Journal of Current Medical Research and Opinion
Received 19-01-2024| Revised 24-01-2024| Accepted 11-02-2024| Published Online 12-02-2024

DOI: https://doi.org/10.52845/CMRO/2024/7-2-8 ISSN (O) 2589-8779 | (P) 2589-8760
CMRO 07 (02), 2015-2122 (2024)
Original Articles

Clinical Applications of Liquid Chromatography-Mass Spectrometry (LC-MS) for Metabolites, Toxicology and Therapeutic Drug Analysis

Asmaa Hussein Obaid

University of Thi-Qar, College of Science, Iraq.

Abstract:

As a potent quantitative and qualitative analytical technique, LC-MS/MS is useful in many fields, including toxicology, endocrinology, paediatrics, microbiology, therapeutic drug monitoring (TDM), and the new field of proteomics. It is also possible to use LC-MS/MS to analyse biological materials, such as blood and urine. The recent founding of the Mass Spectrometry and Separation Sciences Division of the AACC and the increasing number of LC-MS/MS papers and conferences demonstrate a growing interest in the clinical use of mass spectrometry. Analysing complex combinations is the basic idea behind tandem mass spectrometry, which involves connecting several mass spectrometers in a chain.

This method employs a collision cell sandwiched between two sequentially organised mass filters. You can use the filters in either a static or scanning mode to choose a specific mass-to-charge (m/z) ratio or m/z range. Precursor ions, which are bigger, smash with gas molecules in the collision cell, causing them to split into product ions, which are smaller. One of several possible scan modes for a tandem mass spectrometer is determined by the therapeutic application. Toxicology is an interdisciplinary science that seeks to clarify the quantitative and qualitative connections between poisonous substances and the physiological and behavioural impacts they have on living organisms. There are a number of important and prominent aspects of toxicology, including the study of poisons' mechanisms of action and the creation of treatments and therapies for the associated harmful effects. As a result of these efforts, mass spectrometry (MS), an analytical technology with a wide variety of applications, has developed into a strong tool that is applied in the toxicological analysis of medications, poisons, and the metabolites of both. Even now, MS finds use in every subfield of toxicology, from environmental toxicology and forensic toxicology to clinical toxicology. Nowadays, toxicology labs rely on mass spectrometry and its hyphenated applications, which include GC-MS, LC-MS, ICP-MS, and tandem mass spectrometry / MS and MSn. Despite the widespread use of many analytical tools in these domains, this remains the case. The hyphenated MS technology and their toxicological applications will be the focus of this review.

Keywords: LC-MS, Metabolites, Toxicology, Therapeutic, Drug

Introduction:

As a result of the LC-MS/MS's status as a strategic technology, an increasing number of clinical laboratories have begun employing it in place of various other approaches. Historically, immunoassays have been utilized primarily for the purpose of measuring substances with a low molecular weight. However, they have a number of drawbacks, such as issues with specificity, a lack of concordance between the assays of various manufacturers, and variance from lot to lot from the same manufacturer due to unpredictable cross-reactivity of antibodies. In addition, the dynamic measurement range of many immunoassays could be limited by heterophilic antibodies as well as hook effects. On the other hand [1, 2], MS/MS offers a higher selectivity for a greater number of analytes because it can identify them based on at least two of their physical features, namely their precursor mass and their product ion mass. Ionization of low-molecular-weight compounds in liquid phase is now possible thanks to the development of soft ionization techniques like electrospray ionization and atmospheric pressure chemical ionization. This makes it possible to couple high-performance LC with MS/MS [3].

When paired with LC, MS/MS provides another property to correctly identify the analyte, and this additional property is the retention time. This results in increased specificity. Clinical laboratories have also been subject to scenarios in which manufacturers suddenly remove immunoassays from the market. In these instances, the laboratories are forced to look desperately for alternative methods to transmit test results to the physician who ordered the tests [4, 5]. These experiences have provided laboratories with yet another justification for utilizing LC-MS/MS.

As compared to immunoassays and other approaches [6, 7], the sensitivity of LC-MS/MS may make it possible to detect lower levels of certain analytes, such as steroids, than is possible with other methods. Another advantage of LC-MS/MS is that it enables clinical laboratories to multiplex, which means that they can determine the identity and quantity of many analytes of interest at the same time. The cost of individual tests can be reduced through multiplexing [8]. This is in comparison to more time consuming and expensive sample preparation processes, such as solid phase extraction or derivatization. In older methods, such as gas chromatography (GC), derivatization, also known as the chemical modification of polar molecules, was required since these compounds had to be sufficiently volatile in order for them to be examined. Other procedures, such as mass spectrometry (MS), also required derivatization. The derivatization procedure, on the other hand, increased the amount of time, effort, and cost required for sample preparation.

The Challenges of LC-MS/MS The LC-MS/MS technique, while its myriad advantages, is not without its share of difficulties. In order to build and validate LDTs and troubleshoot the instruments, a high level of technical competence is required for LC-MS/MS, which is a high complexity system [9]. In addition, the newly published draft of a guidance paper by the FDA about the regulation of LDTs has the potential to influence the majority of tests that are created and carried out on LC-MS/MS. At this time, laboratories are developing and validating LC-MS/MS assays in accordance with CLIA, which is an organization that established quality standards for laboratory testing as well as accrediting systems for clinical laboratories. In contrast to FDA-approved tests, laboratory developed tests (LDTs) are subject to additional requirements for analytical validation. These requirements include an assessment of the test's imprecision [10]. When developing a normal technique, one of the many problems that must be overcome is determining the right sample preparation, column choice, mobile phase, and selection of adequate internal standards. These are just some of the numerous challenges that must be overcome.

In addition, because LC-MS/MS assays are not standardized or harmonized, and because commercial calibrators are not readily available, there may be variances from one LC-MS/MS test to the next performed by different users [11, 12]. Despite this, manufacturers have begun to address

these constraints and currently sell kits containing immunosuppressants, steroids, and 25-hydroxyvitamin D. One example of this type of product is the Waters MassTrak Immunosuppressant kit, which is intended to facilitate the quantitative determination of tacrolimus concentrations in patients undergoing liver and kidney transplants.

Clinical Applications of Liquid Chromatography with tandem mass spectrometry LC-MS/MS

Mass spectrometry's early users mostly worked in clinical laboratories to screen newborns and diagnose organic metabolic disorders. Current newborn screening programmes rely on LC-MS/MS as the gold standard due to its specificity, sensitivity, and adaptability [13–16]. Patients with phenylketonuria, medium chain acyl coA dehydrogenase deficiency (MCADD), and other amino acid-related disorders typically undergo this assessment of acylcarnitines.

Therapeutic drug monitoring is another domain that relies heavily on LC-MS/MS analysis for quantitative pharmacological assessment. Immunosuppressant medications such cyclosporine A, tacrolimus, sirolimus, everolimus, and mycophenolic acid were initially measured by several clinical laboratories employing LC-MS/MS [17, 18]. On other occasions, reference labs were compelled to analyse sirolimus or everolimus tests because no other techniques were available. Due to the high sendout costs and the need to meet the turnaround time (TAT) standards set by transplant physicians for patient care, numerous laboratories started using LC-MS/MS.

When compared to immunoassays, LC-MS/MS has the additional benefit of being able to look for numerous analytes in a single analytical run, which is something that immunoassays cannot do. When a patient is administered numerous medicines, such as sirolimus in combination with cycloporin A or tacrolimus [19-21], or when a patient is transitioning from one immunosuppressant to another, laboratories that use LC-MS/MS have the ability to simultaneously quantify both drugs in one study, thereby saving both time and money for the patient. Anti-epileptics (such as lamotrigine and levetiracetam), anti-fungals (such as posaconazole), and antidepressants (such as amitriptyline) are some examples of other medications that are frequently tested for safety [22].

Applications of TDM that are more specific include the measurement of anti-neoplastic drugs such as busulfan. When it comes to busulfan, blood samples are taken at a variety of different time intervals after the medicine has been administered in order to quantify the drug's concentration. This pharmacokinetic testing is performed to quantify the drug clearance and offer an anticipated dose in order to prevent toxicity while simultaneously obtaining optimal concentrations prior to hematopoietic stem cell transplantation in both adults and children [23]. The goal of this testing is to adequately ablate the bone marrow in order to prepare for the transplantation. The TAT needs for busulfan can be met by pharmacists and oncologists with the assistance of LC-MS/MS, which offers a solution that is both very cost-effective and very quick. In the field of toxicology, the GC-MS technique has become the method that is applied the most frequently for confirmatory testing of drugs of abuse and prescription pharmaceuticals such as benzodiazepines and opioids [24].

Now, many toxicology labs are switching from GC-MS to LC-MS/MS, which has resulted in significant gains in throughput in addition to cost savings. Even though the upfront cost of an LC-MS/MS system is significantly more than that of a GC-MS system, laboratories are able to use sample preparation methods that are quick, easy, and inexpensive without incurring the additional costs and time required to derivatize samples [25]. The capacity and throughput of laboratories are increased as a result of these advancements, which also result in reduced analytic times and the capability to multiplex. Endocrinology is yet another field in which LC-MS/MS plays a significant part in clinical testing. The clinical value of immunoassays that are employed in

endocrinology is negatively impacted by a number of constraints, including cross-reactivity and specificity difficulties, matrix effects, and heterophilic antibodies, to name just a few. Because of the molecular similarities between testosterone and dehydroepiandrosterone (DHEAS), for instance, DHEAS is known to cause false positive results in testosterone immunoassays [26].

Additionally, certain sex steroids, including estradiol and testosterone in females, have shown that LC-MS/MS has better performance and sensitivity at low concentrations. Publications of the Endocrine Society and the American Urological Association point out the limitations of immunoassays for sex steroids and advocate for the use of mass spectrometric methods instead. Immunoassays are not as precise as mass spectrometric methods, according to these standards.

The capacity to test many steroids at once, often known as comprehensive steroid profiles, is one of the other features of LC-MS/MS. Individual hormones are traditionally measured using immunoassays, whereas LC-MS/MS is able to assess numerous steroids at once. Measurement of thyroxine, urinary cortisol [27], and 25-hydroxyvitamin D are some examples of additional applicable LC-MS/MS applications. Plasma free metanephrines are another essential test that is frequently carried out with LC-MS/MS in order to make a provisional diagnosis of catecholamine-secreting pheochromocytomas or paragangliomas.

Another rapidly expanding area of application for LC-MS/MS is proteomics. It is possible to digest larger proteins or peptides before running the LC-MS/MS analysis, even though the instrument typically has size limits on the molecules it can detect (e.g., a maximum of 2,000 m/z). Some compounds, such immunoglobulins, require digestion prior to LC-MS/MS analysis because their molecular weights are higher than 150 kDa. Conversely, they play a crucial role in determining immune system function, cancer detection, autoimmune diseases, and immunity itself [28]. One such therapeutic agent that the pharmaceutical industry is using to treat a variety of diseases is monoclonal antibodies. Clinical response and improved prognosis have been linked to therapeutic concentrations; infliximab, for instance, is used to treat ulcerative colitis and Crohn's disease. The use of LC-MS/MS techniques allows for the circumvention of potential interference produced by endogenous antibodies directed against infliximab in current immunoassays.

A GC-MS analysis of pharmaceuticals Gas chromatography-mass spectrometry integration opened the door to the prospect of developing everyday applications with MS-level specificity and sensitivity. One analytical method for separating molecules is the gas chromatograph (GC), which uses gas mobile phases and stationary phases to partition the molecules. A gaseous or liquid material makes up the mobile phase, whereas a polymer or liquid might constitute the stationary phase. Elevated temperatures (up to 350 degrees Celsius) or temperature gradients are frequently necessary for the process to enable chemical elution into the mobile gas phase.

The analytes are first separated by column time, and then they are injected into the gas phase of the mass spectrometer to be ionised. This is commonly done [29] with the help of EI sources to facilitate MS detection. Using the kinetic energy of a stream of high-energy electrons (usually 70 eV) to remove electrons from analyte molecules, EI ionisation is carried out at high temperatures. By using this method, organic substances can be transformed into a uniform fragmentation pattern. with this reason, extensive EI-GC-MS libraries have been assembled with the aim of spectrum matching-based identification, and EI-GC-MS data is suitable for inter-laboratory spectral comparisons. When used in conjunction with "in-house" libraries, these collections greatly enhance GC-MS's ability to detect previously unseen compounds. This analytical benefit has propelled EI-GC-MS to the forefront of MS specificity-preserving untargeted detection and quantification of tiny molecules.

Because it may be used with almost any type of material, EI-GC-MS is still used in general unknown screening applications. Further, GC-MS is often used in clinical toxicology to validate IA positive results in drug screens. Analytes must be both heat stable and volatile for GC-MS to work, which is a major limitation of the technique. Because of this need, certain analytes need to be chemically derivatized so that the drugs become sufficiently volatile for GC-MS detection. This raises the number of stages and the expenses involved with GC-MS, and it also limits its extension to the examination of a limited number of medications.

Mass Spectrometry Application for Toxicology

The hyphenated forms of mass spectrometry, including gas chromatography, liquid chromatography, and inductively coupled plasma mass spectrometry (GC/LC/ICP-MS), have evolved into powerful analytical tools with potential uses in toxicology. Elements can be determined with ICP-MS, with metals being the most common target. Compounds that are heat-stable and volatile can be analysed using GC-MS, while compounds that are heat-labile and nonvolatile can be analysed using LC-MS. Compounds that are heat-labile and nonvolatile can also be analysed using GC-MS. Toxicological investigations of pharmaceuticals and poisons rely heavily on MS applications due to the analytical adaptability of MS methods, which include exceptional specificity, sensitivity, dynamic range, and the ability to screen large numbers of unrelated compounds.

This is because MS applications can screen vast numbers of chemicals that are not related to one another. In addition to its usage in focused applications (like pain management and TDM), screening applications (including DOA, forensic toxicology, environmental toxicology, and clinical toxicology), and pharmacokinetics and pharmacodynamics (PK/PD), drug analysis is also finding a place in clinical research. In this section, we will focus on the capabilities of GC-MS, LC-MS, ICP-MS, and MS/MS, as well as the numerous uses these types of instruments have in the field of toxicology.

Vitamins and Related Metabolites

When it comes to assessing vitamin D and the metabolites that it creates, LC-MS is the method of choice. Using LC-MS methods, 25-hydroxyvitamin D2 and D3 in plasma and serum may be accurately measured. Vitamins K and E, both of which are fat-soluble, have their own analogues of these analyses.

Liquid Chromatography Mass Spectrometry LC-MS/MS Applications for Drug Analysis

Direct analysis of non-volatile and heat-labile compounds is now possible in toxicology laboratories thanks to LC-MS applications. This is because GC-MS can only be used to analyze compounds that are heat-stable or volatile. Mass spectrometry and liquid chromatography could be coupled for the first time when API-ESI sources became widely available in the 1990s. This allowed for the direct injection of ions for MS analysis after materials had been ionized while still in the condensed phase.

When compared to EI, a procedure used in GC, ESI stands out as a gentle ionisation technique that avoids fragmentation. Proton transfer processes cause intact molecules to produce charged ions, either singly or in groups, through ESI. Before spraying the solvent aerosol into the MS vacuum, ESI employs a capillary tube to transport it over a voltage potential. The next step is to spritz the solvent into the vacuum of the MS system. After the droplets have dried, a hot gas, such as nitrogen, is used to liberate the ions from the gas phase, which are then detected by MS. This process does not necessitate a hoover. On the other hand, depending on the voltage applied and the protonation and deprotonation processes that occur, the aerosol droplets can be positively or negatively charged, delivering intact [M+H]+ or [M-H]- ions for MS analysis. The precise process by which ESI produces ions remains a bit of a mystery. It would appear that the maximum size of a molecule that can be ionised by ESI in biological samples is still unknown. It appears to be the case,

at least. Multiple m/z peaks can be produced by ESI because a single molecule can experience numerous protonation and deprotonation processes.

This factor can add difficulty to the MS analysis, make it easier to see m/z from targets with MWs outside the instrument's sensitivity range, or both. The mass spectra of a given molecule could fluctuate based on the settings of the instrument, such as the capillary diameter, the sample flow rate, and the voltage that is being applied. This restriction is associated with the ESI procedure and, by extension, LC-MS.

Because of this, ESI mass spectra are instrument dependant, which means that compound analysis needs the building of in-house derived spectrum libraries. Despite this, LC-MS has significantly expanded MS applications to targeted drug investigation of non-volatile and heat-labile compounds such drug metabolites, whereas GC-MS was limited in this regard. One of these limitations is that drug metabolites cannot be analyzed. LC-MS has surpassed GC-MS as the preferred technique for evaluating drug and toxin metabolites in toxicology. This is in part because most drugs and toxins ingested undergo biotransformation during phase I (functionalization) and phase II (conjugation with hydrophilic endogenous molecules) of the metabolic reactions they undergo in the body before being excreted.

Conclusion:

In general, the future of LC-MS/MS seems promising because manufacturers are continuously working to advance, automate, and simplify the technology. This is helping to make the very complex instrumentation more similar to automated chemical analyzers that are approved by the FDA. Eventually, the availability of multiple operators will be enabled by LC-MS/MS devices that are easier to use and more automated. LC-MS/MS will become more accessible to more labs as a result of the availability of additional ready-to-use reagent kits that have been approved by the FDA. These kits will reduce the amount of effort required for method development and lengthy validation. More clinical applications will be possible with further increases in sensitivity, specificity, and throughput.

New applications in metabolomics and metallomics are now being researched, despite the fact that LC-MS/MS is already a cornerstone in several different areas of clinical laboratories. In addition, some organizations are already utilizing MS/MS outside of the clinical laboratory. One such organization is the Imperial College in London, which uses it in the operating room to discriminate between normal and malignant tissue using a surgeon's knife that is connected to the MS. These advancements point to an interesting and promising future for LC-MS/MS as well as for clinical laboratory personnel who are willing to accept the challenge of coping with the field's ongoing scientific and technological development.

ESI is used to introduce ions from liquid samples into the MS so that nonvolatile and heat-labile substances can be analyzed using LC-MS. This allows LC-MS to circumvent the constraints described above. As a result, GC-MS is being gradually replaced by LC-MS as the method of choice for analyzing toxins, medicines, and their metabolites. The LC-MS method has a number of drawbacks, the most notable of which are its expensive price tag and its inability to employ inter-laboratory spectra for compound identification. To this day, advanced laboratories make use of both GC and LC-MS, in addition to MS/MS and MSn applications, with the purpose of increasing the level of specificity in drug identification, drug metabolite analysis, and structural determination. In conclusion, ICP-MS is frequently utilized in toxicology laboratories for the analysis of trace as well as dangerous metals.

The capability of ICP-MS to perform multi-element panels in toxicological investigation, in addition to the utilization of MS/MS, HR-MS, and DRC applications for the resolution of interfering chemicals, is one of the method's primary benefits. In general, mass spectrometry is a flexible analytical instrument that can be applied to a wide variety of different fields and has the potential to be

automated. In general, the trends for adopting MS applications for toxicology rely on the capability to multiplex quantitative and qualitative chemical assessments as well as hyphenated MS applications with higher mass resolution for increased analytical specificity. This is because multiplexing allows for more analytical specificity.

References:

1. Viette, V., Hochstrasser, D., and Fathi, M. (2012) LC-MS (/MS) in Clinical Toxicology Screening Methods, Chimia 66, 339-342.
2. Goulle, J. P., Saussereau, E., Mahieu, L., and Guerbet, M. (2014) Current role of ICP-MS in clinical toxicology and forensic toxicology: a metallic profile, Bioanalysis 6, 2245-2259.
3. Shannon, M., Cox, M. N., and Baum, C. R. (1998) Toxicology reviews: Immunoassay in detecting drugs of abuse, Pediatr Emerg Care 14, 372-375. 7. Holstege, C. P., and Borek, H. A. (2012) Toxidromes, Crit Care Clin 28, 479
4. Pappas, A. A., Massoll, N. A., and Cannon, D. J. (1999) Toxicology: Past, present, and future, Ann Clin Lab Sci 29, 253-262.
5. Ettre, L. S., and Sakodynskii, K. I. (1993) Tswett,M.S. And the Discovery of Chromatography .2. Completion of the Development of Chromatography (1903-1910), Chromatographia 35, 329-338.
6. Pippenger, C. E. (1979) Therapeutic drug monitoring: an overview, Ther Drug Monit 1, 3-9. Wen, B., and Zhu, M. S. (2015) Applications of mass spectrometry in drug metabolism: 50 years of progress, Drug Metab Rev 47, 71-87.
7. Glish, G. L., and Vachet, R. W. (2003) The basics of mass spectrometry in the twenty-first century, Nat Rev Drug Discov 2, 140-150.
8. Grebe, S. K. G., and Singh, R. J. (2011) LC-MS/MS in the Clinical Laboratory – Where to From Here?, Clin. Biochem. Rev. 32, 5-31.
9. Garg, U., and Zhang, Y. V. (2016) Mass Spectrometry in Clinical Laboratory: Applications in Therapeutic Drug Monitoring and Toxicology, Methods Mol Biol 1383, 1-10.
10. Maurer, H. H., and Meyer, M. R. (2016) High-resolution mass spectrometry in toxicology: current status and future perspectives, Arch Toxicol 90, 2161-2172.
11. Hart-Smith, G., and Blanksby, S. J. (2012) Mass analysis, in Mass Spectrometry in Polymer Chemistry (eds C. Barner-Kowollik, T. Gruendling, J. Falkenhagen and S. Weidner), Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany.
12. Nair, H., Woo, F., Hoofnagle, A. N., and Baird, G. S. (2013) Why GCMS Remains an Invaluable Tool in a Toxicology Lab: A Novel Approach to Clinical Validation of a High Sensitive GCMS Platform for Urine Drug Screening, American journal of clinical pathology 140, 431-431.
13. Ojanpera, I., Kolmonen, M., and Pelander, A. (2012) Current use of high-resolution mass spectrometry in drug screening relevant to clinical and forensic toxicology and doping control, Anal Bioanal Chem 403, 1203-1220.
14. Becker, J. O., and Hoofnagle, A. N. (2012) Replacing immunoassays with tryptic digestion-peptide immunoaffinity enrichment and LC-MS/MS, Bioanalysis 4, 281-290.
15. Strathmann, F. G., and Hoofnagle, A. N. (2011) Current and future applications of mass spectrometry to the clinical laboratory, American journal of clinical pathology 136, 609-616.
16. Jannetto, P. J., and Fitzerald, R. L. (2016) Effective Use of Mass Spectrometry in the Clinical Laboratory, Clin Chem 62, 92-98.

17. Adaway, J. E., Keevil, B. G., and Owen, L. J. (2015) Liquid chromatography tandem mass spectrometry in the clinical laboratory, Ann Clin Biochem 52, 18-38.

18. Lynch, K. L., Breaud, A. R., Vandenberghe, H., Wu, A. H. B., and Clarke, W. (2010) Performance evaluation of three liquid chromatography mass spectrometry methods for broad spectrum drug screening, Clin Chim Acta 411, 1474-1481.

19. Senda N, Kohta K, Takahashi T, Shizukuishi K, Mimura T,Fujita T, Nakayama M. 1995. A highly sensitive method toquantify triazolam and its metabolites with liquid chro-matography±mass spectrometry. Biomed Chromatogr9:48±51.

20. Joyce JR, Ardrey RE, Lewis AS. 1985. Thermospray LC/MSapplied to the analysis of complex mixtures of non-polarmaterials. Biomed Mass Spectrom 12:588±592.

21. Heeremans CEM, Stijen AM, Van der Hoeven RAM, NiessenWMA, Danhof M, Van der Greef J. 1991. Liquidchromatography-thermospray mass spectrometry for iden-ti®cation of a heptabarbital metabolite and sample work-upartifacts. J Chromatogr 554:205±214.

22. Henion JD, Thomson BA, Dawson PH. 1982. Determination ofsulfa drugs in biological ¯uids by liquid chromatography/mass spectrometry/mass spectrometry. Anal Chem 54:451±456.

23. Hieda Y, Kashimura S, Hara K, Kageura M. 1995. Higlysensitive and rapid determination of theophylline, theo-bromine and caffeine in human plasma and urine bygradient capillary high-performance liquid chromatography-frit-fast atom bombardment mass spectrometry. JChromatogr 667:241±246.

24. Higton DM, Oxford JM. 1995. Mass spectrometry: pharmaceutical sciences. Appl Spectroscopy Rev 30:81±118.Hill RE. 1990. The widening horizons of bioanalytical mass-spectrometry. Clin Chim Acta 194:1±18.

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

26. Jeanville PM, EstapeÂES, Needham SR, Cole MJ. 2000. Rapidcon ®rmation/quantitation of cocaine and benzoylecgoninein urine utilizing high performance liquid chromatography and tandem mass spectrometry. J Am Soc Mass Spectrom11:257±263.

27. Katagi M, Nishikawa M, Tatsuno M, Miyazawa T, TsuchihashiH, Suzuki A, Shirota O. 1998. Direct analysis of methamphetamine and amphetamine enantiomers inhuman urine by semi-microcolumn HPLC electrospray ionization mass spectrometry. Jpn J Toxicol EnvironHealth 44:107±115

28. Sato K, Moore CM, Kobayashi K, Mizuno Y, Katsumata Y.1992b. Analyses of drugs by capillary high performanceliquid chromatography/fast atom bombardment-mass spec-trometry, Part 2 Identi®cation of chlordiazepoxide and itsmetabolites in human serum. Jpn J Forensic Toxicol10:23

29. Singh G, Arora V, Fenn PT, Mets B, Blair I. 1999. A validatedstable isotope dilution liquid chromatography tandemmass spectrometry assay for the trace analysis of cocaineand its major metabolites in plasma. Anal Chem 71:2021±2027.