In-source CID mass spectral libraries for the "general unknown" screening of drugs and toxicants

P. Marquet[1]*, N. Venisse[1], É. Lacassie[1] and G. Lachâtre[1,2]

[1]*Department of Pharmacology and Toxicology, University Hospital, Limoges, France*
[2]*Laboratory of Toxicology, Faculty of Pharmacy, University of Limoges, France*

A "general unknown" screening procedure for drugs and toxicants using LC-ES-MS was developed. It involved in-source collision induced dissociation of the molecular ions (in the negative-ion mode) or protonated molecules (in the positive-ion mode) generally obtained from electrospray sources. Spectra were reconstructed by adding, on one hand, a pair of positive spectra, one without and one with fragmentation, and on the other a pair of negative spectra acquired in similar conditions. These reconstructed spectra showed at least as many fragments as MS/MS spectra, and sometimes as many as electron-ionisation spectra. They were repeatable and reproducible enough to be used for the specific identification of hundreds of molecules. Libraries of about 1100 positive spectra and 500 negative spectra were constructed and are used routinely, together with chromatographic separation involving a Nucleosil C18, 5 µm column (150 × 1 mm i.d.) and a gradient of acetonitrile in 2 mM, pH 3 ammonium formate as a screening technique complementary to GC-MS and HPLC-DAD.

Introduction

The identification of xenobiotics in biological fluids generally involves a panel of automated immunoassays for the most common drugs, and of chromatographic techniques ideally coupled to specific detectors (mass spectrometers or UV-diode array detectors). Nevertheless, failures are not uncommon, particularly when polar compounds, with no or little UV absorbency are involved. However, mass spectrometry (MS) is more specific and reliable than DAD and should always be preferred when possible. As GC is limited to volatile and thermally stable compounds, the coupling of MS with HPLC has long been considered as a possible means of increasing the range of compounds amenable to MS [1].

Because electron ionisation (El) produces universally reproducible mass spectra, this ionisation 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 [2], but both suffered from an unavoidable volatilisation step by heating and thus were not suitable for polar or thermally labile compounds.

More recently, atmospheric pressure ionisation sources of the electrospray (ES) or APCI type have superseded all the other types of interfaces/ionisation sources for LC-MS. Unfortunately, ES and APCI are not compatible with EI and, on the contrary, involve a soft ionisation process. This limitation can be bypassed by using collision-induced dissociation (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 specialised "collision cell" or in the intermediate pressure part of the mass spectrometer, between the atmospheric pressure source and the high vacuum of the mass analyser (so called "in-source CID"). The first solution, necessitating tandem mass-spectrometers, cannot be readily applied to a "general unknown" screening procedure because it supposes that a limited number of parent ions are selected in the first MS stage (which is not applicable to *a priori* unknowns), before being submitted to fragmentation in the collision cell. It can be easily used, with or without chromatographic separation, to confirm the identity of suspected compounds as long as fragmentation energy is standardised (in terms of nature and pressure of collision gas, and ion kinetic energy) and a library of mass spectra of compounds of interest is built [3,4]. As an alternative we [5] and others [6] have suggested using in-source CID. The fragments produced by in-source CID, generally used as confirmation ions for quantitative methods using single-quadrupole instruments, are most generally the same as those produced by conventional CID in the collision cell of a MS/MS instrument. Moreover, contrary to the latter where a precursor ion is selected before fragmentation, in-source CID needs to be preceded by a thorough separation of compounds for good selectivity (no interference), good sensitivity (no ion suppression) and reproducible fragmentation (fragmentation

Article available at http://analusis.edpsciences.org or http://dx.doi.org/10.1051/analusis:2000280925

efficiency being dependent on the ion density in the transition zone [7]). Therefore, a "general unknown" screening procedure using LC-ES-MS should involve an efficient chromatographic separation procedure.

This study was intended to optimise and standardise the mass spectral conditions for obtaining rich and reproducible mass spectra, to evaluate the advantages and limitations of these mass spectra with respect to EI or triple quadrupole MS/MS mass spectra, to create mass spectrum libraries of compounds relevant to human toxicology and to establish a procedure to compare an *a priori* unknown spectrum to those in these libraries.

Material and methods

Reagents

Organic solvents and reagents were all of HPLC purity. Acetonitrile and methanol were purchased from Carlo Erba (Milan, ltaly), formic acid and ammonium formate from Sigma (St. Louis, MO, U.S.A.). Deionised water was prepared on a MilliQ laboratory plant (Millipore, Bedford, MA, U.S.A.).

Apparatus

The chromatographic system consisted of a Series 200 LC micro-flow-rate, high pressure gradient pumping system and a Series 200 autosampler (Perkin-Elmer Instruments, Les Ulis, France) including a Rheodyne model 7725 injection valve equipped with a 5 µl internal loop (Rheodyne, Cotati, CA, U.S.A.). The mass spectrometer was an API 100 single quadrupole instrument (Applied Biosystem – Sciex, Concord, Canada), equipped with a pneumatically assisted electrospray (Ionspray®) ionisation source.

Chromatographic and mass spectral conditions

The chromatographic separation was performed on a Nucleosil C18, 5 µm (150 × 1 mm i.d.) column (LC-Packings, Marseille, France). The mobile phases tested were different gradient profiles of acetonitrile in pH3, 2 mM ammonium formate, delivered at a flow rate of 50 µl/min. All chromatographic solvents were filtered (0.46 µm) prior to mixing and degassed with helium thereafter. A good compromise between chromatographic separation, correct retention of both polar and non-polar compounds and acceptable analytical run-over time was obtained with the following gradient of acetonitrile (A) in 2 mM, pH 3.0 ammonium formate (B): 0-2 min, 5 % A; 2-42 min, 5 to 95 % linear gradient of A in B; 42-44 min, 95 % A; 44-45 min, from 95 to 5 % A; 45-50 min, equilibration of the column with 5 % A. Ionisation was performed in the positive and negative ion modes and masses were scanned between *m/z* 100 and *m/z* 1100, with a 0.2 a.m.u. step-size.

The main ionisation and fragmentation conditions were as follows: in the positive mode, ionisation voltage + 5500 V and orifice voltages + 20 and + 80 V (in order to obtain both the protonated molecule and fragment ions for the majority of the compounds tested); in the negative mode, ionisation voltage – 4500 V and orifice voltages – 20 and – 80 V. These four experimental settings were alternated with every scan, for a total turn-over time of 5.8 s. The resulting recordings were automatically separated into 4 different chromatograms with respect to polarity and orifice voltage value. "Reconstructed" positive and negative spectra were obtained by adding spectra at + 20 and + 80 V on one hand and spectra at – 20 and – 80 V on the other.

Distance between the ionisation needle and the mass-spectrometer entrance orifice was finely tuned between ca. 5.8 and 6.2 mm in order to obtain a standard fragmentation pattern for glafenine (test compound).

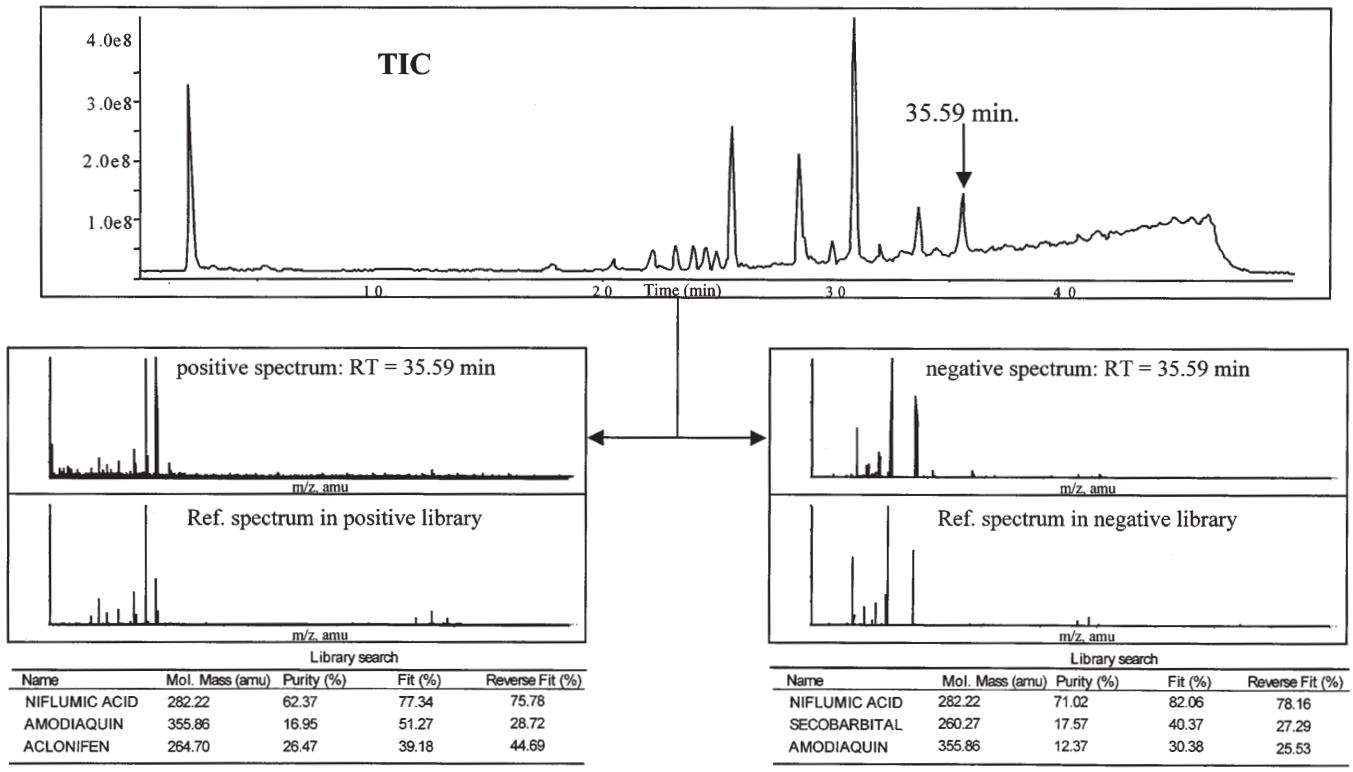
Validation

Intra- and inter-assay variability of the reconstructed mass spectra was tested on a mixture of nine compounds, including the I.S., representing a wide range of polarity, molecular weight and nature: chlorproethazine, antipyrine, bacampicilline, benorylate, aminophenazole, altretamine, althiazide, almitrine, glafenine. A mixture of pure standards of these compounds at 10 µg/l was injected on-column 6 times within 6 hours for the intra-assay study, and once a day for 6 days for the inter-assay study.

Results and discussion

A typical chromatogram obtained from an extract of a patient's serum sample is shown in figure 1. The scanning range chosen was limited to *m/z* 100 at its lower end because of too high a background noise below this value, whereas it was limited to *m/z* 1100 at its upper end because there are very few xenobiotics of toxicological interest with a higher *m/z* ratio. The compounds of toxicological interest with the highest masses studied with the present procedure were digitalics (*e.g.* lanatoside C, MW = 1001.5).

In order to obtain both pseudo-molecular and fragment ions for the majority of the compounds tested, different fragmentation conditions were compared: constant acceleration potential (so-called "orifice voltage" or OR) values, between + 10 and + 100 V and between – 100 and – V with a step of + 10 V; linearly decreasing (OR from + 100 to + 20 V, + 80 to + 10 V, – 100 to – 20 V and – 80 to – 10 V) and increasing (opposite conditions) energy. For this experiment, a selection of 50 test compounds was made on the basis of xenobiotics with a large range of polarity, molecular weight, known chromatographic retention and included acids and bases (data not shown). Moreover, they included therapeutic drugs, drugs of abuse, pesticides and other toxicants. The best constant-voltage fragmentation conditions (OR values) for obtaining molecular or pseudomolecular ions for the



Name	Mol. Mass (amu)	Purity (%)	Fit (%)	Reverse Fit (%)
NIFLUMIC ACID	282.22	62.37	77.34	75.78
AMODIAQUIN	355.86	16.95	51.27	28.72
ACLONIFEN	264.70	26.47	39.18	44.69

Name	Mol. Mass (amu)	Purity (%)	Fit (%)	Reverse Fit (%)
NIFLUMIC ACID	282.22	71.02	82.06	78.16
SECOBARBITAL	260.27	17.57	40.37	27.29
AMODIAQUIN	355.86	12.37	30.38	25.53

Figure 1. Principle of the identification of an unknown compound from a typical "screening" chromatogram obtained by analysing a patient's serum sample.

majority of the 50 compounds studied were + 20 V in the positive mode and – 20 V in the negative mode. The best conditions for obtaining detectable fragments in the scanning range (*m/z* 100 to *m/z* 1100) were respectively + 80 V and – 80 V on average. The use of a linearly increasing fragmentation energy synchronised with mass scanning (the higher the *m/z* ratio scanned the higher the fragmentation energy) was not found convenient: it was unfavourable to the detection of fragments, that were poorly generated (low fragmentation energy) at the time of their detection (low *m/z* scanning). More surprisingly, a linearly decreasing acceleration potential was not found convenient either as it generally induced too high a fragmentation of molecular ions or protonated molecules, probably due to the difference between the highest *m/z* ratio scanned (*m/z* 1100) and the average molecular weight of the xenobiotics of interest (300 to 500 Da): though the fragmentation energy was low for the highest *m/z* ratios scanned (OR voltage: + 10 or + 20 V, – 10 or – 20 V), it was between 2 and 3 times higher when scanning the molecular or pseudomolecular ion of most of the compounds studied; moreover, it was not possible to further decrease the fragmentation energy at the high-mass end, as xenobiotics with a molecular weight close to 1100 Da (which are generally not amenable to GC-MS either) would not be detected at all.

Therefore, the best solution found to obtain sufficient spectral information was to reconstruct a spectrum for each polarity by adding a spectrum acquired with a low fragmentation energy (+ 20 or – 20) and a spectrum resulting from a high fragmentation energy (+ 80 V or – 80 V). The total turn-over time of 5.8 s 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).

Similar or close fragmentation conditions were reported by other teams [8-10], except that all of them used three different fragmentation voltages in the positive-ion mode and none in the negative-ion mode. 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 the present study, the reconstructed spectra of a large majority of compounds exhibited both fragments and the protonated molecule or molecular ion (Fig. 2 to 4) and the addition of an intermediate fragmentation condition equivalent to the OR value of 50 V proposed by others [8,9] added no further useful information. Some compounds were only detected in the negative-ion mode and would probably be

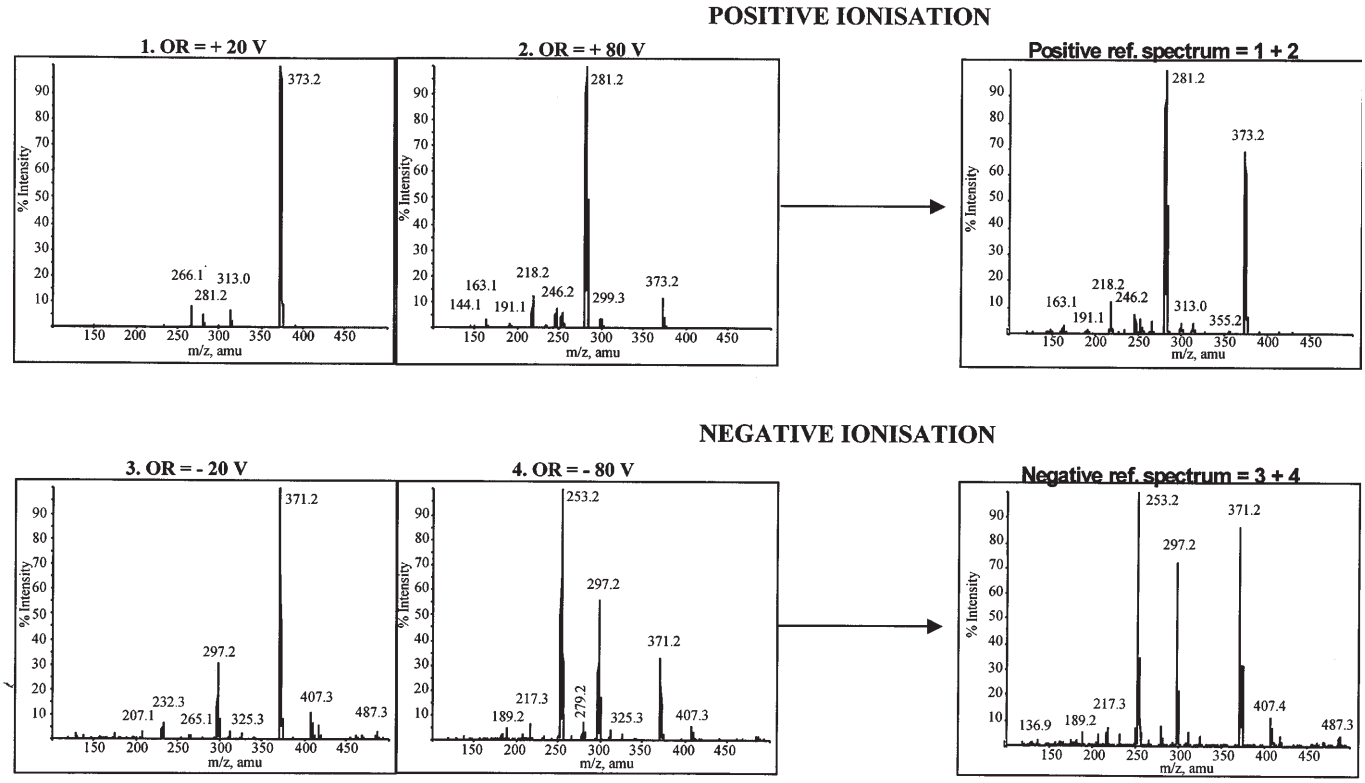


Figure 2. Individual mass spectra of glafenine (IS) at + 20, + 80, – 20 and – 80 V and reconstructed positive and negative mass spectra (reference spectra for fragmentation standardisation).

missed by the procedures proposed in the above-mentioned papers. Moreover, some compounds gave both positive and negative ions and could thus be identified with increased reliability (Fig. 1).

To study the influence of geometrical configuration of the ionisation source on the process of collision-induced dissociation, an extract of blank serum spiked with glafenine (I.S.) was successively analysed, on a same day, at needle-orifice distances of 4, 6, 8, 10 and 12 mm. Fragmentation efficiency was evaluated from the ratio between one of the major fragment ions and the molecular ion (in the negative-ion mode) or protonated molecule (in the positive-ion mode), on the mass spectrum obtained under each polarity and fragmentation condition: *m/z* 281.2 over *m/z* 373.2 in the positive ionisation mode, *m/z* 297.2 or 253.2 over *m/z* 371.2 in the negative ionisation mode (Tab. I). At + 20 V, virtually no fragment was detectable. At + 80 V the intensity of the protonated molecule (*m/z* 373.2) relatively to that of its fragment (*m/z* 281.2) decreased when the distance increased. The same tendency was observed at – 20 V and – 80 V: the abundance of fragment ions *m/z* – 297.2 and – 253.2 increased with respect to that of the molecular ion (*m/z* – 371.2) when the distance increased, except for the longest distance (12 mm) at – 20 V where the fragment/molecular ion ratio decreased.

This phenomenon was confirmed for the nine molecules in the test mixture. This is probably the result of two opposite mechanisms when the distance is increased: (i) in-source CID is favoured, presumably because ion kinetic energy is increased, being proportional to the square of velocity which is, itself, the product of acceleration (electric field) and time elapsed; (ii) transmission of primary and fragment ions to the mass spectrometer is decreased, as the spray enlarges and the electric field decreases. Moreover, these mechanisms may affect the different ions to different extents. This is the reason why we decided to set this distance at approximately 6 mm with the API 100 Ionspray® source used, then to tune it finely (ca. between 5.8 and 6.2 mm) in order to standardise ionisation and in source-CID fragmentation, using a test compound (glafenine, I.S.) whose reference spectra played the role of "gold standard". This rendered the whole procedure transferable to other instruments with electrospray sources, even with different geometry, as we were able to verify on other models of single or triple quadrupole LC-MS instruments of the same manufacturer (data not shown). This standardisation consists, when applied for the first time to a different instrument: first, on adjusting the geometry of the source (needle position, gas pressure, etc.) in order to obtain the best compromise between sensitivity and signal-to-noise ratio for glafenine, infused as a 10 mg/L pure

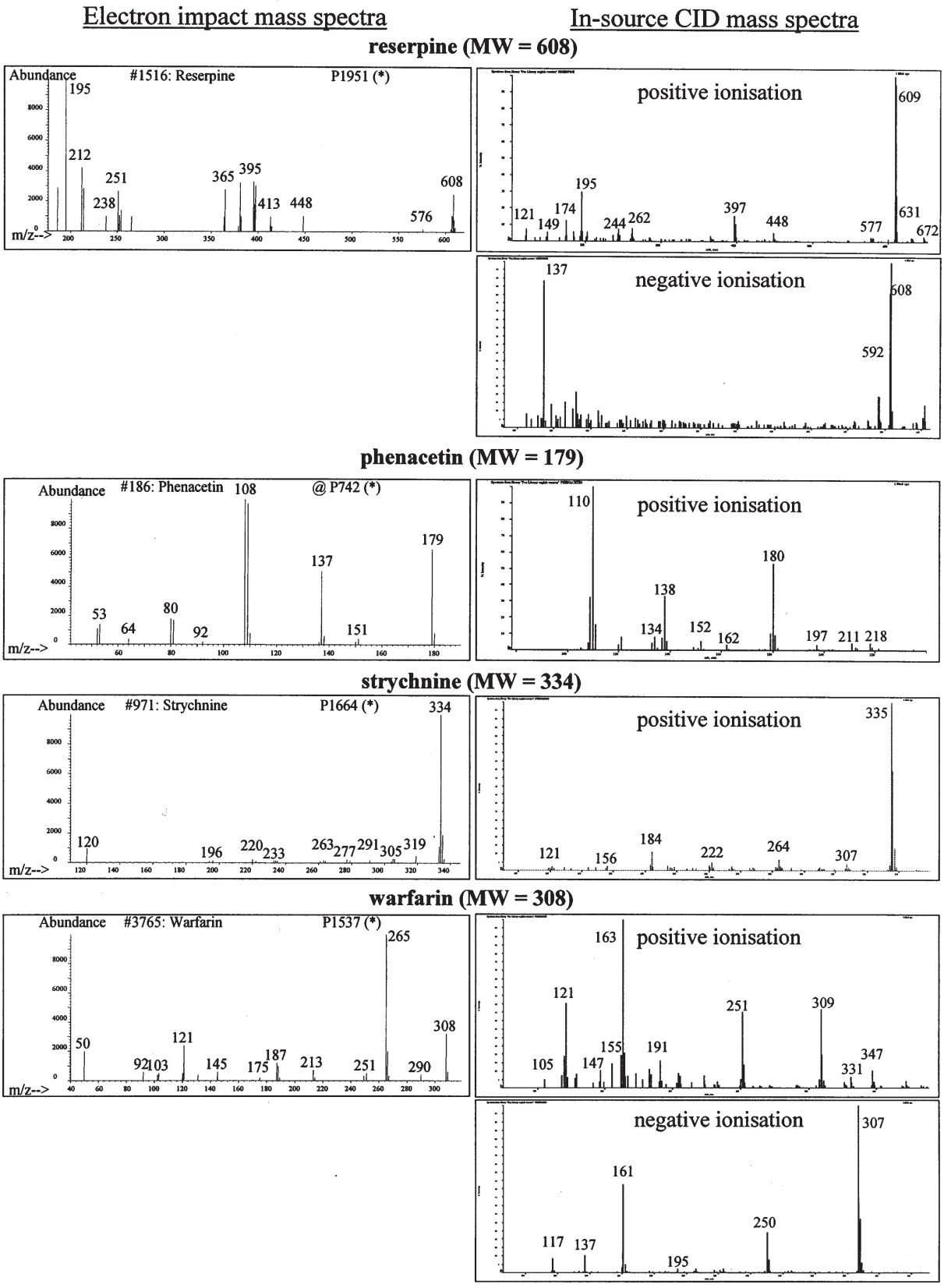


Figure 3. Comparison of CID-MS and EI mass spectra of four test compounds, reserpine, phenacetin, strychnine and warfarin.

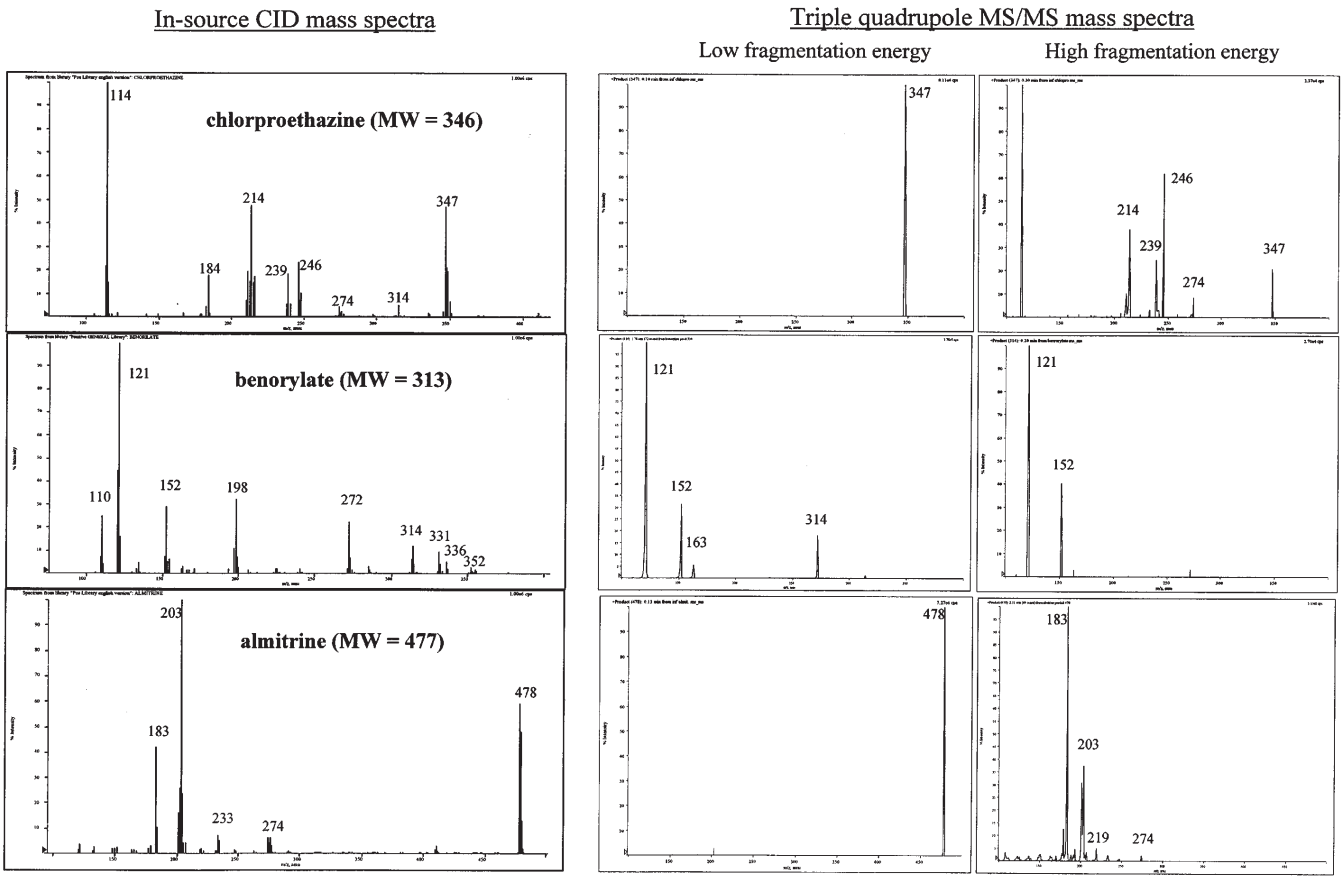


Figure 4. Comparison of in-source CID-MS and triple quadrupole MS/MS spectra of three test compounds, chlorproethazine, benorylate and almitrine.

Table I. Influence of the distance on in-source CID of a test compound, glafenine (I.S.).

Polarity and orifice voltage	m/z	*Needle-orifice distance*				
		4 mm	*6 mm*	*8 mm*	*10 mm*	*12 mm*
+ 20 V	373.2	100 %	100 %	100 %	100 %	100 %
	281.2	< 2 %	2.7 %	2.5 %	2.8 %	4.9 %
+ 80 V	281.2	100 %	100 %	100 %	100 %	100 %
	373.2	47.6 %	7.8 %	6.5 %	6.0 %	4.3 %
– 20 V	–371.2	100 %	100 %	100 %	100 %	100 %
	–297.2	10.6 %	31.0 %	32.5 %	41.5 %	36.6 %
– 80 V	–253.2	100 %	100 %	100 %	100 %	100 %
	–371.2	54.6 %	20.4 %	19.5 %	18.9 %	17.6 %

solution at 50 µl/min; secondly adjusting each fragmentation energy (positive low and high, negative low and high) in order to obtain spectra as close as possible to the individual reference spectra displayed in figure 2; finally, precisely adjusting needle position or source geometry in order to obtain reconstructed positive and negative spectra similar to the reference reconstructed spectra (Fig. 2), with the following relative intensities:

- in the positive ion mode: *m/z* + 281 = 100 %; *m/z* + 373 = 70 ± l0 %; *m/z* + 2l8 = l4 ± 20 %;
- in the negative ion mode: *m/z* – 253 = 100 %; *m/z* – 371 = 90 % ± 5 %; *m/z* – 297 = 72 ± l0 %; *m/z* – 217 = 8 ± 25 %.

For subsequent use of an apparatus for which this procedure has been followed, only the source geometry should be slightly adjusted to accurately tune glafenine fragmentation. This procedure ensures mass spectral reproducibility, as illustrated below.

The intra- and inter-assay variability of the relative intensity of a significant ion with respect to the most intense ion in the spectrum (generally a major fragment and the molecular or pseudo-molecular ion, or *vice versa*) on reconstructed mass spectra of the nine compounds studied is presented in tables II and III, respectively. Intra-assay precision CVs were less than 25 % for all, whether in the positive or in the negative mode, and even less than 10 % for most of them. Inter-assay precision CVs were between 5.8 and 22.0 % in the positive ion mode and between 11.9 % and 33.3 % in the negative mode. Ionisation polarity and the intensity of ion ratios seemed to influence this variability, which was higher in the negative mode and generally increased in both modes when the ratio value decreased; however, some of the low ratios tested led to very good reproducibility, even in the negative mode (*e.g.* benorylate in the negative mode: ratio = 15.2 % and CV = 11.9 %). This variability, including that of the chromatographic background noise, was found acceptable for correct identification of compounds, inasmuch as we attributed a low weight to this intensity ratio in the "library search" procedure. Moreover, apart from these figures concerning a single ion-ratio per compound, visual inspection of the spectra recorded over 6 consecutive days showed a very satisfactory reproducibility of the spectrum pattern, including *m/z* ratios of low intensity. Furthermore, the reconstructed mass spectra were not dependent on concentration in the range 10 ng/ml to 10 µg/ml, as we were able to verify during a

Table II. Repeatability (*n* = 6) of the mass spectra of nine test compounds.

Compounds	*RT* *min*	*Ionisation mode*	*Base peak ion* m/z	*Second most intense ion* m/z	*Mean ratio (%)*	*Variability CV (%)*
Aminophenazole	13.25	Pos	117.0	192.4	82.3	3.1
		Neg	N.D.	-	-	-
Antipyrine	18.85	Pos	189.2	146.3	7.3	6.9
		Neg	N.D.	-	-	-
Glafenine (IS)	20.1	Pos	281.2	373.2	67.3	9.2
		Neg	253.2	297.2	70.0	11.1
Altretamine	22.2	Pos	211.2	195.2	4.7	10.4
		Neg	249.2	175.0	14.0	15.0
Althiazide	24.3	Pos	N.D.	-	-	-
		Neg	249.0	382.4	19.0	19.0
Bacampicilline	24.9	Pos	276.4	466.4	39.0	9.2
		Neg	175.2	382.2	11.7	24.7
Benorylate	25.8	Pos	121.2	272.4	21.3	5.4
		Neg	174.8	312.4	19.7	5.9
Chlorproethazine	31.41	Pos	347.4	214.0	70	4.9
		Neg	N.D.	-	-	-
Almitrine	39.43	Pos	478.4	203.4	94.0	8.4
		Neg	N.D.	-	-	-

N.D. : not detected

Table III. Reproducibility (*n* = 6) of the mass spectra of 9 test compounds.

Compounds	*RT* (*min*)	*Ionisation mode*	*Base peak ion* m/z	*Second most intense ion* m/z	*Mean ratio* (%)	*Variability CV* (%)
Aminophenazole	13.25	Pos	117.0	192.4	96.2	13.2
Antipyrine	18.85	Pos	189.2	146.3	6.8	20.0
Glafenine	20.1	Pos	281.2	373.2	70.5	5.8
		Neg	253.2	297.2	68.0	16.2
Altretamine	22.2	Pos	211.2	197.1	5.2	14.8
		Neg	249.2	174.8	12.9	18.1
Althiazide	24.3	Neg	249.0	382.4	19.3	24.0
Bacampicilline	24.9	Pos	276.2	466.4	42.5	22.0
		Neg	175.2	382.2	15.5	33.3
Benorylate	25.8	Pos	121.2	272.4	23.5	15.4
		Neg	174.8	312.4	15.2	11.9
Chlorproethazine	31.41	Pos	347.4	214.0	68.7	11.3
Almitrine	39.43	Pos	478.4	203.4	92.8	7.0

study of the detection limits of more than 100 compounds [11]. Experience showed that only a dirty interface (between the source and the mass filter) could significantly modify these spectra, probably due to bad transmission of high masses towards the spectrometer, exactly the same as with GC/MS in the EI mode.

Figure 3 compares the richness of the reconstructed mass spectra of a few molecules with that of their respective EI mass spectra as recorded in the Pflegger-Maurer-Weber library [12]. Negative-ion reconstructed spectra, when available, increased further structural information provided by in-source CID-MS. Figure 4 shows comparisons between in-source CID/MS and triple quadrupole MS/MS spectra for three other randomly chosen compounds; for this experiment, an API 2000 LC-ES-MS/MS instrument (Sciex) was used in the product-ion scan mode. Both types of comparisons illustrate the richness of the reconstructed CID-MS mass spectra in terms of number and intensity of ions in the spectrum. However, when using electrospray ionisation 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 on one hand (because of too strong fragmentation conditions) and with MS/MS on the other (because no single fragmentation energy would provide both molecular or pseudomolecular ion and fragments for most molecules).

Under these chromatographic and mass spectral conditions, one library comprised of about 1100 reconstructed mass spectra was built in the positive ionisation mode, and another library comprised of about 500 reconstructed spectra was built in the negative ionisation mode. Less than half the molecules were ionised in the negative mode, but most acidic compounds, hardly amenable to GC-MS without convenient derivatisation, were detected using this polarity. These libraries include spectra from therapeutic drugs, drugs of abuse, pesticides, plant, industrial and domestic toxicants, and are regularly updated. They are available from the authors upon request.

Finally, the "library search" function of the software used for visualising and integrating the chromatograms (Multiview®, Sciex) was optimised to give the highest weight to the nature of the *m/z* ratios present in a mass spectrum rather than to their relative intensity ("Intensity factor" = 8). Using these conditions, the mass spectra of *a priori* unknown compounds could be easily matched with the corresponding spectra in the library (Fig. 1). However, the major drawback of the whole procedure is the loss of spectral information linked to the library spectrum format: all *m/z* ratios are rounded up or down to the nearest mass units, while we chose to monitor full spectra with a 0.2 a.m.u. step-size. As a result, the comparison of a full informative spectrum with its very own image stored in the library generally gives a "fit" index not better than 80 to 85 %. Figure 5 shows the chromatograms of an extract of a commercial quality control for common therapeutic drugs: most compounds present were identified with good similarity indices, given the limits mentioned. Software

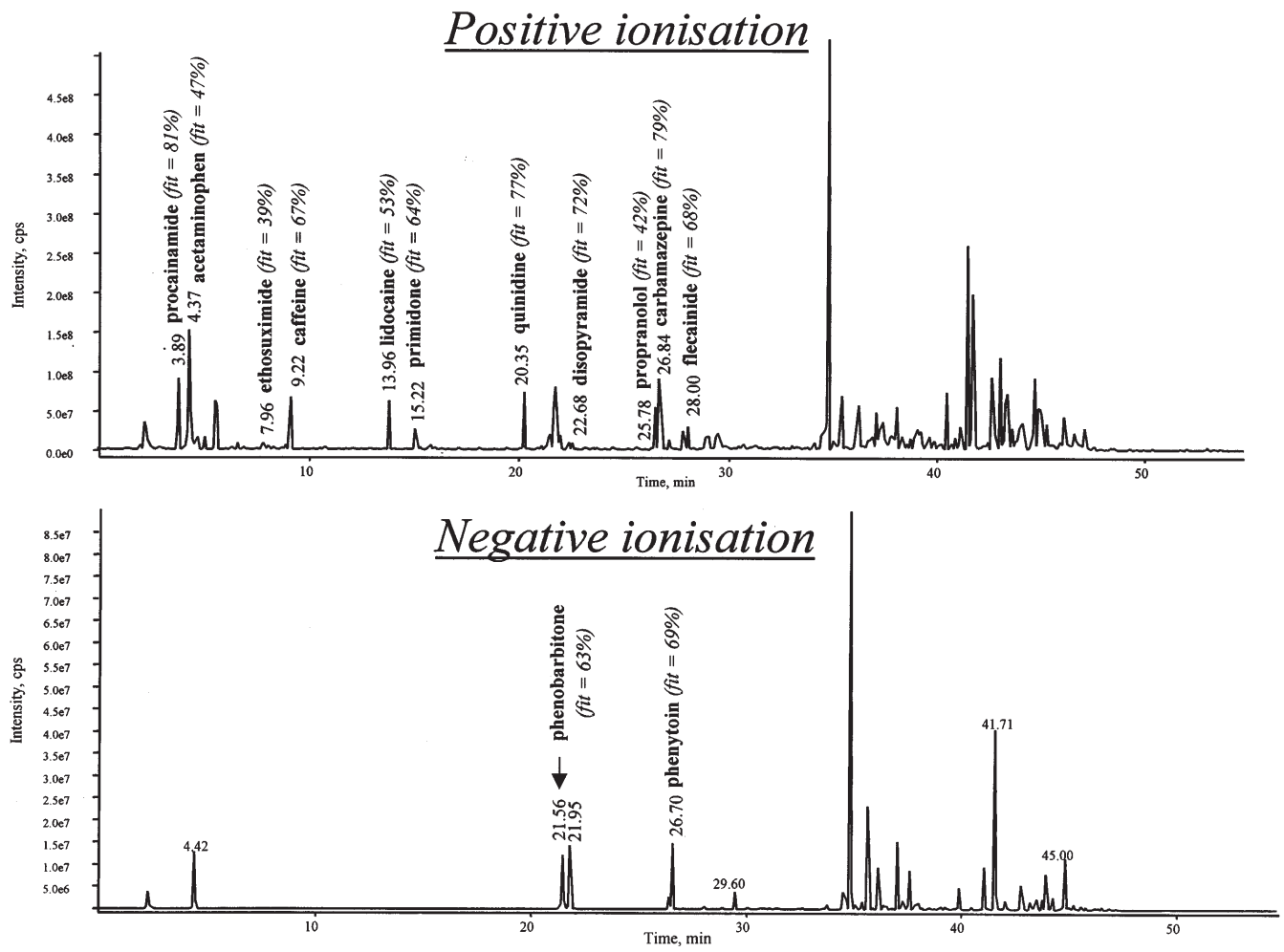


Figure 5. Application example of the present "general unknown" screening procedure: positive and negative ion chromatograms and similarity ("fit") indices of the spectra recorded with the respective reference spectra in the libraries.

improvement is expected with this respect. The whole procedure is now routinely used for the identification of drugs and toxicants in clinical samples, as a complement to GC-MS and HPLC-DAD procedures used in the laboratory.

Conclusion

The present study demonstrated the feasibility of using in-source CID for the mass spectral identification of drugs and toxicants, provided the main instrumental settings are standardised. This ionisation/fragmentation process is obviously not as universal as electron ionisation, but contrary to what was concluded by other authors using other instruments [13], we think it can be shared by users of the same make of instruments and maybe, as we seek to demonstrate, by users of most of the modern electrospray-quadrupole types of instruments.

Based on this technique, we designed a complete "general unknown" screening procedure including optimised sample preparation and automatic interpretation that will be described elsewhere.

Acknowledgements

We thank M. Jean-Louis Dupuy for excellent technical assistance, Ms. Eva Duchoslav, and M. Bori Shushan (Sciex, Canada) for their technical support. A part of this study was sponsored by Applied-Biosystem France and by Sciex, Canada.

References

1. Köppel, C.; Tenczer, J. *J. Am. Soc. Mass Spectrom.* **1995**, *6*, 995-1003.
2. Marquet, P.; Lachâtre, G. *J. Chromatogr. B.* **1999**, *733*, 93-118.
3. Weinmann, W.; Svoboda, M. *J. Anal. Toxicol.* **1998**, *22*, 319-28.
4. Baumann, C.; Cintora, M.A.; Eichler, M.; Lifante, E.; Cooke, M.; Przyborowska, A.; Halket J.M. *Rapid Comm. Mass Spectrom.* **2000**, *14*, 349-56.
5. Marquet, P.; Dupuy, J.L.; Lachâtre, G.; Shushan, B.; Duchoslav, E.; Monasterios, C.; Ilisieu, P.; Anacleto, J. *Communication at the Meeting of the American Society of Mass Spectrometry*, Orlando, FL, 1-3 VI 1998.
6. Weinmann, W.; Wiedemann, A.; Eppinger, B.; Renz, M.; Svoboda, M. *J. Am. Soc. Mass Spectrom.* **1999**, *10*, 1028-37.
7. Niessen, W.M.A. *J. Chromatogr. A.* **1998**, *812*, 53-75.
8. Weinmann, W., Wiedmann A., Eppinger B., Renz M. Svoboda M. *J. Am. Soc. Mass Spectrom.* **1999**, *10*, 1028-1037.
9. Schreiber, A.; Efer, J.; Engewald W. *J. Chromatogr. A* **2000**, *869*, 411-25.
10. Hough, J.M.; Haney, C.A.; Voyksner, R.D.; Bereman, R.D. *Anal. Chem.* **2000**, *72*, 2265-70.
11. Shushan, B.I.; Marquet, P.; Dupuy, J.L.; Anacleto, J.; Lachâtre, G.; Duchoslav, E.; Chan, M. *Ther. Drug Monit.* **1999**, *21*, 467.
12. Pfleger, K.; Maurer,H.H.; Weber, A. *Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and their Metabolites*; Weinheim: VCH, 1992.
13. Bogusz, M.J.; Maier, R.D.; Kruger, K.D.; Webb, K.S.; Romeril, J.; Miller, M.L. *J. Chromatogr. A.* **1999**, *844*, 409-18.