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# Sensitive and specific determination of eight antiretroviral agents in plasma by high-performance liquid chromatography–mass spectrometry

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**Abstract**

Therapeutic drug monitoring of antiretroviral drugs has become more and more important. Therefore, a highly specific method is presented, which is capable of quantifying the different proteinase inhibitors (amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir) and non-nucleoside reverse transcriptase inhibitors (efavirenz, nelfinavir). The antiretroviral agents were separated and detected using LC–MS and atmospheric pressure chemical ionization. After solid-phase extraction, the antiretrovirals were separated within 21 min using gradient elution. The calibration range of each drug was linear including the expected minimum and maximum concentrations measured in plasma after the administration of the different drugs. All within-day and between-day coefficients of variation were below 10% and the recovery rates were between 34.8 and 124%. The respective quantification limits were 1 μg/l (indinavir), 10 μg/l (amprenavir, efavirenz), 50 μg/l (saquinavir), 90 μg/l (nelfinavir), 200 μg/l (nevirapine, ritonavir) and 250 μg/l (lopinavir).


*Keywords:* Indinavir; Amprenavir; Efavirenz; Saquinavir; Nelfinavir; Nevirapine; Ritonavir; Lopinavir

## 1. Introduction

The benefit and toxicity of any drug are a function of the drug's concentration in the patient. In the simplest of all circumstances, e.g., if no drug is present, there is no benefit (or toxicity), and if some drug is present, there is measurable benefit (or toxicity) [1].

In the last 2 years the number of publications on analytical methods for therapeutic drug monitoring of antiretroviral drugs has enormously increased. There exist three different classes of antiretroviral drugs: (a) nucleoside reverse transcriptase inhibitors (NRTIs), (b) non-nucleoside reverse transcriptase inhibitors (NNRTIs) and (c) proteinase inhibitors (PIs). The number of drugs belonging to each of these classes increases every year, the drugs which were registered in Switzerland in 2002 are listed in Table 1. From a pharmacological point of view the NRTIs have to be activated (phosphorylated) intracellularly to exert their effect. These drugs are not metabolized by the cytochrome P450 system and are mainly eliminated as phosphorylated metabolites in urine. In the literature there are only few reports until now on the determination of the phosphorylated

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Table 1
Antiretroviral drugs registered in Switzerland in 2001

| Nucleoside reverse transcriptase inhibitors | Non-nucleoside reverse transcriptase inhibitors | Proteinase inhibitors |
|---|---|---|
| Abacavir | Efavirenz | Amprenavir |
| Didanosine | Nevirapine | Indinavir |
| Lamivudine | | Lopinavir |
| Stavudine | | Nelfinavir |
| Zalcitabine | | Ritonavir |
| Zidovudine | | Saquinavir |

NRTIs although it is obvious that the pharmacodynamics should be more closely related to the metabolites instead of plasma concentrations of the inactive parent drug. Therefore, the triphosphorylated compounds have to be determined intracellularly in order to monitor the NRTIs in vivo. This needs special analytical techniques and therefore separate assays for these drugs have to be established.

In contrast to the NRTIs, the NNRTIs and the PIs are intensively metabolized by the cytochrome P450 system, mainly by CYP3A4 and CYP2D6. These drugs have a high interaction potential if they are prescribed in combination, as it is usually performed. First, these drugs may cause toxicity by competing for CYP450-dependent metabolism during co-administration. Second, the metabolism of these drugs may be hampered in the presence of a better CYP3A4 substrate or inhibitor [2]. There may be an increase or decrease of a drug's serum concentration of $>100\%$ if given in combination.

One of the five metabolic pathways of nelfinavir is catalyzed by CYP2C19 and leads to the pharmacologically active M8 (hydroxy-*tert*.-butylamidenelfinavir). The activity of CYP2C19 and thus the formation of M8 is genetically controlled. Poor metabolizers with CYP2C19 possess two defective alleles resulting in inactive CYP219 protein and are expected to have lower or no M8 concentration. In a recent publication [3] it was shown that also after administration of CYP2C19 inhibitors or CYP3A4 inducers, nelfinavir and total nelvfinavir plus M8 concentrations were only marginally affected [3]. Another reason that there is no need to measure M8 is the fact, that a CYP2C19 poor metabolizer will have elevated levels of parent compound. It has been shown that the two chemical species are apparently similar with respect to antiviral activity and therefore, the antiviral efficacy would not be expected to change significantly due to alterations in this CYP2C19-mediated metabolic pathway. This hypothesis has been clinically confirmed in that no significant differences were observed in the antiretroviral responses achieved in two groups of patients likely to be poor and extensive CYP2C19 metabolizers who formed little or no M8 and appreciable levels of M8, respectively [4].

To date there is no common evidence that the antiretroviral therapy is more effective if drug monitoring is performed on a regular basis. But there are different scenarios where knowledge of the concentration of a protease inhibitor may be clinically useful: (a) lack of initial response, (b) loss of response or a new toxicity in a previously stable patient, (c) management of drug–drug interactions, (d) documentation of medication compliance [5]. Especially with the new treatment regimens using small amounts of ritonavir to inhibit CYP3A4 activity, the levels of the co-administered drugs have to be monitored carefully.

There are many publications on the determination of a single [6–9] or different PIs and NNRTIs [5,10–19] with HPLC. With one exception [19] all methods are using UV-detection, not all are using specific wavelengths for the corresponding compounds. The run-time of the methods detecting several drugs varies between 20 and 55 min with UV-detection and is 10 min for the published methods using mass spectrometry.

In order to offer the analyses of the NNRTIs and PIs registered in Switzerland, a rapid, specific and sensitive method was needed. HIV patients need often not only the antiretroviral drugs but also diverse drugs treating concomitant infections or are applying methadone, heroin or other drugs of abuse. Therefore, the method of choice was HPLC with mass spectrometry with the aim to combine the analyses of as many drugs as possible in a single run.

## 2. Experimental

### *2.1. Chemicals and reagents*

HPLC-grade acetonitrile, ethanol, methanol and ammonium carbonate (p.a.) were obtained from

Scharlau (Barcelona, Spain); potassium dihydrogen phosphate (p.a.), disodium hydrogen phosphate (p.a.), phosphoric acid (p.a.) and sodium hydroxide (p.a.) were obtained from Merck ABS (Dietikon, Switzerland). A-86093 ({(5*S*,8*S*,10*S*,11*S*)-9-hydroxy-2-cyclopropyl-5-(1-methylethyl)-1-[(2-1-methylethyl)-4-thiazolyl]-3,6-dioxo-8,11-bis(phenylmethyl)-2,4,7,12-tetraazatridecan-13-oic acid, 5-thiazolylmethyl ester}, internal standard), lopinavir and ritonavir were generous gifts from Abbott (Baar, Switzerland), nevirapine from Boehringer Ingelheim (Basel, Switzerland), amprenavir from Glaxo Wellcome (Schönbühl/Berne, Switzerland), efavirenz and indinavir from Merck Sharp and Dohme-Chibret (Glattbrugg, Switzerland) and nelfinavir and saquinavir from Roche (Basel, Switzerland), respectively. The chemical and physical characterization of the different antiretroviral drugs are depicted in Table 2.

### 2.2. Liquid chromatography–mass spectrometry

The HPLC system consisted of a Rheos 2000 pump (Flux Instruments, Basel, Switzerland), an A200S autosampler (CTC, Zwingen, Switzerland) and a LCQ ion trap mass spectrometer (ThermoQuest, San Jose, CA, USA). The ionization mode was positive atmospheric pressure chemical ionization (APCI) for lopinavir, nelfinavir, ritonavir, saquinavir and the internal standard and negative APCI for nevirapine in method A. Negative APCI mode was applied for the detection of amprenavir, efavirenz, indinavir and the internal standard in method B. In the positive APCI mode in method A the vaporizer was operated at 500 °C, the discharge current fixed at 10 μA and the capillary voltage set at 38 V. In the negative APCI mode in method A the respective values are 500 °C, 10 μA and −27 V. In the negative APCI mode in method B the vaporizer was operated at 450 °C, the discharge current fixed at 10 μA and the capillary voltage set at −34 V. Amprenavir is detected by its most intensive fragment ion (*m*/*z* 416.0), efavirenz by its monoanion (*m*/*z* 314.0), indinavir by its monoanion (*m*/*z* 612.4), nelfinavir by its monocation (*m*/*z* 568.3), nevirapine by its monoanion (*m*/*z* 265.3), ritonavir by its monocation (*m*/*z* 721.1), saquinavir by its monocation (*m*/*z* 671.4). In the negative ionization mode the internal standard was detected by its de-5-thiazolylmethanolated monoanion (*m*/*z* 630.2), in the positive ionization mode by its monocation (*m*/*z* 747.2), respectively.

Table 2
Chemical and physical characterization of the different antiretroviral drugs

| Name | Molecular formula | Molecular mass | CAS number |
|---|---|---|---|
| Amprenavir | $C_{25}H_{35}N_3O_6S$ | 505.6 | 161814-49-9 |
| Efavirenz | $C_{14}H_9ClF_3NO_2$ | 315.7 | 154598-52-4 |
| Indinavir | $C_{36}H_{47}N_5O_4$ | 613.8 | 150378-17-9 |
| Lopinavir | $C_{37}H_{48}N_4O_5$ | 628.8 | 192725-17-0 |
| Nelfinavir | $C_{32}H_{45}N_3O_4S$ | 567.8 | 159989-64-7 |
| Nevirapine | $C_{15}H_{14}N_4O$ | 266.3 | 129618-40-2 |
| Ritonavir | $C_{37}H_{48}N_6O_5S_2$ | 721.0 | 155213-67-5 |
| Saquinavir | $C_{38}H_{50}N_6O_5$ | 670.9 | 127779-20-8 |

The antiretroviral agents were separated using a Nucleosil $C_{18}$ HD, 5-μm particle size column (12.5 cm×2 mm, Macherey-Nagel, Oensingen, Switzerland), protected with a guard column (8×2 mm, Macherey-Nagel).

Eluent A consisted of acetonitrile containing 30% methanol and ammonium carbonate buffer pH 9.3 (5:95, v/v), eluent B of acetonitrile containing 30% methanol and ammonium carbonate buffer, pH 9.3 (95:5, v/v). The mobile phase was linearly mixed in a gradient system starting with 60% eluent B, 40% eluent A. After 2 min the eluents were changing to 98% eluent B, 2% eluent A during 18 min, during 0.5 additional minutes eluent B was decreased to 60% and then maintained for 5.5 min for equilibration of the column. The flow-rate of the mobile phase was set at 200 μl/min. To enhance the ionization process after separation of the compounds, methanol was added to the eluent as sheath liquid, applying a flow-rate of 0.5 ml/min.

### 2.3. Sample preparation

Because of the different ionization efficiencies of the antiretroviral drugs, two different extraction procedures had to be applied. The difference of these procedures was the amount of plasma, which was used. For amprenavir, efavirenz and indinavir, procedure A was applied, using 1 ml of plasma. For

lopinavir, nelfinavir, nevirapine, ritonavir and saquinavir procedure B was applied, using 0.1 ml plasma.

With procedure A, 1 ml plasma was mixed with 0.9 ml buffer I containing 413 ml of a 0.0733 *M* potassium dihydrogen phosphate solution and 587 ml of a 0.0669 *M* disodium hydrogen phosphate solution. A total of 100 μl of internal standard (1 μg A-86093) was added and the resulting solution vortexed. The clean-up procedure of the diluted plasma samples was performed by solid-phase extraction (SPE) using a 20 vacuum manifold (Varian, Zug, Switzerland). The $C_{18}$ cartridges (500 mg, Bond Elut, Varian, Zug, Switzerland) were conditioned with 2 ml methanol and 2 ml buffer II, containing 0.1% phosphoric acid, neutralized with sodium hydroxide to pH 7.0. The entire diluted plasma was applied on the cartridge only using gravity, afterwards the cartridge was washed with 1 ml buffer II and dried under vacuum during 5 min. The antiretroviral drugs were desorbed with 1.5 ml methanol in glass vials using gravity. The eluted substances were dried by evaporation (Rotavapor, Büchi, Flawil, Switzerland) and the residue was dissolved in 80 μl methanol and 40 μl eluent A.

With procedure B 0.1 ml plasma was mixed with 0.275 ml buffer I and 0.2 ml blank plasma (obtained from volunteers in the laboratory). Forty μl of internal standard (0.4 μg A-86093) were added and the resulting solution vortexed. The clean-up procedure of the diluted plasma samples was performed by SPE as describe above with the following difference: the $C_{18}$ cartridges only contained 200 mg of sorbent and the washing step was performed with only 0.5 ml buffer II. The residue was dissolved in 300 μl methanol and 200 μl eluent A.

For quantification standard samples were prepared by adding the appropriate amount of working solutions of the different antiretroviral drugs to heparin plasma (obtained from volunteers in the laboratory). Stock solutions (1 μg/μl) of amprenavir, efavirenz, lopinavir, nelfinavir, nevirapine and saquinavir were made in methanol, of indinavir in methanol–water (50:50), and of A-86093 and ritonavir in absolute ethanol, respectively. The stock solutions were further diluted with methanol–water (50:50), resulting in working solutions in the range of 1–100 ng/μl.

### *2.4. Assessment of performance characteristics*

#### *2.4.1. Linearity*

Corresponding known amounts of a amprenavir and indinavir solutions in diluted methanol were added to human plasma for the preparation of seven standards of which the concentrations were ranging from 10 to 12 000 μg/l in plasma, the respective concentrations for efavirenz were 200 to 6000 μg/l, for lopinavir 940 to 15 800 μg/l, for nelfinavir and saquinavir 94 to 3940 μg/l and for nevirapine and ritonavir 470 to 11 800 μg/l, respectively. The concentration range for each compound was adapted to the through and peak concentration described in the literature. These standard samples were extracted as described above and the standard curves plotted as the peak area ratio of the respective compound to the internal standard versus the concentration. To assess linearity, the line of best fit was determined by least square regression.

#### *2.4.2. Precision and accuracy*

To determine the analytical precision, plasma samples were prepared by addition of the corresponding amount of each antiretroviral drug (details see Table 4). On five different days a calibration curve for each type of analysis was recorded and two samples were analyzed once. For the determination of the within-day precision, two samples were run five times on the same day. To obtain the within-day and between-day coefficients of variation, mean and standard deviations were calculated for each series of analyses.

The accuracy of the method was assessed by expressing the mean of the assayed concentration for the precision samples as percent of the weighed-in concentration.

#### *2.4.3. Recovery*

For the determination of the recovery corresponding amounts of all antiretroviral drugs were added to 1 ml or 0.1 ml of plasma (‘‘sample’’) or in a clean vial (‘‘standard’’), respectively (details see Table 5). The samples were extracted as described above, the standards only evaporated and afterwards samples and standards were diluted in the corresponding amount of methanol and eluent A. These

analyses were performed four times and the average peak area of each compound of the sample was compared to the corresponding peak area of the standard.

#### 2.4.4. Quantification limit

The quantification limit of the method was calculated using a signal-to-noise ratio of 5. For this purpose, the noise signal was obtained as the amplitude of the peaks from a segment of the chromatogram that preceded each peak on the on the corresponding chromatogram.

### 2.5. Interferences

Several commonly co-administered drugs were added to blank plasma in concentrations at the upper therapeutic range. These spiked plasma samples were extracted and analyzed as described above. The appearance of a peak at the same retention time and with the same $m/z$ of one of the antiretroviral drugs was the parameter to decide that there was an interference of the corresponding drug.

## 3. Results

### 3.1. Chromatographic separation

Representative chromatograms of standards for amprenavir, efavirenz and indinavir and lopinavir, nelfinavir, nevirapine, ritonavir and saquinavir, respectively are shown in Fig. 1, demonstrating the absence of interfering endogenous substances and baseline separation of all compounds. All peaks were symmetrical and well resolved.

The retention times were 11.3 min for amprenavir, 16.3 min for efavirenz, 12.4 min for indinavir, 17.6 min for lopinavir, 19.6 min for nelfinavir, 3.6 min for nevirapine, 16.8 min for ritonavir, 18.6 min for saquinavir and 18.1 min for the internal standard.

### 3.2. Performance characteristics

#### 3.2.1. Linearity

The standard curves for amprenavir, efavirenz, indinavir, lopinavir, nelfinavir, nevirapine, ritonavir and saquinavir in plasma were linear in the calibration range. Least square regression data of the calibration curves are summarized in Table 3.

#### 3.2.2. Precision and accuracy

The results of the precision and accuracy experiments are summarized in Table 4. The validation data of all analytes prove that the extraction procedures and the HPLC method were precise and accurate in the calibration range of each compound. All between-day and within-day coefficients of variation were below 10%.

#### 3.2.3. Recovery

The recoveries of the different antiretroviral drugs are shown in Table 5. The recoveries for procedure A (amprenavir, efavirenz, indinavir, internal standard) were between 96.6 and 124%, for procedure B (lopinavir, nelfinavir, nevirapine, ritonavir, saquinavir, internal standard) between 34.8 and 55.1%, respectively.

#### 3.2.4. Quantification limit

The quantification limit (signal-to-noise ratio, 10) for plasma samples of 1 ml (procedure A) or 0.1 ml (procedure B) was 10 μg/l for amprenavir, 10 μg/l for efavirenz, 10 μg/l for indinavir, 250 μg/l for lopinavir, 90 μg/l for nelfinavir, 200 μg/l for nevirapine, 200 μg/l for ritonavir and 50 μg/l for saquinavir.

### 3.3. Interferences

The drugs tested for interferences are shown in Table 6. There was no interference of any of these drugs with one of the antiretroviral drugs.

### 3.4. Application of the method

The applicability of the method was proven by analyzing more than 1000 patient samples in our hospital. Representative chromatograms of different patient samples are shown in Fig. 2. These patients have been treated with different comedications (e.g.,

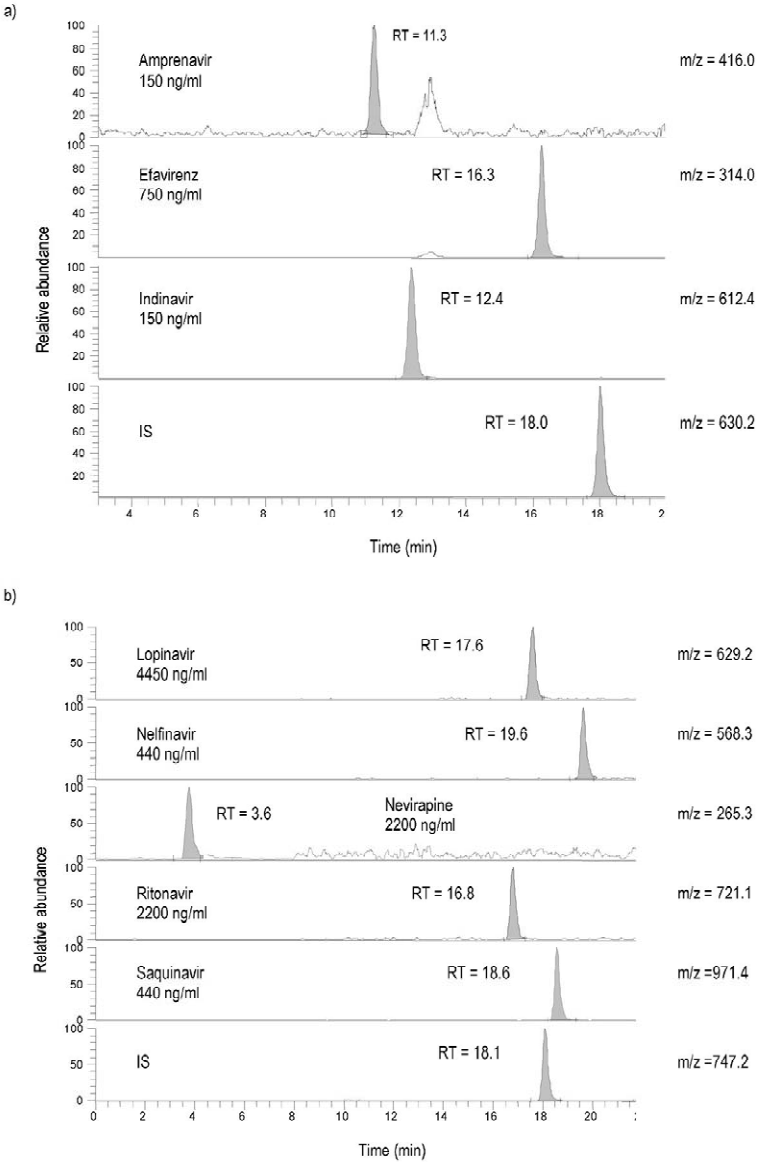


Fig. 1. Chromatograms of plasma standards spiked with the given amounts of amprenavir, efavirenz and indinavir (a) or lopinavir, nelfinavir, nevirapine, ritonavir and saquinavir (b).

abacavir, didanosine, lamivudine, methadone, rifampicine, stavudine, zalcitabine, zidovudine) and in none of the samples any interference was observed. The results will be evaluated separately in order to establish therapeutic ranges for the different antiretroviral drugs.

## 4. Discussion

The HPLC method described provides a simple and fast procedure for the determination of six currently used PIs and two NNRTIs. Due to the highly specific detection method, the procedure was

Table 3
Least square regression data the antiretroviral drugs ($n=5$)

| Drug | Slope (mean±SD) | Intercept (mean±SD) | Correlation coefficient (mean±SD) |
|---|---|---|---|
| Amprenavir | 0.00018±0.00003 | 0.0015±0.0017 | 0.9994±0.0009 |
| Efavirenz | 0.00043±0.00004 | 0.0477±0.0166 | 0.9978±0.0015 |
| Indinavir | 0.00194±0.00035 | 0.0347±0.0545 | 0.9991±0.0009 |
| Lopinavir | 0.00018±0.00004 | −0.0196±0.0050 | 0.9975±0.0022 |
| Nelfinavir | 0.00035±0.00005 | −0.0057±0.0040 | 0.9990±0.0006 |
| Nevirapine | 0.00011±0.00006 | −0.0102±0.0070 | 0.9986±0.0010 |
| Ritonavir | 0.00025±0.00004 | −0.0848±0.0250 | 0.9956±0.0019 |
| Saquinavir | 0.00062±0.00016 | −0.0262±0.0117 | 0.9983±0.0010 |

Table 4
Precision and accuracy data of amprenavir, efavirenz, indinavir, lopinavir, nelfinavir, nevirapine, ritonavir and saquinavir

| Drug | | Concentration (μg/l) | $n$ | Mean (μg/l) | SD (μg/l) | C.V. (%) | Accuracy (%) |
|---|---|---|---|---|---|---|---|
| Amprenavir | Within-day: | 50 | 5 | 53.8 | 1.1 | 2.1 | 108 |
| | | 2000 | 5 | 2132 | 68 | 3.2 | 107 |
| | Between-day: | 50 | 5 | 54.6 | 5.2 | 9.5 | 109 |
| | | 550 | 5 | 546 | 41 | 7.5 | 99.3 |
| Efavirenz | Within-day: | 550 | 5 | 578 | 25 | 4.3 | 105 |
| | | 2500 | 5 | 2663 | 71 | 2.7 | 107 |
| | Between-day: | 550 | 5 | 531 | 38 | 7.2 | 96.6 |
| | | 2500 | 5 | 2592 | 202 | 7.8 | 104 |
| Indinavir | Within-day: | 50 | 5 | 48.9 | 1.7 | 3.5 | 97.8 |
| | | 2000 | 5 | 2202 | 59 | 2.7 | 110 |
| | Between-day: | 50 | 5 | 47.5 | 2.4 | 5.1 | 95.1 |
| | | 550 | 5 | 540 | 29 | 5.4 | 98.1 |
| Lopinavir | Within-day: | 2500 | 5 | 2741 | 67 | 2.5 | 110 |
| | | 9000 | 5 | 9588 | 922 | 9.6 | 107 |
| | Between-day: | 2500 | 5 | 2608 | 237 | 9.1 | 104 |
| | | 9000 | 5 | 9266 | 583 | 6.3 | 103 |
| Nelfinavir | Within-day: | 250 | 5 | 290 | 21 | 7.2 | 116 |
| | | 1350 | 5 | 1447 | 54 | 3.7 | 107 |
| | Between-day: | 250 | 5 | 243 | 23 | 9.6 | 97.0 |
| | | 1350 | 5 | 1464 | 139 | 9.5 | 109 |
| Nevirapine | Within-day: | 950 | 5 | 896 | 51 | 5.7 | 94.1 |
| | | 6000 | 5 | 5624 | 407 | 7.2 | 93.7 |
| | Between-day: | 950 | 5 | 884 | 75 | 8.5 | 93.1 |
| | | 6000 | 5 | 6493 | 286 | 4.4 | 108 |
| Ritonavir | Within-day: | 950 | 5 | 889 | 57 | 6.4 | 94.3 |
| | | 6500 | 5 | 6178 | 202 | 3.3 | 95.1 |
| | Between-day: | 950 | 5 | 896 | 22 | 2.4 | 94.1 |
| | | 6500 | 5 | 6217 | 561 | 9.0 | 95.6 |
| Saquinavir | Within-day: | 225 | 5 | 219 | 14 | 6.3 | 97.5 |
| | | 1250 | 5 | 1240 | 37 | 2.9 | 99.5 |
| | Between-day: | 225 | 5 | 226 | 14 | 6.2 | 100 |
| | | 1250 | 5 | 1240 | 68 | 5.5 | 99.2 |

Table 5
Recoveries of the antiretroviral drugs ($n=5$)

| Drug | | |
|---|---|---|
| Amprenavir (procedure A) | 50 μg/l | 2000 μg/l |
| Mean | 124% | 99.1% |
| SD | 2.2% | 1.9% |
| Efavirenz (procedure A) | 50 μg/l | 2000 μg/l |
| Mean | 96.6% | 97.9% |
| SD | 4.0% | 2.7% |
| Indinavir (procedure A) | 500 μg/l | 2500 μg/l |
| Mean | 119% | 97.1% |
| SD | 2.9% | 1.9% |
| Lopinavir (procedure B) | 2359 μg/l | 8970 μg/l |
| Mean | 43.0% | 37.8% |
| SD | 7.1% | 4.9% |
| Nelfinavir (procedure B) | 236 μg/l | 1350 μg/l |
| Mean | 44.1% | 39.1% |
| SD | 6.2% | 3.8% |
| Nevirapine (procedure B) | 943 μg/l | 6730 μg/l |
| Mean | 46.2% | 49.5% |
| SD | 6.6% | 4.5% |
| Ritonavir (procedure B) | 943 μg/l | 6730 μg/l |
| Mean | 44.0% | 34.8% |
| SD | 8.6% | 6.2% |
| Saquinavir (procedure B) | 236 μg/l | 1350 μg/l |
| Mean | 48.8% | 55.1% |
| SD | 8.5% | 9.1% |
| Internal standard | 4000 μg/l (procedure B) | 1000 μg/l (procedure A) |
| Mean | 76.3% | 93.0% |
| SD | 5.8% | 3.9% |

very insensitive to interferences from metabolites and other drugs. Therefore, the purity of the extracts and the exact reproducibility of the time of elution of the different drugs did not have any impact of the performance of the assay.

Due to the structural differences between the antiretroviral drugs, the ionization mode had to be switched between negative and positive ionization in order to obtain optimal sensitivity. Nevertheless the chromatographic conditions were similar in procedure A and B, due to the different ionization conditions, two different detection procedures had to be applied. If the concentration of amprenavir and indinavir are high enough, their analysis can be included in procedure B. The retention time of efavirenz is similar to that of ritonavir and efavirenz urgently needs negative ionization to get the molecule ionized. Therefore efavirenz cannot be included in the combination of the detection procedures A and B.

In order to decrease the risk of any interference of metabolites or concomitant medication our aim was to separate the different peaks at least partially despite detection with mass spectrometry. The run time of the method was 21 min which had to be followed by an equilibration phase of 5 min due to the gradient elution of the different drugs. This was a reasonable compromise between a short runtime enabling a high throughput and a certain separation of the peaks to decrease co-elution of interfering substances.

Table 6
Drug which did not show any interference with the HPLC method described

| | | |
|---|---|---|
| Acetaminophen and metabolites | Diazepam and metabolites | Midazolam and metabolites |
| Alprazolam and metabolites | Diphenhydramine and metabolites | Morphine and metabolites |
| Amitriptyline and metabolites | Flunitrazepam and metabolites | Nicotine and metabolites |
| Bromazepam and metabolites | Fluoxetine and metabolites | Olanzapine and metabolites |
| Clobazam and metabolites | Flurazepam and metabolites | Phenytoin and metabolites |
| Clomipramine and metabolites | Haloperidol and metabolites | Pyrimethamine and metabolites |
| Clonazepam and metabolites | Hydrocodon and metabolites | Quinine and metabolites |
| Clozapine and metabolites | Imipramine and metabolites | Rifampicin and metabolites |
| Cocaine and metabolites | Lamotrigine and metabolites | Risperidone and metabolites |
| Codein and metabolites | Lorazepam and metabolites | Trimethoprim and metabolites |
| Coffein and metabolites | MDMA and metabolites | Sulfamethoxazole and metabolites |
| Dextromethorphan and metabolites | Methadone and metabolites | |
| Diacetylmorphine and metabolites | Methaqualone and metabolites | |

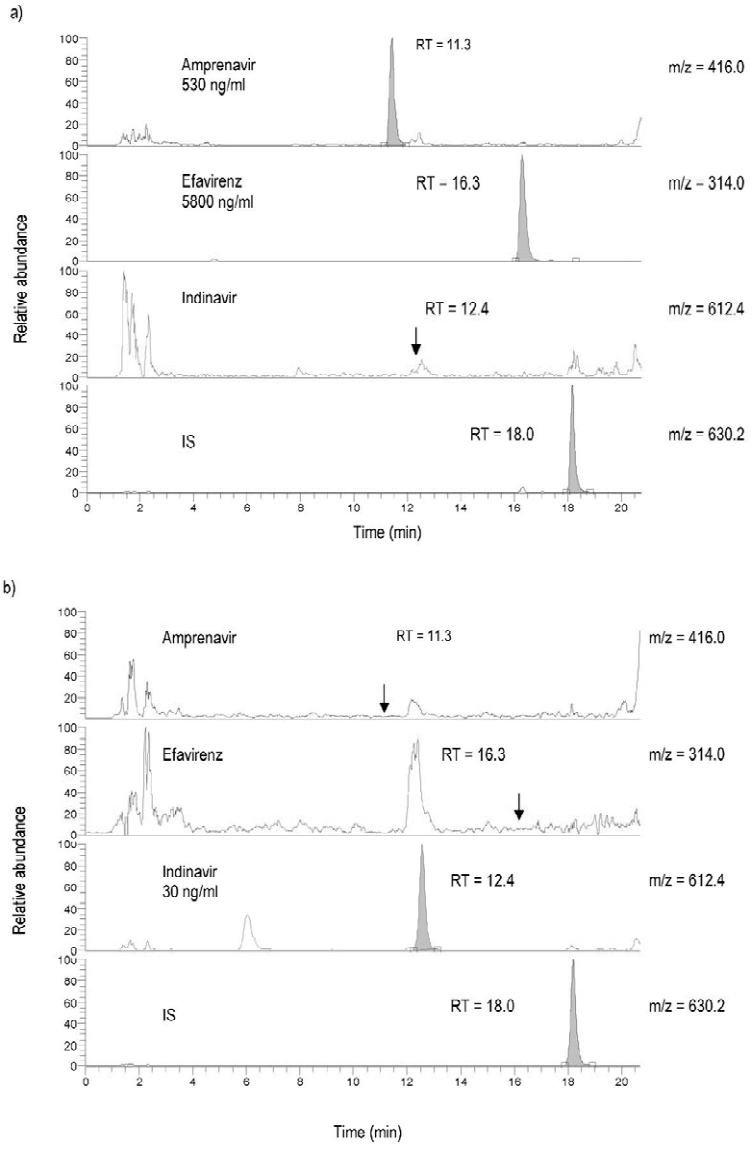


Fig. 2. Chromatograms of plasma samples of patients treated with indinavir (a), amprenavir, ritonavir, efavirenz (b), efavirenz, lopinavir, ritonavir (c), nevirapine, ritonavir, saquinavir (d) and nelfinavir (e), respectively.

The calibration range of each drug included the expected minimum and maximum concentrations measured in plasma after the application of the different drugs. Until now it has not been defined at which time point the blood should be sampled.

There are potentially three major ways in which therapeutic drug monitoring may contribute to HIV treatment: first, by improving potency following initiation of antiretroviral therapy; second, by improving durability of the regimen once maximal virological suppression has been achieved; and, finally, by minimizing drug toxicity [20].

One attempt is to monitor trough concentrations in order to be sure to exceed concentrations in plasma above the minimum efficacy concentration between the dosing interval [21]. It has been shown, that the

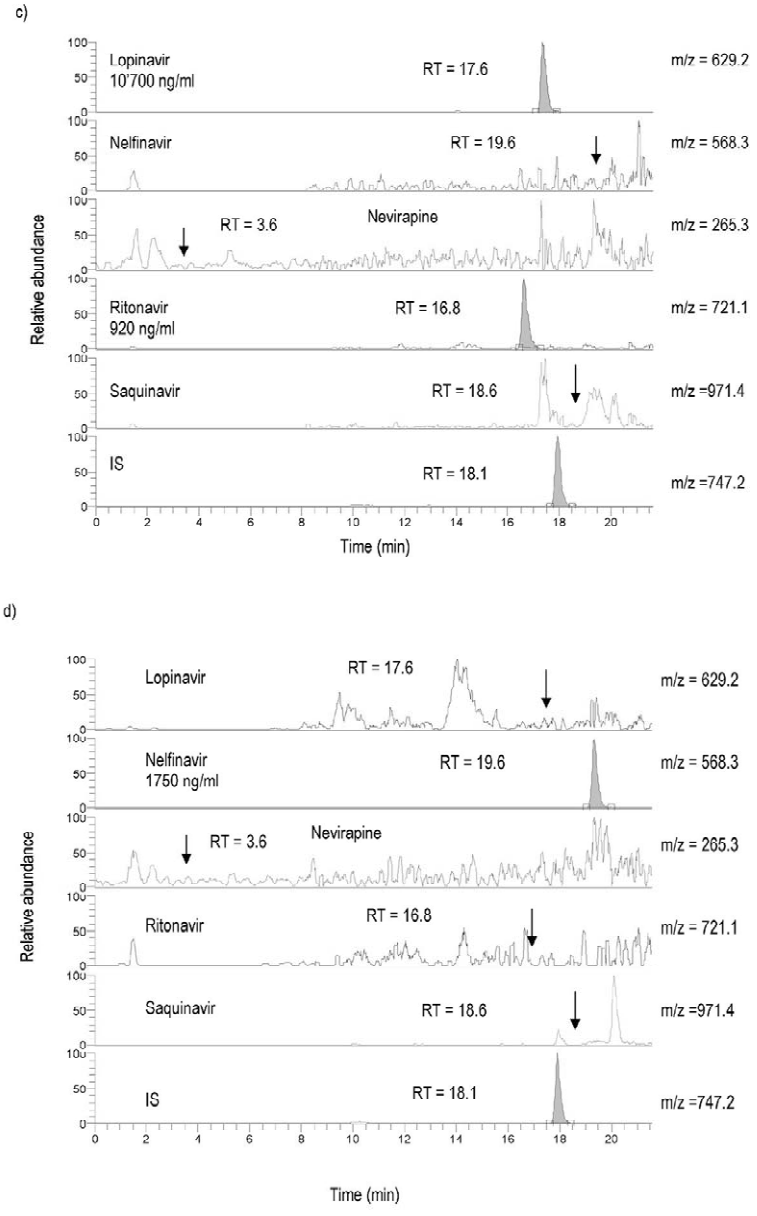


Fig. 2. (*continued*)

increase in plasma triglyceride concentrations (160% at 4 weeks) significantly correlated with the through concentration of saquinavir and ritonavir on twice-daily ritonavir (500 mg) and saquinavir (hard gel, 400 mg) [22]. In contrast, gastrointestinal intolerance and circumoral parasthesia may be related to the maximal concentration of ritonavir [23]. Twelve of 17 patients receiving twice daily ritonavir (600 mg) reported adverse effects. The gastrointestinal symptoms were abolished in two patients by administering

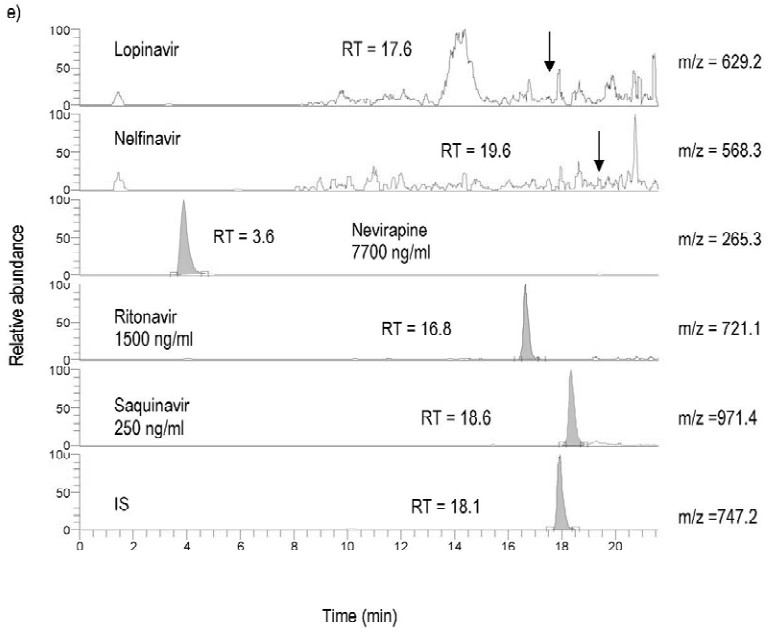


Fig. 2. (*continued*)

300 mg ritonavir every 6 h and therefore, reducing the maximal concentration while maintaining adequate troughs.

In conclusion, the method presented in this paper is an excellent tool for the determination the PIs and NRTIs used in Switzerland in a high number of patient samples. The method has a wide calibration range, thus enabling the quantification of minimal and maximal plasma concentrations. Because mass spectrometry is used as detection method, no interference of other drugs or metabolites of the antiretrovirals has been shown until now.

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