REVIEW

Therapeutic drug monitoring by LC–MS–MS with special focus on anti-infective drugs

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Abstract Liquid chromatography coupled to mass spectrometry nowadays plays an important role in the field of therapeutic drug monitoring (TDM), especially of new compounds for which no immunoassays are available. This paper reviews LC–MS(–MS) methods published recently for anti-infective drugs: antiretroviral drugs, other antiviral drugs, antibacterial drugs, antihelmintic drugs, antimalarial drugs, and other antiprotozoal drugs. An overview of the different methods is given, with special focus on selection of the internal standard and validation procedures.

Keywords Therapeutic drug monitoring · LC–MS(–MS) · Antiviral drugs · Antibacterial drugs · Antihelmintic drugs · Antimalarial drugs · Antiprotozoal drugs

Introduction

LC–MS(–MS) nowadays plays a major role in the field of therapeutic drug monitoring (TDM). The number of instruments available in clinical laboratories is increasing tremendously and a large number of methods have been published in recent years. Nevertheless, most of the drug concentration measurements performed for clinical purposes are determined by immunoassays. Because only few immunoassays for TDM have been developed in the last 20 years, for the quantification of drugs introduced in this

period chromatographic methods have to be applied. When HPLC coupled to mass spectrometry became more popular in clinical laboratories, the advantage of a shorter run-time compared with UV or fluorescence detection, increased specificity, and the higher sensitivity led to the application of LC–MS(–MS) instruments all over the world.

Because of these advantages, the drugs most often analysed by LC–MS(–MS) in clinical laboratories belong to the drug classes immunosuppressants, antidepressants, antipsychotics, or antiretroviral compounds. Saint-Marcoux et al. [1] published, in 2007 a very comprehensive review on the current role of LC–MS(–MS) in TDM focusing mainly on immunosuppressants, antifungal drugs, antiretroviral drugs, antidepressants, and antipsychotics. In this review we will focus on drugs with anti-infective activities, mainly antiviral drugs (including an update on antiretroviral drugs), antibacterial drugs, antihelmintic drugs, and antiprotozoal drugs. With the exception of the aminoglycosides and glycopeptides, the need for TDM is still discussed controversially for all of these compounds. Nevertheless there are many clinical situations which make the quantification of these drugs necessary, e.g. patients on haemofiltration, pharmacokinetic interactions, or pharmacogenetic questions.

Internal standards are widely used for quantitative LC–MS(–MS) methods, because they can compensate for variability during sample preparation and, optimally, also for fluctuations in the ionisation efficiency. The optimum internal standard would have chemical and physical properties very similar to those of the drug of interest during the entire process of sample preparation and LC–MS(–MS) analysis. This gives rise to the conclusion that stable isotope-labelled analogues of the analytes are the optimum internal standards. In recent years however it has become clear that these stable isotope-labelled drug analogues may

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lead to ion suppression and their use must, therefore, be carefully validated [2].

Also, in general, all authors should validate their methods according to conventional validation guidelines (e.g. FDA, EMEA) and manuscript reviewers should also focus on complete validation including ion suppression analysis in LC–MS(–MS) methods.

For this review we have searched the literature since 2000 for antiviral drugs, antibacterial drugs, antihelmintic drugs, and antiprotozoal drugs. As TDM of antiretroviral drugs in plasma by LC–MS(–MS) were reviewed in 2007 [1], we only included articles concerning quantification in plasma published since 2007 for our article. Nevertheless, the number of papers published for this drug class is by far the highest. Special emphasis is put on the selection of internal standards and on the validation procedures which have been applied.

Antiviral drugs

Antiretroviral drugs

The need for TDM in highly active antiretroviral therapy is still discussed controversially. There are many laboratories in developed countries which measure the concentrations of the protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) regularly for their patients and physicians guide the dose according to the measured drug concentrations. Nevertheless, scientific evidence of the need for TDM is still missing, which has also been concluded by Kredo et al. in their review for the Cochrane library [3]. In addition to TDM for guidance of therapy for all patients, there are other indications for the measurement of concentrations of antiretroviral drugs. Some of these indications are pharmacokinetic interactions, testing for compliance, treatment of viruses with resistance genes, or pregnant patients. Since 2007, sixteen papers have been published describing quantitative analyses of PIs and NNRTIs in serum, plasma, or hair [4–19] using LC–MS(–MS). Nucleoside reverse transcriptase inhibitors (NRTIs) do not exert any pharmacokinetic interactions, but their concentrations are still helpful for checking compliance or treating patients infected with viruses having resistance genes. These drugs need to be activated intracellularly (namely phosphorylated) which then gives the rationale for quantification of the active metabolites of these compounds in peripheral blood mononuclear cells (PBMCs). Since 2000, 14 papers have been published describing quantification of the metabolites of NRTIs or the intracellular concentrations of PIs and NNRTIs by LC–MS–MS in PBMCs [5, 14, 20–31]. Very recently a method has been described which enables the concomitant quantification of intracellular natural and antiretroviral nucleosides and nucleotides in PBMCs [32]. The method has not been applied in the context of TDM but for cell-culture assays and should be adaptable for TDM in patient samples. In addition, six papers have been presented describing the quantification of the parent compounds of NRTIs in serum or plasma [33–38]. In the time period considered for this review most of the methods were using tandem mass spectrometry as detection method for quantification of antiretroviral drugs. An overview on these methods can be seen in Table 1. Several authors described methods using UV detection, mainly focusing on laboratories which cannot afford a very expensive mass spectrometer [39–44].

PIs and NNRTIs usually were quantified with LC–MS(–MS) methods enabling the simultaneous quantification of several drugs. Nevertheless, in the last three years six papers have been published describing the determination of only one substance, namely lopinavir [4], raltegravir [5–8] or saquinavir [9]. In all these methods different sample-preparation techniques were applied including online sample clean-up [8]. Most of the authors used isotope-labelled analogues of the compound or another structural analogue, except ter Heine et al. [5]. In this paper dibenzepine was used as internal standard. Unfortunately, few authors included ion suppression or matrix effects in their validation procedures [5, 6, 8, 9]. Chromatography was always performed using a C_{18} column, except the method described by Wagner et al. [9] which did not use chromatography but applied the extracted sample directly to a MALDI (matrix assisted laser desorption ionisation) target. Detection has always been performed by selected reaction monitoring (SRM) on triple-stage quadrupole instruments after, mostly, electrospray ionisation. Only Difrancesco et al. and Merschmann et al. used atmospheric pressure chemical ionisation (APCI), which has shown to be less prone to ion suppression.

Fayet et al. [10] published, in 2009, the first multianalyte procedure also including two substances of new antiretroviral drug classes—raltegravir, an integrase inhibitor, and maraviroc, a CCR5 antagonist. Darunavir and etravirine were the other two compounds included in the method. Separation was performed on a C_{18} column with a mobile phase consisting of aqueous ammonium acetate, formic acid and acetonitrile with a run time of 20 min. The analytical range for all the compounds was 0.0025–10 mg L^{-1}.

In the last three years four papers have been published describing the quantification of lopinavir and ritonavir [11–14], which are always given to patients concomitantly. The sample volume needed for the different methods varies between 25 μL [14] and 200 μL plasma [13] and sample preparation has been performed by solid-phase extraction (SPE) [11], liquid–liquid extraction (LLE) [13], and protein

Table 1 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
Lopinavir [4]	LLE (400 μL CSF, 50 μL plasma ultrafiltrate)	A-86093	Symmetry C_{18}	Ammonium acetate buffer and acetonitrile	Triple-stage, APCI, positive mode, SRM	11	Linearity, accuracy and precision, stability (RT, freeze–thaw)	0.313–25.0 μgL^{-1} (CSF), 1.25–100 μgL^{-1} (ultrafiltrate)
Raltegravir [5]	Protein precipitation (50 μL plasma), extraction (15 μL dried blood spot), cell lysis (PBMCs from 8 mL blood)	Dibenzepine	Gemini C_{18}	Aqueous ammonium acetate, acetic acid, and methanol	Triple-stage, ESI, positive mode, SRM	10	Selectivity, linearity, accuracy and precision, recovery, stability (patient samples, 4 °C, RT, autosampler) matrix effect, ion suppression	0.05–10 mgL^{-1}
Raltegravir [6]	LLE (200 μL plasma), 96-well plate	Raltegravir-13C6	Ace C_{18}	EDTA, formic acid, methanol	Triple-stage, APCI, positive mode, SRM	3.5	Linearity, accuracy and precision, matrix effects, extraction recovery, cross-talk, stability (60 °C, RT, freeze–thaw, long-term)	0.02–10 mgL^{-1}
Raltegravir [7]	LLE (200 μL plasma)	Raltegravir-13C6	Ace C_{18}	Formic acid, methanol	Triple-stage, ESI, positive mode, SRM	7	Linearity, accuracy and precision	0.01–30 mgL^{-1}
Raltegravir [8]	Protein precipitation (25 μL plasma), followed by on-line sample clean-up	Raltegravir-13C6	Poros R1/20, Phenomenex Luna	Aqueous ammonium acetate, acetic acid, and methanol	Triple-stage, ESI, positive mode, SRM	4	Selectivity, linearity, accuracy and precision, recovery, matrix effects	0.01–3.0 mgL^{-1}
Saquinavir [9]	LLE (250 μL plasma)	Saquinavir-d5	No chromatography	No chromatography	Triple-stage, linear ion trap, MALDI, SRM	0.2	Selectivity, accuracy and precision, extraction recovery, matrix effects	0.005–10.0 mgL^{-1}
Tenofovir diphosphate [20]	SPE (PBMCs from 25 mL blood)	Tenofovir-13C5	Oasis HLB and Synergi Polar RP	Acetic acid, water, and acetonitrile	Triple-stage, ESI, positive mode, SRM	5	Selectivity, linearity, accuracy and precision, recovery, stability (–80 °C, RT, freeze–thaw, autosampler), matrix effects	50–10'000 fmol/sample
Zidovudine triphosphate [21]	Immunoaffinity extraction (PBMCs from 7 mL blood)	2-Chloroadenosine 5'-triphosphate	Supelcogel ODP-50	Ammonium formate buffer, dimethylhexylamine, and acetonitrile	Triple-stage, ESI, negative mode, SRM	26	Linearity, LOQ, accuracy and precision, recovery, stability (autosampler)	93.0–3,360 fmol/sample
Zidovudine triphosphate [22]	SPE (PBMCs from 25 mL blood)	Zidovudine-13C, 15N2	Waters QMA and Waters XTerra RP18	Aqueous acetic acid and acetonitrile	Triple-stage, ESI, positive mode, SRM	10	Linearity, accuracy and precision, recovery, stability (RT, autosampler, freeze–thaw)	50.0–6,400 fmol/sample
1 II, 1 CCR5, 1 PI and 1 NNRTI: raltegravir, maraviroc, darunavir, and etravirine [10]	Protein precipitation (100 μL plasma)	Darunavir-d9	Atlantis-dC18	Aqueous ammonium acetate, formic acid, and acetonitrile	Triple-stage, ESI, positive mode, SRM	20	Linearity, accuracy and precision, recovery, stability (RT, 4 °C, –20 °C, freeze–thaw), matrix effects, recovery, dilution effect	All analytes: 0.0025–10 mgL^{-1}

Table 1 (continued)

Compound(s)	Sample preparation	Internal standard	Stationary phase	Mobile phase	Detection mode	Run time (min)	Validation data	Analytical range
2 NRTIs: didanosine triphosphate, stavudine triphosphate [23]	Cell lysis (PBMCs from 8 mL blood)	2-Chloroadenosine 5′-triphosphate	Supelcogel ODP 50	Ammonium formate buffer, dimethylhexylamine, and acetonitrile	Triple-stage, ESI, SRM Pos./neg.?	26	Selectivity, linearity, accuracy and precision, LOQ, recovery, stability (sample preparation, autosampler, −80 °C), matrix effects	Didanosine triphosphate: 53.39–2,670 fmol/sample, stavudine triphosphate: 61.45–3,073 fmol/sample
2 NRTIs: emtricitabine and tenofovir [34]	SPE (200 μL plasma)	Lamivudine	Oasis MCX and Chromolith Speed Rod RP18	Aqueous ammonium acetate and acetonitrile	Triple-stage, ESI, positive mode, SRM	2	Selectivity, linearity, accuracy and precision, recovery, stability (RT, autosampler, dry extract, freeze–thaw), matrix effects	Emtricitabine: 0.025–2.5 mg L^{-1}, tenofovir: 0.01–0.6 mg L^{-1}
2 NRTIs: emtricitabine and tenofovir [35]	Protein precipitation (250 μL plasma)	Emtricitabine-13C,15 N2, tenofovir-13C5	Synergi Polar RP	Acetic acid, water, and acetonitrile	Triple-stage, ESI, positive mode, SRM	11	Selectivity, linearity, accuracy and precision, recovery, stability (RT, autosampler, freeze–thaw), matrix effects	Both analytes: 0.010–1.5 mg L^{-1}
2 PIs: lopinavir and ritonavir [11]	SPE (100 μL plasma)	Lopinavir-d8, ritonavir-d6	Oasis HLB and Acquity UPLC BEH C$_{18}$	Ammonium formate buffer and methanol	Triple-stage, ESI, positive mode, SRM	1.2	Selectivity, linearity, accuracy and precision, LOQ, recovery, stability (autosampler, bench top, dry extract, freeze–thaw), matrix effects	Lopinavir: 0.0297–14.379 mg L^{-1}, ritonavir: 0.0029–1.452 mg L^{-1}
2 PIs: lopinavir and ritonavir [12]	Protein precipitation (50 μL plasma)	Lopinavir-d8, ritonavir-d6	Zorbax Extend-C$_{18}$ Rapid resolution	Formic acid, water, and acetonitrile	Triple-stage, ESI, positive mode, SRM	1	Selectivity, linearity, accuracy and precision, LOQ, recovery, matrix effects, carryover	Lopinavir: 0.0192–16 mg L^{-1}, ritonavir: 0.00973–8.11 mg L^{-1}
2 PIs: lopinavir and ritonavir [13]	LLE (200 μL plasma, semen, saliva, ultrafiltered plasma)	A-86093	LiChrospher 100 RP-18	Ammonium acetate formic acid, and methanol	Triple-stage, ESI, positive mode, SRM	4.5	Selectivity, linearity, accuracy and precision, LOQ, recovery, stability (freeze–thaw)	Lopinavir: 0.001–2.0 mg L^{-1} (plasma), 0.001–0.2 mg L^{-1} (semen, ultrafiltrate), 0.001–0.1 mg L^{-1} (saliva,); ritonavir: 0.001–0.2 mg L^{-1} (plasma), 0.001–0.05 mg L^{-1} (semen, saliva, ultrafiltrate)
2 PIs: lopinavir and ritonavir [14]	Protein precipitation (25 μL plasma, 500 μL ultrafiltered plasma, PBMCs from 8 mL blood)	Saquinavir-d5	Jupiter Proteo C12	Ammonium acetate, acetic acid, and acetonitrile	Triple-stage, ESI, positive mode, SRM	6	Selectivity, linearity, accuracy and precision, LOQ, recovery, stability (freeze–thaw), matrix effects	Both analytes: 0.004–10 mg L^{-1} (plasma), 0.20–500 μg L^{-1} (ultrafiltrate), 0.10–250 ng/3×10^{6} cells (PBMCs)

Analytes	Sample preparation	Internal standard	Column	Mobile phase	MS	Run time	Validation	LOQ / range
2 PIs and 1 NNRTI: lopinavir, ritonavir, and efavirenz [15]	Pulverization, LLE (2 mg hair)	Ritonavir-d6, celecoxib	BDS-C$_{18}$	Aqueous ammonium acetate, acetic acid, and acetonitrile	Triple-stage, ESI, positive mode (lopinavir, ritonavir), negative mode (efavirenz), SRM	5	Selectivity, linearity, accuracy and precision, recovery, matrix effects	Lopinavir: 0.05–20 ng mg^{-1} hair, ritonavir: 0.01–4.0 ngmg^{-1} hair, efavirenz: 0.05–20 ngmg^{-1} hair
2 PIs and 1 NNRTI: darunavir, ritonavir, and etravirine [16]	LLE (100 µL plasma)	Alprazolam	Zorbax XDB C-8	Formic acid, water, and acetonitrile	Single-stage, ESI, positive mode, SIM	10	Selectivity, linearity, accuracy and precision, LOQ, recovery, stability (heat inactivation, freeze–thaw, RT, 4 °C, autosampler)	All analytes: 0.02–2.0 mg L^{-1}
2 N(t)RTIs and 1 NNRTI: efavirenz, emtricitabine, and tenofovir [36]	SPE (100 µL plasma)	Rosuvastatin, propranolol	Oasis HLB and Chromolith Performance RP-18e	Water, formic acid, and acetonitrile	Triple-stage, ESI, positive mode, SRM	7	Linearity, accuracy and precision, LOQ, recovery, stability (RT, −50 °C, freeze–thaw)	Efavirenz: 0.02–2.0 mgL^{-1}, emtricitabine: 0.002–0.2 mgL^{-1}, tenofovir: 0.02–2.0 mgL^{-1}
2 NRTIs and 1 NNRTI: lamivudine, stavudine, and nevirapine [37]	Protein precipitation (150 µL plasma)	Zidovudine	Shiseido C$_8$	Aqueous ammonium acetate and methanol	Triple-stage, ESI, positive mode, SRM	6	Selectivity, linearity, accuracy and precision, stability (−20 °C, RT, autosampler, freeze–thaw)	Lamivudine: 0.025–3.0 mgL^{-1}, stavudine: 0.02–2.0 mgL^{-1}, nevirapine: 0.05–5.0 mgL^{-1}
2 NRTIs and 1 NNRTI: lamivudine, stavudine, and nevirapine [33]	SPE (500 µL plasma)	Metaxalone	DVB-HLB and Symmetry C$_{18}$	Glacial acetic acid, water, and acetonitrile	Triple-stage, ESI, positive mode, SRM	4.5	Selectivity, linearity, accuracy and precision, LOQ, stability (bench-top, process, freeze–thaw, long-term), recovery, matrix effects, dilution integrity	Lamivudine: 0.025–5.0 mgL^{-1}, stavudine: 0.02–4.0 mgL^{-1}, nevirapine: 0.025–5.0 mgL^{-1}
3 NRTIs: stavudine triphosphate, zidovudine monophosphate, and zidovudine triphosphate [24]	Cell lysis (PBMCs from 7 mL blood)	2-Chloroadenosine 5'-triphosphate	Supelcogel ODP-50	Ammonium formate buffer, dimethylhexylamine, and acetonitrile	Triple-stage, ESI, negative mode, SRM	9.5	Selectivity, linearity, LOQ, accuracy and precision, recovery, stability (autosampler), matrix effects	Stavudine triphosphate: 63–3,200 fmol/sample, zidovudine monophosphate: 300–25,000 fmol/sample, zidovudine triphosphate: 150–5,000 fmol/sample
3 NRTIs: carbovir triphosphate, lamivudine triphosphate, and tenofovir triphosphate [25]	Cell lysis (PBMCs)	2-Chloroadenosine 5'-triphosphate	Supelcogel ODP-50	Ammonium formate buffer, dimethylhexylamine, acetonitrile and, methanol	Triple-stage, ESI, positive mode, SRM	12	Selectivity, linearity, LOQ, accuracy and precision, recovery, stability (autosampler, −80 °C), matrix effects	Carbovir triphosphate: 0.2–6.0 pmol/sample, lamivudine triphosphate: 1.0–300 pmol/sample, tenofovir triphosphate: 0.1–7.0 pmol/sample
3 NRTIs: lamivudine triphosphate, didanosine triphosphate, and stavudine triphosphate [26]	Cell lysis (PBMCs from 7 mL blood)	2-Chloroadenosine 5'-triphosphate	Supelcogel ODP-50	Ammonium formate buffer, dimethylhexylamine, and acetonitrile	Triple-stage, ESI, negative mode, SRM	26	Linearity	Lamivudine triphosphate: 0.1879–375.7 pmol/sample, didanosine triphosphate: 53.39–2,670 fmol/sample, stavudine triphosphate: 61.45–3,073 fmol/sample
3 NRTIs: carbovir triphosphate lamivudine triphosphate, zidovudine triphosphate [27]	SPE (PBMCs from 7 mL blood)	Zidovudine-13C	QMA Sep Pak and Aquasil C$_{18}$	Acetic acid, water, and methanol	Triple-stage, ESI, positive mode, SRM	17	Selectivity, linearity, accuracy and precision, LOQ, matrix effects	Carbovir triphosphate: 0.05–5.00 ngL^{-1}, lamivudine triphosphate: 2.5–250 ngL^{-1}, zidovudine triphosphate: 0.1–10.0 ngL^{-1}

Table 1 (continued)

Compound(s)	Sample preparation	Internal standard	Stationary phase	Mobile phase	Detection mode	Run time (min)	Validation data	Analytical range
7 NRTIs: abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, and zidovudine [38]	Protein precipitation (100 μL plasma)	6-beta-Hydroxy-theophylline	Atlantis T3	Water and acetonitrile containing formic acid	Triple-stage, ESI, positive mode, SRM	14	Selectivity, linearity, accuracy and precision, recovery, stability (−20 °C), matrix effects	Abacavir: 0.02–3.00 mg L^{-1}, didanosine: 0.01–1.50 mg L^{-1}, emtricitabine: 0.01–1.50 mg L^{-1}, lamivudine: 0.01–1.50 mg L^{-1}, stavudine: 0.02–3.00 mg L^{-1}, tenofovir: 0.0050–0.7500 mg L^{-1}, zidovudine: 0.02–2.50 mg L^{-1}
4 PIs and 1 NNRTI: amprenavir, lopinavir, ritonavir, saquinavir, and efavirenz [28]	LLE (PBMCs from 8 mL blood)	A-86093	X-Terra MS C_{18}	Buffer (formic acid, ammonium hydroxide) and acetonitrile	Single stage, APCI, positive mode, SIM	15	Linearity, accuracy and precision, LOQ, recovery	Amprenavir, lopinavir, efavirenz: 2–200 ng/3×10^6 cells, ritonavir, saquinavir: 1.6–128 ng/3×10^6 cells
6 PIs and 3 NNRTIs: amprenavir, indinavir, lopinavir, nelfinavir and metabolite, ritonavir, saquinavir, delavirdine, efavirenz, and nevirapine [29]	Cell lysis (PBMCs from 7 mL blood)	Ketoconazole	Nova Pak C_{18}, 60Å	Ammonium acetate buffer and acetonitrile	Triple-stage, ESI, positive mode except efavirenz (negative mode), SRM	20	Selectivity, linearity, accuracy and precision, LOQ, recovery, matrix effects	All analytes: 0.5–250 ng/sample
7 PIs and 2 NNRTIs: amprenavir, atazanavir, nelfinavir, indinavir, lopinavir, saquinavir, ritonavir, efavirenz, nevirapine [30]	Cell lysis and extraction (PBMCs from 8 mL blood)	Clozapine	SymmetryShield RP18	Aqueous ammonium acetate, acetic acid, formic acid, acetonitrile	Triple-stage, ESI, positive mode except efavirenz (negative mode), SRM	20	Selectivity, linearity, accuracy and precision, LOQ, stability (RT, freeze–thaw, autosampler), recovery, cell number variability, dilution effects	All analytes: 0.0005–0.1 mg L^{-1}
8 PIs and 2 NNRTIs: amprenavir, atazanavir, nelfinavir, indinavir, lopinavir, saquinavir, ritonavir, tipranavir, efavirenz, nevirapine [31]	Cell lysis and extraction (PBMCs from 8 mL blood)	Atazanavir-d5, lopinavir-d4, efavirenz-d4, indinavir-d6, lopinavir-d8, saquinavir-d5	SymmetryShield RP18	Aqueous ammonium acetate, formic acid, and acetonitrile	Triple-stage, ESI, positive mode except efavirenz (negative mode), SRM	25	Selectivity, linearity, accuracy and precision, LOQ, recovery, matrix effects	All analytes: 0.00025–0.125 mg L^{-1}

Analytes	Sample preparation	Internal standard	Analytical column	Mobile phase	MS	n	Validation	Concentration ranges
9 PIs and 2 NNRTIs: amprenavir, atazanavir, darunavir, nelfinavir+ metabolite, indinavir, lopinavir, saquinavir, ritonavir, tipranavir, efavirenz, nevirapine [17]	Protein precipitation (50 μL plasma)	Quinoxaline	Atlantis dC-17	Water, formic acid, and acetonitrile	Triple-stage, ESI, positive mode, except efavirenz (negative mode), SRM	25	Selectivity, linearity, accuracy and precision, LOQ, recovery, matrix effects	Amprenavir: 0.039–10 mgL⁻¹, atazanavir: 0.023–6.0 mgL⁻¹, darunavir: 0.039–10 mgL⁻¹, nelfinavir: 0.0312–8.0 mgL⁻¹, M-8: 0.0156–4.0 mgL⁻¹, indinavir: 0.0312–8.0 mgL⁻¹, lopinavir: 0.0586–15 mgL⁻¹, saquinavir: 0.0273–7.0 mgL⁻¹, ritonavir: 0.0098–2.5 mgL⁻¹, tipranavir: 0.1758–45.0 mgL⁻¹, efavirenz: 0.0312–8.0 mgL⁻¹, nevirapine: 0.0312–8.0 mgL⁻¹
9 PIs and 2 NNRTIs: amprenavir, atazanavir, darunavir, nelfinavir+ metabolite, indinavir, lopinavir, saquinavir, ritonavir, tipranavir, efavirenz, nevirapine [18]	Protein precipitation (100 μL plasma)	Efavirenz-13C, indinavir-d6, saquinavir-d5, dibenzepine	Phenomenex Gemini C₁₈	Ammonium acetate buffer, and methanol	Triple-stage, ESI, positive mode, SRM	10	Selectivity, linearity, accuracy and precision, LOQ, recovery, matrix effects	Amprenavir, atazanavir, efavirenz, indinavir, lopinavir, nelfinavir, M8, nevirapine and ritonavir: 0.1–20 mgL⁻¹, darunavir and saquinavir: 0.05–10 mgL⁻¹, tipranavir: 0.5–100 mgL⁻¹
7 PIs, 7 N(t)RTIs, and 3 NNRTIs: amprenavir, atazanavir, nelfinavir, indinavir, lopinavir, saquinavir, ritonavir, abacavir, didanosine, lamivudine, stavudine, zalcitabine, zidovudine, tenofovir, delavirdine, efavirenz, nevirapine [19]	LLE and protein precipitation (50 μL plasma)	Cimetidine	Aquasil C₁₈	Water, formic acid, and methanol	Triple-stage, ESI, negative and positive mode, SRM	18	Linearity, accuracy and precision, LOQ, stability (freeze-thaw, refrigerator), recovery, matrix effects	All analytes: 0.001–0.5 mgL⁻¹
7 PIs, 8 NRTIs, 3 NNRTIs: amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, abacavir, didanosine, emtiricitabine, lamivudine, stavudine, tenofovir, zalatabine, zidovudine, delavirdine, efavirenz, nevirapine [44]	Protein precipitation (200 μL plasma or cerebrospinal fluid)	S-CH₃-saquinavir, saquinavir-d5, cyclopropyl-ritonavir	Nucleosil C₁₈-100 Nautilus	Aqueous ammonium acetate, formic acid, and acetonitrile	Triple-stage, ESI, negative and positive mode, SRM	10	Selectivity, linearity, accuracy and precision, LOQ, stability (freeze-thaw, refrigerator), recovery, matrix effects	Amprenavir, atazanavir, zidovudine: 50–10 mgL⁻¹; delavirdine, indinavir, lopinavir: 0.01–20 mg/mL; Efavirenz, nelfinavir, nevirapin, saquinavir, zalcitabine: 0.01–10 mgL⁻¹, ritonavir: 0.05–20 mgL⁻¹

APCI, atmospheric pressure chemical ionization; ESI, electrospray ionization; II, integrase inhibitor; LLE, liquid–liquid extraction; LOQ, limit of quantification; MALDI, matrix-assisted laser desorption ionisation; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NtRTI, nucleotide reverse transcriptase inhibitor; PBMCs, peripheral blood mononuclear cells; PI, proteinase inhibitor; RT, room temperature; SIM, single ion monitoring; SRM, selected reaction monitoring

precipitation (PP) [12, 14]. All internal standards used were either deuterated analogues of a PI or a structural analogue. ESI was always used, and detection by SRM on a triple stage quadrupole. The method described by Estrela et al. [13] not only used plasma as sample material but was also validated for ultrafiltered plasma, semen, and saliva, always using 200 μL. The lower limits of quantification were 0.001 mg L^{-1} for lopinavir and ritonavir, irrespective of the sample material. The upper limits of quantification were 2.0 mg L^{-1} for lopinavir in plasma, 0.2 mg L^{-1} for lopinavir in semen and ultrafiltered plasma, and 0.1 mg L^{-1} for saliva. For ritonavir the values were 0.2 mg L^{-1} for plasma and 0.05 mg L^{-1} for semen, saliva, and ultrafiltered plasma. Ehrhardt et al. [14] presented a method using 25 μL plasma, 500 μL ultrafiltered plasma, or PBMCs harvested from 8 mL peripheral blood. The analytical range for both analytes in plasma was 0.004–10 mg L^{-1}; the respective value for ultrafiltered plasma was 0.20–500 μg L^{-1} and the analytical range in PBMCs was 0.10–250 ng per 3×10^6 cells.

Since 2007, three papers have been published describing the simultaneous quantification of up to five different PIs and NNRTIs [15, 16, 28]. Huang et al. [15] described the quantification of efavirenz, lopinavir, and ritonavir in human hair, with the purpose of using a sample material which enables monitoring of long-term drug exposure over a period of weeks to months. Whereas ritonavir-d6 was used as internal standard for quantification of lopinavir and ritonavir, they used celecoxib as internal standard for the quantification of efavirenz; celecoxib has no structural similarity to efavirenz.

In the last three years, seven papers have been published describing the simultaneous quantification of nine to seventeen antiretroviral drugs simultaneously either in plasma [17–19, 44] or in PBMCs [29–31]. The plasma methods included amprenavir, atazanavir, efavirenz, indinavir, lopinavir, nelfinavir (some also its metabolite M8), nevirapine, ritonavir, and saquinavir. D'Avolio et al. [17] and ter Heine et al. [18] included also the newest PIs darunavir and tipranavir in their procedures, whereas Jung et al. [19] included some of the NRTIs (abacavir, didanosine, lamivudine, stavudine, zalcitabine, tenofovir, and delavirdine) in their method. D'Avolio et al. [17] and ter Heine et al. [18] used protein precipitation as sample-preparation method with a similar analytical range but very different run times of 10 min [18] versus 25 min [17]. The method described by Jung et al. [19] used liquid–liquid extraction combined with protein precipitation with a run time of 18 min but a much lower analytical range compared with the other methods, which will not even allow measurement of trough concentrations in patients for all compounds. The internal standards used in these three multi-analyte procedures included quinoxaline [17], cimet-

idine [18], and deuterated analogues of efavirenz, indinavir, saquinavir , and dibenzepine [19]. Gehrig et al. [44] used protein precipitation for sample preparation and included also delavirdine as NNRTI and 2 NRTIs (zalcitabine, zidovudine) for quantification. In addition they also detected six NRTIs (abacavir, didanosine, emtricitabine, lamivudin, stavudine, and tenofovir). The run time was only 10 min, and the analytical range was comparable with those of the other multianalyte procedures.

Since 2000, several methods have been published describing the intracellular concentrations of antiretroviral drugs. The cells which have been used for these quantifications are always PBMCs which are thought to be the target of the different antiretroviral drugs. PBMCs have been used as sample materials for analysis of amprenavir, efavirenz, indinavir, lopinavir, nelfinavir (some also its metabolite M8), nevirapine, and ritonavir. Pelerin et al. [29] also included saquinavir and delavirdine in their procedure, Colombo et al. [30] also determined atazanavir and saquinavir and Elens et al. [31] atazanavir, saquinavir, and tipranavir. Sample preparation consisted always of cell lysis; Colombo et al. [30] and Elens et al. [31] extracted the lysed cells for 3 h or overnight on a horizontal shaker. Only Elens et al. [31] selected internal standards with structural similarities to the compounds to be analysed. The method described by Colombo et al. [30] used clozapine as internal standard whereas Pelerin et al. [29] used ketoconazole. It must, however, be mentioned that these two methods were published in 2005; at that time deuterated analogues of the PIs and NNRTIs were not commercially available. The analytical ranges can hardly be compared as Pelerin et al. [29] described a calibration per sample, namely the total extracted amount of the different drugs out of 7 mL blood. Colombo et al. [30] and Elens et al. [31] calibrated their method according to the concentration of the different drugs in the solution used to spike blank PBMCs.

As NRTIs have to be phosphorylated if they are to exert their antiviral activity, measurements of the phosphorylated metabolites of NRTIs in PBMCs are the optimum means of measuring drug exposure. Therefore, most of the methods published for the quantification of NRTIs use PBMCs as sample material. These cells must be lysed after harvesting; some of the authors then added an additional SPE [20, 22, 27] or immunoaffinity extraction [21] to their extraction method. King et al. [20] described a method for specific quantification of tenofovir diphosphate, using the deuterated analogue as the internal standard. The amount of blood needed for isolation of PBMCs was 25 mL. Becher et al. [21] and King et al. [22] described a quantitative method for analysis of zidovudine triphosphate. Whereas Becher et al. [21] used immunoaffinity extraction with PBMCs from 7 mL blood, King et al. [22] applied SPE with PBMCs out of 26 mL blood. The run time of the ion-pair liquid

chromatographic method described by Becher et al. [21] was 26 min, which is more than twice as long as that described by King et al [22]. Several methods have been published describing the simultaneous analysis of different NRTIs. Becher et al. [23] described a procedure for simultaneous quantification of didanosine triphosphate and stavudine triphosphate. They used PBMCs from 7 mL blood and performed ion-pair liquid chromatography on a reversed phase column. The run time of the second method of this group was again relatively long—26 min. Compain et al. [24] published a procedure for quantification of stavudine triphosphate, zidovudine diphosphate, and zidovudine triphosphate in PBMCs. They also added dimethylhexylamine as ion-pair reagent to the mobile phase and were able to achieve a run time of 9.5 minutes. Pruvost et al. [25] presented a method for quantification of carbovir triphosphate, lamivudine triphosphate, and tenofovir triphosphate using a comparable chromatographic approach. Robbins et al. [27] described a procedure for quantification of the same NRTIs using reversed-phase chromatography after SPE of the lysed PBMCs. Both methods have a similar analytical range and comparable run times of approximately 15 min.

Other antiviral drugs

HPLC in combination with UV detection is often used for TDM of other antiviral drugs, mostly in plasma [45–55] but also in cerebrospinal fluid [56] and—for the same reasons as antiretroviral drugs—intracellularly [57].

Regarding TDM using LC–MS(–MS), several new methods have been published (Table 2). For determination of adefovir, which is mostly used for treatment of chronic hepatitis B, five methods have been published [58–62] since 2000. Most authors used protein precipitation as sample pretreatment, except Liu et al. [61], who used solid-phase extraction. Regarding the choice of the internal standards, Liu et al. [61] applied the most advanced approach with a ^{13}C-labelled analogue of adefovir. Bi et al. [59], even though reporting good accuracy and precision, did not mention the use of an internal standard at all, a procedure which, in our opinion, can no longer be recommended.

Run times of all methods were comparable, with a range from 7.8 [59] to 10 min [62]. In all methods, ESI was used as ionization method, mainly in positive mode, only once reported in negative mode by Liu et al. [61]. Regarding the analytical range, Zou et al. [62] reported the most sensitive method with a limit of quantification of 0.2 $\mu g L^{-1}$.

Since 2000, three LC–MS(–MS) methods have been published for quantification of entecavir and tenofovir, two substances also used for the therapy of chronic hepatitis B. For entecavir, Zhang et al. [63] published a method that was applied to a Phase I clinical study. Using solid-phase extraction of a relatively large sample volume (1 mL plasma), a very low limit of quantification, 0.005 $\mu g L^{-1}$, could be achieved. For tenofovir, two LC–MS(–MS) methods have been published. Yadav et al. [64] recently presented a method with a run time of only 1.8 min using a monolithic column, and solid-phase extraction as sample pretreatment. The method of Delahunty et al. [65] had a run time of 7 min, with protein precipitation in 250 μL plasma as sample preparation.

Ribavirin is an important drug in the therapy of chronic hepatitis C. A method for intracellular analysis of ribavirin in red blood cells was presented by Yeh et al. [66], who used ^{13}C-labelled ribavirin as internal standard. Ribavirin accumulates in red blood cells, which leads over time to rupture of the cells, causing haemolytic anaemia, an important side effect of treatment with this drug. Shou et al. [67] and Zhou et al. [68] both reported a method using protein precipitation in 0.1 mL plasma as sample preparation. Accuracy and precision reported by Shou et al. [67] were slightly better than those reported by Zhou et al. [68], whereas Zhou et al. [68] reported a lower limit of quantification of 1 $\mu g L^{-1}$, a factor of ten lower than the 10 $\mu g L^{-1}$ of Shou et al. [67]. The upper limit of quantification was 1 $mg L^{-1}$ for both methods. Liu et al. [69] presented a method for simultaneous quantification of viramidine, a prodrug of ribavirin, and ribavirin in human plasma, using the ^{13}C-labelled analogues of both drugs as internal standards. The analytical range was 0.001 to 1 $mg L^{-1}$ for both analytes.

For quantitative determination of cidofovir, a nucleotide analogue which is approved for the therapy of CMV retinitis in AIDS patients, Breddemann et al. [70] developed a method which was used for a pharmacokinetic study in paediatric cancer patients. With solid-phase extraction of 0.3 mL serum, an analytical range from 0.0078–10 $mg L^{-1}$ could be achieved.

For determination of penciclovir, valacyclovir, acyclovir, and ganciclovir, a total of four LC–MS(–MS) methods have been presented since 2000 [71–74]. Lee et al. [72] published a method for a bioequivalence study, in which protein precipitation in 250 μL of plasma led to an analytical range of 0.05–10 $mg L^{-1}$. The run time of the method was quite short—2.4 min. Yadav et al. [74] and Kasiari et al. [71] both presented methods for simultaneous determination of valacylovir and acyclovir in human plasma. Yadav et al. [74] used selected reaction monitoring (SRM) with analytical ranges of 0.005 to 1.075 $mg L^{-1}$ for valacyclovir and if 0.0476 to 10.225 $mg L^{-1}$ for acyclovir. Kasiari et al. [71] applied single ion monitoring (SIM) with an analytical range from 0.02 to 0.8 $mg L^{-1}$ for valacyclovir and 0.1 to 20 $mg L^{-1}$ for acyclovir. Ganciclovir, the internal standard chosen by Kaisari et al. [71], has more structural

Table 2 Overview of techniques using LC–MS(–MS) for therapeutic drug monitoring of other antiviral drugs in humans

Compound(s)	Sample preparation	Internal standard	Stationary phase	Mobile phase	Detection mode	Run time (min)	Validation data	Analytical range
Adefovir [58]	Protein precipitation (500 µL serum), dilution (50 µL urine)	Acyclovir	Diamonsil C$_{18}$	Methanol and water with formic acid	Triple-stage, ESI, positive mode, SRM	8	Linearity, LOQ, accuracy and precision, stability (post-extraction, bench top, freeze–thaw, long term), specificity, recovery	1.25–160 µg L^{-1} (serum), 0.05–8 mg L^{-1} (urine)
Adefovir [59]	Protein precipitation (500 µL plasma)	None	Hypersil BDS C$_{18}$	Methanol and water with formic acid	Triple-stage, ESI, positive mode, SRM	7.8	Selectivity, matrix effects, accuracy and precision, recovery, linearity, LOQ, stability (bench top, freeze–thaw, post-extraction, long-term)	1.5–90 µg L^{-1}
Adefovir [60]	Protein precipitation (250 µL plasma)	9-(2-Phosphono methoxypropyl) adenine (PMPA)	Diamonsil C$_{18}$	Methanol and water with formic acid	Triple-stage, ESI, positive mode, SRM	8	Selectivity, matrix effects, linearity, LOQ, accuracy and precision, recovery, stability (bench top, freeze–thaw, post-extraction, long-term)	0.25–100 µg L^{-1}
Adefovir [61]	SPE (500 µL serum)	Adefovir-13C	MCX and XDB-C8	Acetonitrile and water with acetic acid	Triple-stage, ESI, negative mode, SRM	6	Selectivity, LOQ, linearity, accuracy and precision, stability (bench top, freeze–thaw), recovery	0.1–10 µg L^{-1}
Adefovir [62]	Protein precipitation (200 µL plasma)	9-(2-Phosphono methoxypropyl) adenine (PMPA)	XTerra MS-MS C$_{18}$	Methanol and water	Triple-stage, ESI, positive mode, SRM	10	Selectivity, matrix effects, linearity, LOQ, accuracy and precision, recovery, stability (long-term, bench top, post-extraction, freeze–thaw)	0.2–100 µg L^{-1}
Cidofovir [70]	SPE (300 µL serum)	9-(2-Phospho nylmethoxyethyl) guanine (PMEG)	BondElut SAX and Purospher Star RP-18	Methanol and water with ammonia	Triple-stage, ESI, negative mode, SRM	6	Selectivity, accuracy and precision, linearity, recovery, matrix effects, stability (stock solutions, bench top, postextraction, freeze–thaw)	0.078–10.0 mg L^{-1}
Entecavir [63]	SPE (1 mL plasma)	Lobucavir	Oasis HLB and XTerra MS C$_{18}$	Methanol and water with ammonium bicarbonate and ammonia	Triple-stage, ESI, positive mode, SRM	5	Specificity, LOQ, recovery, matrix effects, stability (freeze–thaw, long-term), linearity, accuracy and precision,	0.005–25 µg L^{-1}
Penciclovir [72]	Protein precipitation (250 µL plasma)	Acyclovir	Capcellpak MGII C$_{18}$	Acetonitrile and aqueous ammonium acetate with formic acid	Triple-stage, ESI, positive mode, SRM	2.4	Linearity, selectivity, accuracy and precision, LOD, matrix effects, stability (bench top, freeze–thaw)	0.05–10 mg L^{-1}
Ribavirin [67]	Protein precipitation (100 µL serum or plasma)	Bamethan	Betasil silica	Water and acetonitrile with TFA	Triple-stage, ESI, negative mode, SRM	3	Specificity, accuracy and precision, LOQ, carry-over, stability (freeze–thaw, bench top, stock solution, post-extraction), recovery, matrix effectsX	0.01–10.0 mg L^{-1}

Drug [ref]	Sample preparation	Analyte	Column	Mobile phase	MS	Run time (min)	Validation	Linear range
Ribavirin [68]	Protein precipitation (100 µL plasma)	Aciclovir	SinoChrom ODS-BP	Acetonitrile and aqueous ammonium acetate with formic acid	Triple-stage, ESI, positive mode, SRM	4.5	Linearity, accuracy and precision, stability (stock solutions, freeze–thaw, bench top), specificity, recovery, LOQ	0.001–1.0 mgL⁻¹
Ribavirin [66]	SPE (100 µL RBC)	Ribavirin-13C	Zorbax SB-C$_{18}$	Methanol and water with acetic acid	Triple-stage, ESI, negative mode, SRM	7.5	Accuracy and precision, extraction efficiency, sensitivity, selectivity, linearity, stability	0.1–10.0 mgL⁻¹
Ribavirin, viramidine, [69]	Protein precipitation (100 µL plasma)	Viramidine-13C, ribavirin-13C	Polarity C$_{18}$	Methanol and water with formic acid	Triple-stage, ESI, negative mode, SRM	8	Accuracy and precision, LOQ, specificity, recovery, stability (freeze–thaw, bench top, post-extraction)	Both analytes: 0.001–1.0 mgL⁻¹
Tenofovir [65]	Protein precipitation (250 µL plasma)	Adefovir	Synergi Polar-RP	Acetonitrile and water with acetic acid	Triple-stage, ESI, positive mode, SRM	7	Linearity, accuracy and precision, specificity, matrix effect, LOQ, stability (freeze–thaw, bench top, post-extraction)	0.010–0.75 mgL⁻¹
Tenofovir [64]	SPE (200 µL plasma)	Adefovir	Oasis MCX and Chromolith C$_{18}$	Acetonitrile and water with formic acid	Triple-stage, ESI, positive mode, SRM	1.8	Carryover, linearity, LOQ, selectivity, accuracy and precision, matrix effect, recovery, stability, dilution integrity	0.003–1.0 mgL⁻¹
Valacyclovir and acyclovir [74]	SPE (500 µL plasma)	Fluconazole	Oasis HLB and Gemini C$_{18}$	Methanol and water with formic acid	Triple-stage, ESI, positive mode, SRM	3	Carryover (autosampler), selectivity, linearity, LOQ, accuracy and precision, recovery, matrix effects, stability (bench top, post-extraction, freeze–thaw, stock solution), dilution integrity	Valacyclovir 0.005–1.075 mgL⁻¹, acyclovir 0.0476–10.225 mgL⁻¹
Valacyclovir, and acyclovir [71]	Protein precipitation (500 µL plasma)	Ganciclovir	Porous graphitized carbon column	Acetonitrile, water with diethylamine	Single stage, ESI, negative mode, SIM	4	Linearity, accuracy and precision, recovery, stability (bench top, freeze–thaw)	Valacyclovir 0.02–0.8 mgL⁻¹, acyclovir 0.1–20 mgL⁻¹
Valganciclovir and ganciclovir [73]	Protein precipitation (50 µL plasma)	Acyclovir	Aquasil C$_{18}$	Methanol and water with formic acid	Triple-stage, ESI, positive mode, SRM	5.5	Linearity, accuracy and precision, specificity, matrix effects, stability (freeze–thaw, bench top, post-extraction)	Valganciclovir 0.004–0.512 mgL⁻¹, ganciclovir 0.1–12.8 mgL⁻¹

APCI, atmospheric pressure chemical ionization; ESI, electrospray ionization; ; LLE, liquid–liquid extraction; LOQ, limit of quantification; RT, room temperature; SIM, single ion monitoring; SRM, selected reaction monitoring

similarities to the analytes than does fluconazole, which was used in the method described by Yadav et al. [74]. Xu et al. [73] presented a method for simultaneous quantification of valganciclovir and ganciclovir in plasma. The lower limit of quantification for valganciclovir was 0.004 mg L^{-1} which was even slightly lower than that one reported by Yadav et al. [74], even though only 50 µL plasma was used for protein precipitation.

Antibacterial drugs

TDM of aminoglycosides and glycopeptides is performed routinely nowadays. This has helped to greatly reduce the number of patients having the severe side effects of these drugs. For all drugs which are clinically used, immunoassays are available and enable rapid and reliable quantification. For other antibiotic drugs, e.g. drugs against tuberculosis, no immunoassays are available and, therefore, chromatographic methods have to be applied to check a patient's adherence to therapy or to monitor drug levels in cases of suspected pharmacokinetic interactions.

Many methods published for quantification of antibacterial drugs still use HPLC with UV detection, e.g. for quantification of linezolid [75–77], daptomycin [78], tobramycin [79], or the simultaneous determination of several antibiotic drugs [80]. Since 2000, five papers have been published on use of LC–MS(–MS) for quantification of tobramycin [81, 82] and vancomycin [83] specifically, or methods for simultaneous quantification of several antibacterial drugs [84–86]. All the methods used protein precipitation for sample preparation [81, 82, 84, 86], except that described by Zhang et al., for which SPE was used [83]. Concerning the selection of internal standards, the structural analogue sisomycin was used in two methods [81, 82]. Lu et al. [84] used one of the therapeutically used aminoglycoside antibiotic drugs (tobramycin) which may be used for well controlled clinical studies but should not be used routinely when analysing real clinical samples. Concerning detection of the analytes, Lu et al. [84] described a combination of nanospray ESI and Q-TOF mass spectrometry. Data for precision and accuracy, and the analytical range were presented convincingly. Unfortunately, the authors only validated their assay for amikacin and did not include any data on matrix effects or ion suppression. The procedures published by de Velde et al. [85] and Baietto et al. [86] were fully validated for all the compounds included in the method, namely clarithromycin and its metabolite and rifampicin and its metabolite [85], and daptomycin, amikacin, gentamicin, and rifampicin [86]. Both authors also included matrix effects in their validation process.

Concerning the quantification of tuberculostatic drugs, the situation is the same as for the other antibacterial drugs. Several methods using UV or diode array detection have been reported [87–90]. In the last few years four papers have been published describing the quantification of tuberculostatic drugs, only, by LC–MS(–MS). The drugs measured included rifampicin [91, 92], isoniazid [92, 93], ethambutol [92–94], and pyrazinamid [92, 94]. All methods used protein precipitation as extraction method with a sample (plasma) volume ranging from 50 to 100 µL. The method described by Song et al. [92] included the determination of the four first-line drugs for treatment of tuberculosis with an analytical range covering trough and peak concentrations. Unfortunately isoniazid, ethambutol, and pyrazinamide eluted close to the void volume of the column. The authors then added aminonicotinic acid as second internal standard which coeluted with the three compounds and therefore underwent the same ion suppression processes. They demonstrated that the method was accurate and precise and applied it to patients taking, mostly, four different tuberculostatic drugs without any problems. Nevertheless special care must be taken if compound peaks elute close to the void volume, and the addition of an internal standard cannot always compensate for ion suppression as the ionisation efficiency might be much better than for the analyte (Table 3).

Antihelmintic drugs

TDM of antihelmintic drugs is most often done for benzimidazoles, e.g. the active metabolite of albendazole. Other compounds which are sometimes analysed are, e.g., ivermectin or praziquantel. For TDM of antihelmintic drugs, two main techniques have been used: capillary electrophoresis [95, 96] and HPLC. Only a few methods using LC–MS(–MS) have been published [97–99]; notably, two of the three were from the same research group (Table 4). Perhaps because of the economic situation in countries using antihelmintic drugs, methods using UV detection [100, 101], sometimes in combination with fluorescence detection [102], have been published.

Two of the published LC–MS(–MS) methods used liquid–liquid extraction of a relatively large volume of plasma (1 mL) with ESI detection in positive mode [97, 98]. The first paper of Bonato et al. [97] covered the quantification of the two main metabolites of albendazole, albendazole sulfoxide and albendazole sulfone, with an analytical range of 5–2,500 µg L^{-1} for the first metabolite and 0.5–250 µg L^{-1} for the second. In the second paper, Bonato et al. [98] presented a method, which, besides the two albendazole metabolites, also included praziquantel

Table 3 Overview of techniques using LC–MS(–MS) for therapeutic drug monitoring of antibiotic drugs in humans

Compound(s)	Sample preparation	Internal standard	Stationary phase	Mobile phase	Detection mode	Run time (min)	Validation data	Analytical range
Tobramycin [81]	Protein precipitation (20 μL plasma)	Sisomycin	Security Guard C$_{18}$	Aqueous ammonium acetate, formic acid, methanol, heptafluorobutyric acid	Triple-stage, ESI, positive mode, SRM	2.5	Selectivity, linearity, accuracy and precision, LOQ, stability (autosampler), matrix effects	0.1–50.0 mgL^{-1}
Tobramycin [82]	Protein precipitation (200 μL plasma)	Sisomicin	Pathfinder MR	Aqueous ammonium acetate and acetonitrile	Triple-stage, ESI, positive mode, SRM	3	Selectivity, linearity, accuracy and precision, LOQ, stability (−20 °C, freeze–thaw, autosampler), matrix effects	0.05–1.0 mgL^{-1}
Vancomycin [83]	SPE (200 μL plasma)	Atenolol	Strata-X and ACE-3-C8	Formic acid, water, and acetonitrile	Ion trap/Orbitrap, ESI, positive mode, full scan	5	Linearity, accuracy and precision, LOQ, stability (freeze–thaw, autosampler)	0.05–10 mgL^{-1}
Amikacin, gentamicin, kanamycin and neomycin [84]	Protein precipitation (10 μL plasma)	Tobramycin	C$_{18}$ capillary column	Heptafluorobutyric acid (HFBA), water, and methanol	Q-TOF, nanospray ESI, positive mode, SRM	5	Selectivity, accuracy and precision, recovery	Amikacin: 1–30 mgL^{-1}
Rifampicin [91]	Protein precipitation (50 μL sample)	Rifamycin SV	Betasil Phenyl-Hexyl	Aqueous ammonium acetate and acetonitrile	Triple-stage, APCI, positive mode, SIM	6	Linearity, recovery, stability (heat inactivation, freeze–thaw), matrix effects, accuracy and precision	0.1–12.8 mgL^{-1}
Isoniazid and ethambutol [93]	Protein precipitation (100 μL plasma)	Metformin	Atlantis dC$_{18}$	Water and methanol with formic acid	Triple-stage, APCI, positive mode, SRM	3	Selectivity, matrix effects, linearity, LOQ, accuracy and precision, recovery, stability (post extraction, storage, stock solution)	Both analytes: 0.01–5.0 mgL^{-1}
Ethambutol, pyrazinamide [94]	Protein precipitation (50 μL plasma)	Ethambutol-d4, pyrazinamide-d3	Chromolith SpeedROD RP-18e	Water, methanol, and TFA	Triple-stage, APCI, positive mode, SRM	3.8	Linearity, accuracy and precision, selectivity, recovery, matrix effects, stability (bench-top, freeze–thaw, post-extraction, long-term, stock solution)	Ethambutol 0.01–5.0 mgL^{-1}, pyrazinamide 0.05–2.5 mgL^{-1}
Isoniazid, acetylisoniazid, rifampicin, 25-desacetylrifampicin, pyrazinamide, and ethambutol [92]	Protein precipitation (50 μL serum)	Rifabutin, 6-aminonicotinic acid	Hydrosphere C$_{18}$	Water and methanol with formic acid	Triple-stage, ESI, positive mode, SRM	4	Accuracy and precision, linearity, LOD, LOQ, matrix effects, recovery	Isoniazid and ethambutol 0.5–8. mgL^{-1}, rifampicin and pyrazinamide 5–80 mgL^{-1}
Clarithromycin, 14-hydroxyclarithromycin, rifampicin, and 25-desacetylrifampicin [85]	Protein precipitation (10 μL plasma)	Cyanoimipramine	HyPurity Aquastar	Aqueous ammonium acetate, acetic acid, trifluoroacetic anhydride, and acetonitrile	Triple-stage, ESI, positive mode, SRM	3.6	Selectivity, linearity, accuracy and precision, matrix effects, dilution integrity, recovery, stability (freeze–thaw, long term storage, bench top, post-extraction)	Clarithromycin and 14-hydroxyclarithromycin 0.1–10 mgL^{-1}, rifampicin and 25-desacetylrifampicin 0.2–5 mgL^{-1}

Table 3 (continued)

Compound(s)	Sample preparation	Internal standard	Stationary phase	Mobile phase	Detection mode	Run time (min)	Validation data	Analytical range
Daptomycin, amikacin, gentamicin and rifampicin [86]	Protein precipitation (200 µL plasma)	Quinoxaline	Synergy Hydro-RP	Formic acid, water and acetonitrile	Single stage, ESI, positive mode, SRM	20	Selectivity, accuracy and precision, LOQ, recovery, stability (−20 °C, bench top, autosampler, freeze-thaw), matrix effects	Daptomycin: 1.6–130 mg L⁻¹, amikacin: 2.3–150 mg L⁻¹, gentamicin: 0.6–40 mg L⁻¹, rifampicin: 0.6–40 mg L⁻¹

APCI, atmospheric pressure chemical ionization; ESI, electrospray ionization; ; LLE, liquid–liquid extraction; LOQ, limit of quantification; RT, room temperature; SIM, single ion monitoring; SRM, selected reaction monitoring

Table 4 Overview of techniques using LC–MS(–MS) for therapeutic drug monitoring of antihelmintic drugs in humans

Compound(s)	Sample preparation	Internal standard	Stationary phase	Mobile phase	Detection mode	Run time (min)	Validation data	Analytical range
Albendazole sulfoxide, albendazole sulfone [97]	LLE (1 mL plasma)	Phenacetin	LiChrospher CN	Methanol, water with acetic acid	Triple-stage, ESI, positive mode, SRM	15	Recovery, linearity, LOQ, selectivity, accuracy and precision, stability	Albendazole sulfoxide 5–2,500 µg L⁻¹, albendazole sulfone 0.5–250 µg L⁻¹
Albendazole metabolites, praziquantel and metabolite [98]	LLE (1 mL plasma)	Phenacetin	LiChrospher CN	Methanol, water with acetic acid	Triple-stage, ESI, positive mode, SRM	12	Linearity, recovery, accuracy and precision, LOQ, stability (freeze–thaw, bench top), matrix effects	All analytes: 5–2,500 µg L⁻¹
Ivermectin [99]	Protein precipitation and filtration (100 µL plasma)	Emamectin	BetaBasic-8	Acetonitrile, aqueous ammonium acetate with formic acid	Triple-stage, ESI, positive mode, SRM	4.5	Accuracy and precision, linearity, selectivity, LOQ, recovery, stability (bench top, post-extraction), matrix effects	1–2,000 µg L⁻¹

APCI, atmospheric pressure chemical ionization; ESI, electrospray ionization; ; LLE, liquid–liquid extraction; LOQ, limit of quantification; RT, room temperature; SIM, single ion monitoring; SRM, selected reaction monitoring

Table 5 Overview of techniques using LC–MS(–MS) for therapeutic drug monitoring of antimalarial drugs in humans

Compound(s)	Sample preparation	Internal standard	Stationary phase	Mobile phase	Detection mode	Run time (min)	Validation data	Analytical range
Amodiaquine and metabolite [123]	Protein precipitation (200 µL whole blood)	Hydroxychloroquine	Venusil MP-C$_{18}$	Acetonitrile, water with pentafluoropropionic acid	Triple-stage, APCI, positive mode, SRM	2	Selectivity, matrix effect, linearity, accuracy and precision, recovery, stability (freeze–thaw, bench top)	Amodiaquine 0.15–100 µg L^{-1}, N-desethylamodiaquine 1.5–1,000 µg L^{-1}
Artemether, artesunate, dihydroartemisinin, amodiaquine, N-desethyl-amodiaquine, lumefantrine, desbutyl-lumefantrine, piperaquine, pyro naridine, meflo quine, chloroquine, quinine, pyrimethamine, and sulfadoxine [115]	Protein Precipitation (200 µL plasma)	Trimipramine-d$_3$ and artemisinin	Atlantis dC$_{18}$	Aqueous ammonium formate and acetonitrile with formic acid	Triple-stage, ESI, positive mode, SRM	21	Accuracy and precision, LOQ, LOD, stability (bench top, freeze–thaw), matrix effect, extraction yield, recovery, selectivity, memory effect, dilution integrity	Piperaquine 2–4,000 µg L^{-1}, desethyl-amodiaquine 0.3–600 µg L^{-1}, chloroquine 2.5–5,000 µg L^{-1}, amodiaquine 0.3–600 µg L^{-1}, pyronaridine 1–1,000 µg L^{-1}, quinine 2.5–5,000 µg L^{-1}, sulfadoxine 0.5–1,000 µg L^{-1}, pyrimethamine 0.5–1,000 µg L^{-1}, mefloquine 2.5–5,000 µg L^{-1}, dihydroartemisinin 1–2,000 µg L^{-1}, artesunate 2–2,000 µg L^{-1}, artemether 5–2,000 µg L^{-1}, lumefantrine 4–4,000 µg L^{-1}, desbutyl-lumefantrine 4–4,000 µg L^{-1}
Artemether and dihydroartemisinin [117]	SPE (500 µL plasma)	Artemisinin	Oasis HLB and Symmetry C$_{18}$	Aqueous ammonium formate, acetonitrile with formic acid	Triple-stage, ESI, positive mode, SRM	6	LOQ, accuracy and precision, recovery, matrix effect, stability (freeze–thaw, bench top)	Both analytes: 2–200 µg L^{-1}
Artemether and dihydroartemisinin [116]	LLE (500 µL plasma)	Artemisinin	Alltima C$_{18}$	Acetonitrile, water with acetic acid	Triple-stage, positive mode, APCI, SIM	18	Selectivity, recovery, linearity, accuracy and precision, stability (freeze–thaw, bench top)	Both analytes: 5–200 µg L^{-1}
Artemisinin [122]	SPE (50 µL plasma)	Artesunate	Oasis HLB and Hypersil Gold C$_{18}$	Acetonitrile, water with ammonium acetate	Triple-stage, ESI, positive mode, SRM	5.1	Linearity, accuracy and precision, LOQ, carryover, over-curve dilution, stability (stock solutions, freeze–thaw, bench top), recovery, matrix effects	1.03–762 µg L^{-1}

Table 5 (continued)

Compound(s)	Sample preparation	Internal standard	Stationary phase	Mobile phase	Detection mode	Run time (min)	Validation data	Analytical range
Artensunate and dihydroartemisinin [118]	SPE (500 µL plasma)	Artemisinin	Oasis HLB and Synergi Max-RP	Acetonitrile, methanol with 0.1% acetic acid	Single stage, APCI, positive mode, SIM	21	Selectivity, accuracy and precision, recovery, stability (freeze–thaw, bench top, post-extraction), linearity	Both analytes: 1–3,000 µg L^{-1}
Artesunate, dihydroartemisinin [120]	LLE (1 mL plasma)	Artemisinin	XTerra RP18	Acetonitrile and water with formic acid	Triple-stage, APCI, positive mode, SIM	14	Selectivity, linearity, recovery, accuracy and precision, stability (bench top, post-extraction)	Artesunate 25–1,000 µg L^{-1}, dihydroartemisinin 10–1,000 µg L^{-1}
Artesunate and dihydroartemisinin [119]	LLE2	Artemisinin	Eclipse XDB-C$_{18}$	Acetonitrile, water with acetic acid	Ion trap, ESI, positive mode	12	LOD, LOQ, linearity, recovery,	Both analytes: 10–3,200 µg L^{-1}
Artesunate and dihydroartemisinin [121]	LLE (50-100 µL of plasma)	Indomethacin	Pursuit C$_{18}$	Aqueous ammonium acetate and acetonitrile	Triple-stage, ESI, positive mode	7	Linearity, accuracy and precision, recovery	Both analytes: 2–500 µg L^{-1}

APCI, atmospheric pressure chemical ionization; ESI, electrospray ionization; ; LLE, liquid-liquid extraction; LOQ, limit of quantification; RT, room temperature; SIM, single ion monitoring; SRM, selected reaction monitoring

Table 6 Overview of techniques using LC–MS(–MS) for therapeutic drug monitoring of other antiprotozoal drugs in humans

Compound(s)	Sample preparation	Internal standard	Stationary phase	Mobile phase	Detection mode	Run time (min)	Validation data	Analytical range
Metronidazole and spiramycin I [126]	LLE (500 µL plasma, saliva or gingival crevicular fluid)	Ornidazole	Kromasil C$_{18}$	Acetonitrile and water with formic acid	Triple-stage, ESI, positive mode, SRM	10	Accuracy and precision, linearity, recovery, stability (freeze–thaw, bench top, long-term, post-extraction), specificity, matrix effects	Metronidazol 0.05–5 mg L^{-1}, spiramycin I 0.015–2 µg L^{-1}
Metronidazole [127]	LLE (250 µL plasma)	Zidovudine	Varian C$_{18}$ microsorb	Aqueous ammonium acetate with formic acid, acetonitrile	Triple-stage, ESI, positive mode, SRM	5	Specificity, linearity, LOQ, accuracy and precision, recovery, stability (freeze–thaw, post-extraction, bench top)	0.05–8 mg L^{-1}

APCI, atmospheric pressure chemical ionization; ESI, electrospray ionization; ; LLE, liquid-liquid extraction; LOQ, limit of quantification; RT, room temperature; SIM, single ion monitoring; SRM, selected reaction monitoring

and its main metabolite, *trans*-4-hydroxypraziquantel. For all the analytes, the analytical range was 5–2,500 µgL^{-1}. Matrix effects, an important factor lacking in the validation of the procedure reported by Bonato et al. 2003 [97] was added to the validation procedure of the method from the same authors in 2007 [98]. For determination of ivermectin, Pereira et al. [99] published a method using a semi-automated protein-precipitation approach, using ESI in positive mode. The analytical range for ivermectin was 1–2,000 µgL^{-1}.

Antiprotozoal drugs

Antimalarial drugs

The oldest antimalarial drug still in use, quinine, has a favourable feature, namely its strong fluorescence upon activation with UV light. This feature has been used for detection of quinine and metabolites and other antimalarial drugs in human samples, either with classical HPLC [103, 104] or with capillary–LC [105]. Several different extraction techniques were used for methods using HPLC with UV detection for the quantification of antimalarial drugs: liquid-phase microextraction [106], solid-phase microextraction [107], automated solid-phase extraction [108], and extraction with restricted-access materials [109]. As sample material, many methods use dried blood spots, either with UV detection [110–113], or fluorescence detection [114].

Chromatographic methods for the quantification of newer antimalarial drugs usually applied SRM mass spectrometry as detection tool (Table 5). An LC–MS(–MS) procedure enabling simultaneous determination of 14 antimalarial drugs and their metabolites in plasma was recently published by Hodel et al. [115]. The method used 200 µL plasma, a fast protein precipitation procedure, positive mode ESI, and SRM detection; the runtime was 21 min. The method was extensively validated, including a check of the stability of endoperoxide-containing drugs in haemolysed plasma. Nevertheless, a disadvantage of the method could be that only two internal standards, trimipramine-d3 and artemisinin (itself an antimalarial drug), were used. Regarding the distribution of the retention times in this multi-analyte procedure (approximately 4–16 min), two internal standards with retention times of 8.8 min (trimipramine-d3) and 11.1 min (artemisinin) would not be able to compensate for all fluctuations, e.g. of the ionization efficiency, for all the analytes.

The newer substances, artemether and artesunate, on which many pharmacokinetic trials have been performed, dominate the picture of newly developed LC–MS(–MS) methods for antimalarial drugs. For artemether, three LC–MS(–MS) methods have been published since 2000, including the method by Hodel et al. [115] discussed

earlier. In 2002, Souppart et al. [116] presented a method for quantification of artemether and its main metabolite, dihydroartemisinin, with liquid–liquid extraction of 0.5 mL plasma and positive mode APCI, monitoring single fragment ions for both analytes with the same mass-to-charge ratio of 267. The analytical range was 5–200 µgL^{-1} for both analytes; evaluation of matrix effects was missing from the validation study. The method was applied to a pharmacokinetic interaction study. Huang et al. [117] presented, in 2009, a method with a slightly wider analytical range, 2–200 µgL^{-1} for artemether and dihydroartemisinin. The method used solid-phase extraction, again 0.5 mL plasma, ESI in positive mode, and SRM, monitoring of the transitions *m/z* 316→267 for artemether and *m/z* 302→267 for dihydroartemisinin. In this method, the runtime was 6 min, which is only one third of that for the method by Souppart et al. [116]. The applicability of the method was shown by analysing samples from healthy volunteers taking artemether for three days.

For artesunate and its metabolite dihydroartemisinin, a total of five LC–MS(–MS) methods, including the method by Hodel et al. [115] discussed earlier, have been introduced since 2000. Three of these methods used artemisinin as internal standard [118–120], one used indomethacin [121]. The run times of the methods differed quite substantially, from 7 min [121] to 21 min [118]. Also the analytical ranges differed, with a lower limit of 1 µgL^{-1} for both analytes [118] up to 25 µgL^{-1} for artesunate [120] and 10 µgL^{-1} for dihydroartemisinin [119, 120]. The upper limit of quantification was 3,200 µgL^{-1} for both analytes [119]. For extraction, the sample volume required ranged from 50 µL [121] to 1 mL [120]. Naik et al. [118] used solid-phase extraction, the others liquid–liquid extraction [119–121]. For ionization, APCI [118, 120] and ESI [119, 121] were applied equally. Accuracy and precision were comparable for all methods.

The quantification of artemisinin using artesunate as internal standard was performed by Lindegardh et al. [122]. The method used a very small amount of sample with only 50 µL plasma. The analytical range of the method was 1.03–762 µgL^{-1}.

For quantification of amodiaquine and its active metabolite *N*-desethylamodiaquine in whole blood, Chen et al. [123] developed and validated a method using ion-pair liquid chromatography. The analytical range of the method was 0.15–100 µgL^{-1} for amodiaquine and 1.5–1,000 µg L^{-1} for *N*-desethylamodiaquine. This method was also applied to a pharmacokinetic study.

Others

Besides antimalarial drugs, TDM is used for a few other antiprotozoal drugs only, the most important being metronidazole, a nitroimidazole derivative. HPLC was mainly

used for quantification of antiprotozoal drugs, with either diode-array [124] or UV [125] detection. In the time period considered for this review two papers have been published describing quantification of antiprotozoal drugs by LC–MS (–MS) (Table 6) [126, 127].

The LC–MS–MS method developed by Sagan et al. [126] enabled determination of metronidazole and spiramycin I in plasma, saliva, and gingival crevicular fluid, using liquid–liquid extraction of a 500-µL sample. Silva et al. [127] presented a method for metronidazole only, and for which less sample (250 µL) was needed for the liquid–liquid extraction. The analytical range was also extended, and ranged from 0.05–8 mg L^{-1} compared with 0.05–5 mg L^{-1} for the method by Sagan et al. [126]; also, the run-time was shortened by 50% to 5 min. Precision and accuracy of both intra-day and inter-day assays were comparable. After complete validation, the method of Silva et al. [127] was applied to a bioequivalence study.

Conclusions

This paper illustrates the increasing role of LC–MS(–MS) for measurement of anti-infective drugs. For each drug category, methods have been published enabling quantification of several drugs in a single run. Because most of the drugs discussed in this article are not used in big patient groups, this approach helps the laboratory to enable a short turn-around-time also with low sample numbers for each compound. Nevertheless it should not be forgotten that LC–MS(–MS) methods need a very careful validation, taking special care to minimise ion suppression and to select appropriate internal standards.

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