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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, extraction recovery, matrix effects, 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-dC_{18}	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 mgL^{-1}, tenofovir: 0.01–0.6 mgL^{-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 mgL^{-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 mgL^{-1}, ritonavir: 0.0029–1.452 mgL^{-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 mgL^{-1}, ritonavir: 0.00973–8.11 mgL^{-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 mgL^{-1} (plasma), 0.001–0.2 mgL^{-1} (semen, ultrafiltrate), 0.001–0.1 mgL^{-1} saliva),; ritonavir: 0.001–0.2 mgL^{-1} (plasma), 0.001–0.05 mgL^{-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 mgL^{-1} (plasma), 0.20–500 μgL^{-1} (ultrafiltrate), 0.10–250 $ng/3\times10^6$ cells (PBMCs)

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 mgL^{-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 mgL^{-1}, emtricitabine: 0.01–1.50 mgL^{-1}, lamivudine: 0.01–1.50 mgL^{-1}, stavudine: 0.02–3.00 mgL^{-1}, tenofovir: 0.0050–0.7500 mgL^{-1}, zidovudine: 0.02–2.50 mgL^{-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 mgL^{-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 mgL^{-1}

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 C_{18}	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 C_{18}	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, emtirictabine, lamivudine, stavudine, tenofovir, zalatabine, zidovudine, delavirdine, efavirenz, nevirapine [44]	Protein precipitation (200 μL plasma or cerebrospinal fluid)	*S*-CH_3-saquinavir, saquinavir-d5, cyclopropyl-ritonavir	Nucleosil C_{18}-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; CCR5, CCR5 antagonist; DDA, data-dependent acquisition; 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 mgL^{-1} for lopinavir and ritonavir, irrespective of the sample material. The upper limits of quantification were 2.0 mgL^{-1} for lopinavir in plasma, 0.2 mgL^{-1} for lopinavir in semen and ultrafiltered plasma, and 0.1 mgL^{-1} for saliva. For ritonavir the values were 0.2 mgL^{-1} for plasma and 0.05 mgL^{-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 mgL^{-1}; the respective value for ultrafiltered plasma was 0.20–500 μgL^{-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], cimetidine [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 $\mu g L^{-1}$ (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 $\mu g L^{-1}$
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 $\mu g L^{-1}$
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 $\mu g L^{-1}$
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 $\mu g L^{-1}$
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 $\mu g L^{-1}$
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 $mg L^{-1}$

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}
Valacylovir, 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 mgL^{-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 μgL^{-1} for the first metabolite and 0.5–250 μgL^{-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 mgL^{-1}, amikacin: 2.3–150 mgL^{-1}, gentamicin: 0.6–40 mgL^{-1}, rifampicin: 0.6–40 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

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 μgL^{-1}, albendazole sulfone 0.5–250 μgL^{-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 $\mu g L^{-1}$
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 μgL^{-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 $\mu g L^{-1}$, *N*-desethylamodiaquine 1.5–1,000 $\mu g L^{-1}$
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 $\mu g L^{-1}$, desethyl-amodiaquine 0.3–600 $\mu g L^{-1}$, chloroquine 2.5–5,000 $\mu g L^{-1}$, amodiaquine 0.3–600 $\mu g L^{-1}$, pyronaridine 1–1,000 $\mu g L^{-1}$, quinine 2.5–5,000 $\mu g L^{-1}$, sulfadoxine 0.5–1,000 $\mu g L^{-1}$, pyrimethamine 0.5–1,000 $\mu g L^{-1}$, mefloquine 2.5–5,000 $\mu g L^{-1}$, dihydroartemisinin 1–2,000 $\mu g L^{-1}$, artesunate 2–2,000 $\mu g L^{-1}$, artemether 5–2,000 $\mu g L^{-1}$, lumefantrine 4–4,000 $\mu g L^{-1}$, desbutyl-lumefantrine 4–4,000 $\mu g L^{-1}$
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 $\mu g L^{-1}$
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 $\mu g L^{-1}$
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 $\mu g L^{-1}$

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 $\mu g L^{-1}$
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 $\mu g L^{-1}$, dihydroartemisinin 10–1,000 $\mu g L^{-1}$
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 $\mu g L^{-1}$
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 $\mu g L^{-1}$

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 $mg L^{-1}$
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 $\mu g L^{-1}$. 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 $\mu g L^{-1}$.

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 $\mu g L^{-1}$ 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 $\mu g L^{-1}$ 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 $\mu g L^{-1}$ for both analytes [118] up to 25 $\mu g L^{-1}$ for artesunate [120] and 10 $\mu g L^{-1}$ for dihydroartemisinin [119, 120]. The upper limit of quantification was 3,200 $\mu g L^{-1}$ 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 $\mu g L^{-1}$.

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 $\mu g L^{-1}$ for amodiaquine and 1.5–1,000 $\mu g L^{-1}$ 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.

References

1. Saint-Marcoux F, Sauvage FL, Marquet P (2007) Current role of LC–MS in therapeutic drug monitoring. Anal Bioanal Chem 388:1327–1349
2. Remane D, Wissenbach DK, Meyer MR, Maurer HH (2010) Systematic investigation of ion suppression and enhancement effects of fourteen stable-isotope-labeled internal standards by their native analogues using atmospheric-pressure chemical ionization and electrospray ionization and the relevance for multi-analyte liquid chromatographic/mass spectrometric procedures. Rapid Commun Mass Spectrom 24:859–867
3. Kredo T, Van der Walt JS, Siegfried N, Cohen K (2009) Therapeutic drug monitoring of antiretrovirals for people with HIV. Cochrane Database Syst Rev: CD007268
4. Difrancesco R, DiCenzo R, Vicente G, Donnelly J, Martin TM, Colon LA, Schifito G, Morse GD (2007) Determination of lopinavir cerebral spinal fluid and plasma ultrafiltrate concentrations by liquid chromatography coupled to tandem mass spectrometry. J Pharm Biomed Anal 44:1139–1146
5. Ter Heine R, Hillebrand MJX, Rosing H, van Gorp ECM, Mulder JW, Beijnen JH, Huitema ADR (2009) Quantification of the HIV-integrase inhibitor raltegravir and detection of its main metabolite in human plasma, dried blood spots and peripheral blood mononuclear cell lysate by means of high-performance liquid chromatography tandem mass spectrometry. J Pharm Biomed Anal 49:451–458
6. Merschman SA, Vallano PT, Wenning LA, Matuszewski BK, Woolf EJ (2007) Determination of the HIV integrase inhibitor, MK-0518 (raltegravir), in human plasma using 96-well liquid–liquid extraction and HPLC–MS–MS. J Chromatogr B 857:15–24
7. Long MC, Bennetto-Hood C, Acosta EP (2008) A sensitive HPLC–MS–MS method for the determination of raltegravir in human plasma. J Chromatogr B 867:165–171
8. Jourdil JF, Bartoli M, Stanke-Labesque F (2009) Lack of specificity for the analysis of raltegravir using online sample clean-up liquid chromatography–electrospray tandem mass spectrometry. J Chromatogr B 877:3734–3738
9. Wagner M, Varesio E, Hopfgartner G (2008) Ultra-fast quantitation of saquinavir in human plasma by matrix-assisted laser desorption/ionization and selected reaction monitoring mode detection. J Chromatogr B 872:68–76
10. Fayet A, Béguin A, Zanolari B, Cruchon S, Guignard N, Telenti A, Cavassini M, Günthard HF, Buclin T, Biollaz J, Rochat B, Decosterd LA (2009) A LC–tandem MS assay for the simultaneous measurement of new antiretroviral agents: Raltegravir, maraviroc, darunavir, and etravirine. J Chromatogr B 877:1057–1069
11. Yadav M, Rao R, Kurani H, Singhal P, Goswami S, Shrivastav PS (2009) Application of a rapid and selective method for the simultaneous determination of protease inhibitors, lopinavir and ritonavir in human plasma by UPLC–ESI-MS/MS for bioequivalence study in Indian subjects. J Pharm Biomed Anal 49:1115–1122
12. Myasein F, Kim E, Zhang J, Wu H, El-Shourbagy TA (2009) Rapid, simultaneous determination of lopinavir and ritonavir in human plasma by stacking protein precipitations and salting-out assisted liquid/liquid extraction, and ultrafast LC–MS–MS. Anal Chim Acta 651:112–116
13. Estrela RCE, Ribeiro FS, Seixas BV, Suarez-Kurtz G (2008) Determination of lopinavir and ritonavir in blood plasma, seminal plasma, saliva and plasma ultra-filtrate by liquid chromatography/tandem mass spectrometry detection. Rapid Commun Mass Spectrom 22:657–664
14. Ehrhardt M, Möck M, Haefeli WE, Mikus G, Burhenne J (2007) Monitoring of lopinavir and ritonavir in peripheral blood mononuclear cells, plasma, and ultrafiltrate using a selective and highly sensitive LC–MS–MS assay. J Chromatogr B 850:249–258
15. Huang Y, Gandhi M, Greenblatt RM, Gee W, Lin ET, Messenkoff N (2008) Sensitive analysis of anti-HIV drugs, efavirenz, lopinavir and ritonavir, in human hair by liquid chromatography coupled with tandem mass spectrometry. Rapid Commun Mass Spectrom 22:3401–3409
16. Rezk NL, White NR, Jennings SH, Kashuba ADM (2009) A novel LC–ESI-MS method for the simultaneous determination of etravirine, darunavir and ritonavir in human blood plasma. Talanta 79:1372–1378
17. D'Avolio A, Siccardi M, Sciandra M, Baietto L, Lorena B, Bonora S, Trentini L, Di Perri G (2007) HPLC–MS method for the simultaneous quantification of the new HIV protease inhibitor darunavir, and 11 other antiretroviral agents in plasma of HIV-infected patients. J Chromatogr B 859:234–240
18. ter Heine R, Alderden-Los CG, Rosing H, Hillebrand MJX, van Gorp ECM, Huitema ADR, Beijnen JH (2007) Fast and

simultaneous determination of darunavir and eleven other antiretroviral drugs for therapeutic drug monitoring: method development and validation for the determination of all currently approved HIV protease inhibitors and non-nucleoside reverse transcriptase inhibitors in human plasma by liquid chromatography coupled with electrospray ionization tandem mass spectrometry. Rapid Commun Mass Spectrom 21:2505–2514
19. Jung BH, Rezk NL, Bridges AS, Corbett AH, Kashuba ADM (2007) Simultaneous determination of 17 antiretroviral drugs in human plasma for quantitative analysis with liquid chromatography–tandem mass spectrometry. Biomed Chromatogr 21:1095–1104
20. King T, Bushman L, Kiser J, Anderson PL, Ray M, Delahunty T, Fletcher CV (2006) Liquid chromatography–tandem mass spectrometric determination of tenofovir-diphosphate in human peripheral blood mononuclear cells. J Chromatogr B 843:147–156
21. Becher F, Schlemmer D, Pruvost A, Nevers M-C, Goujard C, Jorajuria S, Guerreiro C, Brossette T, Lebeau L, Créminon C, Grassi J, Benech H (2002) Development of a direct assay for measuring intracellular AZT triphosphate in humans peripheral blood mononuclear cells. Anal Chem 74:4220–4227
22. King T, Bushman L, Anderson PL, Delahunty T, Ray M, Fletcher CV (2006) Quantitation of zidovudine triphosphate concentrations from human peripheral blood mononuclear cells by anion exchange solid-phase extraction and liquid chromatography–tandem mass spectroscopy; an indirect quantitation methodology. J Chromatogr B 831:248–257
23. Becher F, Pruvost A, Gale J, Couerbe P, Goujard C, Boutet V, Ezan E, Grassi J, Benech H (2003) A strategy for liquid chromatography/tandem mass spectrometric assays of intracellular drugs: application to the validation of the triphosphorylated anabolite of antiretrovirals in peripheral blood mononuclear cells. J Mass Spectrom 38:879–890
24. Compain S, Durand-Gasselin L, Grassi J, Benech H (2007) Improved method to quantify intracellular zidovudine mono- and triphosphate in peripheral blood mononuclear cells by liquid chromatography–tandem mass spectrometry. J Mass Spectrom 42:389–404
25. Pruvost A, Théodoro F, Agrofoglio L, Negredo E, Bénech H (2008) Specificity enhancement with LC–positive ESI-MS/MS for the measurement of nucleotides: application to the quantitative determination of carbovir triphosphate, lamivudine triphosphate and tenofovir diphosphate in human peripheral blood mononuclear cells. J Mass Spectrom 43:224–233
26. Becher F, Pruvost A, Goujard C, Guerreiro C, Delfraissy J-F, Grassi J, Benech H (2002) Improved method for the simultaneous determination of d4T, 3TC and ddl intracellular phosphorylated anabolites in human peripheral-blood mononuclear cells using high-performance liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 16:555–565
27. Robbins BL, Poston PA, Neal EF, Slaughter C, Rodman JH (2007) Simultaneous measurement of intracellular triphosphate metabolites of zidovudine, lamivudine and abacavir (carbovir) in human peripheral blood mononuclear cells by combined anion exchange solid-phase extraction and LC–MS–MS. J Chromatogr B 850:310–317
28. Rouzes A, Berthoin K, Xuereb F, Djabarouti S, Pellegrin I, Pellegrin JL, Coupet AC, Augagneur S, Budzinski H, Saux MC, Breilh D (2004) Simultaneous determination of the antiretroviral agents: amprenavir, lopinavir, ritonavir, saquinavir and efavirenz in human peripheral blood mononuclear cells by high-performance liquid chromatography–mass spectrometry. J Chromatogr B 813:209–216
29. Pèlerin H, Compain S, Duval X, Gimenez F, Bénech H, Mabondzo A (2005) Development of an assay method for the detection and quantification of protease and non-nucleoside reverse transcriptase inhibitors in plasma and in peripherical blood mononuclear cells by liquid chromatography coupled with ultraviolet or tandem mass spectrometry detection. J Chromatogr B 819:47–57
30. Colombo S, Beguin A, Telenti A, Biollaz J, Buclin T, Rochat B, Decosterd LA (2005) Intracellular measurements of anti-HIV drugs indinavir, amprenavir, saquinavir, ritonavir, nelfinavir, lopinavir, atazanavir, efavirenz and nevirapine in peripheral blood mononuclear cells by liquid chromatography coupled to tandem mass spectrometry. J Chromatogr B 819:259–276
31. Elens L, Veriter S, Yombi JC, Di Fazio V, Vanbinst R, Lison D, Wallemacq P, Vandercam B, Haufroid V (2009) Validation and clinical application of a high performance liquid chromatography tandem mass spectrometry (LC–MS–MS) method for the quantitative determination of 10 anti-retrovirals in human peripheral blood mononuclear cells. J Chromatogr B 877:1805–1814
32. Fromentin E, Gavegnano C, Obikhod A, Schinazi RF (2010) Simultaneous quantification of intracellular natural and antiretroviral nucleosides and nucleotides by liquid chromatography–tandem mass spectrometry. Anal Chem 82:1982–1989
33. Mistri HN, Jangid AG, Pudage A, Gomes N, Sanyal M, Shrivastav P (2007) High throughput LC–MS–MS method for simultaneous quantification of lamivudine, stavudine and nevirapine in human plasma. J Chromatogr B 853:320–332
34. Gomes NA, Vaidya VV, Pudage A, Joshi SS, Parekh SA (2008) Liquid chromatography–tandem mass spectrometry (LC–MS–MS) method for simultaneous determination of tenofovir and emtricitabine in human plasma and its application to a bioequivalence study. J Pharm Biomed Anal 48:918–926
35. Delahunty T, Bushman L, Robbins B, Fletcher CV (2009) The simultaneous assay of tenofovir and emtricitabine in plasma using LC–MS–MS and isotopically labeled internal standards. J Chromatogr B 877:1907–1914
36. Nirogi R, Bhyrapuneni G, Kandikere V, Mudigonda K, Komarneni P, Aleti R, Mukkanti K (2009) Simultaneous quantification of a non-nucleoside reverse transcriptase inhibitor efavirenz, a nucleoside reverse transcriptase inhibitor emtricitabine and a nucleotide reverse transcriptase inhibitor tenofovir in plasma by liquid chromatography positive ion electrospray tandem mass spectrometry. Biomed Chromatogr 23:371–381
37. Li Z, Ding C, Ge Q, Zhou Z, Zhi X, Liu X (2010) Simultaneous determination of lamivudine, stavudine and nevirapine in human plasma by LC–MS–MS and its application to pharmacokinetic study in clinic. Biomed Chromatogr. doi:10.1002/bmc.1387
38. Le Saux T, Chhun S, Rey E, Launay O, Weiss L, Viard J-P, Pons G, Jullien V (2008) Quantification of seven nucleoside/nucleotide reverse transcriptase inhibitors in human plasma by high-performance liquid chromatography with tandem mass-spectrometry. J Chromatogr B 865:81–90
39. Choi SO, Rezk NL, Kashuba ADM (2007) High-performance liquid chromatography assay for the determination of the HIV-protease inhibitor tipranavir in human plasma in combination with nine other antiretroviral medications. J Pharm Biomed Anal 43:1562–1567
40. Ramachandran G, Hemanthkumar AK, Kumaraswami V, Swaminathan S (2006) A simple and rapid liquid chromatography method for simultaneous determination of zidovudine and nevirapine in plasma. J Chromatogr B 843:339–344
41. Rezk NL, White N, Kashuba ADM (2008) An accurate and precise high-performance liquid chromatography method for the rapid quantification of the novel HIV integrase inhibitor raltegravir in human blood plasma after solid-phase extraction. Anal Chim Acta 628:204–213

42. Sentenac S, Fernandez C, Thuillier A, Lechat P, Aymard G (2003) Sensitive determination of tenofovir in human plasma samples using reversed-phase liquid chromatography. J Chromatogr B 793:317–324
43. Tarinas A, Tápanes RD, Ferrer G, Pérez J (2007) Validation of high-performance liquid chromatography methods for determination of zidovudine, stavudine, lamivudine and indinavir in human plasma. Farm Hosp 31:243–247
44. Gehrig AK, Mikus G, Haefeli WE, Burhenne J (2007) Electrospray tandem mass spectroscopic characterisation of 18 antiretroviral drugs and simultaneous quantification of 12 antiretrovirals in plasma. Rapid Commun Mass Spectrom 21:2704–2716
45. Asberg A, Hansen CN, Reubsaet L (2007) Determination of ganciclovir in different matrices from solid organ transplanted patients treated with a wide range of concomitant drugs. J Pharm Biomed Anal 43:1039–1044
46. Bahrami G, Mirzaeei S, Kiani A (2005) Determination of acyclovir in human serum by high-performance liquid chromatography using liquid–liquid extraction and its application in pharmacokinetic studies. J Chromatogr B 816:327–331
47. Bangaru RA, Bansal YK, Rao AR, Gandhi TP (2000) Rapid, simple and sensitive high-performance liquid chromatographic method for detection and determination of acyclovir in human plasma and its use in bioavailability studies. J Chromatogr B Biomed Sci Appl 739:231–237
48. D'Avolio A, Ibañez A, Sciandra M, Siccardi M, de Requena DG, Bonora S, Di Perri G (2006) Validation of liquid/liquid extraction method coupled with HPLC–UV for measurement of ribavirin plasma levels in HCV-positive patients. J Chromatogr B 835:127–130
49. Fernández M, Sepúlveda J, Aránguiz T, von Plessing C (2003) Technique validation by liquid chromatography for the determination of acyclovir in plasma. J Chromatogr B 791:357–363
50. Larrat S, Stanke-Labesque F, Plages A, Zarski J-P, Bessard G, Souvignet C (2003) Ribavirin quantification in combination treatment of chronic hepatitis C. Antimicrob Agents Chemother 47:124–129
51. Loregian A, Scarpa MC, Pagni S, Parisi SG, Palù G (2007) Measurement of ribavirin and evaluation of its stability in human plasma by high-performance liquid chromatography with UV detection. J Chromatogr B 856:358–364
52. Morello J, Rodríguez-Nóvoa S, Cantillano ALR, González-Pardo G, Jiménez I, Soriano V (2007) Measurement of ribavirin plasma concentrations by high-performance liquid chromatography using a novel solid-phase extraction method in patients treated for chronic hepatitis C. Ther Drug Monit 29:802–806
53. Pan Y, Gao X, Guo N, Li J, Guo X (2008) HPLC method for the determination of penciclovir in human plasma and application to a bioequivalence study. J Chromatogr Sci 46:819–822
54. Teshima D, Otsubo K, Yoshida T, Itoh Y, Oishi R (2003) A simple and simultaneous determination of acyclovir and ganciclovir in human plasma by high-performance liquid chromatography. Biomed Chromatogr 17:500–503
55. Weller DR, Balfour HH, Vezina HE (2009) Simultaneous determination of acyclovir, ganciclovir, and (R)-9-[4-hydroxy-2-(hydroxymethyl)butyl]guanine in human plasma using high-performance liquid chromatography. Biomed Chromatogr 23:822–827
56. Darville JM, Lovering AM, MacGowan AP (2007) Development, evaluation and application of an isocratic high-performance liquid chromatography (HPLC) assay for the simultaneous determination of aciclovir and its metabolite 9-carboxymethoxymethylguanine in human serum and cerebrospinal fluid. Int J Antimicrob Agents 30:30–33
57. Inoue Y, Homma M, Matsuzaki Y, Shibata M, Matsumura T, Ito T, Mitamura K, Tanaka N, Kohda Y (2004) Liquid chromatography assay for routine monitoring of cellular ribavirin levels in blood. Antimicrob Agents Chemother 48:3813–3816
58. Sun D, Wang H, Wang B, Guo R (2006) Development and validation of a sensitive LC–MS–MS method for the determination of adefovir in human serum and urine. J Pharm Biomed Anal 42:372–378
59. Bi H-C, Zhong G-P, Zhou S, Chen X, Huang M (2005) Determination of adefovir in human plasma by liquid chromatography/tandem mass spectrometry: application to a pharmacokinetic study. Rapid Commun Mass Spectrom 19:2911–2917
60. Chen X, Liu D, Zhu L, Zhong D (2005) Development and validation of a liquid chromatography/tandem mass spectrometry procedure for the quantification of adefovir in human plasma. Rapid Commun Mass Spectrom 19:1893–1898
61. Liu Y, Xu G, Xu C, Garcia L, Lin C-C, Yeh L-T (2004) Ultra sensitive method for the determination of 9-(2-phosphonylmethoxyethyl)adenine in human serum by liquid chromatography–tandem mass spectrometry. J Chromatogr B 803:293–298
62. Zou J, Di B, Zhang J, Dai L, Ding L, Zhu Y, Fan H, Xiao D (2009) Determination of adefovir by LC–ESI-MS–MS and its application to a pharmacokinetic study in healthy Chinese volunteers. J Chromatogr Sci 47:889–894
63. Zhang D, Fu Y, Gale JP, Aubry AF, Arnold ME (2009) A sensitive method for the determination of entecavir at picogram per milliliter level in human plasma by solid-phase extraction and high-pH LC–MS–MS. J Pharm Biomed Anal 49:1027–1033
64. Yadav M, Mishra T, Singhal P, Goswami S, Shrivastav PS (2009) Rapid and specific liquid chromatographic tandem mass spectrometric determination of tenofovir in human plasma and its fragmentation study. J Chromatogr Sci 47:140–148
65. Delahunty T, Bushman L, Fletcher CV (2006) Sensitive assay for determining plasma tenofovir concentrations by LC–MS–MS. J Chromatogr B 830:6–12
66. Yeh L-T, Nguyen M, Lin C-C (2003) A sensitive and specific method for the determination of total ribavirin in human red blood cells by liquid chromatography–tandem mass spectrometry. J Chromatogr Sci 41:255–260
67. Shou WZ, Bu HZ, Addison T, Jiang X, Naidong W (2002) Development and validation of a liquid chromatography/tandem mass spectrometry (LC–MS–MS) method for the determination of ribavirin in human plasma and serum. J Pharm Biomed Anal 29:83–94
68. Zhou Z-L, Yu X-Y, Yang M, Peng H-Y, Chen T-F, Lin Q-X, Shan Z-X, Liu X-Y, Deng C-Y, Zhu P, Huang X-Z, Lin S-G (2008) Rapid and sensitive HPLC–MS–MS method for pharmacokinetic assessment of ribavirin in healthy Chinese. Biomed Chromatogr 22:1409–1415
69. Liu Y, Xu C, Yan R, Lim C, Yeh L-T, Lin C-C (2006) Sensitive and specific LC–MS–MS method for the simultaneous measurements of viramidine and ribavirin in human plasma. J Chromatogr B 832:17–23
70. Breddemann A, Hsien L, Tot E, Läer S (2008) Quantification of cidofovir in human serum by LC–MS–MS for children. J Chromatogr B 861:1–9
71. Kasiari M, Gikas E, Georgakakou S, Kazanis M, Panderi I (2008) Selective and rapid liquid chromatography/negative-ion electrospray ionization mass spectrometry method for the quantification of valacyclovir and its metabolite in human plasma. J Chromatogr B 864:78–86
72. Lee H-W, Seo J-H, Lee K-T (2007) Development and validation of a high-performance liquid chromatography–tandem mass spectrometry for the determination of penciclovir in human

plasma: application to a bioequivalence study. J Chromatogr B 852:382–388
73. Xu H-R, Li X-N, Chen W-L, Liu G-Y, Chu N-N, Yu C (2007) A sensitive assay for simultaneous determination of plasma concentrations of valganciclovir and its active metabolite ganciclovir by LC–MS–MS. J Chromatogr B 848:329–334
74. Yadav M, Upadhyay V, Singhal P, Goswami S, Shrivastav PS (2009) Stability evaluation and sensitive determination of antiviral drug, valacyclovir and its metabolite acyclovir in human plasma by a rapid liquid chromatography–tandem mass spectrometry method. J Chromatogr B 877:680–688
75. Borner K, Borner E, Lode H (2001) Determination of linezolid in human serum and urine by high-performance liquid chromatography. Int J Antimicrob Agents 18:253–258
76. Ehrlich M, Trittler R, Daschner FD, Kümmerer K (2001) A new and rapid method for monitoring the new oxazolidinone antibiotic linezolid in serum and urine by high performance liquid chromatography–integrated sample preparation. J Chromatogr B Biomed Sci Appl 755:373–377
77. Boak LM, Li J, Nation RL, Rayner CR (2006) High-performance liquid chromatographic method for simple and rapid determination of linezolid in human plasma. Biomed Chromatogr 20:782–786
78. Martens-Lobenhoffer J, Kielstein JT, Oye C, Bode-Böger SM (2008) Validated high performance liquid chromatography–UV detection method for the determination of daptomycin in human plasma. J Chromatogr B 875:546–550
79. Feng C-H, Lin S-J, Wu H-L, Chen S-H (2002) Trace analysis of tobramycin in human plasma by derivatization and high-performance liquid chromatography with ultraviolet detection. J Chromatogr B 780:349–354
80. Baietto L, D'Avolio A, De Rosa FG, Garazzino S, Patanella S, Siccardi M, Sciandra M, Di Perri G (2009) Simultaneous quantification of linezolid, rifampicin, levofloxacin, and moxifloxacin in human plasma using high-performance liquid chromatography with UV. Ther Drug Monit 31:104–109
81. Keevil BG, Lockhart SJ, Cooper DP (2003) Determination of tobramycin in serum using liquid chromatography–tandem mass spectrometry and comparison with a fluorescence polarisation assay. J Chromatogr B 794:329–335
82. Attema-de Jonge ME, Bekkers JM, Oudemans-van Straaten HM, Sparidans RW, Franssen EJF (2008) Simple and sensitive method for quantification of low tobramycin concentrations in human plasma using HPLC–MS–MS. J Chromatogr B 862:257–262
83. Zhang T, Watson DG, Azike C, Tettey JNA, Stearns AT, Binning AR, Payne CJ (2007) Determination of vancomycin in serum by liquid chromatography–high resolution full scan mass spectrometry. J Chromatogr B 857:352–356
84. Lu C-Y, Feng C-H (2007) Micro-scale analysis of aminoglycoside antibiotics in human plasma by capillary liquid chromatography and nanospray tandem mass spectrometry with column switching. J Chromatogr A 1156:249–253
85. de Velde F, Alffenaar J-WC, Wessels AMA, Greijdanus B, Uges DRA (2009) Simultaneous determination of clarithromycin, rifampicin and their main metabolites in human plasma by liquid chromatography–tandem mass spectrometry. J Chromatogr B 877:1771–1777
86. Baietto L, D'Avolio A, De Rosa FG, Garazzino S, Michelazzo M, Ventimiglia G, Siccardi M, Simiele M, Sciandra M, Di Perri G (2010) Development and validation of a simultaneous extraction procedure for HPLC–MS quantification of daptomycin, amikacin, gentamicin, and rifampicin in human plasma. Anal Bioanal Chem 396:791–798
87. Moussa LA, Khassouani CE, Soulaymani R, Jana M, Cassanas G, Alric R, Hüe B (2002) Therapeutic isoniazid monitoring using a simple high-performance liquid chromatographic method with ultraviolet detection. J Chromatogr B 766:181–187
88. Calleja I, Blanco-Príeto MJ, Ruz N, Renedo MJ, Dios-Viéitez MC (2004) High-performance liquid-chromatographic determination of rifampicin in plasma and tissues. J Chromatogr A 1031:289–294
89. Khuhawar MY, Rind FMA (2002) Liquid chromatographic determination of isoniazid, pyrazinamide and rifampicin from pharmaceutical preparations and blood. J Chromatogr B 766:357–363
90. Espinosa-Mansilla A, Acedo-Valenzuela MI, Muñoz de la Peña A, Cañada Cañada F, Salinas López F (2002) Determination of antitubercular drugs in urine and pharmaceuticals by LC using a gradient flow combined with programmed diode array photometric detection. Talanta 58:273–280
91. Hartkoorn RC, Khoo S, Back DJ, Tjia JF, Waitt CJ, Chaponda M, Davies G, Ardrey A, Ashleigh S, Ward SA (2007) A rapid and sensitive HPLC–MS method for the detection of plasma and cellular rifampicin. J Chromatogr B 857:76–82
92. Song SH, Jun SH, Park KU, Yoon Y, Lee JH, Kim JQ, Song J (2007) Simultaneous determination of first-line anti-tuberculosis drugs and their major metabolic ratios by liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 21:1331–1338
93. Chen X, Song B, Jiang H, Yu K, Zhong D (2005) A liquid chromatography/tandem mass spectrometry method for the simultaneous quantification of isoniazid and ethambutol in human plasma. Rapid Commun Mass Spectrom 19:2591–2596
94. Gong Z, Basir Y, Chu D, McCort-Tipton M (2009) A rapid and robust liquid chromatography/tandem mass spectrometry method for simultaneous analysis of anti-tuberculosis drugs–ethambutol and pyrazinamide in human plasma. J Chromatogr B 877:1698–1704
95. Procházková A, Chouki M, Theurillat R, Thormann W (2000) Therapeutic drug monitoring of albendazole: determination of albendazole, albendazole sulfoxide, and albendazole sulfone in human plasma using nonaqueous capillary electrophoresis. Electrophoresis 21:729–736
96. Shen J, Tong J, Jiang H, Rao Q, Li N, Guo L, Ding S (2009) Simultaneous determination of five benzimidazoles in feeds using high-performance capillary electrophoresis. J AOAC Int 92:1009–1015
97. Bonato PS, Lanchote VL, Takayanagui OM (2003) Simultaneous liquid chromatography–tandem mass spectrometric determination of albendazole sulfoxide and albendazole sulfone in plasma. J Chromatogr B 783:237–245
98. Bonato PS, de Oliveira ARM, de Santana FJM, Fernandes BJD, Lanchote VL, Gonzalez AE, Garcia HH, Takayanagui OM (2007) Simultaneous determination of albendazole metabolites, praziquantel and its metabolite in plasma by high-performance liquid chromatography–electrospray mass spectrometry. J Pharm Biomed Anal 44:558–563
99. Pereira T, Chang SW (2004) Semi-automated quantification of ivermectin in rat and human plasma using protein precipitation and filtration with liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 18:1265–1276
100. Hanpitakpong W, Banmairuroi V, Kamanikom B, Choemung A, Na-Bangchang K (2004) A high-performance liquid chromatographic method for determination of praziquantel in plasma. J Pharm Biomed Anal 36:871–876
101. Ridtitid W, Wongnawa M, Mahatthanatrakul W, Punyo J, Sunbhanich M (2002) LC determination of praziquantel in human plasma. J Pharm Biomed Anal 28:181–186
102. Mirfazaelian A, Dadashzadeh S, Rouini MR (2002) A high performance liquid chromatography method for simultaneous

determination of albendazole metabolites in human serum. J Pharm Biomed Anal 30:1249–1254
103. Mirghani RA, Ericsson O, Cook J, Yu P, Gustafsson LL (2001) Simultaneous determination of quinine and four metabolites in plasma and urine by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 754:57–64
104. Samanidou VF, Evaggelopoulou EN, Papadoyannis IN (2005) Simultaneous determination of quinine and chloroquine antimalarial agents in pharmaceuticals and biological fluids by HPLC and fluorescence detection. J Pharm Biomed Anal 38:21–28
105. Ibrahim H, Bouajila J, Siri N, Rozing G, Nepveu F, Couderc F (2007) Very small injected samples to study chloroquine and quinine in human serum using capillary-LC and native fluorescence. J Chromatogr B 850:481–487
106. Magalhães IRdS, Bonato PS (2009) Two-step liquid-phase microextraction and high-performance liquid chromatography for the simultaneous analysis of the enantiomers of mefloquine and its main metabolite carboxymefloquine in plasma. Anal Bioanal Chem 393:1805–1813
107. de Oliveira AR Moraes, Sueli Bonato P (2007) Stereoselective determination of hydroxychloro-quine and its major metabolites in human urine by solid-phase microextraction and HPLC. J Sep Sci 30:2351–2359
108. Lindegårdh N, Bergqvist Y (2000) Automated solid-phase extraction method for the determination of atovaquone in plasma and whole blood by rapid high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 744:9–17
109. Gordi T, Nielsen E, Yu Z, Westerlund D, Ashton M (2000) Direct analysis of artemisinin in plasma and saliva using coupled-column high-performance liquid chromatography with a restricted-access material pre-column. J Chromatogr B Biomed Sci Appl 742:155–162
110. Lejeune D, Souletie I, Houzé S, Le bricon T, Le bras J, Gourmel B, Houzé P (2007) Simultaneous determination of monodesethylchloroquine, chloroquine, cycloguanil and proguanil on dried blood spots by reverse-phase liquid chromatography. J Pharm Biomed Anal 43:1106–1115
111. Gitau EN, Muchohi SN, Ogutu BR, Githiga IM, Kokwaro GO (2004) Selective and sensitive liquid chromatographic assay of amodiaquine and desethylamodiaquine in whole blood spotted on filter paper. J Chromatogr B 799:173–177
112. Kolawole JA, Mustapha A (2000) Improved RP-HPLC determination of quinine in plasma and whole blood stored on filter paper. Biopharm Drug Dispos 21:345–352
113. Ntale M, Ogwal-Okeng JW, Mahindi M, Gustafsson LL, Beck O (2008) A field-adapted sampling and HPLC quantification method for lumefantrine and its desbutyl metabolite in whole blood spotted on filter paper. J Chromatogr B 876:261–265
114. Jansson A, Gustafsson LL, Mirghani RA (2003) High-performance liquid chromatographic method for the determination of quinine and 3-hydroxyquinine in blood samples dried on filter paper. J Chromatogr B 795:151–156
115. Hodel EM, Zanolari B, Mercier T, Biollaz J, Keiser J, Olliaro P, Genton B, Decosterd LA (2009) A single LC–tandem mass spectrometry method for the simultaneous determination of 14 antimalarial drugs and their metabolites in human plasma. J Chromatogr B 877:867–886
116. Souppart C, Gauducheau N, Sandrenan N, Richard F (2002) Development and validation of a high-performance liquid chromatography–mass spectrometry assay for the determination of artemether and its metabolite dihydroartemisinin in human plasma. J Chromatogr B 774:195–203
117. Huang L, Jayewardene AL, Li X, Marzan F, Lizak PS, Aweeka FT (2009) Development and validation of a high-performance liquid chromatography/tandem mass spectrometry method for the determination of artemether and its active metabolite dihydroartemisinin in human plasma. J Pharm Biomed Anal 50:959–965
118. Naik H, Murry DJ, Kirsch LE, Fleckenstein L (2005) Development and validation of a high-performance liquid chromatography–mass spectroscopy assay for determination of artesunate and dihydroartemisinin in human plasma. J Chromatogr B 816:233–242
119. Thuy LTD, Hung LN, Danh PT, Na-Bangchang K (2008) Development and validation of a liquid chromatography–mass spectrometry method for the simultaneous quantification of artesunate and dihydroartemisinin in human plasma. Southeast Asian J Trop Med Public Health 39:963–977
120. Van Quekelberghe SAA, Soomro SA, Cordonnier JA, Jansen FH (2008) Optimization of an LC–MS method for the determination of artesunate and dihydroartemisinin plasma levels using liquid–liquid extraction. Journal of analytical toxicology 32:133–139
121. Gu Y, Li Q, Melendez V, Weina P (2008) Comparison of HPLC with electrochemical detection and LC–MS–MS for the separation and validation of artesunate and dihydroartemisinin in animal and human plasma. J Chromatogr B 867:213–218
122. Lindegardh N, Tarning J, Toi PV, Hien TT, Farrar J, Singhasivanon P, White NJ, Ashton M, Day NPJ (2009) Quantification of artemisinin in human plasma using liquid chromatography coupled to tandem mass spectrometry. J Pharm Biomed Anal 49:768–773
123. Chen X, Deng P, Dai X, Zhong D (2007) Simultaneous determination of amodiaquine and its active metabolite in human blood by ion-pair liquid chromatography–tandem mass spectrometry. J Chromatogr B 860:18–25
124. do Nascimento TG, Oliveira EdJ, Macêdo RO (2005) Simultaneous determination of ranitidine and metronidazole in human plasma using high performance liquid chromatography with diode array detection. J Pharm Biomed Anal 37:777–783
125. Klimowicz A, Bielecka-Grzela S, Tomaszewska U (2002) A simple and rapid liquid chromatographic method for the determination of metronidazole and its hydroxymetabolite in plasma and cutaneous microdialysates. Acta Pol Pharm 59:327–331
126. Sagan C, Salvador A, Dubreuil D, Poulet PP, Duffaut D, Brumpt I (2005) Simultaneous determination of metronidazole and spiramycin I in human plasma, saliva and gingival crevicular fluid by LC–MS–MS. J Pharm Biomed Anal 38:298–306
127. Silva M, Schramm S, Kano E, Koono E, Porta V, Serra C (2009) Development and validation of a HPLC–MS–MS method for quantification of metronidazole in human plasma. J Chromatogr Sci 47:781–784