for overlapping compounds) using the former trace.

In a preliminary experiment, the whole procedure described above was compared with GC-MS and HPLC-DAD for the analysis of clinical serum samples after nonselective SPE. It was found that LC-ES-CID-MS was able to detect approximately 44% of all the compounds detected and that 20% of all compounds were detected only by this procedure, either because their concentration was too low or because they were not detectable by GC-MS or HPLC-DAD under the conditions used (108). The inconveniences of this are summarized as follows:

- A rather long chromatographic time (50 min), giving a total turnover time of about 1 h 30 min for one
compound (this may be even longer than GC-MS after short microwave-assisted derivatization, a procedure still not widespread in toxicology laboratories)

- Relatively high LODs (100 ng/mL in the best cases at this stage) not convenient for drugs of abuse, some benzodiazepines, or antipsychotic drugs (108).

However, polar drugs often present higher therapeutic and/or toxic thresholds than nonpolar drugs due to their low capacity to cross cell membranes. For example, during our preliminary experiments for determining the limits of detection of about 150 drugs and toxic compounds as test molecules, this procedure allowed the detection of subtoxic levels of oral anticoagulants (acenocoumarol), rodenticides (crimidine, chloralose), or antibiotics (e.g., ciprofloxacin, cephalosporins, aztreonam) that were not accessible to GC-MS. For a comprehensive overview of the efficiency of this procedure, extraction recoveries and LODs should be determined for a wider range of compounds of different lipid solubility, polarity, pKa, molecular mass, and so forth, and mainly for polar compounds, toxic at relatively low concentrations, because they are not accessible to GC-MS. Moreover, systematic comparison of LC-MS, GC-MS, and HPLC-DAD general unknown screening procedures should be conducted on a larger number of clinical samples. However, this is probably too much work for a single laboratory, in addition to the building of large libraries of mass spectra. Collaborative studies are now necessary, provided it is first verified that this procedure is robust enough to be transferred to other laboratories, equipped with other types of instruments.

Using a triple-quadrupole instrument from the same manufacturer, Gergov et al (103) also built a library of reconstructed CID-MS spectra obtained by the addition of spectra recorded respectively at +25 and +90 V, in addition to a library of single CID-MS spectra obtained at +40 V orifice voltage. Each contained mass spectra from about 400 therapeutic or illicit drugs. The reconstructed mass spectra were claimed to maximize the information content of the library entry. The reproducibility coefficient of variation of the reconstructed spectra of 17 β-blocking drugs over a 1-week period was found to be 0.9%. No other details were given with respect to the use of these procedures and libraries, except that the library search results for spiked urine samples were better using the previously mentioned MS-MS library than this reconstructed spectra CID-MS library. However, apparently no attempt was made to standardize the fragmentation energy or to extract the significant signal from noise.

Other teams used three different fragmentation voltages, in the positive-ion mode only for all of them (102, 110,114,115) but one (112). Moreover, as no final spectra were reconstructed by addition of the different spectra generated for a given compound, different libraries corresponding to the different fragmentation conditions had to be built and searched, sometimes leading to confusing results. In particular, Weinmann et al (102) used fragmentation energies (OR voltages) of +20, +50, and +80 V in the in-source CID mode and built a mass spectra library for about 400 compounds, including these three spectra per compound. In my experience, the spectra obtained at +50 V added no further useful information to that already brought by the pair of spectra obtained at +20 and +80 V. The rest of their experimental conditions were very similar to those of my own group, except that they used a chromatographic column of larger ID

**FIG. 4.** Influence of signal processing ("enhance" procedure) on the total ion chromatogram (TIC) of an extract of spiked serum.
ticides gave negative or positive mass spectra, or both. Mainly detected in the negative-ion mode, whereas pesticides, probably owing to heating. Explosives were mass spectra than ES because it induces more fragmentation energy, because the bond between the glucuronide moiety and lorazepam was broken when the highest fragmentation energy was applied (102). This is a good illustration of the potential of LC-MS general unknown screening procedures to directly identify conjugated metabolites of drugs, opening a new detection window (i.e., for almost totally metabolized compounds). However, the whole procedure can hardly be called general unknown screening because the authors applied different and sometimes very selective extraction procedures, as well as different internal standards depending on the drugs analyzed (102), and even selective ion extraction to locate the compounds in the background noise of the chromatogram (110,114). When interfering compounds or background noise impeded the identification of the suspected compounds, they used MS-MS fragmentation of selected ions and MS-MS library searching (102,114). Although the combination of in-source CID-MS and MS-MS library searching is obviously a valuable tool, it is a rather time-consuming and cumbersome procedure for it to be used routinely as a complement to GC-MS and HPLC-DAD in forensic toxicology, as claimed by the authors (102). Finally, they admitted that for acidic compounds, analysis should be conducted in the negative-ion mode, but they did not actually apply it (110).

Schreiber et al (112) used similar conditions to build spectral libraries for 42 pesticides and 48 explosive residues, to be applied to the analysis of environmental samples. They used a Sciex API 100 LC-MS system, ES as well as APCI sources, in-source CID in the positive- and negative-ion modes, and three fragmentation (OR) voltages in each mode: ± 10, ± 50, and ± 100 V. The mass spectral conditions were optimized and the library entries obtained by flow injection (i.e., no chromatographic separation) of pure solutions of the compounds of interest. They found that APCI gave slightly different mass spectra than ES because it induces more fragmentation, probably owing to heating. Explosives were mainly detected in the negative-ion mode, whereas pesticides gave negative or positive mass spectra, or both. The intensity of MS signals was dependent on the number and position of functional groups, and only a few substances were not detectable in either mode. Finally, the authors pointed out the difficulty in differentiating isomeric compounds by their respective mass spectra, emphasizing the need for their chromatographic separation. Although not applied to human samples, this procedure confirms the reproducibility of mass spectra and the potential efficiency of LC-ES-CID-MS as a general unknown screening technique, provided all the chain links are optimized and standardized, beginning with chromatography. However, in addition to the lack of any standardization of the fragmentation energy, some of the previously mentioned limitations appear in this work, compared with the first method reported (107). These include the complexity of handling several mass spectra for each compound, when each spectrum of an unknown has to be compared with the library entries acquired with the same respective fragmentation energy, and the difficulty of detecting chromatographic peaks against the background noise.

Finally, Hough et al (115) reported a preliminary study that concerned an in-source CID-MS library of 22 benzodiazepines and 16 sulfonylurea herbicides for which they applied a tuning of the instrument using a reference compound to define what they called “performance-based conditions.” However, the relative intensity criteria presented seemed somewhat imprecise (e.g., intensity of m/z 290 >80% and intensity of m/z 260 <20% with respect to the reference ion m/z 586). Moreover, the authors actually found a range of fragmentation voltages in which the tuning criteria for each level were acceptable and chose the median of this range as a standard setting for each energy level, a procedure they had previously reproached others for using. They argued that their “performance-based” optimization was the solution to transferring the technique from one instrument to another of a different type, but the tuning compound they used was only identified as “the Hewlett-Packard ESI Tune Mixture.” Benzodiazepines were analyzed using flow-injection-ES-MS and sulfonylurea using LC-ES-MS, but no chromatogram was shown. Mass spectra were acquired in the m/z 50 to 800 range, with an unspecified step size (probably 1 u based on the mass spectra presented), at three collision energies, giving rise to three libraries. A positive identification was defined by a similar search result with high confidence for at least two of the three spectra generated for each peak. The search algorithm used was derived from the NIST Mass Spectral Search Program version 1.6, but no indication was given with respect to the relative weights of the nature.

Ther Drug Monit. Vol. 24, No. 2, 2002
workers have attempted to use the separation power of depending on the compounds and applications. Some sensitivity and S/N, can be either increased or decreased, stages, and the limits of detection, resulting from both sensitivity and S/N, can be either increased or decreased, depending on the compounds and applications. Some workers have attempted to use the separation power of

**DISCUSSION**

Within the past 5 years, the potential and then the routine applicability of LC coupled to MS via an API interface with ES or an APCI source have been shown. The relative merits of ES and APCI are not so clear-cut, except for the most polar (for ES) or nonpolar (for APCI) compounds. However, as far as the most hydrophobic compounds are concerned, such as some pesticides (e.g., organophosphorus pesticides), very few or even no convenient LC-MS technique could be found in the literature, as is of course the case for the analysis of gases. At the other end of the polarity range, some difficulties are encountered when one wants to analyze very polar compounds such as phosphorylated metabolites of nucleoside analogs (e.g., some anticancer or antiviral drugs), for example, which may be due to chromatographic separation issues rather than to MS detection. Indeed, the mobile-phase composition compatible with API sources is very restrictive, with neither nonvolatile salts or counterions nor aggressive modifiers such as alkylamines being acceptable. In such cases, capillary electrophoresis coupled to ES-MS could be an interesting solution.

The potential of triple-quadrupole or Q-TOF MS-MS detection over single MS is largely exemplified in the present review. MS-MS in toxicology brings higher specificity and selectivity (higher S/N) as well as more structural information when an unknown chromatographic peak is to be explored. However, its sensitivity (as number of counts per second) is generally less than that of MS due to low ion transmission through the three stages, and the limits of detection, resulting from both sensitivity and S/N, can be either increased or decreased, depending on the compounds and applications. Some workers have attempted to use the separation power of the first MS step to dispense with chromatography. As shown by a few applications reviewed here, flow-injection analysis can sometimes be used successfully for particular drugs and matrices (mainly urine), provided deuterated analogs that compensate for any ionization competition phenomena are available. Indeed, infusion- or flow-injection-API-MS is subject to the ion-suppression phenomenon, the main reason why attempts are being made to use very rapid chromatographic techniques, mainly intended to separate the compounds of interest from salts and proteins, preceded by automated or even no sample preparation.

In this review I did not insist on the validation criteria of the quantitative methods analyzed because the data were often incomplete. Indeed, the novelty of the various applications probably allowed publication of many of these methods despite the lack of careful validation. This approach is actually changing as the technique is gaining in maturity, and this will help promote LC-API-MS to the status of the gold standard as an alternative to GC-MS.

As far as instrumental improvements are concerned, manufacturers continuously optimize the design of their interfaces with respect to the shape of the ion-focusing parts or to prevent source contamination (6). One of the recent improvements is the development of multi-ES sources (i.e., with multiple ES needles, each receiving the effluent of a different chromatographic column), allowing multiplexed (or time-shared) acquisition of several analyses, performed in parallel, using a single MS. This will obviously benefit from the speed of TOF and Q-TOF scanning. Despite various attempts, to date there does not seem to be any new type of ionization source that can compete with ES or APCI. Indeed, the main improvements are now expected from the MS part of the coupling (6). In particular, in addition to the impressive mass precision achieved, the “all-ion-detection” capability of orthogonal-acceleration reflectron TOF (oa-TOF) mass spectrometers provides a 20- to 100-fold improvement in sensitivity compared with the triple-quadrupole systems used in the product ion scan mode, whereas the latter will provide better sensitivity in the MRM mode, at the expense of the information content. The combination of a quadrupole, a collision cell, and an oa-TOF (Q-TOF) leads to unsurpassed sensitivity and specificity that greatly facilitates the identification of unknown compounds. This, combined with signal processing and data-dependent acquisition (i.e., software improvements to detect a chemical signal in the chromatographic noise and switch from single to tandem MS modes), will probably help design even better general unknown screening procedures, thus truly combining the relative universality
of both HPLC and MS. However, a limitation of oa-TOF already alluded to here is its limited dynamic range, which could be compensated for in the future by hardware and software developments. In the meantime, QqQ and Q-TOF are bound to coexist, and QqQ instruments will still offer the largest span of applications to toxicologists interested by LC-MS.

CONCLUSIONS

A review paper from my group in 1999 concluded that the potential of LC-MS in human toxicology had been shown during the previous few years, mainly using API. During the past 2 years, the situation has evolved toward the disappearance of other types of interfaces/ionization sources and the exponential increase in the number of applications in the field of clinical or forensic toxicology. Almost all drugs of abuse can be (and were) analyzed in biologic fluids or tissues with LC-MS or LC-MS-MS instruments, with equal or better specificity and generally better sensitivity than GC-MS, using simpler sample preparation. The number of therapeutic drugs to which LC-MS was applied in the past 5 years, mainly by the pharmaceutical industry, probably exceeds that of drugs for which GC-MS methods were published in the past few decades. However, only a limited number of LC-MS methods were published for the analysis of pesticides in biologic samples, maybe due to the high lipid solubility of many pesticides. Indeed, despite the complementarities of ES and APCI sources, LC-MS is not always convenient for very lipophilic or very hydrophilic compounds (and of course not for the analysis of the gases of highly volatile compounds).

LC-API-MS is now a mature technique, not expected to be revolutionized in the near future. The only limitation to its widespread use in toxicology laboratories is still its high price, which, in the best case, is twice that of GC-MS instruments (and much more for tandem mass spectrometers). It is probably only a matter of time before these prices decrease owing to the large number of instruments being sold today (mainly in the richer analytic fields) and the competition between manufacturers.

REFERENCES


