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High-performance liquid chromatographic method for the simultaneous determination of HIV-1 protease inhibitors indinavir, saquinavir and ritonavir in plasma of patients during highly active antiretroviral therapy

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Abstract

A new high-performance liquid chromatographic method for the simultaneous determination of indinavir, saquinavir and ritonavir in human plasma is described. Quantitative recovery following liquid–liquid extraction with diethyl ether from 500 μl of human plasma was achieved. Subsequently, the assay was performed with a linear gradient starting at 67 m*M* potassium dihydrogenphosphate–acetonitrile (65:35 to 40:60, v/v) as a mobile phase, a Phenomenex C_{18} column and UV detection at 240 and 258 nm, respectively. Linear standard curves were obtained for concentrations ranging from 75 to 20 000 ng/ml for indinavir, from 10 to 6000 ng/ml for saquinavir, and from 45 to 30 000 ng/ml for ritonavir. The calculated intra- and inter-day coefficients of variation were below 6%.

Keywords: Indinavir; Saquinavir; Ritonavir

1. Introduction

In addition to therapy with reverse transcriptase inhibitors, patients with HIV-infection are frequently treated with combinations of various HIV-1 protease inhibitors such as indinavir, saquinavir and ritonavir.

Saquinavir is characterized by a low (4%) bioavailability [1]. Indinavir and saquinavir are predominantly metabolized by the cytochrome P450 3A4 [1,2].

In the small intestine and in the liver ritonavir acts as a potent inhibitor of cytochrome P450 3A4. Thus ritonavir is responsible for elevated plasma concentrations of saquinavir and indinavir [3–5].

Increasing evidence for pharmacokinetic and pharmcodynamic interactions of protease inhibitors as well as patient compliance are important factors limiting the efficacy of antiretroviral therapy. In view of this, therapeutic drug monitoring may be warranted to manage a patients medication regimen.

Therefore, the aim of the present study is to develop a valid, reliable and convenient HPLC-based

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method for the simultaneous determination of indinavir, saquinavir and ritonavir levels in human plasma samples.

2. Experimental

2.1. Chemicals and reagents

Indinavir sulphate [1(1*S*,2*R*),5(*S*)]-2,3,5-trideoxy-*N*-(2,3-dihydro-2-hydroxy-1*H*-inden-1-yl)-5-[2-[[(1,1-dimethylethyl)amino]carbonyl]-4-(3-pyridinylmethyl)-1-piperazinyl]-2-(phenylmethyl)-D-*erythro*-pentonamidsulfat (1:1) was a kind gift from Merck Sharp and Dohme (Hoddesdon, UK).

Saquinavir mesylate (Ro 31-8959) (*cis-N-tert*.-butyl-decahydro-2-[2(*R*)-hydroxy-4-phenyl-3(*S*)-([(*N*-2-quinolylcarbonyl)-L-asparaginyl]amino]-butyl]-(4aS,8aS)-isoquinoline-3(*S*)-carboxamide methylsulfonate) was provided by Hoffmann–La Roche (Basel, Switzerland). Ritonavir (ABT-538) (10-hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-3,6-dioxo-8,11-bis-(phenylmethyl),4,7,12-tetraazatridecan-13-oic-acid) and the internal standard (A-86093) were kindly supplied by Abbott Laboratory (North Chicago, USA).

Diethylether, acetonitrile, methanol, *n*-hexane, distilled water, potassium dihydrogenphosphate, sodium carbonate anhydrous, and sodium bicarbonate were products of Merck (Darmstadt, Germany). Blank, drug-free plasma was obtained from Sigma–Aldrich Chemie (Deisenhofen, Germany).

All chemicals were of analytical reagent grade and all solvents were of HPLC grade.

2.2. Chromatographic equipment and conditions

The HPLC system consisted of a Beckman System Gold (Beckman Instruments, München, Germany), a 126 solvent pump module, and a 502 e autoinjector. A 167 programmable detector module was used for peak determination and a Beckman System Gold software for peak identification and integration.

The chromatographic separation was performed with an analytical column system Phenomenex Luna C_{18} (150×2.0 mm I.D., 5 μm, 100 Å) (Phenomenex, Hösbach, Germany) protected with a security guard™ C_{18} (4×2.0 mm) (Phenomenex).

A millipore vacuum filtration system equipped with a 0.45-μm filtration disk membrane was used for filtration and degassing of the mobile phase. All analytical compounds were detected simultaneously by UV absorbance detection at wavelength 240 nm in channel A for saquinavir, ritonavir and the internal standard and at wavelength 258 nm in channel B for indinavir.

The two mobile phases used for gradient HPLC elution were 67 m*M* potassium dihydrogenphosphate, pH 4.6 (A), and acetonitrile (B). Phosphate buffer was an aqueous solution of 9.07 g/l of potassium dihydrogenphosphate. Mobile phase flow was maintained at 0.2 ml/min, starting with 65% A and 35% B (v/v). B was increased to 60% from 0 to 15 min. The isocratic plateau (40:60, v/v, A/B) was remained for 10 min. The total analytical run time was 37 min. The column was then re-equilibrated with the initial conditions within 1 min for 12 min before the next injection was started. The injection volume was 100 μl.

2.3. Standard preparation

The initial stock solution of indinavir, saquinavir and ritonavir was prepared by dissolving 10 mg of indinavir, 5 mg of saquinavir and 15 mg of ritonavir in a 100-ml volumetric flask containing a mixture of 67 m*M* potassium dihydrogenphosphate–methanol (1:1, v/v). Standard solutions of different concentrations were obtained by serial dilution of the stock solution.

A stock solution of the internal standard (A-86093) was similarly prepared in a mixture of 1/15 *M* potassium dihydrogenphosphate–methanol (1:1, v/v) to yield a final concentration of 10 mg per 100 ml. Each solution was stored at 4°C and was stable for at least 3 months.

For preparation of the plasma standard samples to achieve calibration concentrations as described, an appropriate amount of the stock solutions and the internal standard (5 μg/ml) were added to blank plasma.

2.4. Sample preparation

A 500-μl aliquot of plasma was mixed with an equal volume of carbonate buffer (0.1 *M* sodium carbonate–sodium bicarbonate pH 9.4), and an

aliquot of 100 μl solution of internal standard (A-86039) was added to a 15-ml glass tube. The sample was vortexed for 10 s and extracted twice with 3 ml diethyl ether for 5 min, followed by centrifugation at 3000 *g* (4°C). The organic layers were transferred to a glass centrifuge tube and evaporated to dryness with a gentle stream of nitrogen at 37°C. The residue was reconstituted in 300 μl of 1/15 *M* potassium dihydrogenphosphate–methanol (1:1, v/v) and washed for 5 min with 1.5 ml *n*-hexane. The aqueous layer was transferred to autosampler vials with glass micro-inserts for HPLC analysis. A 100-μl aliquot was injected into the chromatograph.

2.5. Specificity and selectivity

In order to evaluate levels of endogenous compounds with potential for interference with the analytical method, analysis of six different blank samples was performed. The following compounds were determined: amprenavir, delavirdine, didanosine, fluconazole, ganciclovir, lamivudine, methadone, methotrexate, nelfinavir, M8-metabolite of nelfinavir, nevirapine, oxazepam, prazinamide, pyrimethamine, stavudine, sulfamethoxazole, sulfadoxin, trimethoprim, zalcitabine, and zidovudine. Three spiked plasma samples were analysed for each substrate.

2.6. Limit of detection and limit of quantitation

The limit of detection in plasma (LOD) was defined by the lowest detectable concentration yielding a signal-to-noise ratio of three, indicating a significant difference between spiked and blank samples in plasma samples of three individuals as determined by the two-tailed, paired Student's *t*-test. For the concentration to be accepted as the lower limit of quantitation (LOQ), the measure of accuracy (percent deviation from the nominal concentration) and precision (relative standard deviation) have to be less than 20%. All samples were assayed in triplicate.

2.7. Accuracy, precision, linearity and recovery

Intra-day accuracy and precision of the method were determined by measuring three replicate plasma samples at three different concentrations of indinavir (400, 4000 and 10 000 ng/ml), saquinavir (300, 2000 and 5000 ng/ml), or ritonavir (1000, 6000 and 15 000 ng/ml), respectively. To obtain the inter-day accuracy and precision, duplicates of each concentration were analyzed at five different days as described above. Accuracy was calculated as the relative error of the nominal concentration. Precision was expressed in terms of relative standard deviation and obtained by analysis of variance (ANOVA) for each test concentration using the analytical run as the grouping variable.

Daily standard curves were evaluated by duplicate analysis of seven spiked plasma samples for indinavir (range, 75–20 000 ng/ml), for saquinavir (range, 10–6000 ng/ml), and for ritonavir (range, 45–30 000 ng/ml), respectively. A linear weighted (one per concentration squared) least squares regression analysis to plot the observed peak area/internal standard ratio of indinavir, saquinavir, or ritonavir against the concentrations of indinavir, saquinavir or ritonavir was performed. Linearity and assay reproducibility were determined by measuring the standard concentration in five separate assay runs on five separate days.

The linearity of five calibration curves was tested with the *F*-test for lack of fit, using a weight factor of (one per concentration). The recovery of indinavir, saquinavir and ritonavir in the extraction procedure was determined by comparing the detected concentrations of indinavir (400 ng/ml), saquinavir (300 ng/ml) and ritonavir (1000 ng/ml) in three extracted spiked plasma samples to those of non-processed standard solutions. The procedure was performed three times for each drug alone, and for the combination of all three substances in order to investigate whether the presence of indinavir and/or saquinavir and/or ritonavir influenced the results of the the recovery or the assay.

2.8. Stability

Blank plasma samples were spiked with aliquots of diluted protease inhibitor stock solution resulting in three different concentrations of indinavir (400 ng/ml, 4000 ng/ml, 10 000 ng/ml), saquinavir (300 ng/ml, 2000 ng/ml, 5000 ng/ml) and ritonavir (1000 ng/ml, 6000 ng/ml, 15 000 ng/ml), respectively. Three replicates of these samples were ana-

lyzed after storage periods of 48 h, 7 days and 14 days at 25°C, 4°C and −20°C.

2.9. Analysis of patient samples

Plasma samples derived from HIV-infected patients during antiretroviral therapy with a combination of zidovudine, lamivudine and a protease inhibitor were taken prior to and 3 h after ingestion of 800 mg of indinavir, or 600 mg of saquinavir, or 400 mg of ritonavir. Plasma samples of all patients were obtained by a standardized procedure. Plasma was separated by centrifugation at 3000 *g* for 10 min at 4°C and was immediately stored at −20°C until further analysis.

2.10. Calculation and data analysis

All statistical calculations were performed with the Statistical Product and Service Solutions (SPSS) for Windows, version 7.5.2.dt. (SPSS, Chicago, IL, USA). Correlation was considered statistically significant if the calculated value of p was 0.05 or less.

3. Results and discussion

3.1. Chromatography and detection

Sample preparation by diethyl ether extraction was performed as described previously [6]. The recovery observed with solid-phase extraction (as described by van Heeswijk et al. [7]) was similar to the recovery from the sample preparation with liquid–liquid extraction. Indinavir exhibits a maximum UV absorption at 258 nm, whereas saquinavir, ritonavir and the internal standard show a maximum absorbance at 240 nm. Therefore, simultaneous UV detection of indinavir in channel B (at 258 nm), and of saquinavir, ritonavir and the internal standard in channel A (at 240 nm) was performed. UV detection of the levels of the respective compounds was reported previously by other groups [6,8–12].

Peak shape, separation from endogenous compounds, and separation from indinavir, saquinavir and ritonavir in one run were therefore optimized by improving the gradient of the mobile phase with 67 m*M* potassium dihydrogenphosphate–acetonitrile 65:35 (v/v) to 40:60 (v/v).

The use of A-86093 as an internal standard with a retention time of 23.5 mm was found to be convenient. A chromatogram of a blank plasma sample (Fig. 1) showed no interfering endogenous peaks. Representative HPLC runs of different spiked plasma samples are shown in Figs. 2–4. Combination therapy with saquinavir at 1.2 g per day and ritonavir at 0.8 g per day results in plasma levels of the respective compounds (saquinavir, 761 ng/ml and ritonavir, 2224 ng/ml) detected in the HPLC run shown in Fig. 5.

A HPLC run of a plasma sample derived from a patient receiving combination therapy with indinavir

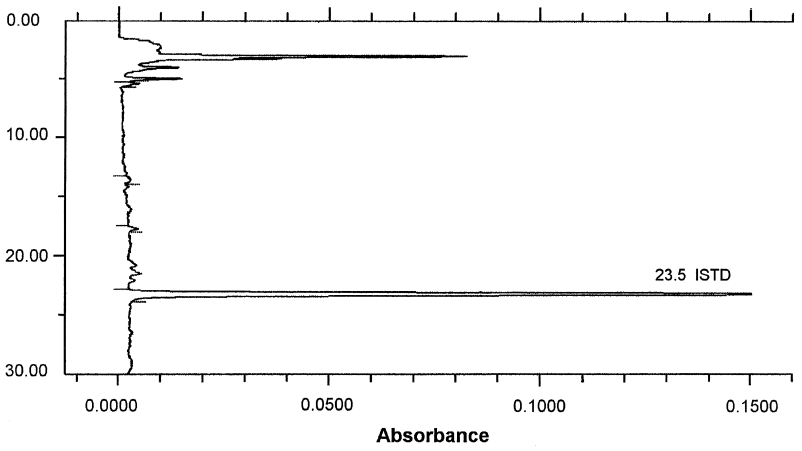

Fig. 1. Chromatogram of a blank human plasma spiked with 5 μg/ml internal standard A-86093 (23.5 min). UV detection at 240 nm.

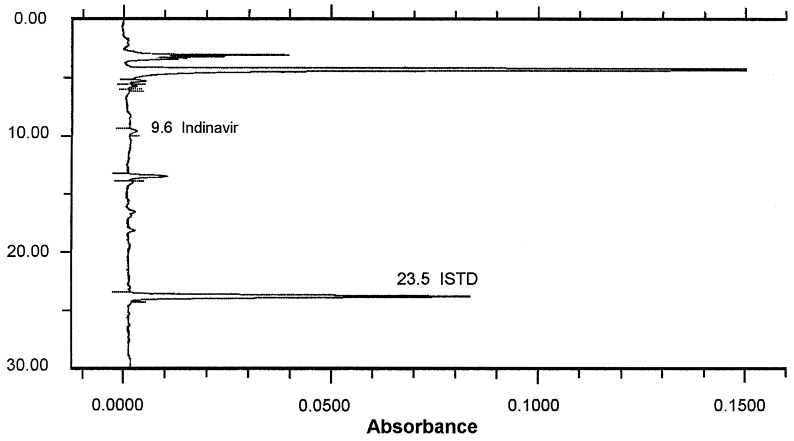

Fig. 2. Chromatogram of a blank human plasma spiked with 5 μg/ml internal standard A-86093 (23.5 min) and indinavir 75 ng/ml (9.60 min). UV detection at 258 nm.

(2×0.8 g per day) and ritonavir (2×0.4 g per day) is shown in Fig. 6. Retention time of indinavir is 9.6 min, of ritonavir 21.5 min, respectively. Trough plasma concentrations of 3895 ng/ml of indinavir and 7365 ng/ml of ritonavir were detected.

3.2. Specificity and selectivity

Drug-free plasma samples obtained from healthy individuals were devoid of interference near the retention times of indinavir, saquinavir, ritonavir and the internal standard. The analysis of plasma samples containing amprenavir, delavirdine, didanosine, fluconazole, ganciclovir, lamivudine, methadone, methotrexate, nelfinavir, M8-metabolite of nelfinavir, nevirapine, oxazepam, pyrazinamide, pyrimethamine stavudine, sulfamethoxazole, sulfadoxin, trimethoprim, zalcitabine, or zidovudine showed no interference with the extraction procedure nor with the analytical method. Circulating metabolites of

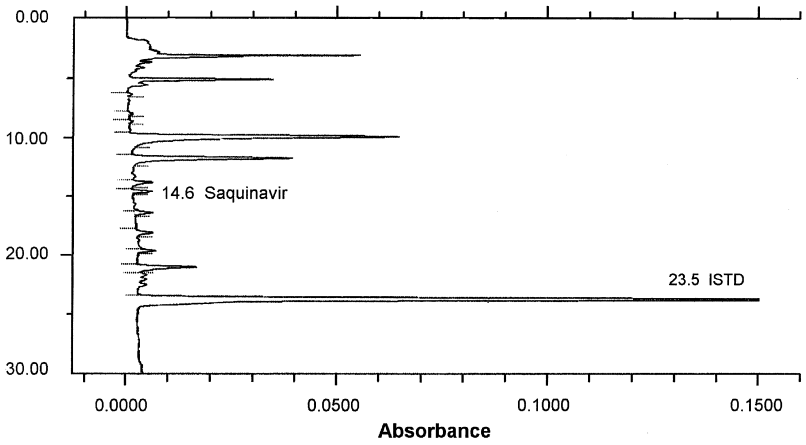

Fig. 3. Chromatogram of a blank human plasma spiked with 5 μg/ml internal standard A-86093 (23.5 min) and saquinavir 15 ng/ml (14.60 min). UV detection at 240 nm.

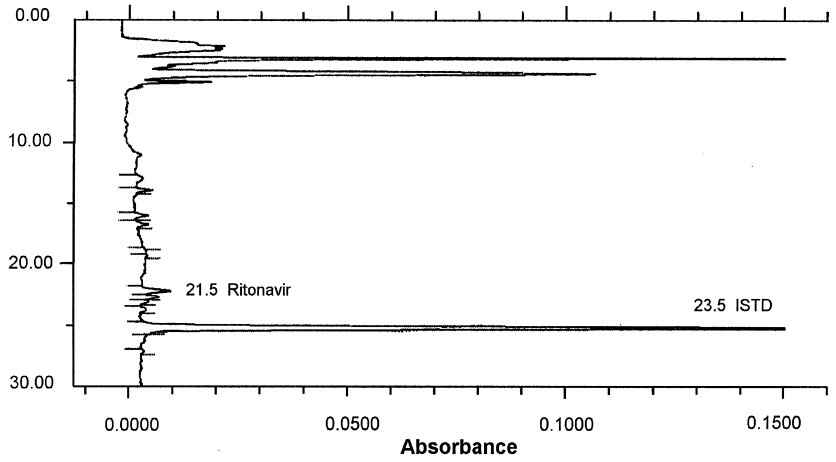

Fig. 4. Chromatogram of a blank human plasma spiked with 5 μg/ml internal standard A-86093 (23.5 min) and ritonavir 50 ng/ml (21.50 min). UV detection at 240 nm.

ritonavir or saquinavir do not appear to interfere with the indinavir assay and metabolites of ritonavir do not interfere with the saquinavir assay.

3.3. Limit of detection and limit of quantitation

The detection limit of indinavir, saquinavir, or ritonavir in plasma was determined at 2 ng/ml, 1 ng/ml and 2.5 ng/ml, respectively. The lower limit of quantitation was reached at a concentration of 75 ng for indinavir, 10 ng/ml for saquinavir and 45 ng/ml for ritonavir.

3.4. Accuracy, precision, linearity and recovery of the assay

The intra-day accuracy and precision of analysis was determined in three analytical runs. Precision ranged from 3.4% to 6.9% for indinavir, 0.9% to 1.8% for saquinavir and 2.8% to 10.7% for ritonavir. Intra-day accuracy was −5.0% to 4.5% for indinavir,

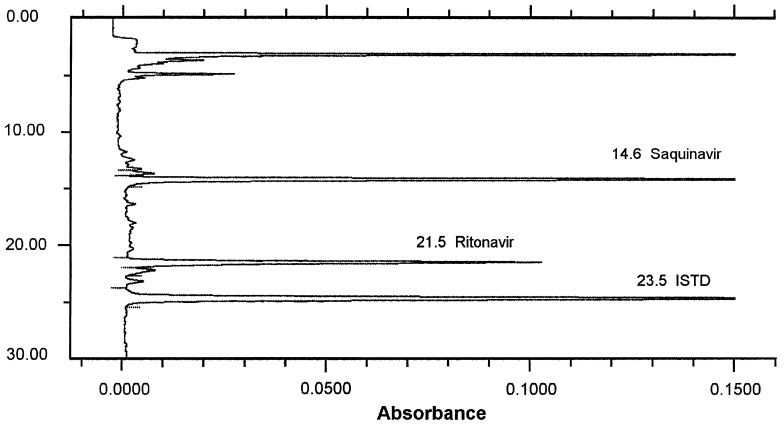

Fig. 5. Chromatogram of a patient sample during combination therapy with saquinavir 2×600 mg per day and ritonavir 2×400 mg per day. Trough plasma concentration of saquinavir is 761 ng/ml (14.6 min) and trough level of ritonavir is 2224 ng/ml (21.5 min). Internal standard A-86093 (23.5 min). UV detection at 240 nm.

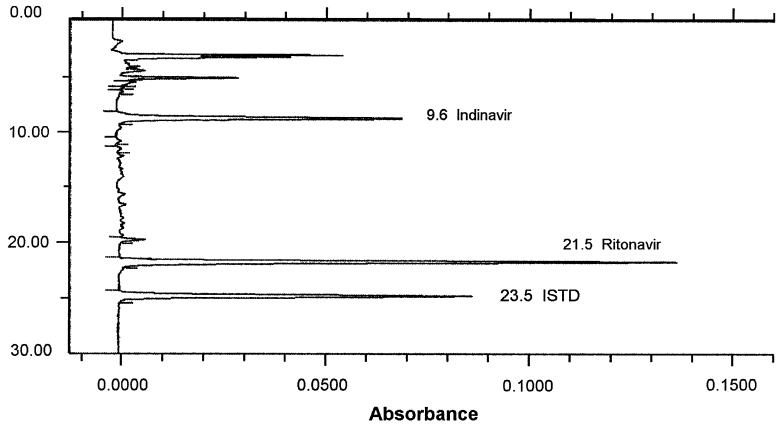

Fig. 6. Chromatogram of a patient sample during combination therapy with indinavir 2×800 mg per day and ritonavir 2×400 mg per day. Through plasma concentration of indinavir is 3895 ng/ml (9.6 min) and trough level of ritonavir is 7365 ng/ml (21.5 min). Internal standard A-86093 (23.5 min). UV detection at 258 nm.

−9.7% to 0.1% for saquinavir, and 1.9% to 7.9% for ritonavir. The results of indinavir, saquinavir and ritonavir intra-day validation are presented in Table 1. The inter-day accuracy and precision of the three protease inhibitors shown in Table 2 were below 10%.

Table 1
Simultaneous determination of indinavir (IDV), saquinavir (SQV) and ritonavir (RIT) in human plasma: intra-day precision (C.V.%) and accuracy (R.E.%)

Concentration found (ng/ml)	Sample concentration (ng/ml)		
	400	4000	10 000
IDV			
Mean	420	4002	9550
±S.D.	29	178	323
C.V. (%)	6.9	4.4	3.4
R.E. (%)	−5.0	−0.1	4.5
n=	3	3	3
SQV	300	2000	5000
Mean	329	1998	5027
±S.D.	6	18	57
C.V. (%)	1.8	0.9	1.1
R.E. (%)	−9.7	0.1	−0.5
n=	3	3	3
RIT	1000	6000	15 000
Mean	922	5527	14 712
±S.D.	99	194	410
C.V. (%)	10.7	3.5	2.8
R.E. (%)	7.8	7.9	1.9
n=	3	3	3

Table 2
Simultaneous determination of indinavir (IDV), saquinavir (SQV) and ritonavir (RIT) in human plasma: inter-day precision (C.V.%) and accuracy (R.E.%)

Concentration found (ng/ml)	Sample concentration (ng/ml)		
	400	4000	10 000
IDV			
Mean	378	3984	10 183
±S.D.	30	155	788
C.V. (%)	8.0	3.9	7.7
R.E. (%)	5.4	0.4	−1.8
n=	5	5	5
SQV	300	2000	5000
Mean	306	2084	5248
±S.D.	14	92	472
C.V. (%)	4.5	4.4	9.0
R.E. (%)	−2.0	−42	−5.0
n=	5	5	5
RIT	1000	6000	15 000
Mean	1052	5991	15 494
±S.D.	68	209	967
C.V. (%)	6.5	3.5	6.2
R.E. (%)	−5.2	0.2	−3.3
n=	5	5	5

Using the ratios of the observed peak areas for indinavir, saquinavir or ritonavir and the internal standard in seven spiked plasma samples analyzed in duplicate, the standard curves showed a correlation coefficient of 0.998 for indinavir (range, 75 ng/ml to 20 000 ng/ml), 0.995 for saquinavir (range, 10 ng/ml to 6000 ng/ml) as determined by least-square analysis. The calibration curve for ritonavir (range, 45 ng/ml to 30 000 ng/ml) exhibited a correlation coefficient of 0.998. All calibration curves proved to be linear in the respective ranges listed above in the *F*-test for lack of fit, which was performed to assess the linearity of the regression model. The C.V.% of five slopes was 4%.

Stability studies were conducted with samples containing an aliquot of diluted stock solution to yield an initial indinavir concentration of 400 ng/ml, 4000 ng/ml and 10 000 ng/ml, a saquinavir concentration of 300 ng/ml, 2000 ng/ml and 5000 ng/ml and a ritonavir concentration of 1000 ng/ml, 6000 ng/ml and 15 000 ng/ml. The stability of indinavir, saquinavir and ritonavir at various conditions is shown in Table 3. There was no evidence for a degradation of indinavir, saquinavir or ritonavir. Recovery of each compound was estimated by comparison of peak areas in extracted spiked drug-free plasma with those of standard solutions. Recovery was found to be 92.0%±5.6 (n=3) for indinavir, 92.8%±3.7 (n=3) for saquinavir and 94.9%±3.0 (n=3) for ritonavir, respectively.

3.5. Analysis of patient samples

Plasma samples derived from HIV-infected patients undergoing antiretroviral therapy with a combination of zidovudine, lamivudine and a protease inhibitor were obtained prior to and 3 h after ingestion of the PI. Trough plasma concentrations of 1005±1285 ng/ml during the steady state of patients treated with 800 mg indinavir three times a day increased to peak concentrations of 3906±2023 ng/ml 3 h after ingestion of 800 mg indinavir. Trough plasma concentrations of saquinavir in five patients treated with 600 mg thrice a day were 71±68 ng/ml. Three hours after ingestion of 600 mg of saquinavir, a peak concentration of 550±266 ng/ml was detected. In patients treated with 600 mg of ritonavir twice a day, trough levels of 2462±919 ng/ml were

Table 3
Stability of indinavir (IDV), sacuinavir (SQV) and ritonavir (RIT) in spiked human plasma

Storage condition	Nominal concentration (ng/ml)	Measured concentration (ng/ml; mean±S.D.)	C.V. (%)	*n*
Indinavir (IDV)				
48 h at 25°C	400	427.7±15.3	3.6	3
	4000	3826.3±72.7	1.9	3
	10 000	9547.0±226.7	2.4	3
7 days at 4°C	400	417.7±31.2	7.5	3
	4000	4033.0±85.4	2.1	3
	10 000	10056.0±265.0	2.6	3
14 days at −20°C	400	409.0±32.6	8.0	3
	4000	3768.7±58.3	1.5	3
	10 000	9741.7±200.5	2.1	3
Saquinavir (SQV)				
48 h at 25°C	300	270.0±25.2	9.3	3
	2000	1884.0± 24.2	1.3	3
	5000	4810.7±122.9	2.6	3
7 days at 4°C	300	273.3±16.7	6.1	3
	2000	2088.3±42.5	2.0	3
	5000	5093.3±150.8	3.0	3
14 days at −20°C	300	295.3±26.8	9.1	3
	2000	1926.3±57.8	3.0	3
	5000	4987.7±107.5	2.2	3
Ritonavir (RIT)				
48 h at 25°C	1000	1011.3±17.1	1.7	3
	6000	5313.7±68.6	1.2	3
	15 000	15086.0±478.2	3.2	3
7 days at 4°C	1000	1033.3±30.9	3.0	3
	6000	6044.0± 162.3	2.7	3
	15 000	15747.7±741.5	4.7	3
14 days at −20°C	1000	1101.0±81.1	7.4	3
	6000	5956.7±160.5	2.7	3
	15 000	15423.3±325.5	2.1	3

determined. Three hours after ingestion of 600 mg of ritonavir, peak ritonavir levels of 6407±2656 ng/ml were measured. The presented results, shown in Fig. 7, demonstrate the applicability of the assay for pharmacokinetic monitoring in HIV-infected patients.

4. Conclusion

We present here a validated, reliable and convenient assay for the simultaneous determination of indinavir, saquinavir and ritonavir levels in human plasma. The described HPLC assay can readily be used in a standard hospital laboratory. In our hands, the described procedure was most suitable. No significant loss of quality in more than 1000 runs performed in our laboratory in 500 different patients was observed.

Calibration curves for indinavir (75–20 000 ng/ml), saquinavir (10 ng/ml to 6000 ng/ml) and ritonavir (45–30 000 ng/ml) are appropriate for clinical drug monitoring, and are especially suitable for assessment of patients compliance. The practicability of the assay is demonstrated by individual patients plasma levels as trough and peak concentration of the PI. This HPLC method is preferencially used for drug monitoring in patients treated with HIV-1 protease inhibitors in combination with other

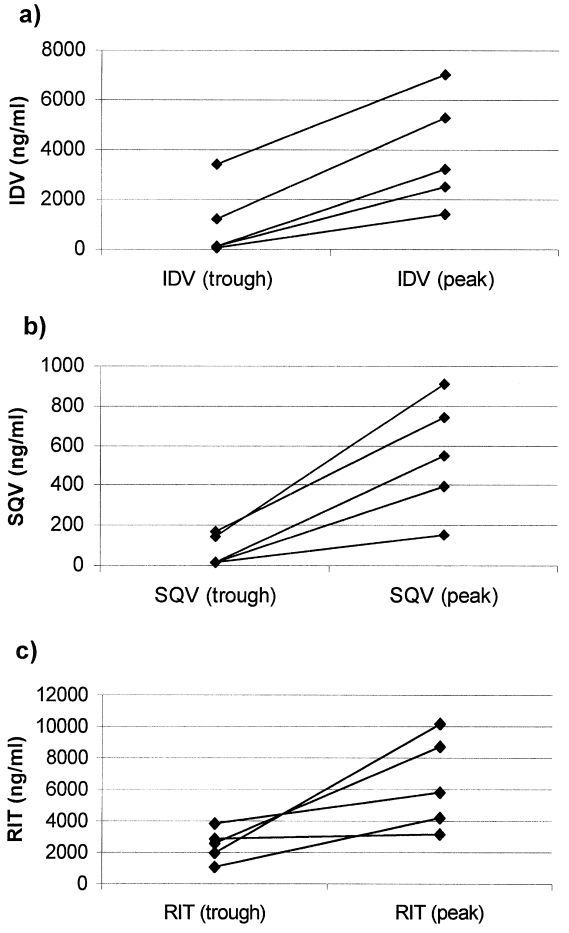

Fig. 7. Steady state trough and peak plasma concentrations of indinavir (IDV), saquinavir (SQV) and ritonavir (RIT) in five patients before and 3 h after oral ingestion of one protease inhibitor: (a) indinavir 800 mg, (b) saquinavir 600 mg or (c) ritonavir 600 mg.

antiretroviral agents. This type of drug monitoring may be essential for the estimation of interaction with other hepatically metabolized co-medications and subsequent modification of the administered dose.

References

[1] J.M. Schapiro, M.A. Winters, F. Stewart, B. Efron, J. Norris, M.J. Kozal et al., Ann. Intern. Med. 124 (1996) 1039.
[2] C. Merry, M.G. Barry, F. Mulcahy, M. Ryan, J. Heavy, J.F. Tjia et al., AIDS 11 (1997) F29.
[3] H.R. Ha, F. Follath, Y. Bloemhard, S. Krähenbühl, J. Chromatogr. B 694 (1997) 427.
[4] K.C. Marsh, E. Eiden, E. McDonald, J. Chromatogr. B 704 (1997) 307.
[5] R.M.W. Hoetelmans, M. van Essenberg, M. Profijt, P.L. Meenhorst, J.W. Mulder, J.H. Beijnen, J. Chromatogr. B 705 (1998) 119.
[6] D.J. Kempf, K.C. Marsh, G. Kumar, A.D. Rodrigues, J.F. Denissen, E. McDonald et al., Antimicrob. Agents Chemother. 41 (1997) 654.
[7] R.P.G. van Heeswijk, R.M.W. Hoetelmans, R. Harms, P.L. Meenhorst, J.W. Mulder et al., J. Chromatogr. B 719 (1998) 159.
[8] S. Frappier, D. Breilh, E. Diarte, B. Ba, D. Ducint, J.L. Pellegrin, M.C. Saux, J. Chromatogr. B 714 (1998) 384.
[9] E. Woolf, T. Au, H. Haddix, B. Matuszewsi, J. Chromatogr. A 714 (1995) 45.
[10] D.M. Burger, M. de Graaff, E.W. Wui, P.P. Koopmans, Y.A. Hekster, J. Chromatogr. B 703 (1997) 235.
[11] A.L. Jayewardene, F. Zhu, F.T. Aweeka, J.G. Gamertoglio, J. Chromatogr. B 707 (1998) 203.
[12] K.C. Yeh, P.J. Deutsch, H. Haddix, M. Hesney, V. Hoagland, W.D. Ju, S.J. Justice et al., Antimicrob. Agents Chemother. 42 (1998) 332.