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REVIEW

Current role of LC-MS in therapeutic drug monitoring

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Abstract The role of liquid chromatography coupled with mass spectrometry (LC-MS) techniques in routine therapeutic drug monitoring activity is becoming increasingly important. This paper reviews LC-MS methods published in the last few years for certain classes of drugs subject to therapeutic drug monitoring: immunosuppressants, antifungal drugs, antiretroviral drugs, antidepressants and antipsychotics. For each class of compounds, we focussed on the most interesting methods and evaluated the current role of LC-MS in therapeutic drug monitoring.

Keywords Therapeutic drug monitoring · Liquid chromatography · Mass spectrometry

Introduction

Theoretically, therapeutic drug monitoring (TDM) concerns only a small number of classes of drugs that represent a few dozen compounds in total. Improvement to patient care based on blood concentration measurements of these drugs has been clearly established and consequently the analytical methods used for quantification have to provide accuracy, specificity, selectivity and productivity. Nowadays, immunoassays are used worldwide, mainly for the TDM of certain immunosuppressants, antibiotics or anticonvulsants. However, these technologies are unspecific for certain drugs and are subject to interferences. Moreover, the costs of reagents and consumables are substantial. Apart from immunoassays, TDM can be performed using liquid chromatographic techniques coupled to specific detectors such as UV-diode array detectors (LC-DAD) which are able to cover a very large panel of relevant compounds. Nevertheless, the limited specificity of UV spectra, their variability as a function of pH and the fact that a lot of compounds present poor UV absorbance, render LC-DAD that are neither very specific, reliable nor universal. Initially, the LC-MS techniques were mainly used in research, due to the high financial investments involved and the technical difficulties encountered in setting up procedures for routine activity. Since then, these technologies have been made available for an increased number of users as the cost has been significantly reduced and the application has been simplified. Obviously, the question for the head of a laboratory is no longer whether he can afford to invest in an LC-MS system or not, but to decide which methods should be transferred from classical immunoassays or other chromatographic techniques to LC-MS, and which new quantification procedures should be developed using this technology. As an illustration of this tendency, numerous LC-MS procedures dedicated to TDM have been published in the last few years.

In this paper, we focus on the most recent studies reported for the improvement of TDM of immunosuppressants, antifungal drugs, antiretroviral drugs, antidepressants and antipsychotics, and discuss the current role of LC-MS in the management of these drugs.

Immunosuppressants

The TDM of immunosuppressants currently concerns cyclosporine A (CsA), everolimus, sirolimus, tacrolimus and mycophenolic acid (MPA). The therapeutic ranges of CsA and above all MPA are much higher than those of everolimus, sirolimus and tacrolimus. Three principal

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analytical technologies are widely used in their TDM: immunoassays, liquid chromatography coupled with diode-array detection (LC-DAD), and liquid chromatography coupled with single or tandem mass spectrometry (LC-MS/(MS)) and recent reviews underline the increasing importance of LC-MS [1–3]. Indeed, numerous studies comparing performances between immunoassays and other chromatographic techniques showed that results obtained using immunoassays are overestimated due to cross-reactivity of antibodies with metabolites.

We will only consider here papers published since these lastest reviews, including methods describing the quantification of metabolites (see Table 1). Brandhorst et al. recently reported a simple LC-MS/MS method for the determination of MPA and its glucuronide metabolites after protein precipitation of 100 μL of serum [4]. Quantification of MPA in saliva [5] or plasma [6, 7] after simple protein precipitation with a similar sample volume has also been described. These techniques involve chromatographic runs which do not exceed 8 min. Previous techniques [9–13] investigated in a former review [2] generally required longer chromatographic runs and sample preparation procedures. However, as signalled in a short paper [14], though analytical run time can be shortened, appropriate chromatographic separation between MPA and its glucuronide derivative (MPAG) is essential, due to in-source fragmentation of MPAG into the MPA aglycone. LC-DAD is widely used in the clinical laboratory for TDM of MPA [15–20]. It involves longer extraction procedures (generally liquid–liquid extraction (LLE)) [17, 18] and chromatographic runs. Solid-phase extraction (SPE) tends to be used more often now [15, 19]. Interestingly, MPA was investigated in a recent paper presenting an on-line sample preparation procedure and UV detection at 306 nm [15]. Furthermore, among the most recent papers, an easier sample preparation procedure, i.e. protein precipitation, seems to be applicable upfront to this mode of detection [16].

Few methods [21–26] concerning the determination of CsA (sometimes with its metabolites) have been published since the last reviews [1–3]. Koseki et al. [21] developed a method using 100 μL of blood sample that allows the simultaneous LC-MS/MS determination of CsA and its main metabolites in 10 min. These authors used a triple quadrupole with an APCI source and selected reaction monitoring (SRM) in the positive mode and reported a 1–2,500 μg/L analytical range for each analyte. Vollenbroeker et al. [24] presented an LC-MS method for the determination of CsA and some of its metabolites in blood sample using single ion monitoring (SIM) but with a very long chromatographic run (65 min). Salm et al. [22] published a method for CsA with a chromatographic run of 2 min, linear from 10 to 2,000 μg/L using a simple protein precipitation of 50 μL of blood sample followed by SPE on a C18 cartridge, SRM detection being performed on a triple quadrupole instrument equipped with an electrospray interface working in the positive mode. Keevil et al. [26] had previously published a method with a 1.5-min chromatographic run time obtained by using a triple quadrupole mass spectrometer fitted with a Z Spray ion source.

Although CsA has no chromophores it can also be measured by HPLC with ultraviolet detection at relatively low wavelengths (i.e. <230 nm) [1]. Of note, this technology was considered as the gold standard throughout the world for CsA measurement a decade ago [27]. However, longer extraction procedures (e.g. liquid–liquid extraction) are necessary to remove interferences from matrix, and the lack of specificity of the technique when using low wavelength is obvious.

Due to its low therapeutic concentrations and weak UV absorbency, UV detection seems to be inadequate for tacrolimus. To the best of our knowledge, no new method allowing the specific quantification of tacrolimus has been published since the last reviews [1–3], except a recent paper presenting a quantitative method for three isomeric metabolites of tacrolimus with a run time of 10 min [31]. Older techniques were also characterized by short chromatographic runs, limits of quantification which did not exceed 0.2 μg/L [28, 29] and sometimes included metabolites [30].

The recent LC-MS(/MS) techniques for the quantitation of sirolimus or everolimus in blood were characterized by the small sample volumes needed, fast sample preparation and short chromatographic runs [32, 33]. Using only 100 μL of blood, Taylor et al. [32] described an SRM method with a 2-min run time in positive mode using a triple quadrupole equipped with an electrospray ionization source that allows the quantitation of everolimus from 0.5 to 40 μg/L. Moreover, Korecka et al. [33] recently published an LC-MS/MS method using a triple quadrupole mass spectrometer working in the positive mode for measurement of everolimus in 2.8 min with a limit of quantification of 1 μg/L. This procedure was based on-line extraction and cleanup of the injected sample with subsequent introduction into the mass spectrometer by using a built-in Valco switching valve.

Methods using UV detection have recently been published for sirolimus and everolimus [34, 35]. Despite good analytical results, they often rely on time-consuming LLE procedures and much longer analytical runs than above-mentioned LC-MS/MS procedures.

As patients' treatment often consists in a combination of different immunosuppressants, there is great interest in developing methods which include several drugs in a single procedure. Some studies reporting the simultaneous determination of combined immunosuppressants have been published [36–44]. Briefly, the following strategies were

Table 1 Overview of techniques using LC-MS(/MS) for therapeutic drug monitoring of immunosuppressants in humans

Compound(s)	Sample preparation	Internal standard	Stationary phase	Mobile phase	Detection mode	Run time (min)	Validation data	Analytical range
MPA and its phenol glucuronide, (MPAG) and acyl glucuronide (AcMPAG) metabolites [4]	Protein precipitation of 100 μL of serum followed by on-line solid-phase extraction	Carboxyether of mycophenolic acid (MPAC)	Oasis HLB and Aqua Perfect C_{18}	Aqueous ammonium acetate, methanol and acetonitrile	Triple stage, ESI, positive mode, SRM	4	Selectivity, linearity, LOQ, accuracy and precision, recovery, applicability	MPA 0.1–50 mg/L, MPAG 1–500 mg/L, AcMPAG 0.05–10 mg/L
MPA [5]	Protein precipitation of 100 μL of saliva	Indomethacin	Zorbax Rx C_8	Formic acid in water and methanol	Triple stage, ESI, negative mode, SRM	8	Selectivity, linearity, LOD, LOQ, accuracy and precision, recovery, stability (bench-top, freeze/thaw), applicability	MPA 2.5–800 μg/L
MPA and MPAG [6, 7]	Protein precipitation of 100 μL of plasma	Indomethacin	Nucleosil C18	Aqueous ammonium formate and acetonitrile	Triple stage, ESI, negative mode, SRM	6	Selectivity, linearity, LOD, LOQ, accuracy and precision, applicability	MPA 0.1–30 mg/L, MPAG 1–300 mg/L.
MPA and MPAG [8]	SPE (50 μL of serum)	Carboxybutoxy ether of MPA	Atlantis dC18	Aqueous ammonium acetate and methanol	Triple stage, ESI, positive mode, SRM	7	Selectivity, linearity, LOQ, recovery, applicability	MPA 0.1–16 mg/L, MPAG 1–200 mg/L
MPA and MPAG [38]	On-line sample extraction using 50 μL of plasma	Cyclosporin D	POROS perfusion	Ammonium acetate and methanol with acetic acid	Triple stage, ESI, positive mode, SRM	5	Linearity, accuracy and precision, recovery, LOQ, applicability	MPA 0.05–50 mg/L, MPAG 0.1–120 mg/L
CsA and metabolites [21]	Protein precipitation using 100 μL of whole blood	PSC833	Symmetry C8	Acetic acid in water and methanol	APCI, positive mode, SIM	10	Selectivity, linearity, LOQ, accuracy and precision, stability (bench-top, processed sample, freeze/thaw, long term), applicability	CsA 1–2,500 μg/L
CsA [22]	Protein precipitation using 50 μL of whole blood followed by a solid-phase extraction	Cyclosporin D12	Sep-Pak C18 (100 mg) and Zorbax Bonus C18	Aqueous ammonium acetate and methanol	Triple stage, ESI, positive mode, SRM	2	Selectivity, linearity, accuracy and precision, recovery, stability (processed sample), applicability	CsA 10–2,000 ng/mL
CsA [23]	On-line solid phase extraction using 50 μL of plasma	Cyclosporin C	Zorbax C18	Aqueous ammonium acetate and THF	Triple stage, ESI, positive mode, SRM	3	Linearity, accuracy and precision, applicability	CsA 0.977–4,000 ng/mL
CsA and metabolites [24]	SPE (250 μL of whole blood)	Cyclosporin D	Hypersil MOS	Acetonitrile and water	ESI, positive mode, SIM	65	Not available	Not available
CsA [25]	Solid-phase extraction using 500 μL of saliva	Cyclosporin C	Aqua Perfect C18	Aqueous ammonium acetate and methanol	Triple stage, ESI, positive mode, SRM	5	Linearity, LOD, LOQ, accuracy and precision, recovery, stability (bench-top, freeze/thaw), applicability	CsA 1–300 ng/mL
Three metabolites of tacrolimus [31]	LLE (250 μL of whole blood or plasma)	FR298701 FR290918	Genesis C18	Aqueous ammonium acetate and methanol with formic acid	Triple stage, ESI, positive mode, SRM	10	Specificity, linearity, accuracy and precision, recovery, LOQ, stability (bench-top, freeze/	Each metabolite 0.2–20 ng/mL

Table 1 (continued)

Compound(s)	Sample preparation	Internal standard	Stationary phase	Mobile phase	Detection mode	Run time (min)	Validation data	Analytical range
							thaw, long term), applicability	
Everolimus [32]	Protein precipitation using 100 μL of whole blood	40-*O*-(3′-Hydroxy) propyl-rapamycin	TDM C18	Aqueous ammonium acetate and methanol with formic acid	Triple stage, ESI, positive mode, SRM	2	Selectivity, linearity, LOQ, accuracy and precision, recovery, stability (bench-top), applicability	Everolimus 0.5–40 ng/mL
Everolimus [33]	On-line sample extraction using whole blood after protein precipitation	SDZ RAD 223–756 or ascomycizn	Nova-Pak C18	Aqueous ammonium acetate and methanol	Triple stage, ESI, positive mode, SRM	2.8	Linearity, accuracy and precision, LOQ, recovery, applicability	Everolimus 1–50 ng/mL
Sirolimus, tacrolimus and CsA [41]	On-line solid-phase extraction using 500 μL of whole blood	Ascomycin, desmetho-xysirolimus, and cyclosporine G	Allure C18	Acetonitrile and water	ESI, positive mode, SIM	3	Linearity, accuracy and precision, LOQ, recovery, applicability	Tacrolimus, sirolimus 1.0–80.0 ng/mL, CsA 25–2,000 ng/mL
Tacrolimus and sirolimus R[44]	Protein precipitation using 80 μL of whole blood	Ascomycin and desmethoxy-rapamycin	C18	Aqueous ammonium acetate and methanol with formic acid	Triple stage, ESI, positive mode, SRM	2.5	Linearity, accuracy and precision, recovery, applicability	Tacrolimus 0.52–155.5 ng/mL, sirolimus 0.47–94.8 ng/mL

AcMPAG acyl mycophenolic acid glucuronide, *CsA* cyclosporin A, *ESI* electrospray ionisation, *LOD* limit of detection, *LOQ* limit of quantification, *MPA* mycophenolic acid, *MPAG* mycophenolic acid glucuronide, *SPE* solid-phase extraction, *SRM* selected reaction monitoring

employed: (i) direct injection of the supernatant obtained after centrifugation, (ii) injection of a reconstituted sample obtained after drying the supernatant, (iii) on-line sample preparation such as SPE or turbulent flow chromatography. Contrary to the other modes of detection, in these studies the chromatographic separation of all compounds of interest was not as necessary because the authors employed the highly specific tandem mass spectrometry. However, some pitfalls inherent to the use of mass spectrometry, such as matrix effects and/or ion suppression [45], had to be thoroughly investigated when such high-throughput sample preparation was involved.

In conclusion, immunoassays are still widely used for the analysis of immunosuppressants. However, recent studies showed their important drawbacks due to their cross-reactivity with metabolites. Although LC-DAD technologies are less expensive, extraction procedures used are often time-consuming and analytical runs are relatively long. As illustrated by the recent tendency to transfer immunosuppressant measurements from immunoassays to LC and owing to the possibility of simultaneous analysis of several immunosuppressants, LC-MS/MS may become the mainstay technology for this TDM in the near future. The main advantages of the methods are minimum sample preparation, short analytical runs, lower limits of detection and high selectivity (theoretically, absence of interfering compounds). Moreover, although the investment cost is important, profitability can be rapidly obtained because of the minor cost of reagents.

Antifungal drugs

Numerous antifungal drugs have been commercialized in the last few years, with new azole derivatives (posaconazole, voriconazole), new classes of compounds (echinocandins) or new galenic formulations (lipidic forms of amphotericin B). Among antifungal drugs, only those used for the treatment of invasive and disseminated fungal infections are eligible for TDM, namely flucytosine, amphotericin B, caspofungin and azole derivatives (itraconazole, fluconazole, voriconazole and posaconazole). The pharmacokinetics and pharmacodynamics of these compounds have been recently reviewed [46]. Though pharmacokinetic–pharmacodynamic relationships were clearly identified in experimental models, few target plasma concentrations could be determined from studies performed in patients [47]. Thus, the relation between plasma concentrations and efficacy is still ambiguous for most antifungals. Nevertheless, multiple analytical techniques have been published in the last few years for the determination of antifungals in human fluids (see Table 2), and preliminary pharmacokinetic studies suggest that TDM can be useful in most populations, especially patients suffering from major burns or cystic fibrosis [46].

For azole derivatives, only a few LC-MS(/MS) methods have been published up to now, and most of them concerned itraconazole. Numerous methods have been reported to quantify itraconazole and its coactive metabolite hydroxyitraconazole (OH-ITZ) in biological fluids using HPLC coupled to different detection modes. Among them, a method based on SPE and fluorescence detection with a sensitivity of 10 μg/L [48], one using LLE and spectrofluorimetric detection with a sensitivity of 20 μg/L [49], and one based on a sample preparation using protein precipitation and UV detection with a sensitivity of 25 μg/L [50] are available. However, these methods largely used for TDM often lack the selectivity needed for these analyses. Only a few methods for the determination of itraconazole with higher sensitivity using LC-MS(/MS) have been published. Yao M et al. proposed a method with a single mass spectrometer where the detection of ITZ was performed in SIM mode after protein precipitation of 100 μL of plasma [51]. The authors reported that standard curves were linear over the concentration range 4–1,000 μg/L with a recovery of 96%. However, the validated assay was only applied to a pharmacokinetic study of ITZ in rats following administration of a single dose of itraconazole (15 mg/kg). Recently, Young et al. reported a method using a triple quadrupole mass spectrometer operated in the positive mode and using MRM to quantify ITZ [52]. The authors reported good precision and accuracy but the upper limit of the concentration range was 100 μg/L. Vogeser et al. [53] reported an LC-MS/MS method that involves automated SPE for the quantification of ITZ and its active metabolite in human samples, whereas Kousoulos et al. reported a semi-automated procedure based on a LLE [54]. In this last application the extraction process was performed using two liquid-handling robotic workstations, the detection with a triple quadrupole operated in MRM mode, and ranges of concentrations 2–500 μg/L for ITZ and 4–1,000 μg/L for OH-ITZ were obtained. Apart from the gain in sensitivity and selectivity, the main interest of these LC-MS/MS procedures was to offer run times of less than 5 min when coupled to robotic workstations for the extraction procedure.

Numerous clinical reports have been published about voriconazole pharmacokinetics. In these studies various analytical HPLC methods were employed for the determination of voriconazole in serum [55–58]. The first LC-MS method was described by Zhou et al. [59] to study the penetration of voriconazole in the eye of rabbits, and so no sample preparation was required because of the clean aqueous humor samples that were employed. Keevil et al. [60] developed an LC-MS/MS method involving precipitation of proteins with zinc sulfate and acetonitrile before on-

Table 2 Overview of techniques using LC-MS(/MS) for therapeutic drug monitoring of antifungal drugs in humans

Compound(s)	Sample preparation	Internal standard	Stationary phase	Mobile phase	Detection mode	Run time (min)	Validation data	Analytical range
Itraconazole [51]	Protein precipitation on 100 μL of plasma	Nefazodone	BDS Hypersil C_{18}	Aqueous ammonium formate and acetonitrile	ESI, positive mode, SIM	4	Selectivity, linearity, LOQ, accuracy and precision, recovery, stability (autosampler), applicability	Itraconazole 4–1,000 μg/L
Itraconazole [52]	LLE (500 μL of plasma)	Loratadine	YMC C18	Aqueous ammonium formate and acetonitrile	Triple stage, ESI, positive mode, SRM	3	Selectivity, linearity, LOQ, accuracy and precision, applicability	Itraconazole 0.2–100 μg/L
Itraconazole and hydroxyitraconazole [53]	Protein precipitation on 50 μL of plasma	R51012	LiChrospher C_{18}	Aqueous ammonium acetate and acetonitrile	Triple stage, ESI, positive mode, SRM	5	Linearity, accuracy and precision, applicability	Itraconazole 10–10,000 μg/L, hydroxyitraconazole 10–10,000 μg/L
Itraconazole and hydroxyitraconazole [54]	Automated LLE (150 μL of plasma)	R51012	YMC Pack ODS-A (C_{18})	Aqueous ammonium acetate and acetonitrile	Triple stage, ESI, positive mode, SRM	2	Selectivity, linearity, accuracy and precision, recovery, stability (bench-top, freeze/thaw, long term), applicability	Itraconazole 2–500 μg/L, hydroxyitraconazole 4–1,000 μg/L
Voriconazole [59]	Direct analysis on aqueous humor samples	No internal standard	Delta PAK C_{18}	Trifluoroacetic acid in water and acetonitrile	ESI, positive mode, SIM	10	Linearity, accuracy and precision, recovery	Voriconazole 60–1,000 μg/L
Voriconazole [60]	Protein precipitation on 10 μL of serum	Ketoconazole	SecurityGuard C_{18} cartridge	Aqueous ammonium acetate and methanol	Triple stage, ESI, positive mode, SRM	3	Linearity, accuracy and precision, recovery, stability (freeze/thaw, autosampler)	Voriconazole 0.38–15.3 mg/L
Voriconazole [61]	On-line extraction (5 μL of serum)	No internal standard	LiChrospher ADS C_8 and Nucleodur 100–5	Formic acid in water and acetonitrile	Triple stage, ESI, positive mode, SRM	13	Linearity, LOD, LOQ, accuracy and precision	Voriconazole 0.05–5.0 mg/L
Posaconazole [62]	Protein precipitation on 100 μL of plasma	SCH 56984	Polaris C-18A	Formic acid in water, methanol and acetonitrile	Triple stage, APCI, positive mode, SRM	4	Selectivity, linearity, accuracy and precision, recovery, stability (bench-top, long term), applicability	Posaconazole 5–5,000 μg/L
Caspofungin [65]	On-line extraction (5 μL of serum)	No internal standard	Vertex Eurospher 100 Diol and Vertex Eurospher 100 CN	Formic acid in water and acetonitrile	Triple stage, ESI, positive mode, SRM	30	Linearity, LOD, LOQ, accuracy and precision	Caspofungin 0.2–2.0 and 2.0–20.0 mg/L
Amphotericin B [74]	Dilution of 50 μL of plasma and SPE (50 μL of urine, fecal homogenate or ultrafiltrate)	Natamycin	Symmetry C18	Acetic acid in water and methanol	Triple stage, ESI, positive mode, SRM	4	Linearity, accuracy and precision, stability (bench-top, freeze/thaw, long term), applicability	Amphotericin B 2–150 mg/L (plasma), amphotericin B 1–200 μg/L(ultrafiltrate), amphotericin B 0.05–30 mg/L (urine), amphotericin B 0.40–40 mg/L (fecal homogenate)

ESI electrospray ionisation, *LLE* liquid–liquid extraction, *LOQ* limit of quantification, *SIM* single ion monitoring, *SRM* selected reaction monitoring

line chromatographic cleanup and analysis with a triple quadrupole. However, as reported by the authors themselves, this technique was "found to lack interassay reproducibility with the results of a QC sample (HPLC value of 3.026 mg/L) varying from 2.42 to >6.2 mg/L". Recently, Egle et al. [61] reported a "fully automated analysis of voriconazole from serum by LC-MS/MS with parallel column-switching technique". Working with a triple quadrupole coupled with an orthogonal electrospray ionisation source and an ion trap, the authors described the quantification of voriconazole in crude serum without any other form of pre-treatment. The procedure was based on automated on-line extraction with a total sample preparation and analysis time of 13 min, showing sufficient performance and precision. Voriconazole was detected by isolation and fragmentation of the positive pseudomolecular ion (m/z=350.3) and its specific fragment (m/z=281.3) which results from loss of the triazole moiety. A major point in this method was the very small volume of serum (5 μL) required for the analysis, whereas classical HPLC methods for the detection of voriconazole require at least 500 μL. This could be of interest in some special cases.

Although the routine monitoring of posaconazole plasma levels has not been recommended, an accurate assay could be necessary, at least in the context of clinical trials. Based on earlier toxicokinetic data, an analytical method able to measure concentrations as low as 5 μg/L is needed to accurately characterize the human plasma concentration versus time profile of posaconazole. In a recent study, Shen et al. [62] presented a 96-well sample preparation and LC-MS/MS method that was suitable for quantifying posaconazole over a dynamic range of 5–5,000 μg/L. The positive-ion APCI mass spectrum of posaconazole was dominated by the protonated molecular ion at m/z 701, and only showed a m/z 683 fragment (dehydration). The loss of water is not a recommended choice for a selective MRM transition, but according to the authors the difference in signal to noise ratio between the two transitions was sufficient to allow its selection for quantitation of posaconazole.

Very few experimental or clinical data describing the pharmacokinetics of caspofungin are available [63, 64]. However, a specific and reliable method for the measurement of this drug could be necessary. Such a method should be suitable for the determination of clinically relevant caspofungin levels (i.e. 1.2–10.4 mg/L in serum). Egle et al. [65] published a method using an advanced column-switching technique for fully automated analysis of caspofungin in serum without any pre-treatment. The authors reported an extraction performed automatically on-line, using a diol column, and followed by chromatography on a CN column. MS detection was performed with a triple quadrupole. To the best of our knowledge, no other LC-MS technique has been proposed up to now for the determination of caspofungin. However, the authors did not use any internal standard in their method. In our opinion, this strategy is not pertinent. An internal standard is supposed to compensate for the variability in sample preparation, recovery, injection and possible compound degradation. Although it is more the case for GC-MS than for LC-MS, an internal standard is also indispensable to limit detection variability leading to quantification errors. For example, ionization efficiency (i.e. the portion of the total number of molecules ionized when the compound is introduced into the source) varies during day-to-day operation due to variations in several parameters that are impossible to control (the temperature and pressure in the ion source, for instance). Additionally, the signal is further modified by the effect of matrix components present in serum, plasma or urine. Indeed, when the analyte is introduced into the ion source, it competes for ionization with other compounds simultaneously introduced into the source, which decreases the analyte signal (ion suppression) especially with electrospray ionization. The degree of ion suppression caused by matrix components mainly depends on the chemical structure of the analyte. Therefore, analyte and internal standard have to present similar structures to preserve the ratio of analyte and internal standard detector responses. For this reason, internal standards used in quantitative LC-MS(/MS) assays should either be structural analogues or labelled (e.g. deuterated) analogues of the analyte.

Early determination of amphotericin B was performed using microbiological inhibition assays, which lacked selectivity and sensitivity. HPLC methods were then developed for the quantification of amphotericin B in plasma or serum [66–73]. The most sensitive HPLC method reported for amphotericin B in plasma used solid-phase extraction for sample cleanup with a limit of quantitation of 5 μg/L [73]. All these methods concerned the total (free and bound) concentrations of amphotericin B in blood. Lee et al. reported an LC-MS/MS procedure with solid-phase extraction for the determination of total amphotericin B in human plasma and other biologic matrices as well as the free amphotericin B concentration in plasma [74]. Using a triple quadrupole, electrospray interfaced to a C18 analytic column, the authors reported a LOQ of 1 μg/L for ultrafiltrate and LOQs of 2 mg/L in plasma for total amphotericin B, 50 μg/L in urine, and 400 μg/L in fecal homogenate.

The last few years have seen the availability of several new antifungal drugs, providing increased efficacy and safety, particularly in immunosuppressed patients. The choice of the drugs, their combinations and their dose adjustments are still a matter of debate. In this context, the availability of specific and sensitive analytical techniques is probably necessary.

Antiretroviral drugs

The role of TDM for the optimization of antiretroviral therapy has been the focus of previous reviews [75–82]. There is consensus that most antiretroviral agents can be considered as candidates for TDM, as they are characterized by a large inter-individual variability in drug concentrations. Clinical data also suggest a correlation between drug concentrations and toxicity or efficacy. Indeed, plasma determination of antiretroviral drugs [82] is important in relation to: (i) checking compliance, (ii) drug–drug interactions, (iii) physiopathologic status changes, (iv) pregnancy, (v) virological failure, (vi) pediatric patients. Optimal efficacy and prevention of viral resistances require that antiretroviral drugs are administered in combination regimens, which are generally referred to as highly active antiretroviral therapy (HAART). According to current guidelines, HAART generally consists of two nucleoside reverse transcriptase inhibitors (NRTIs) combined with either one or two protease inhibitors (PIs), or with an non-nucleoside reverse transcriptase inhibitors (NNRTI). Additionally, ritonavir is commonly added as a pharmacokinetic enhancer.

Most of the published procedures for the TDM of antiretroviral drugs were developed using LC-DAD [83]. Briefly, large differences amongst assays in lower limits of quantitation for the measurement of PIs have been reported. The review article by Aarnoutse et al. [84] shows that a number of assays are not sensitive enough to measure concentrations below mean trough levels in the population or below the recommended therapeutic ranges. The selectivity of LC-DAD for antiretroviral drugs is another major point in method validation. Apart from endogenous substances, the potential for drug interferences is high, because of the large number of concomitant drugs administered to HIV-infected patients, as well as the presence of numerous metabolites, particularly for PIs. Most of all, in order to increase sensitivity and reduce plasma volume, LC-DAD methods were usually developed using low wavelength ranges, which are not specific enough.

Numerous methods dedicated to the determination of a single compound have been reported [85–88], but, except in particular cases such as pharmacokinetic studies, it seems obvious to develop methods allowing the detection of several antiretroviral drugs in a single procedure. This approach is clearly recommended for a routine activity. In the review of the HPLC methods articles published since 2000 [84, 89, 90], we identified about 25 studies reporting the use of an HPLC technique for the quantification of several NRTIs, NNRTIs or PIs in a single run. Among them, nine were LC-MS or LC-MS/MS techniques (see Table 3) [91–99]. For example, working on a triple quadrupole mass spectrometer, Crommentuyn et al. developed a procedure for the quantification of six PIs (amprenavir, indinavir, lopinavir, nelfinavir, ritonavir and saquinavir) and the pharmacologically active nelfinavir metabolite M8 in plasma [96]. Simple protein precipitation of 100 μL of plasma afforded a linearity over a concentration range 10–10,000 μg/L for indinavir and saquinavir, 100–10,000 μg/L for amprenavir, 50–10,000 μg/L for nelfinavir and ritonavir, 100–20,000 μg/L for lopinavir and 10–5,000 μg/L for M8. The analytical run time was about 5 min. Egge-Jacobsen et al. [91] reported a method for the quantification of six PIs (amprenavir, indinavir, lopinavir, nelfinavir, ritonavir and saquinavir) and three NNRTIs (nevirapine, efavirenz and delavirdine) using a G1946A single quadrupole mass detector. This procedure was based on an on-line extraction with a previous simple precipitation of 100 μL of plasma and presented LOQs ranging from 20 to 100 μg/L for the investigated compounds. The total procedure lasted about 8 min. Volosov et al. [99] developed a method for quantification of most of the marketed anti-HIV drugs in human plasma, using a triple quadrupole. This procedure included an on-line extraction–cleaning step using 80 μL of plasma. Stavudine, zidovudine and efavirenz were analysed in the negative MS/MS mode, while amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, abacavir, didanosine, lamivudine, zalcitabine, delavirdine and nevirapine were analyzed in the positive mode. The procedure allowed the determination of this panel of drugs with a 4.5-min run time. Between-day imprecision was less than 10% for all analytes, accuracy ranged between 95 and 105% (n=20), and the method was linear over the measuring range of each drug (2–2,000 μg/L for stavudine, didanosine, zalcitabine and zidovudine, and 10–10,000 μg/L for all other drugs). Therefore, these examples of LC-MS/MS methods illustrate the feasibility of this technique for the TDM of anti-HIV drugs. LC-MS/MS obviously provides satisfactory results especially when compared with those obtained in recently published LC-DAD methods. For example, a procedure for the determination of 16 anti-HIV drugs using LC-DAD required 600 μL of plasma, for off-line SPE and took 35 min for a single analysis [100], with calibration ranges of, among others, 25–10,000 μg/L for didanosine and lamivudine, and 100–10,000 μg/L for most PIs. In our opinion, such a comparison illustrates the opportunity of a substantial gain in sample volume for the analysis, and in analysis time through the automation of the extraction procedure and simplification of chromatographic separation when using LC-MS/MS.

Total plasma concentrations are currently measured for therapeutic drug monitoring of PIs and NNRTIs. However, the pharmacological target of antiretroviral drugs is inside cells. This suggests that a potential improvement of TDM

Table 3 Overview of techniques using LC-MS(/MS) for therapeutic drug monitoring of antiretroviral drugs in humans

Compound(s)	Sample preparation	Internal standard	Stationary phase	Mobile phase	Detection mode	Run time (min)	Validation data	Analytical range
6 PIs: amprenavir, indinavir, ritonavir, lopinavir, nelfinavir and saquinavir; 3 NNRTIs: nevirapine, efavirenz and delavirdine [91]	Protein precipitation using 100 μL of plasma followed by on-line solid-phase extraction	Pepstatin A	Hypersil MOS \5 μm and Eclipse XDB-C8	Aqueous ammonium acetate and methanol	Positive mode, SIM	9	Selectivity, linearity, LOQ, accuracy and precision, stability and applicability	Amprenavir 0.02–20 mg/L, indinavir 0.04–30 mg/L, ritonavir 0.05–30 mg/L, saquinavir 0.02–10 mg/L, nelfinavir 0.05–20 mg/L, lopinavir 0.04–20 mg/L, nevirapine 0.1–20 mg/L, efavirenz 0.1–20 mg/L, delavirdine 0.1–20 mg/L
4 PIs: indinavir, saquinavir, nelfinavir and amprenavir [92]	LLE (100 μL of plasma)	One of the 4 PIs, depending on the treatment of the patient	QuickSorb ODS	Water and acetonitrile containing acetic acid	APCI, negative mode, SIM	5	Selectivity, linearity, LOQ, accuracy and precision and applicability	Saquinavir 0.003–10 mg/L, nelfinavir 0.005–10 mg/L, amprenavir 0.01–10 mg/L, indinavir 0.05–10 mg/L
6 PIs: amprenavir, indinavir, lopinavir, nelfinavir, ritonavir and saquinavir; 2 NNRTIs: efavirenz and nevirapine [93]	SPE. For amprenavir, efavirenz and indinavir 1 mL of plasma. For lopinavir, nelfinavir, nevirapine, ritonavir and saquinavir 0.1 mL plasma	A-86093	Bond Elut C_{18} and Nucleosil C_{18} HD	Acetonitrile containing methanol and ammonium carbonate	APCI, positive or negative mode, SIM	21	Selectivity, linearity, LOQ, accuracy and precision and applicability	Amprenavir 0.001–12 mg/L, indinavir 0.01–11.8 mg/L, lopinavir 0.94–15.8 mg/L, nelfinavir 0.094–3.94 mg/L, ritonavir 0.47–11.8 mg/L, saquinavir 0.094–3.94 mg/L, efavirenz 0.2–6 mg/L, nevirapine 0.47–11.8 mg/L
5 PIs: amprenavir, indinavir, nelfinavir, ritonavir and saquinavir [94]	Protein precipitation using 100 μL of plasma	Not available	Zorbax XDB C8	Ammonium formate buffer and acetonitrile	Triple stage, ESI, positive mode, SRM	5	Linearity, accuracy and precision, stability (bench-top, freeze/thaw, long term), applicability	5 PIs 0.015–6 mg/L
6 PIs: amprenavir, ritonavir, saquinavir, lopinavir, indinavir and nelfinavir; active metabolite of nelfinavir (M8)[95]	LLE (1,000 μL)	A-86093	Symmetry C18	Acetonitrile and buffer of ammonium acetate	Triple stage, ESI, positive mode, SRM		Linearity, accuracy and precision, stability (bench-top, freeze/thaw, long term), applicability	Amprenavir 0.0163–4 mg/L, indinavir 0.0163–4 mg/L, lopinavir 0.0163–4 mg/L, nelfinavir 0.0163–4 mg/L, saquinavir 0.0163–4 mg/L, ritonavir 0.0512–5 mg/L, M8–nelfinavir 0.0082–5 mg/L
6 PIs: amprenavir, indinavir, lopinavir, nelfinavir, ritonavir and saquinavir; active metabolite of nelfinavir (M8) [96]	Protein precipitation of 100 μL	Indinavir-d6 or saquinavir-d5	Inertsil ODS3	Aqueous ammonium acetate and acetonitrile	Triple stage, ESI, positive mode, SRM	6	Linearity, accuracy and precision, stability (bench-top, freeze/thaw, long term)	Amprenavir 0.05–10 mg/L, indinavir 0.01–10 mg/L, lopinavir 0.1–20 mg/L, nelfinavir 0.05–10 mg/L, ritonavir 0.05–10 mg/L, M8–nelfinavir 0.01–5 mg/L

Table 3 (continued)

Compound(s)	Sample preparation	Internal standard	Stationary phase	Mobile phase	Detection mode	Run time (min)	Validation data	Analytical range
Zidovudine, lamivudine, AZT-5′-glucuronide (AZTG), 3′-amino-3′-deoxythymidine (AMT), AZT-5′-phosphate (AZTP) and 3TC-5′-phosphate (3TCP) [97]	SPE of 10 to 100 μL of plasma	D3-AZT13C2 and 15N33TC	Polaris C18	Formic acid and acetonitrile	Positive and negative modes, SIM	9	Linearity, accuracy and precision, applicability	AZT 0.1–200 mg/L, AZTG 0.1–10 mg/L, AMT 0.1–5 mg/L, 3TC 0.1–20 μg/L
Zidovudine (AZT), lamivudine (3TC) [98]	Fully automated LLE of 250 μL of plasma	Isotope-labelled AZT and isotope-labelled 3TC	Aquasil C18	Acetonitrile and water	Triple stage, ESI, positive and negative modes, SRM	6	Linearity, accuracy and precision, stability (bench-top, freeze/thaw, long term), applicability and cross-validation with an RIA	AZT 0.0025–2.5 mg/L, 3TC 0.0025–5 mg/L
6 PIs: amprenavir, indinavir, lopinavir, nelfinavir, ritonavir and saquinavir; 3 NNTRIs: efavirenz, delavirdine and nevirapine; 6 NTRIs: abacavir, didanosine, lamivudine, stavudine, zalcitabine, zidovudine [99]	On-line SPE of 80 μL of plasma	Cimetidine	LC-18 DB	Not available	Triple stage, ESI, positive and negative modes, SRM	4.5	Selectivity, linearity, LOQ, accuracy and precision and applicability	AZT 0.1–10 mg/L

APCI atmospheric pressure chemical ionisation, *ESI* electrospray ionisation, *LLE* liquid–liquid extraction, *LOQ* limit of quantification, *NNRTI* non-nucleoside reverse transcriptase inhibitor, *NRTI* nucleoside reverse transcriptase inhibitor, *PI* protease inhibitor, *RIA* radio immuno assay, *SIM* single ion monitoring, *SRM* selected reaction monitoring

would be to measure cellular concentrations [101–106]. In this context, the difficulties encountered with sample cleaning and the low concentration ranges targeted required the use of a very sensitive and specific technique. Recently, Colombo et al. [101] studied the variability of cellular accumulation of several PIs and NNRTIs, and evaluated to what extent total plasma concentrations correlate with cellular concentrations in 133 HIV-infected patients. In this study, total plasma concentrations were analysed by LC-DAD but cellular concentrations (peripheral blood mononuclear cells) could only be determined by LC-MS/MS using a triple quadrupole to achieve the analytical sensitivity required for the intracellular measurements (i.e. LOQ <0.4 μg/L). Of note, the best correlations between total plasma concentrations and cellular concentrations were observed for nelfinavir, saquinavir and lopinavir, while no correlation was found for nevirapine, which suggests that monitoring of cellular drug concentrations can be of interest for this compound.

Antidepressants and antipsychotics

Antidepressants are drugs widely prescribed for the treatment of a variety of depressive states and psychiatric disorders. They can be divided in five main classes: tricyclic antidepressants, also called imipraminic antidepressants; monoamine oxidase inhibitors (IMAO); selective serotonin reuptake inhibitors (SSRIs); serotonin-noradrenergic reuptake inhibitors (SNaRIs); and other antidepressants. The wide variety of these compounds increases the difficulty of a reliable TDM.

The analysis of tricyclic antidepressants (especially amitriptyline, nortriptyline, imipramine and desipramine) can be performed using immunoassays [107, 108]. However, the antibodies employed cross-react differently with the different drugs and also with their metabolites, rendering precise quantitation almost impossible. Moreover, there are risks of interferences due to drugs with structural similarity to antidepressants [107, 109].

The TDM of antidepressants is generally performed using chromatographic technologies. Most of the published techniques concerned a single drug (bupropion, citalopram [110–112], doxepin [113], fluoxetine [114–119], fluvoxamine, mianserin [120], milnacipran, mirtazapine [121], paroxetine [122, 123], sertraline [124, 125], venlafaxine [126]), sometimes with its metabolites, and employed GC, LC-DAD or LC-MS(/MS). Sample preparations consisted in most cases in LLEs or SPEs. A few multi-compound techniques [126–128] have also been developed using these same detection modes. Owing to the development of high-throughput technologies such as on-line SPE with column switching or turbulent flow chromatography, new opportunities have appeared. These recent papers present methods allowing the determination of several compounds in a single run and require a small sample volume, contrary to SPE or LLE for which not less than 500 μL is classically necessary. The main published techniques based on LC-MS (/MS) are summarized in Table 4.

According to this table, acquisitions are usually performed in the positive mode, mainly using electrospray ionization sources, either in SIM or MRM modes with run times varying from 3 to 10 min, the shortest run times being characteristic of MRM acquisitions. When LLE and SPE procedures are employed, sample volumes higher than 500 μL are often necessary to reach the therapeutic ranges of the investigated drugs. Newer methods allow the quantitation of several molecules at the same time and, owing to the emergence of automated systems allowing on-line sample extraction, a lower sample volume is needed.

As an example, Kollroser et al. [127] and Souverain et al. [117] developed methods for the determination of antidepressants using on-line sample extraction with column switching coupled with mass spectrometry, requiring only 50 μL of plasma. More recently, Sauvage et al. [128] developed a quantitative method for 14 antidepressants and their respective metabolites using turbulent flow chromatography coupled with a triple quadrupole (Fig. 1). This procedure used only 100 μL serum and presented calibration curves ranging from 10 to 500 μg/L. Working with a triple quadrupole, Kirchherr et al. also published a method for the quantitation of 48 antidepressants and antipsychotics based on a sample of 50 μL of serum, using a monolithic column with injection intervals of 8 min [129]. These last two applications yielded good performances in terms of limit of quantification, accuracy and inter- or intra-assay precision, at least comparable to those of methods using higher sample volume, longer extraction steps and chromatographic runs.

The antipsychotics for which TDM is performed belong to a large variety of chemical families (phenothiazines, butyrophenones, benzamides, thioxanthenes, diphenylbutylpiperazines etc.) and present various modes of action and therapeutic ranges [131–133]. Most of the published methods concerning this therapeutic class concerned a single compound (amisulpride, aripiprazole, clozapine, droperidol, haloperidol, olanzapine, perospirone, perphenazine, pimozide, quetiapine, risperidone and its metabolite, sertindole, sulpiride, sultopride, tiapride, ziprasidone, zuclopenthixol). A lesser number dealt with several compounds. In most cases, LC was used, with different detection modes (UV, single or tandem mass spectrometry, electrochemical or fluorimetric detection).

Less methods using LC-MS(/MS) for antipsychotics have been published compared to antidepressants and these

Table 4 Overview of techniques using LC-MS(/MS) for therapeutic drug monitoring of antidepressants in humans

Compound	Sample preparation	Internal standard	Stationary phase	Mobile phase	Detection mode	Run time (min)	Validation data	Analytical range
Citalopram [110]	LLE (500 μL of plasma)	Imipramine	Hypersil BDS C8	Aqueous ammonium formate and acetonitrile	ESI, positive mode, SIM	10	Linearity, accuracy and precision, recovery, LOQ, stability (bench-top, freeze/thaw, long term), applicability	Citalopram 0.50–250 ng/mL
Escitalopram [111]	LLE (1,000 μL of plasma)	Paroxetine	ODS YMC AQ	Aqueous ammonium acetate and acetonitrile	ESI, positive mode, SIM	7	Selectivity, linearity, accuracy and precision, LOQ, stability (bench-top, freeze/thaw, long term), applicability	Escitalopram 1–200 ng/mL
Citalopram, fluvoxamine and paroxetine [112]	On-line SPE with column switching using 50 μL of plasma	Dibenzepin	Oasis HLB and Symmetry C18	Formic acid in water and acetonitrile	Triple stage, APCI, positive mode, SRM	6	Selectivity, linearity, accuracy and precision, LOD, LOQ, recovery	Citalopram and fluvoxamine 20–800 ng/mL, paroxetine 10–600 ng/mL
Doxepin and nordoxepin [113]	LLE (500 μL of plasma)	Benzoctamine	Luna C_{18}	Formic acid in methanol and water	Triple stage, ESI, positive mode, SRM	3	Linearity, accuracy and precision, LOQ, recovery, applicability	Doxepin 0.32–81.1 ng/mL, nordoxepin 0.18–45.1 ng/mL
Fluoxetine and norfluoxetine [114]	LLE (1,000 μL of plasma)	Flumazenil	Lichrospher 100 RP-8 E	Aqueous ammonium formate and acetonitrile	ESI, positive mode, SIM	6	Linearity, accuracy and precision, recovery, LOD, LOQ, applicability	Fluoxetine 2.5–250 ng/mL, norfluoxetine 10–250 ng/mL
Fluoxetine and norfluoxetine [115]	LLE (200 μL of plasma)	Deuterated fluoxetine	Zorbax SB-C18	Aqueous ammonium formate and acetonitrile	Triple stage, ESI, positive mode, SRM	5	Linearity, accuracy and precision, recovery, applicability	Fluoxetine and norfluoxetine 0.27–22 ng/mL
Fluoxetine and norfluoxetine [116]	LLE (1,000 μL of plasma)	Doxepin	Luna C_{18}	Formic acid in acetonitrile and water	Triple stage, ESI, positive mode, SRM	2.6	Linearity, accuracy and precision, recovery, stability (short term), applicability	Fluoxetine 0.15–79.1 ng/mL, norfluoxetine 0.15–78.8 ng/mL
Fluoxetine and norfluoxetine [117]	On-line extraction using column	Methylfluoxetine	Oasis HLB and Discovery	Formic acid in acetonitrile	ESI, positive mode,	4	Linearity, accuracy and precision, LOQ, applicability	Fluoxetine and norfluoxetine 25–1,000 ng/mL

	switching using 50 μL of plasma		HS C18	and water	SIM			
Fluoxetine [118]	Stir bar sorptive extraction (1,000 μL of plasma)	Desipramine	Luna C_{18}	Aqueous ammonium acetate and methanol	ESI, positive mode, SIM	7	Selectivity, linearity, accuracy and precision, LOD, LOQ, recovery, applicability	Fluoxetine 10–500 ng/mL
Fluoxetine and norfluoxetine [119]	Automated SPE (500 μL of plasma)	Fluvoxamine	XTerra MS C_{18}	Formic acid in methanol and water	Triple stage, ESI, positive mode, SRM	4	Selectivity, linearity, accuracy and precision, LOQ, recovery, stability (bench-top, freeze/thaw, long term), applicability	Fluoxetine and norfluoxetine 0.5–50 ng/mL
Mianserin and *N*-desmethylmianserin [120]	LLE (1,000 μL of plasma)	Imipramine	Kromasil RP-18	Aqueous ammonium formate, acetonitrile and methanol	ESI, positive mode, SIM	3	Linearity, accuracy and precision, LOQ, recovery, stability (bench-top, freeze/thaw, autosampler), applicability	Mianserin 1–60 ng/mL, *N*-desmethylmianserin 0.5–14 ng/mL
Mirtazapine and demethylmirtazapine [121]	LLE (500 μL of plasma)	Imipramine	XTerra MS C8	Aqueous ammonium formate and acetonitrile	Triple stage, ESI, positive mode, SRM	4	Linearity, accuracy and precision, LOD, LOQ, recovery, stability (bench-top, freeze/thaw, long term), applicability	Mirtazapine and demethylmirtazapine 0.10–200 ng/mL
Paroxetine [122]	LLE (500 μL of plasma)	Fluoxetine	Polaris C18	Formic acid in acetonitrile and water	Triple stage, ESI, positive mode, SRM	2.6	Selectivity, linearity, accuracy and precision, recovery, LOQ, stability (freeze/thaw, long term), applicability	Paroxetine 0.2–20.0 ng/mL
Paroxetine and its 4-hydroxy-3-methoxy metabolite [123]	LLE (1,000 μL of plasma)	Methoxyphenamine and pholedrine	Synergi 4u MAX-RP 80A	Formic acid in acetonitrile and water	Triple stage, ESI, positive mode, SRM	6	Selectivity, linearity, accuracy and precision, LOD, LOQ, recovery, stability (freeze/thaw), applicability	Paroxetine 0.75–100 ng/mL, metabolite 5–100 ng/mL
Sertraline [124]	LLE (250 μL of plasma)	Diphenhydramine	Zorbax Eclipse XDB C18	Formic acid in methanol and water	Triple stage, APCI, positive mode, SRM	4	Linearity, accuracy and precision, LOD, LOQ, recovery, stability (bench-top, freeze/thaw, long term), applicability	Sertraline 0.10–100 ng/mL
Sertraline [125]	SPE (475 μL of plasma)	Imipramine	Beta Basic C-8	Aqueous ammonium formate and	Triple stage, ESI,	3	Selectivity, linearity, accuracy and precision, LOQ, recovery, stability	Sertraline 0.5–60.0 ng/mL

Table 4 (continued)

Compound	Sample preparation	Internal standard	Stationary phase	Mobile phase	Detection mode	Run time (min)	Validation data	Analytical range
				acetonitrile	positive mode, SRM		(bench-top, autosampler, freeze/thaw, long term), applicability	
Venlafaxine and *O*-desmethylvenlafaxine [126]	SPE (500 μL of plasma)	Escitalopram	Betasil C18	Aqueous ammonium formate and acetonitrile	Triple stage, ESI, positive mode, SRM	3	Selectivity, linearity, accuracy and precision, LOQ, recovery, stability (bench-top, freeze/thaw, long term), applicability	Venlafaxine 3–300 ng/mL, *O*-desmethylvenlafaxine 6–600 ng/mL
7 antidepressants: amitriptyline, nortriptyline, doxepin, dosulepin, dibenzepin, opipramol and melitracen [127]	On-line extraction using column switching using 50 μL of plasma	Lofepramine	Oasis HLB and Symmetry C18	Formic acid in acetonitrile and water	Triple stage, APCI, positive mode, SRM	12	Linearity, accuracy and precision, LOD, LOQ, recovery, applicability	Dosulepin, doxepin, amitriptyline, nortriptyline and melitracen 10–800 ng/mL, dibenzepin 50–800 ng/mL, opipramol 50–1500 ng/mL
20 antidepressants: amoxapine, amitriptyline, citalopram, clomipramine, dothiepin, doxepin, fluoxetine, imipramine, maprotiline, mianserin, paroxetine, sertraline, trimipramine, nortriptyline, monodesmethylcitalopram, desmethylclomipramine, desipramine, norfluoxetine, desmethylmianserin, *N*-desmethylsertraline [128]	On-line extraction using column switching using 100 μL of serum	Amitriptyline-D6, clomipramine-D3, imipramine-D3	Cyclone and Xterra MS C18	Ammonium acetate in water, formic acid in acetonitrile and water	Triple stage, ESI, positive mode, SRM	6.5	Selectivity, linearity, accuracy and precision, recovery, applicability	All compounds 10–500 ng/mL
Amitriptyline, citalopram, clomipramine, desipramine, doxepin, fluoxetine, fluvoxamine, imipramine, maprotiline, mianserine, mirtazapine, moclobemide, norclomipramine, nordoxepin, norfluoxetine, nortriptyline, *O*-desmethylvenlafaxine, paroxetine, reboxetine, sertraline,trazodone, trimipramine, venlafaxine, viloxazine [129]	Protein precipitation of 100 μL of serum	Clonidine, metha-benzthiazurone, dehydromethylris-peridone	Chromolith Speed ROD C18	Ammonia in water and acetic acid in methanol	Triple stage, ESI, positive mode, SRM	8	Linearity, accuracy and precision, LOD, LOQ, recovery,	Amitriptyline 10–1,000 ng/mL, citalopram 10–1,000 ng/mL, clomipramine 100–10,000 ng/mL, desipramine 5–500 ng/mL, doxepin 10–1,000 ng/mL, fluoxetine 10–1,000 ng/mL, fluvoxamine 10–1,000 ng/mL, imipramine 10–1,000 ng/mL, maprotiline 100–10,000 ng/mL, mianserin 10–1,000 ng/mL, mirtazapine 10–1,000 ng/mL, moclobemide 100–10,000 ng/mL, norclomipramine 100–1,000, nordoxepin 10–1,000 ng/mL, norfluoxetine 10–1,000 ng/mL, nortriptyline 5–500 ng/mL, *O*-desmethylvenlafaxine 100–10,000 ng/mL, paroxetine 5–500 ng/mL, reboxetine 10–1,000 ng/mL, sertraline 5–500 ng/mL, trazodone 100–10,000 ng/mL, trimipramine 100–10,000 ng/mL,

								venlafaxine 100–10,000 ng/mL, viloxazine 10–1,000 ng/mL
Fluoxetine, citalopram, paroxetine and venlafaxine [130]	SPE (500 μL of plasma)	Fluvoxamine	C18	Aqueous ammonium acetate and acetonitrile	ESI, positive mode, SIM	9	Linearity, accuracy and precision, recovery, applicability	Fluoxetine, citalopram, paroxetine, venlafaxine 5.0–1,000.0 ng/mL

APCI atmospheric pressure chemical ionisation, *ESI* eletrospray ionisation, *LLE* liquid–liquid extraction, *LOD* limitation of detection, *LOQ* limit of quantification, *SIM* single ion monitoring, *SPE* solid-phase extraction, *SRM* selected reaction monitoring

are summarized in Table 5. They were meant for the TDM of a single compound, sometimes with its active metabolite (amisulpride [134], aripiprazole [135, 136], clozapine [137, 138], haloperidol [139, 140], olanzapine [141–146], risperidone [147–150], sulpiride [151], sultopride [152], tiapride [153], ziprasidone [154]), or for several compounds [129, 155–157]. Generally, sample extraction procedures were not considerably simplified when compared to those applied with other techniques. However, as described above, Kirchherr et al. [129] proposed a method allowing the determination of several antipsychotics after protein precipitation of 50 μL of serum. The authors reported good accuracy, linearity and inter-and intra-assay precision but each compound was monitored using a single MRM transition. Kollroser et al. [156] also reported a method for three antipsychotics using on-line sample preparation based on Oasis HLB extraction cartridges, and a six-port switching valve coupled with an ion-trap mass spectrometer. The authors reported that the whole procedure lasted 6 min and was accurate enough for a TDM application. Kratzsch et al. [155] described a method for 15 neuroleptic drugs and three of their relevant metabolites with a limit of detection lower than 1 μg/L, allowing the determination of those active at low concentrations. After SPE of 500 μL of plasma, the presence of neuroleptics was screened for in the full-scan mode using a single quadrupole with an APCI source. Positive peaks in the traces were identified by comparing the spectra obtained with those registered in an in-house library. Finally, quantification was performed in the SIM mode.

When analysing results of the Heathcontrol-Therapeutic Drugs Scheme (Cardiff Bioanalytical Services Ltd), which circulates quality control samples containing several antidepressants and antipsychotics, LC-MS and LC-MS/MS do not seem to be widely used yet. Indeed, for the majority of compounds investigated, less than 20% of all laboratories used these technologies. However, future developments of quantitative methods for antidepressants and antipsychotics TDM should obviously be focussed on technologies characterized by low quantification limits, necessitating less sample volume, easier and less time-consuming sample preparation. Therefore, the number of methods using automated extraction techniques, either on-line solid-phase extraction or turbulent flow chromatography, constantly increases. Nevertheless, developers should be informed of the risks of misusing such on-line sample extraction systems. Indeed, due to carry-over phenomena, the signal observed for a sample could be affected by carry-over of the drugs, fixed either on the extraction column or on the different switching valves from a former injected sample. Furthermore, the shorter analysis run time the higher the risks of ion suppression or cross-talking, i.e. when a signal caused by another compound with another MRM transition interferes with the investigated compound. For example, a

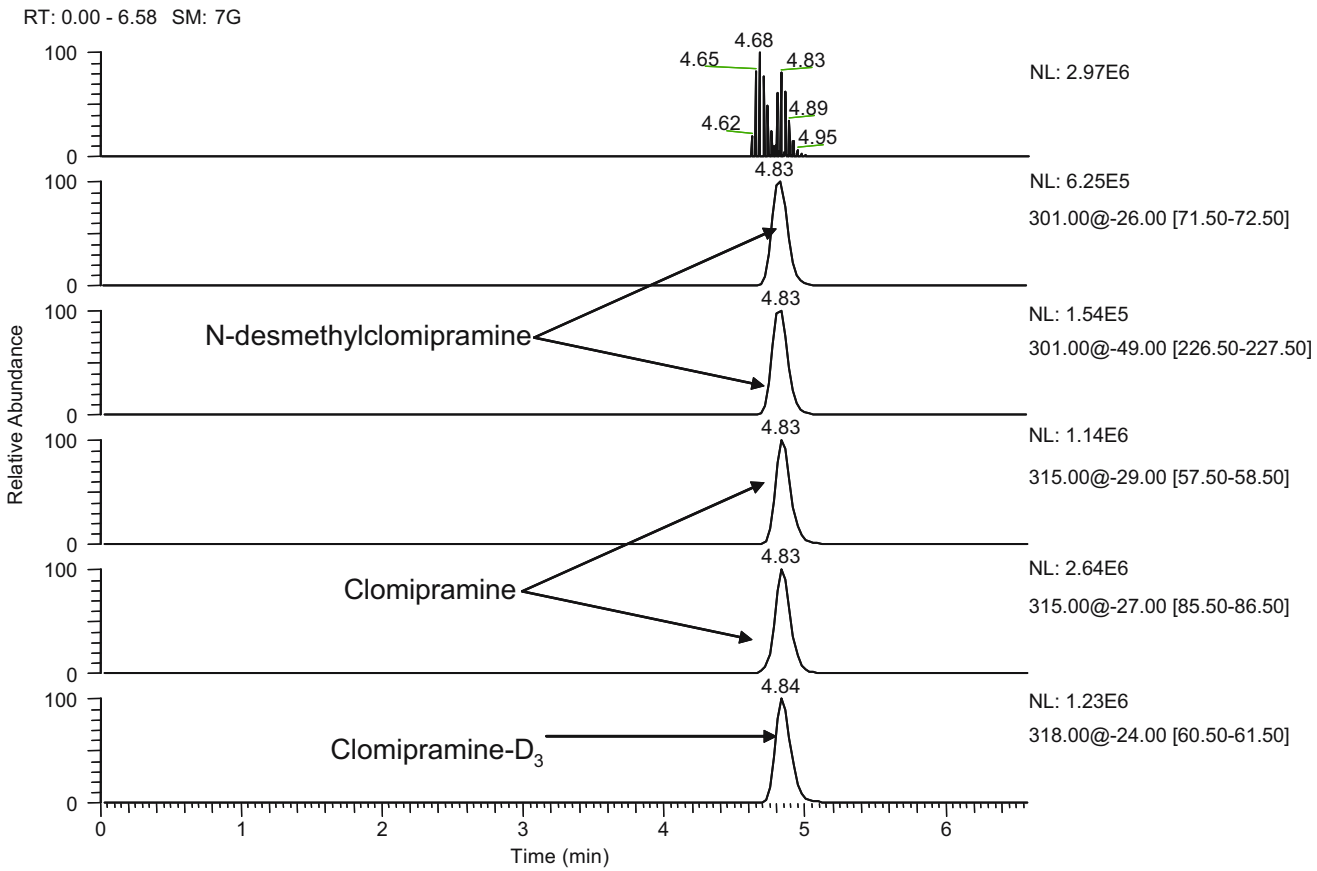

Fig. 1 Example of the application of LC-MS/MS procedure proposed by Sauvage et al. [128] for the quantification of antidepressants in human serum. The chromatogram was obtained from a patient receiving clomipramine. The calculated concentrations were 202.5 and 85.2 μg/L for clomipramine and its demethylated metabolite, respectively

recent paper described an LC-MS method allowing the simultaneous determination of fluoxetine, citalopram, paroxetine and venlafaxine in plasma [130]. Indeed, amitripyline, maprotiline and venlafaxine are characterized by the same *m*/*z* 278 pseudo-molecular ion, but apparently no investigation considering this obvious interference was carried out. Consequently, appropriate chromatographic separation of these potentially interfering compounds should be effective and afford accurate quantitation, though this point was not discussed in the article.

Moreover, as reported in a paper describing the interference of tramadol with a metabolite of venlafaxine [158], tandem mass spectrometry does not avoid such problems, and even with this very selective technique, the choice of appropriate transitions is of the utmost importance. Monitoring only one transition is now advised against, as it is often not sufficient if metabolites had not been considered beforehand. Indeed, for most antidepressants being characterized by a similar tricyclic structure and by an amine-containing side chain, some fragments may not be specific to a given drug. For example, the imipramine transition 281.3/86.1 can interfere with that of a demethylated metabolite of trimipramine, which can have similar retention properties [129]. The same risks apply to nortriptyline and a demethylated metabolite of maprotiline, as well as to phenothiazines that present similar chemical structures and are extensively metabolized, and so induce interferences in the case of co-administrated drugs (personal communication, unpublished data). Therefore, appropriate chromatographic conditions must be chosen to confirm that the signals studied are specific to the investigated compounds. From our own experience, although the selection of a single MRM transition improves the LOQ—as it increases the dwell time, or the number of points acquired over a chromatographic peak—it can be the source of errors even for a TDM method where the drugs to be analysed are known beforehand. In two recent papers [159, 160], although an extensive validation procedure was conducted, problems of isobaric compounds had not been investigated or documented, and potential risks of mass interferences with amitriptyline or maprotiline were not discussed. As a consequence it seems obligatory to use at least two transitions per compound and to check the respective abundances of the confirmation and quantitation transitions, especially when analytical run times become very short. Even if putatively interfering compounds had been extensively studied during method development, metabolites from concomitant administered drugs might interfere.

Table 5 Overview of techniques using LC-MS(/MS) for therapeutic drug monitoring of antipsychotics in humans

Compound	Sample preparation	Internal standard	Stationary phase	Mobile phase	Detection mode	Run time (min)	Validation data	Analytical range
Amisulpride [134]	LLE (250 μL of plasma)	Sulpiride	Synergi Polar-RP	Aqueous ammonium formate and acetonitrile	Triple stage, ESI, positive mode, SRM	4	Selectivity, linearity, accuracy and precision, LOD, LOQ, recovery, stability (bench-top, freeze/thaw, long term, autosampler), applicability	Amisulpride 0.50–500.5 ng/mL
Aripiprazole [135]	LLE (400 μL of plasma)	OPC-14714	Chemcobond ODS-W	Acetic acid in water and acetonitrile	Triple stage, ESI, positive mode, SRM	7.5	Selectivity, linearity, accuracy and precision, LOQ, recovery, stability (bench-top, autosampler, freeze/thaw)	Aripiprazole 0.10–100 ng/mL
Aripiprazole [136]	LLE (150 μL of plasma)	Estazolam	Hypersil Gold C18	Aqueous ammonium acetate and acetonitrile	ESI, positive mode, SIM	5	Selectivity, linearity, LOQ, accuracy and precision, recovery, stability (bench-top, autosampler, long term), applicability	Aripiprazole 19.9–1,119.6 ng/mL
Clozapine [137]	LLE (500 μL of plasma)	Congener of risperidone	C-18	Aqueous ammonium acetate, methanol and acetonitrile	Triple stage, ESI, positive mode, SRM	4	Linearity, accuracy and precision, recovery, applicability	Clozapine 1–1,000 ng/mL
Clozapine [138]	Automated on-line SPE using 50 μL of plasma	Mirtazapine	Zorbax Eclipse XDB-C18	Aqueous ammonium acetate and methanol	ESI, positive mode, SIM	2.2	Linearity, accuracy and precision, LOD, LOQ, recovery, applicability	Clozapine 10–1,000 ng/mL
Haloperidol [139]	Automated SPE (250 μL of plasma)	Haloperidol-D4	Symmetry C18	Formic acid in water and methanol	Triple stage, ESI, positive mode, SRM	3	Selectivity, linearity, accuracy and precision, recovery, stability (autosampler, freeze/thaw), applicability	Haloperidol 0.1–50.0 ng/mL
Haloperidol and reduced haloperidol [140]	LLE (2 mL of plasma)	Chlorohaloperidol	Nucleosil C18	Aqueous ammonium formate and acetonitrile	ESI, positive mode, SIM	10	Linearity, accuracy and precision, recovery, applicability	Haloperidol 0.1–50 ng/mL, reduced haloperidol 0.25–50 ng/mL
Olanzapine [141]	SPE (500 μL of plasma)	LY170222	MetaChem Monochrom	Ammonium acetate in water and propan-1-ol in methanol	Triple stage, APCI, positive mode, SRM	16	Selectivity, linearity, accuracy and precision, recovery, stability (bench-top, freeze/thaw, long term)	Olanzapine 0.25–50 ng/mL
Olanzapine [142]	LLE (250 μL of whole blood)	LY170158	Metachem Monochrom	Ammonium acetate in	Triple stage, APCI, positive	9	Selectivity, linearity, accuracy and precision,	Olanzapine 5–500 ng/mL

Table 5 (continued)

Compound	Sample preparation	Internal standard	Stationary phase	Mobile phase	Detection mode	Run time (min)	Validation data	Analytical range
				water and propan-1-ol in methanol	mode, SRM		recovery, stability (bench-top, freeze/thaw, autosampler)	
Olanzapine [143]	SPE (1 mL of serum)	LY170222	Superspher RP 18	Aqueous ammonium formate and acetonitrile	APCI, positive mode, SIM	7	Selectivity, linearity, accuracy and precision, recovery, applicability	Olanzapine 1–1,000 ng/mL
Olanzapine and desmethylolanzapine [144]	Automated SPE (200 μL of serum)	Olanzapine-D3 Desmethylolanzapine-D8	LUNA phenyl hexyl	Aqueous ammonium acetate and formic acid in acetonitrile	Triple stage, ESI, positive mode, SRM	4	Selectivity, accuracy and precision, recovery	Olanzapine 0.05–50 ng/mL, desmethylolanzapine 0.1–100 ng/mL
Olanzapine [145]	LLE (500 μL of plasma)	LY170222	Hypersil BDS C18	Formic acid in water and acetonitrile	ESI, positive mode, SIM	5	Selectivity, linearity, accuracy and precision, applicability	Olanzapine 0.1–200 ng/mL
Olanzapine [146]	LLE (500 μL of plasma)	Loratadine	Inertsil ODS	Aqueous ammonium acetate and acetonitrile	Triple stage, ESI, positive mode, SRM	6	Linearity, accuracy and precision, LOQ, recovery, stability (bench-top, autosampler, freeze/thaw, long term), applicability	Olanzapine 0.1–30 ng/mL
Risperidone and 9-OH risperidone [147]	LLE (500 μL of plasma)	Methylrisperidone	Phenyl-hexyl	Aqueous ammonium acetate, methanol and acetonitrile	Triple stage, ESI, positive mode, SRM	4	Linearity, accuracy and precision, recovery, applicability	Risperidone and 9-OH risperidone 0.1–100 ng mL
Risperidone and 9-OH risperidone [148]	Protein precipitation with acetonitrile (100 μL of plasma)	Methylrisperidone	Betasil C18	Aqueous ammonium acetate and acetonitrile	Triple stage, ESI, positive mode, SRM	3	Selectivity, linearity, accuracy and precision, LOQ, recovery, stability (bench-top, autosampler, freeze/thaw, long term), applicability	Risperidone and 9-OH risperidone 0.1–15 ng/mL
Risperidone and 9-OH risperidone [149]	On-line extraction with column-switching after protein precipitation with acetonitrile of 25 μL of plasma or saliva	Methylrisperidone	Zorbax SB C18	Aqueous ammonium acetate and acetonitrile	Triple stage, ESI, positive mode, SRM	4	Selectivity, linearity, accuracy and precision, recovery, stability (bench-top, freeze/thaw, long term), applicability	Risperidone and 9-OH risperidone 1–100 ng/mL
Risperidone and 9-OH risperidone [150]	SPE (500 μL of plasma)	R068809 (desfluororisperidone)	C BDS-Hypersil	Aqueous ammonium formate and acetonitrile	Triple stage, ESI, positive mode, SRM	7.5	Selectivity, linearity, accuracy and precision, recovery, stability (bench-top, freeze/thaw,	Risperidone and 9-OH risperidone 0.1–250 ng/mL

	SPE (500 μL of plasma)	R215640 ($^{2}H_3$-$^{13}C_2$-risperidone) and R215639 ($^{2}H_3$-$^{13}C_2$-9-hydroxyrisperidone)	C BDS-Hypersil	Aqueous ammonium formate and acetonitrile			long term), applicability	
Sulpiride [151]	LLE (100 μL of plasma)	Tiapride	Atlantis HILIC silica	Aqueous ammonium formate and acetonitrile	Triple stage, ESI, positive mode, SRM	7	Linearity, accuracy and precision, LOQ, recovery, stability (bench-top, freeze/thaw), applicability	Sulpiride 1–200 ng/mL
Sultopride [152]	LLE (1,000 μL of plasma)	Tiapride	Hypersil silica	Aqueous ammonium acetate and acetonitrile	Particle beam mass spectrometry operating in negative ion chemical ionisation (NICI) mode	15	Linearity, accuracy and precision, LOD, LOQ, recovery, applicability	Sultopride 10–1,000 ng/mL
Tiapride [153]	LLE (100 μL of plasma)	Metoclopramide	Atlantis HILIC Silica	Aqueous ammonium formate and acetonitrile	Triple stage, ESI, positive mode, SRM	6	Linearity, accuracy and precision, LOQ, recovery, stability (bench-top, freeze/thaw)	Tiapride 1–200 ng/mL
Ziprasidone [154]	LLE (1,000 μL of plasma)	*N*-Methylziprasidone	Symmetry C8	Aqueous ammonium acetate and acetonitrile	Triple stage, ESI, positive mode, SRM	2.5	Linearity, accuracy and precision, LOD, recovery	Ziprasidone 0.5–200 ng/mL
Amisulpride, bromperidol, clozapine, droperidol, flupenthixol, fluphenazine, haloperidol, melperone, olanzapine, perazine, pimozide, risperidone, sulpiride, zotepine, zuclopenthixol, norclozapine, clozapine *N*-oxide and 9-hydroxyrisperidone [155]	SPE (500 μL of plasma)	Trimipramine-*d*3	LiChroCART with Superspher 60 RP Select B	Aqueous ammonium formate and acetonitrile	APCI, positive mode, SIM	8	Selectivity, linearity, LOD, LOQ, recovery, accuracy and precision, stability (bench-top, autosampler, freeze/thaw), applicability	Amisulpride 2.5–500 ng/mL, bromperidol 0.5–25 ng/mL clozapine 50–1,000 ng/mL, norclozapine 25–500 ng/mL, clozapine *N*-oxide 25–500 ng/mL, droperidol 1–75 ng/mL, flupenthixol 0.1–2.5 ng/mL, fluphenazine 0.1–25 ng/mL, haloperidol 1–125 ng/mL, melperone 20–500 ng/mL, olanzapine 5–125 ng/mL, perazine 10–500 ng/mL, pimozide 2–12 ng/mL, risperidone 5–125 ng/mL, 9-hydroxyrisperidone 5–125 ng/mL, sulpiride 20–500 ng/mL, zotepine 2–375 ng/mL, zuclopenthixol 1–125 ng/mL
Olanzapine, clozapine and *N*-desmethylclozapine [156]	On-line extraction with column-switching using 50 μL of plasma	Dibenzepin	Oasis HLB and Symmetry C18	Formic acid in water and acetonitrile	Triple stage, ESI, positive mode, SRM	6	Linearity, accuracy and precision, recovery	Olanzapine 5–300 ng/mL, clozapine and *N*-desmethylclozapine 10–800 ng/mL
Clozapine, olanzapine, risperidone and quetiapine [157]	LLE (500 μL of plasma)	Diazepam	C18	Aqueous ammonium acetate and acetonitrile	ESI, positive mode, SIM	10	Linearity, accuracy and precision, LOD, recovery, stability (bench-top, freeze/thaw,	Olanzapine and risperidone 1–50 ng/mL, clozapine and quetiapine 20–1,000 ng/mL

Table 5 (continued)

Compound	Sample preparation	Internal standard	Stationary phase	Mobile phase	Detection mode	Run time (min)	Validation data	Analytical range
Amisulpride, aripiprazole, benperidol, chlorpromazine, chlorprothixene, clozapine, flupentixol, fluphenazine, haloperidol, hydroxyrisperidone, levomepromazine, olanzapine, opipramol, perazine, perphenazine, pimozide, pipamperone, quetiapine, risperidone, sulpiride, thioridazine, ziprasidone, zotepine and zuclopenthixol [129]	Protein precipitation of 100 μL of serum	Clonidine, methabenzthiazurone, dehydromethylris-peridone	Chromolith Speed ROD C18	Ammonia in water and acetic acid in methanol	Triple stage, ESI, positive mode, SRM	8	long term), applicability Linearity, accuracy and precision, LOD, LOQ, recovery	Amisulpride 100–10,000 ng/mL, aripiprazole 10–1,000 ng/mL, benperidol 1–20 ng/mL, chlorpromazine 10–1,000 ng/mL, chlorprothixene 5–500 ng/mL, clozapine 100–10,000 ng/mL, flupentixol 1–100 ng/mL, fluphenazine 1–100 ng/mL, haloperidol 1–20 ng/mL, hydroxyrisperidone 5–200 ng/mL, levomepromazine 10–1,000 ng/mL, olanzapine 10–1,000 ng/mL, opipramol 10–1,000 ng/mL, perazine 100–10,000 ng/mL, perphenazine 1–20 ng/mL, pimozide 1–50 ng/mL, pipamperone 10–1,000 ng/mL, quetiapine 10–1,000 ng/mL, risperidone 1–50 ng/mL, sulpiride 100–10,000 ng/mL, thioridazine 100–10,000 ng/mL, ziprasidone 10–1,000 ng/mL, zotepine 5–500 ng/mL, zuclopenthixol 1–100 ng/mL

APCI atmospheric pressure chemical ionisation, *ESI* eletrospray ionisation, *LLE* liquid–liquid extraction, *LOD* limitation of detection, *LOQ*limit of quantification, *SIM*single ion monitoring, *SPE*solid-phase extraction, *SRM*selected reaction monitoring

Conclusion

This paper illustrates the increasing role of LC-MS in the clinical laboratory routine activity. Nowadays, it seems obvious that almost all the drugs subject to TDM can be analysed using LC-MS(/MS). Therefore, it is no longer a question of evaluating the performance or the feasibility of this technology in routine activity, but to determine which classes of compounds have to be analysed with priority using it. The best example of current applications is the tendency to transfer the routine activity of immunosuppressants from immunoassays to LC-MS which allows the automation of sample preparation, shortens analytical run times and is probably cost-effective despite heavy investment costs. This single example illustrates most of the advantages of LC-MS techniques and suggests their suitability for routine TDM.

However, this expansion, not to speak of vulgarisation, implicates a necessary reassessment of the function of the TDM specialist (PharmD, clinical chemists, etc.) and the development of new positions such as analytical engineers in the laboratory. Indeed, whereas analysis automation using immunoassays permitted one to obtain results only by "pushing a button", these new methods necessitate a higher degree of analytical competence. Knowledge and skills should be upgraded and criteria for assessing an applicable and accurate method have to be clearly defined and respected. When constraints and limitations (e.g. the use of at least two specific transitions per compound, the research of potential interferences and the ion suppression phenomenon) are ignored or incompletely understood, quantification or even identification errors can occur, and the advantages of LC-MS are lost.

References

1. Taylor PJ (2004) Ther Drug Mon 26:215–219
2. Yang Z, Peng Y, Wang S (2005) Clin Appl Immunol Rev 5:405–430
3. Deters M, Kaever V, Kirchner G (2003) Anal Chim Acta 492:133–145
4. Brandhorst G, Streit F, Goetze S, Oellerich M, Armstrong VW (2006) Clin Chem 52:1962–1964
5. Mendonza AE, Gohh RY, Akhlaghi F (2006) Ther Drug Monit 28:402–406
6. Prémaud A, Rousseau A, Picard N, Marquet P (2006) Ther Drug Monit 28:274–277
7. Prémaud A, Rousseau A, Le Meur Y, Lachâtre G, Marquet P (2004) Ther Drug Monit 26:609–619
8. Annesley TM, Clayton LT (2005) Clin Chem 51:872–877
9. Willis C, Taylor PJ, Salm P, Tett SE, Pillans PI (2000) J Chromatogr B 748:151–156
10. Patel CG, Mendonza AE, Akhlaghi F, Majid O, Trull AK, Lee T, Holt DW (2004) J Chromatogr B 813:287–294
11. Atcheson B, Taylor PJ, Mudge DW, Johnson DW, Pillans PI, Tett SE (2004) J Chromatogr B 799:157–163
12. Streit F, Shipkova M, Armstrong VW, Oellerich M (2004) Clin Chem 50:152–159
13. Platzer M, Jahn K, Wohlrab J, Neubert RH (2001) J Chromatogr B 755:355–359
14. Vogeser M, Zachoval R, Spohrer U, Jacob K (2001) Ther Drug Monit 23:722–724
15. Daurel-Receveur M, Titier K, Picard SP, Ducint D, Moore N, Molimard M (2006) Ther Drug Monit 28:505–511
16. Westley IS, Sallustio BC, Morris RG (2005) Clin Biochem 38:824–829
17. Bahrami G, Mohammadi B (2006) Clin Chim Acta 370:185–190
18. Zhong Y, Jiao Z, Yu Y (2006) Biomed Chromatogr 20:319–326
19. Patel CG, Akhlaghi F (2006) Ther Drug Monit 28:116–122
20. Hosotsubo H, Takahara S, Imamura R, Kyakuno M, Tanaka T, Yazawa K, Hanafusa T, Matsumiya K, Nonomura N, Okuyama A, Sugimoto H (2001) Ther Drug Monit 23:669–674
21. Koseki N, Nakashima A, Nagae Y, Masuda N (2006) Rapid Commun Mass Spectrom 20:733–740
22. Salm P, Taylor PJ, Lynch SV, Warnholtz CR, Pillans PI (2005) Clin Biochem 38:667–673
23. Li AC, Li Y, Guirguis MS, Caldwell RG, Shou WZ (2007) J Pharm Biomed Anal 43:277–284
24. Vollenbroeker B, Koch JH, Fobker M, Suwelack B, Hohage H, Müller U (2005) Transplant Proc 37:1741–1744
25. Mendonza A, Gohh R, Akhlaghi F (2004) Ther Drug Monit 26:569–575
26. Keevil BG, Tierney DP, Cooper DP, Morris MR (2002) Clin Chem 48:69–76
27. Shaw LM, Holt DW, Keown P, Venkataramanan R, Yatscoff RW (1999) Clin Ther 21:1632–1652
28. Lensmeyer GL, Poquette MA (2001) Ther Drug Monit 23:239–249
29. Ramakrishna NV, Vishwottam KN, Puran S, Manoj S, Santosh M, Wishu S, Koteshwara M, Chidambara J, Gopinadh Sumatha B (2004) J Chromatogr B 805:13–20
30. Feng Y, Zhang S, Poloyac S, Strom S, Venkataramanan R (2005) J Chromatogr B 821:31–37
31. Chen YL, Hirabayashi H, Akhtar S, Pelzer M, Kobayashi M (2006) J Chromatogr B 830:330–341
32. Taylor PJ, Franklin ME, Graham KS, Pillans PI (2007) J Chromatogr B 848(2):208–214
33. Korecka M, Solari SG, Shaw LM (2006) Ther Drug Monit 28:484–490
34. Andrade MC, Di Marco GS, Felipe CR, Alfieri F, Junior HT, Pestana JO, Casarini DE (2005) Transpl Int 18:354–359
35. Khoschsorur G (2005) Clin Chem 51:1721–1724
36. Baldelli S, Zenoni S, Merlini S, Perico N, Cattaneo D (2006) Clin Chim Acta 364:354–358
37. Vogeser M, Spohrer U (2006) Clin Chem Lab Med 44:1126–1130
38. Ceglarek U, Casetta B, Lembcke J, Baumann S, Fiedler GM, Thiery J (2006) Clin Chim Acta 373:168–171
39. Ceglarek U, Lembcke J, Fiedler GM, Werner M, Witzigmann H, Hauss JP, Thiery J (2004) Clin Chim Acta 346:181–190
40. Koal T, Deters M, Casetta B, Kaever V (2004) J Chromatogr B 805:215–222
41. Poquette MA, Lensmeyer GL, Doran TC (2005) Ther Drug Monit 27:144–150
42. Streit F, Armstrong VW, Oellerich M (2002) Clin Chem 48:955–958
43. Deters M, Kirchner G, Resch K, Kaever V (2002) Clin Chem Lab Med 40:285–292
44. Wang S, Magill JE, Vicente FB (2005) Arch Pathol Lab Med 129:661–665
45. Annesley TM (2003) Clin Chem 49:1041–1044
46. Tod M (2006) Therapie 61:219–225
47. Jullien V, Lortholary O (2006) Therapie 61:209–217
48. Gubbins PO, Gurley BJ, Bowman J (1998) J Pharm Biomed Anal 16:1005–1012

49. Koks CHW, Sparidans RW, Lucassen G, Crommentuyn ML, Beijnen JH (2002) J Chromatogr B 767:103–110
50. Poirier JM, Lebot M, Descamps P, Levy M, Cheymol G (1994) Ther Drug Monitor 16:596–601
51. Yao M, Chen L, Srinivas NR (2001) J Chromatogr B 752:9–16
52. Young WC, Nam D, Kyoung HK, Kyung WH, In HH, Byung KC, Yoon M, Lee J (2006) Bull Korean Chem Soc 27:291–294
53. Vogeser M, Spohrer U, Schiel X (2003) Clin Chem Lab Med 41:915–920
54. Kousoulos C, Tsatsou G, Apostolou C, Dotsikas Y, Loukas YL (2006) Anal Bioanal Chem 384:199–207
55. Walsh TJ, Karlsson MO, Driscoll T, Arguedas AG, Adamson P, Saez-Llorens X, Vora AJ, Arrieta AC, Blumer J, Lutsar I, Milligan P, Wood N (2004) Antimicrob Agents Chemother 48:2166–2172
56. Gage R, Stopher DA (1998) J Pharm Biomed Anal 17:1449–1453
57. Perea S, Pennick GJ, Modak A, Fothergill AW, Sutton DA, Sheehan DJ, Rinaldi MG (2000) Antimicrob Agents Chemother 44:1209–1213
58. Stopher DA, Gage R (1997) J Chromatogr B 691:441–448
59. Zhou L, Glickman RD, Chen N, Sponsel WE, Graybill JR, Lam KW (2002) J Chromatogr B 776:213–220
60. Keevil BG, Newman S, Lockhart S, Howard SJ, Moore CB, Denning DW (2004) Ther Drug Monitor 26:650–657
61. Egle H, Trittler R, Konig A, Kummerer K (2005) J Chromatogr B 814:361–367
62. Shen JX, Krishna G, Hayes RN (2007) J Pharm Biomed Anal 43:228–236
63. Neely M, Blumer J (2003) Curr Ther Res Clin Exp 64:127–136
64. Groll AH, Gullick BM, Petraitiene R, Petraitis V, Candelario M, Piscitelli SC, Walsh TJ (2001) Antimicrob Agents Chemother 45:596–600
65. Egle H, Trittler R, Kummerer K (2004) Rapid Commun Mass Spectrom 18:2871–2877
66. Nilsson-Ehle I, Yoshikawa TT, Edwards JE, Schotz MC, Guze LB (1977) J Infect Dis 135:414–422
67. Mayhew JW, Fiore C, Murray T, Barza M (1983) J Chromatogr 274:271–279
68. Leclercq M, Fouillit M, Panteix G (1985) J Chromatogr 337:423–428
69. Bach PR (1984) Antimicrob Agents Chemother 26:314–317
70. Golas CL, Prober CG, Macleod SM, Soldin SJ (1983) J Chromatogr 278:387–395
71. Brassinne C, Laduron C, Coune A, Sculier JP, Hollaert C, Collette N, Meunier F (1987) J Chromatogr 419:401–407
72. Lue LP, Hadman ST, Vancura A (2002) J AOAC Int 85:15–19
73. Wang LH, Smith PC, Anderson KL, Fielding RM (1992) J Chromatogr 579:259–268
74. Lee JW, Petersen ME, Lin P, Dressler D, Bekersky I (2001) Ther Drug Monitor 23:268–276
75. Back D, Gatti G, Fletcher C, Garaffo R, Haubrich R, Hoetelmans R, Kurowski M, Luber A, Merry C, Perno CF (2002) AIDS 16 (Suppl 1):S5–S37
76. Burger DM, Aarnoutse RE, Hugen PW (2002) Curr Opin Infect Dis 15:17–22
77. Van Heeswijk RPG (2002) Ther Drug Monitor 24:323–331
78. Acosta EP, Gerber JG (2002) AIDS Res Hum Retro 18:825–834
79. Aarnoutse RE, Schapiro JM, Boucher CA, Hekster YA, Burger DM (2003) Drugs 63:742–753
80. Kappelhoff BS, Crommentuyn KM, de Maat MM, Mulder JW, Huitema AD, Beijnen JH (2004) Clin Pharmacokinet 43:845–853
81. Boffito M, Acosta E, Burger D (2005) Antivir Ther 10:375–392
82. Boffito M, Acosta E, Burger D, Fletcher CV, Flexner C, Garaffo R, Gatti G, Kurowski M, Perno CF, Peytavin G, Regazzi M, Back D (2005) Antivir Ther 10:469–477
83. Back D, Gibbons S, Khoo S (2006) Ther Drug Monitor 28:468–473
84. Aarnoutse RE, Verweij-van Wissen CP, Underberg WJ, Kleinnijenhuis J, Hekster YA, Burger DM (2001) J Chromatogr B 764:363–384
85. Burhenne J, Riedel KD, Martin-Facklam M, Mikus G, Haefeli WE (2003) J Chromatogr B 784:233–242
86. Jemal M, Rao S, Gatz M, Whigan D (2003) J Chromatogr B 795:273–289
87. Hua Y, Fan-Havard P, Chan KK (2004) J Chromatogr B 809:1–7
88. Chi J, Jayewardene AL, Stone JA, Aweeka FT (2003) J Pharm Biomed Anal 31:953–959
89. Crommentuyn KM, Huitema AD, Beijnen JH (2005) J Pharm Biomed Anal 38:139–147
90. Pereira AS, Tidwell RR (2001) J Chromatogr B 764:327–347
91. Egge-Jacobsen W, Unger M, Niemann CU, Baluom M, Hirai S, Benet LZ, Christians U (2004) Ther Drug Monitor 26:546–562
92. Gao W, Kishida T, Kimura K, Kageyama M, Sumi M, Yoshikawa Y, Shibata N, Takada K (2002) Biomed Chromatogr 16:267–273
93. Rentsch KM (2003) J Chromatogr B 788:339–350
94. Chi J, Jayewardene AL, Stone JA, Motoya T, Aweeka FT (2002) J Pharm Biomed Anal 30:675–684
95. Frerichs VA, DiFrancesco R, Morse GD (2003) J Chromatogr B 787:393–403
96. Crommentuyn KM, Rosing H, Nan-Offeringa LG, Hillebrand MJ, Huitema AD, Beijnen JH (2003) J Mass Spectrom 38:157–166
97. Williams LD, Von Tungeln LS, Beland FA, Doerge DR (2003) J Chromatogr B 798:55–62
98. Kenney KB, Wring SA, Carr RM, Wells GN, Dunn JA (2000) J Pharm Biomed Anal 22:967–983
99. Volosov A, Alexander C, Ting L, Soldin SJ (2002) Clin Biochem 35:99–103
100. Notari S, Bocedi A, Ippolito G, Narciso P, Pucillo LP, Tossini G, Donnorso RP, Gasparrini F, Ascenzi P (2006) J Chromatogr B 831:258–266
101. Colombo S for the Swiss HIV Cohort Study (2006) Ther Drug Monitor 28:332–338
102. Ford J, Boffito M, Wildfire A, Hill A, Back D, Khoo S, Nelson M, Moyle G, Gazzard B, Pozniak A (2004) Antimicrob Agents Chemother 48:2388–2393
103. Hennessy M, Clarke S, Spiers JP, Kelleher D, Mulcahy F, Hoggard P, Back D, Barry M (2004) Antivir Ther 9:115–122
104. Ford J, Cornforth D, Hoggard PG, Cuthbertson Z, Meaden ER, Williams I, Johnson M, Daniels E, Hsyu P, Back DJ, Khoo SH (2004) Antivir Ther 9:77–84
105. Azoulay S, Nevers MC, Creminon C, Heripret L, Durant J, Dellamonica P, Grassi J, Guedj R, Duval D (2004) Antimicrob Agents Chemother 48:104–109
106. Almond LM, Edirisinghe D, Dalton M, Bonington A, Back DJ, Khoo SH (2005) Clin Pharmacol Ther 78:132–142
107. Linder MW, Keck PE Jr (1998) Clin Chem 44:1073–1084
108. Rao ML, Staberock U, Baumann P, Hiemke C, Deister A, Cuendet C, Amey M, Härtter S, Kraemer M (1994) Clin Chem 40:929–933
109. Dasgupta A, Wells A, Datta P (2006) Ther Drug Monit 28:662–667
110. Pistos C, Panderi I, Atta-Politou J (2004) J Chromatogr B 810:235–244
111. Singh SS, Shah H, Gupta S, Jain M, Sharma K, Thakkar P, Shah R (2004) J Chromatogr B 811:209–215
112. Kollroser M, Schober C (2003) Chromatographia 57:133–138
113. Badenhorst D, Sutherland FC, de Jager AD, Scanes T, Hundt HK, Swart KJ, Hundt AF (2000) J Chromatogr B 742:91–98
114. Djordjevic S, Kovacevic I, Miljkovic B, Vuksanovic J, Pokrajac M (2005) Farmaco 60:345–349

115. Li C, Ji Z, Nan F, Shao Q, Liu P, Dai J, Zhen J, Yuan H, Xu F, Cui J, Huang B, Zhang M, Yu C (2002) Rapid Commun Mass Spectrom 16:1844–1850
116. Sutherland FC, Badenhorst D, de Jager AD, Scanes T, Hundt HK, Swart KJ, Hundt AF (2001) J Chromatogr A 914:45–51
117. Souverain S, Mottaz M, Cherkaoui S, Veuthey JL (2003) Anal Bioanal Chem 377:880–885
118. Fernandes C, Jiayu P, Sandra P, Lanças FM (2006) Chromatographia 64:517–521
119. Green R, Houghton R, Scarth J, Gregory C (2002) Chromatographia 55:S133–S136
120. Chauhan B, Rani S, Guttikar S, Zope A, Jadon N, Padh H (2005) J Chromatogr B 823:69–74
121. Pistos C, Koutsopoulou M, Panderi I (2004) Anal Chim Acta 514:15–26
122. Massaroti P, Cassiano NM, Duarte LF, Campos DR, Marchioretto MA, Bernasconi G, Calafatti S, Barros FA, Meurer EC, Pedrazzoli J (2005) J Pharm Sci 8:340–347
123. Segura M, Ortuño J, Farré M, Pacifici R, Pichini S, Joglar J, Segura J, De la Torre R (2003) Rapid Commun Mass Spectrom 17:1455–1461
124. Chen X, Duan X, Dai X, Zhong D (2006) Rapid Commun Mass Spectrom 20:2483–2489
125. Jain DS, Sanyal M, Subbaiah G, Pande UC, Shrivastav P (2005) J Chromatogr B 829:69–74
126. Bhatt J, Jangid A, Venkatesh G, Subbaiah G, Singh S (2005) J Chromatogr B 829:75–81
127. Kollroser M, Schober C (2002) Ther Drug Monit 24:537–544
128. Sauvage FL, Gaulier JM, Lachâtre G, Marquet P (2006) Ther Drug Monit 28:123–130
129. Kirchherr H, Kühn-Velten WN (2006) J Chromatogr B 843:100–113
130. Juan H, Zhiling Z, Huande L (2005) J Chromatogr B 820:33–39
131. Hiemke C, Dragicevic A, Grunder G, Hatter S, Sachse J, Vernaleken I, Muller MJ (2004) Ther Drug Monit 26:156–160
132. Mitchell PB (2000) Br J Clin Pharmacol 49:303–312
133. Mitchell PB (2001) Br J Clin Pharmacol 52:45S–54S
134. Gschwend MH, Arnold P, Ring J, Martin W (2006) J Chromatogr B 831:132–139
135. Kubo M, Mizooku Y, Hirao Y, Osumi T (2005) J Chromatogr B 822:294–299
136. Zuo XC, Wang F, Xu P, Zhu RH, Li HD (2006) Chromatographia 64:387–391
137. Aravagiri M, Marder SR (2001) J Pharm Biomed Anal 26:301–311
138. Niederländer HA, Koster EH, Hilhorst MJ, Metting HJ, Eilders M, Ooms B, de Jong GJ (2006) J Chromatogr B 834:98–107
139. Hempenius J, Steenvoorden RJ, Lagerwerf FM, Wieling J, Jonkman JH (1999) J Pharm Biomed Anal 20(6):889–898
140. Hoja H, Marquet P, Verneuil B, Lotfi H, Dupuy JL, Penicaut B, Lachâtre G (1997) J Chromatogr B 688:275–280
141. Berna M, Shugert R, Mullen J (1998) J Mass Spectrom 33:1003–1008
142. Berna M, Ackermann B, Ruterbories K, Glass S (2002) J Chromatogr B 767:163–168
143. Bogusz MJ, Krüger KD, Maier RD, Erwoh R, Tuchtenhagen F (1999) J Chromatogr B 732:257–269
144. Chin C, Zhang ZP, Karnes HT (2004) J Pharm Biomed Anal 35:1149–1167
145. Gervasini G, Vizcaino S, Herraiz AG, Benitez J, Carrillo JA (2003) Clin Chem 49:2088–2091
146. Nirogi RV, Kandikere VN, Shukla M, Mudigonda K, Maurya S, Boosi R, Yerramilli A (2006) J Pharm Biomed Anal 41:935–942
147. Aravagiri M, Marder SR (2000) J Mass Spectrom 35:718–724
148. Bhatt J, Subbaiah G, Singh S (2006) Rapid Commun Mass Spectrom 20:2109–2114
149. Flarakos J, Luo W, Aman M, Svinarov D, Gerber N, Vouros P (2004) J Chromatogr A 1026:175–183
150. Remmerie BMM, Sips LLA, De Vries R, De Jong J, Schothuis AM, Hooijschuur EWJ, Van de Merbel NC (2003) J Chromatogr 783:461–472
151. Paek IB, Moon Y, Ji HY, Kim HH, Lee HW, Lee YB, Lee HS (2004) J Chromatogr B 809:345–350
152. Jitsufuchi N, Kudo K, Tokunaga H, Imamura T (1997) J Chromatogr B 690:153–159
153. Moon Y, In BP, Kim HH, Hye YJ, Hye WL, Park HG, Hye SL (2004) Arch Pharm Res 27:901–905
154. Al-Dirbashi OY, Aboul-Enein HY, Al-Odaib A, Jacob M, Rashed MS (2006) Biomed Chromatogr 20:365–368
155. Kratzsch C, Peters FT, Kraemer T, Weber AA, Maurer HH (2003) J Mass Spectrom 38:283–295
156. Kollroser M, Schober C (2002) Rapid Commun Mass Spectrom 16:1266–1272
157. Zhou Z, Li X, Li K, Xie Z, Cheng Z, Peng W, Wang F, Zhu R, Li H (2004) J Chromatogr B 802:257–262
158. Allen KR (2006) Clin Toxicol (Phila) 44:147–153
159. Liu W, Wang F, Li HD (2007) J Chromatogr B (in press)
160. Liu W, Cai HL, Li HD (2007) J Chromatogr B (in press)