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A LC–MS/MS method to quantify the novel cholesterol lowering drug ezetimibe in human serum, urine and feces in healthy subjects genotyped for SLCO1B1

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Abstract

Ezetimibe (Ezetrol®) is a novel cholesterol lowering drug which disposition is not fully understood in man. We developed a selective and high-sensitive assay to measure serum concentration–time profiles, renal and fecal elimination of ezetimibe in pharmacokinetic studies. Ezetimibe glucuronide, the major metabolite of ezetimibe was determined by enzymatic degradation to the parent compound. Ezetimibe was measured after extraction with methyl *tert*-butyl ether using 4-hydroxychalcone as internal standard and liquid chromatography coupled via an APCI interface with tandem mass spectrometry (LC–MS/MS) for detection. The chromatography (column XTerra® MS, C_{18}, 2.1 mm × 100 mm, particle size 3.5 μm) was done isocratically with acetonitrile/water (60/40, v/v; flow rate 200 μl/min). The MS/MS analysis was performed in the negative ion mode (*m/z* transition: ezetimibe 408–271, internal standard 223–117). The validation ranges for ezetimibe and total ezetimibe were as follows: serum 0.0001–0.015 μg/ml and 0.001–0.2 μg/ml; urine and fecal homogenate 0.025–10 μg/ml and 0.1–20 μg/ml, respectively. The assay was successfully applied to measure ezetimibe disposition in two subjects genotyped for the hepatic uptake transporter SLCO1B1.
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1. Introduction

Ezetimibe (1-(4-fluorophenyl)-3(*R*)-[3(*S*)-(4-fluorphenyl)-3-hydroxypropyl]-4(*S*)-(4-hydroxyphenyl)azetidin-2-one, Fig. 1), a synthetic 2-azetidinone, is the first member of a new class of compounds that selectively inhibits intestinal sterol absorption by blocking the recently discovered sterol transporting protein Niemann-Pick C 1L1 (NPC1L1) [1,2]. The drug is widely used in treatment of hypercholesterolemia and of sitosterolemia [3,4]. Combination of ezetimibe with low dose statins provides probably additional reduction in cardiovascular mortality [5].

The drug is rapidly absorbed from the gastrointestinal tract and subjected to nearly complete pre-systemic conjugation by intestinal UDP-glucuronosyltransferases (UGT) [6,7]. It is still unclear whether the cholesterol uptake is blocked by luminal and/or intracellular binding. However, the sterol lowering effect of ezetimibe depends obviously on the complex interplay between ezetimibe uptake into the enterocytes, intestinal glucuronidation, apical and/or basolateral efflux out of the intestinal absorption compartment as well as uptake into the liver and enterohepatic circulation. There is evidence from drug interaction studies with cyclosporine, fibrates and rifampicin which is not plausibly explained by competition with intestinal UGT that active intestinal and hepatic transporter proteins are involved in ezetimibe disposition [8–11]. To evaluate the major pharmacokinetic variables of the ezetimibe effect in man, a validated high-sensitive analytical method is required to quantify concentrations of the parent drug and its metabolite in serum, urine and feces. The methods described so far lacked adequate sensitivity (limit of quantification: 1 ng/ml for serum assay) to describe pharmacokinetics of ezetimibe properly because the expected maximum serum concentrations after a single therapeutic dose is about 5 ng/ml; i.e. less than three half-lives of the terminal slope could be followed up with former methods. Furthermore,

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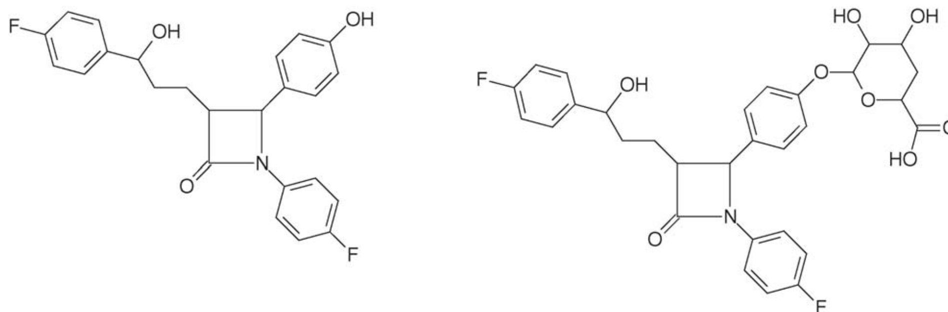

Fig. 1. Ezetimibe (left) and ezetimibe glucuronide (right).

radioactive labeled ezetimibe had to be used in early pharmacokinetic studies in man to quantify elimination of ezetimibe with feces and urine, which is not suitable for analysis of ezetimibe in pharmacokinetic studies [12]. Therefore, we present in this paper details of a new LC–MS/MS method to measure serum concentrations time profiles and intestinal and renal excretion of ezetimibe in two healthy subjects with genetic polymorphisms of the hepatic uptake transporter protein OATP-C (SLCO1B1).

2. Experimental

2.1. Reagents

Acetonitrile was purchased in LC–MS quality (Chromasolv®, Sigma–Aldrich, Taufkirchen, Germany). Deionized water (conductance: ≤0.055 μS/cm, pH 5.0–6.0) was generated with the system SG 2800 (Hamburg, Germany). The internal standard 4-hydroxychalcone (Fig. 2) was purchased from Lancaster (Frankfurt/Main, Germany), β-glucuronidase (>100.000 Fishman units/ml) from Sigma–Aldrich, and methyl *tert*-butyl ether as well as diethyl ether from Merck (Darmstadt, Germany). Ezetimibe was extracted from Ezetrol® tablets (MSD Sharp & Dome, Haar, Germany) as follows: 10 tablets each containing 10 mg ezetimibe were mortared and suspended in 20 ml diethyl ether. The solids were separated using a filter (porosity 5, Schott, Mainz, Germany). The filtrate was evaporated under a gentle nitrogen stream at 40 °C. The residuum was washed twice with 10 ml deionized water. Then, the filter cake was dissolved in 20 ml diethyl ether. After evaporation, this washing step was repeated and the residue was dried for 60 min at 40 °C under a nitrogen stream and stored until use in an exsiccator filled with blue gel at 2–8 °C. Identity, purity and content were confirmed by mass spectrometry, IR-spectroscopy and NMR-spectroscopy as well as by determination of the melting point, by elementary analysis and acidimetric titration. The ascertained content was 99%.

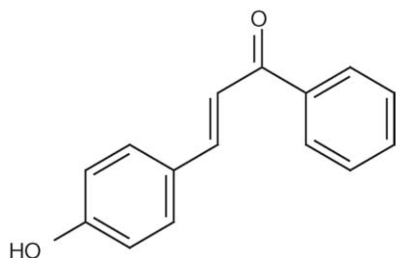

Fig. 2. 4-Hydroxychalcone.

2.2. Genotyping

OATP-C (*SLCO1B1*) polymorphisms were genotyped for G-11187A, A388G (Asn130Asp) and T521C (Val174Ala) by polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP) under the same conditions for each reaction. Five picomoles of specific primers (TIB Molbiol, Berlin, Germany), 1.0 mmol/l of desoxyribonucleoside triphosphate (Biozym, Hessisch Oldendorf, Germany), 2.0 mmol/l $MgCl_2$ and 0.75 units of *Taq* DNA polymerase (Invitrogen, Karlsruhe, Germany) were mixed in a total volume of 25 μl. The initial denaturation was performed for 2 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 58 °C and 30 s at 72 °C. A final elongation was carried out at 72 °C for 7 min. All PCR amplifications were carried out using a GeneAmp® 9700 thermocycler (Applied Biosystems, Darmstadt, Germany). DNA fragments generated after restriction enzyme digestion (New England Biolabs, Frankfurt/Main, Germany) were separated on a 3.0% agarose gel (AppliChem, Darmstadt, Germany) and visualized after ethidium bromide staining by a digital image station (Kodak, Stuttgart, Germany). Haplotype *SLCO1B1* *1a is defined as −11187G, 388A, 521T and *SLCO1B1* *15 as −11187G, 388G, 521C. The following primers were used: G-11187A, 5′-AGT ACA GAC CCT TCT CTC AC as forward primer, 5′-GCA ACC ATA TCA ACA AAT GTC C as reverse primer; T521C, 5′-GAA ATC ATC AAT GTA AGA AAG CCC as forward primer, 5′-ATC TGG GTC ATA CAT GTG GAT ATA C as reverse primer; A388G, 5′-GAC TTT TTA CTG TCA ATA TTA ATT C as forward primer, 5′-TAT TCA GTA GAT AAG CAA AAT G as reverse primer.

2.3. Sample preparation

The samples arrived at the laboratory deeply frozen at −80 °C. All analytical procedures were carried out at room temperature and did not exceed 2 h.

For determination of ezetimibe, 0.5 ml serum, urine or homogenized feces (diluted with deionized water, 25/75, v/v) was mixed with 0.5 ml deionized water and 25 μl 4-hydroxychalcone as internal standard (final concentrations:

serum 1.0 μg/ml; urine and feces 5 μg/ml). Then, the samples were extracted with 4 ml methyl *tert*-butyl ether for 15 min. After centrifugation at 4000 rpm for 2 min, the organic layer was separated and evaporated under a gentle air stream at 50 °C. The residue was dissolved in 100 μl (200 μl for urine and feces) mobile phase (acetonitrile/water: 60/40, v/v) of which 50 μl (10 μl for urine and feces) was used for chromatography. For determination of total ezetimibe (unconjugated ezetimibe plus ezetimibe glucuronide), 450 μl deionized water and 50 μl β-glucuronidase (>5000 Fishman units) were added to 0.5 ml serum, urine or feces and incubated at 50 °C for 60 min according to Patrick et al. [12]. After cooling, 25 μl internal standard solution was added and following the samples were handled as described for unchanged ezetimibe. The enzymatic glucuronide cleavage was optimized as described in Sections 2.5 and 3.2.

2.4. LC–MS/MS analysis

The LC–MS/MS system consisted of the pump series 1100 (Hewlett Packard, Waldbronn, Germany), the autosampler series 200 equipped with an cooling peltier rack tempered at 15 °C (Perkin-Elmer, Applied Biosystems, Darmstadt, Germany), the column thermostat L-5025 (Merck-Hitachi, Darmstadt, Germany) tempered at 50 °C and the PE Sciex API 2000 mass spectrometer equipped with the Analyst 1.2 software (both Applied Biosystems, Darmstadt, Germany). The chromatography was performed isocratically using acetonitrile/water (60/40, v/v) as mobile phase (flow 200 μl/min), the column X Terra® MS (C_{18}, 2.1 mm × 100 mm, particle size 3.5 μm) and the guard column X Terra® MS (C_{18}, 2.1 mm × 10 mm, particle size 3.5 μm, Waters, Milford, USA). The mass spectrometer was used in the multiple reaction monitoring (MRM) mode and equipped with the Heated Nebulizer (atmospheric pressure chemical ionisation, APCI) interface in the negative ion mode. The m/z transition for ezetimibe was monitored from 408 to 271, for the internal standard from 223 to 117. The optimized APCI and MS/MS parameters are given in Table 1.

2.5. Validation

Selectivity of the LC–MS/MS method was confirmed for serum, urine and feces by analysing in each case six blank samples and six samples spiked with 4-hydroxychalcone and varying amounts of ezetimibe, respectively. Recovery of ezetimibe extraction from serum, urine and feces was assessed by comparing the peak areas obtained from accordingly six spiked matrix samples with known concentrations of ezetimibe relative to peak areas from samples obtained without extraction (diluted stock solution). The spiking concentrations to assure selectivity and recovery in serum were 0.2, 2.5 and 10 ng/ml for unchanged ezetimibe and 2.5, 25 and 100 ng/ml for total ezetimibe. The levels for unchanged and total ezetimibe in urine and feces were 0.1, 1.0 and 10 μg/ml.

Table 1
Optimized APCI and MS/MS parameters for the determination of ezetimibe

APCI parameters	MS/MS parameters
Needle current: −2 μA	Declustering potential: −10 V
Nebulizer gas: 75 psi	Focusing potential: −400 V
Auxiliary gas: 85 psi	Entrance potential: −10 V
Temperature: 450 °C	Collision-activated dissociation gas: 3 psi
Curtain gas: 30 psi	Collision energy: −20 V

Nitrogen was used as nebulizer, auxiliary, curtain and collision gas (1 psi = 6894.8 Pa).

Linearity of the calibration function was confirmed by adding increasing amounts of ezetimibe to drug-free serum, urine and feces. The calibration curves ($n = 6$) consisted of a double blank matrix sample without analyte and without internal standard, a blank matrix sample spiked with internal standard and eight (unchanged ezetimibe in serum; feces) or nine (total ezetimibe in serum; urine) calibrants. The validation range for unchanged ezetimibe in serum was 0.0001–0.015 μg/ml, for total ezetimibe 0.001–0.2 μg/ml. The validation range for unchanged and total ezetimibe in urine was 0.025–10 μg/ml and in feces was 0.1–20 μg/ml. To assess matrix effects, which are in common of less relevance using APCI compared to electrospray ionization (ESI), all calibration curves were prepared in blank samples of serum, urine and feces, an internal standard was used and the chromatographic flow of the first two minutes was sent to waste to prevent matrix materials reaching the APCI source. To assess relative matrix effects, the coefficient of variation (CV) of six quality control (QC) sample sets spiked with ezetimibe in low, medium and high concentrations and prepared in independent sources of each matrix (serum, urine, feces) was acquired. For detection of absolute matrix effects, one lot of quality control samples of each matrix was measured according to our developed assay or using the method of standard addition [13].

The chromatograms were evaluated with the internal standard method using peak-area ratios for calculation of the calibration curves using linear regression analysis weighted by $1/x$ (x is the concentration). Concentrations of ezetimibe glucuronide were assessed by subtracting the concentrations of unchanged ezetimibe from the respective total ezetimibe concentrations.

For evaluation of accuracy and precision, QC samples spiked with ezetimibe in low, medium and high concentrations were prepared with serum, urine and feces before the beginning of the study analysis and stored at −80 °C. The spiking concentrations in quality control samples were identical to the concentrations used in selectivity and recovery assessments. Six lots were used for validation.

Between-day accuracy and precision was assessed by comparison of the estimated concentrations in QC and calibrator samples (in each case six separately prepared sets measured on different days) with the respective nominal concentrations, expressed as relative error (accuracy) and the calculation of the respective coefficients of variations (precision). Within-day accuracy and precision was estimated by six-fold measuring of the respective quality control samples on one day.

The stability of ezetimibe was determined using accordingly five QC sample lots for each matrix. Short-term (bench-top) stability was tested after storing these samples before extraction at room temperature for 6 h. Post-preparative stability was assessed by storing extracts in the autosampler at 15 °C for 24 h. To assess freeze-and-thaw stability, the samples were thawed and frozen

two times before extraction. Stock solution stability of ezetimibe and 4-hydroxychalcone was tested at −20 °C for 1 month by measurement of UV absorption (ezetimibe, $\lambda = 232$ nm; 4-hydroxychalcone, $\lambda = 244$ nm). Short-term, post-preparative and freeze-and-thaw stability of ezetimibe glucuronide was investigated as described for unchanged ezetimibe with five different glucuronide containing samples of serum, urine and feces which were obtained in another drug interaction study with ezetimibe, which were five-fold assayed for parent ezetimibe to indicate glucuronide hydrolysis [14].

The enzymatic glucuronide cleavage was optimized using five samples with serum, urine and feces from a drug interaction study with 20 mg ezetimibe in which the ezetimibe concentrations were expected to be maximal according to the literature [12,14]. Each sample was five-fold incubated with 50 μl β-glucuronidase (100.000 Fishman units/ml) at 50 °C for 30, 60 or 90 min. In a second step, the samples were incubated with increasing amounts of β-glucuronidase (2.500–10.000 units) at 50 °C for 1 h. Finally all samples were assayed for ezetimibe as described before

2.6. Pharmacokinetic study

The pharmacokinetic pilot study was performed according to the current international and national regulations and recommendations in two male healthy subjects (age 26 and 27 years; body mass index 24.6 and 24.7 kg/m^2) who gave informed written consent. The study was approved by the local ethical committee. One subjects was carrier of the *SLCO1B1* wild-type allele (*1a), the other was heterozygous for *15 (*SLCO1B1* 388AG/521TC).

On the pharmacokinetic study day, 20 mg ezetimibe (Ezetrol®, MSD Sharp & Dome, Haar, Germany) was swallowed with 200 ml tap water after overnight fasting for at least 10 h. A standard lunch was eaten 5 h and a standard dinner 11 h after the morning administration. Venous blood (5 ml) was sampled from a forearm vein before and 0.33, 0.66, 1, 1.33, 1.66, 2, 2.33, 2.66, 3, 3.5, 4, 5, 6, 7, 8, 10, 12, 16, 24, 36 and 48 h after drug intake. Urine and feces were collected for 7 days. Feces were completely sampled and homogenized with deionized water. Serum, aliquots of urine and homogenized feces were stored before quantitative analysis at least at −80 °C for less than one month.

The pharmacokinetic evaluation to assess the area under the concentration–time curve between 0 and 48 h ($AUC_{0–48}$), maximum serum concentrations (C_{max}), the time of C_{max} (t_{max}), half-life ($t_{1/2}$), total body clearance (CL), renal clearance (CL_R), metabolic clearance (CL_M), intestinal clearance ($CL_{intestinal}$), and the amounts of ezetimibe and its glucuronide excreted (Ae) into urine and feces, respectively, was done with standard methods using the SAS statistical package (SAS 8.02, SAS Institute Inc., Carry, USA).

3. Results and discussion

3.1. LC–MS/MS analysis

Since no stable isotope-labeled ezetimibe was available 4-hydroxychalcone (Fig. 2) was used as the internal standard because of its similar chemical properties to ezetimibe (4-hydroxychalcone: pK_a: 9.75, log *P*: 3.48; ezetimibe pK_a 9.66, log *P* 4.39; both are phenolic compounds). Fig. 3 shows the mass spectra of ezetimibe and the internal standard in the negative ionization mode. Both mass spectra show the respective molecular ion produced by hydride abstraction ($[M-H]^-$). In the positive mode, both substances showed the protonated molecule ($[M+H]^+$). However, ezetimibe was also fragmented to a major fragment ion at *m/z* 392 (loss of one water molecule). A minor additional mass peak occurred at *m/z* 433 representing the sodium adduct ($[M+Na]^+$) (data not shown). The product ion scan of ezetimibe identified in the negative ionization mode a strong fragment at *m/z* 271 with more than three-fold higher intensity than a fragment in the positive mode (*m/z* 392–133). Surprisingly the *m/z* 297 mass peak occurring in the negative Q3 scan was no appropriate fragment for monitoring the parent compound (Fig. 3). Therefore, we used the negative MRM mode from *m/z* 408 to 271 in our ezetimibe assays. APCI and MS parameters were optimized for maximum intensity of the mentioned precursor and product ions (Table 1). The mean retention times of ezetimibe and the internal standard were 2.5 and 2.4 min, respectively. Therefore, one analytical run lasted less than 6 min, i.e. the method enables a daily throughput of about 150–160 samples.

3.2. Validation

The analytical method was selective for ezetimibe. In blank matrix samples, there were neither peaks from ezetimibe nor from the internal standard detectable. Furthermore, there was no analytical interference between ezetimibe and 4-hydroxychalcone (Fig. 4). Ezetimibe can be extracted by liquid extraction using methyl *tert*-butyl ether. The recovery of ezetimibe from serum, urine and feces is given in Tables 2 and 3. The linear regression weighted by $1/x$ (x is the concentration) showed a distribution of the residuals without in-homogeneities and trends, i.e. fits the correlation better than a quadratic regression model (minimum of the mean square error). Correlation coefficients (r) of all calibration curves ($n = 6$) were at least 0.9928 or better. Within-day as well as between-day accuracy and precision of calibration curves and quality controls for serum, urine and feces were within the stipulated range of the current bioanalytical guidelines (Tables 2 and 3) [15,16]. Ezetimibe was stable in all matrices at room temperature for at least 6 h, at 15 °C in the autosampler for at least 24 h and during two freeze–thaw cycles. Ezetimibe glucuronide before hydrolysis was also found to be stable in all matrices at room temperature for at least 6 h, at 15 °C in the autosampler for at least 24 h and during two freeze–thaw cycles. Stock solutions of ezetimibe and 4-hydroxychalcone were stable at −20 °C for at least 1 month. With these stock solutions, working solutions were prepared freshly on the day of analysis.

To overcome matrix effects, all calibration curves were prepared in blank matrix samples, an internal standard was used and the chromatographic flow of the first two minutes was sent to waste. Furthermore we investigated relative and absolute matrix effects as described above. For all matrices and concentrations

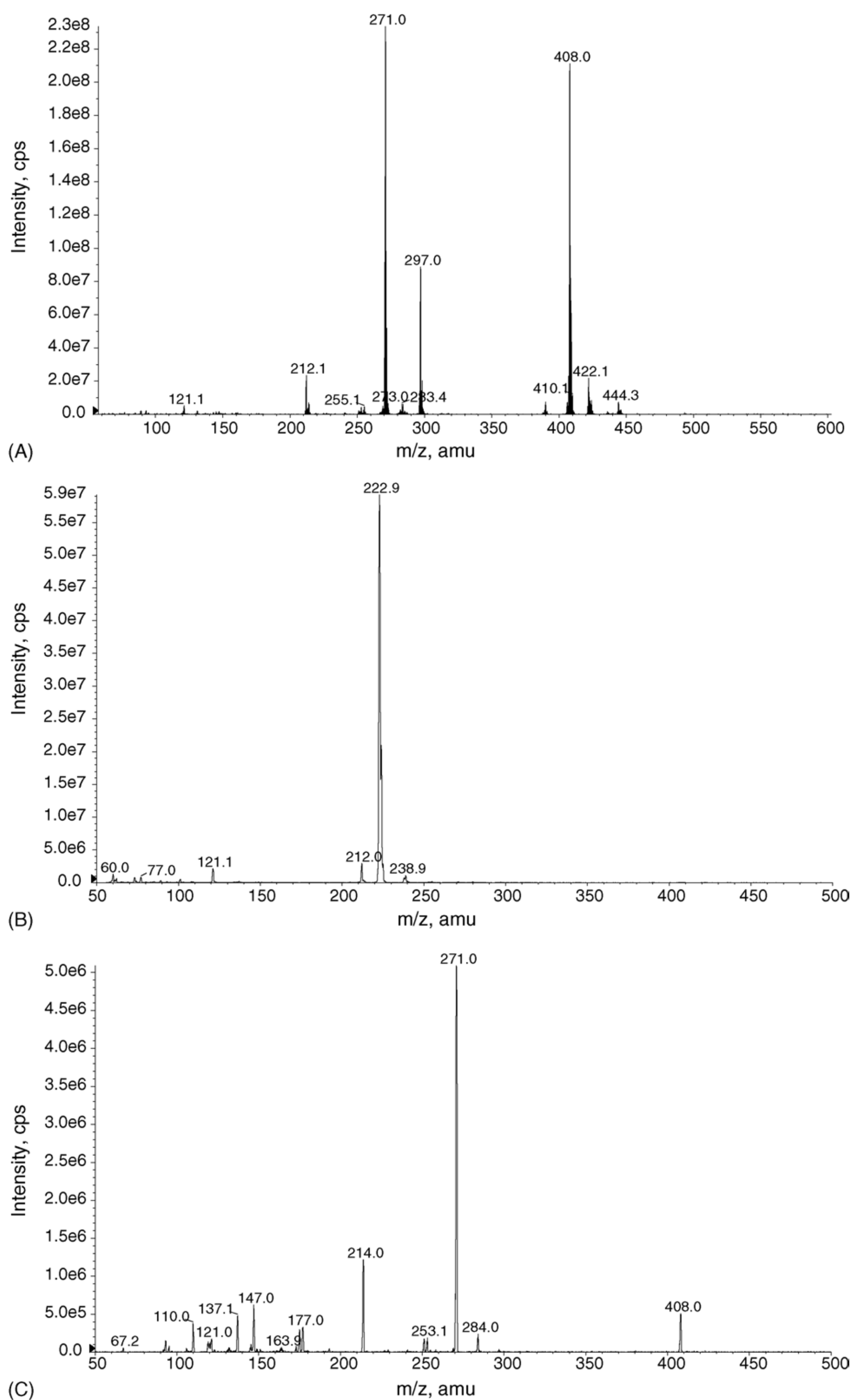

Fig. 3. Mass spectra of ezetimibe (A) and the internal standard 4-hydroxychalcone (B) obtained in the negative ion mode. Product ion scan of ezetimibe (*m/z* 408) in the negative ion mode is shown in (C).

CV according relative matrix effects and deviations in comparison to the method of standard addition were within 15 or 20% at the lower limit of quantification, which suggests that matrix effects in our method are of minor significance.

The enzymatic cleavage process gained maximum ezetimibe levels after incubation of all glucuronide containing matrices with 5.000 Fishman units β-glucuronidase for 60 min at 50 °C. Our results confirmed the information on enzymatic cleavage

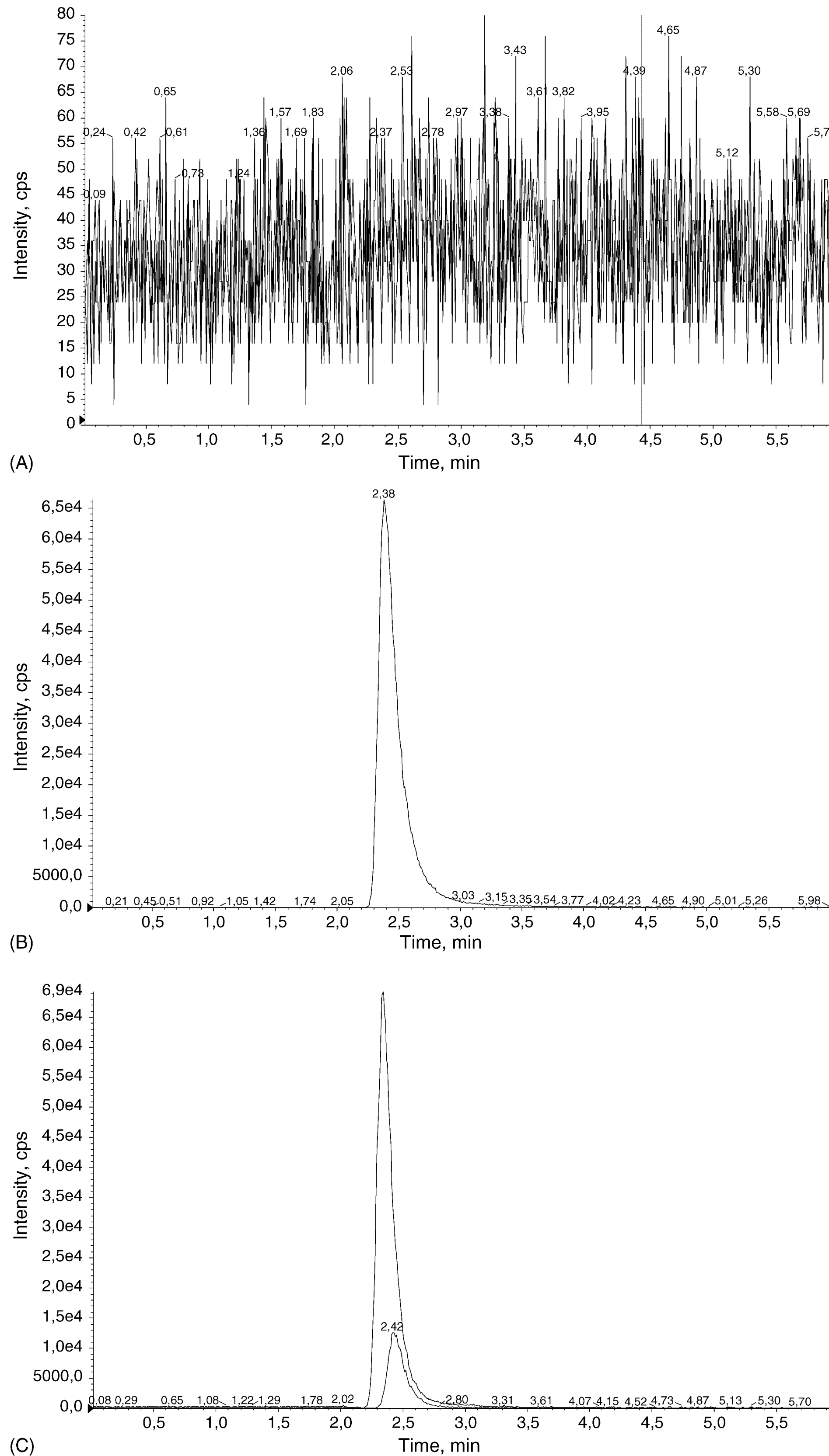

Fig. 4. Mass chromatograms of extracts from blank human urine (A), spiked with internal standard (B) and ezetimibe (25 ng/ml, C). All chromatograms were obtained by monitoring for m/z transitions 408/271 (ezetimibe) and 223/117 (4-hydroxychalcone) in the negative ion mode.

Table 2
Within- and between-day accuracy and precision in percent of the nominal, respectively, mean concentrations of unchanged ezetimibe and total ezetimibe (unchanged ezetimibe plus ezetimibe glucuronide) in quality control samples of human serum. Recovery is given in percent relative to unextracted samples

	n	Ezetimibe			Total ezetimibe		
		Accuracy	Precision	Recovery	Accuracy	Precision	Recovery
Within-day							
0.2/2.5 ng/ml	6	−5.49	13.3	114	8.84	11.9	105
2.5/25 ng/ml	6	−3.20	3.95	101	−0.94	8.74	95.8
10/100 ng/ml	6	−2.37	10.9	91.3	1.01	8.27	92.4
Between-day							
0.2/2.5 ng/ml	6	9.90	18.5	–	11.5	8.47	–
2.5/25 ng/ml	6	9.32	8.50	–	3.47	14.7	–
10/100 ng/ml	6	5.36	7.83	–	2.05	13.0	–

Table 3
Within- and between-day accuracy and precision in percent of the nominal, respectively, mean concentrations of ezetimibe in quality control samples of human urine and feces

	n	Urine			Feces		
		Accuracy	Precision	Recovery	Accuracy	Precision	Recovery
Within-day							
0.1 μg/ml	6	−2.35	10.4	102	2.70	12.6	92.2
1 μg/ml	6	13.9	9.94	87.1	10.9	7.73	83.6
10 μg/ml	6	6.99	9.13	97.7	4.8	9.35	85.6
Between-day							
0.1 μg/ml	6	9.47	6.60	–	4.68	7.88	–
1 μg/ml	6	5.92	7.65	–	5.30	5.09	–
10 μg/ml	6	0.13	8.33	–	−3.47	10.7	–

Recovery is given in percent relative to unextracted samples.

of total ezetimibe from previous papers [6,12]. However, it is unknown whether ezetimibe undergoes also sulfate conjugation in man. Therefore, we cannot exclude that sulfates are destroyed under the conditions of our hydrolysis and that the difference between total and unchanged ezetimibe represents specifically the concentration of the glucuronide. The method was specific, sensitive, precise and accurate for unchanged ezetimibe only. The sensitivity of the LC–MS/MS assay was at least 10 times higher than in a method described previously [12].

3.3. Assay application

The validated analytical assay enabled the quantitative determination of unchanged ezetimibe and total ezetimibe in samples with human serum, urine and feces which were collected in our pharmacokinetic pilot study in two healthy subjects genotyped for *SLCO1B1* (Fig. 5, Table 4). The concentration–time profiles of ezetimibe and the glucuronide were irregular in profile showing at least two distinct concentration peaks. The serum concentrations of both ezetimibe and the glucuronide were several fold

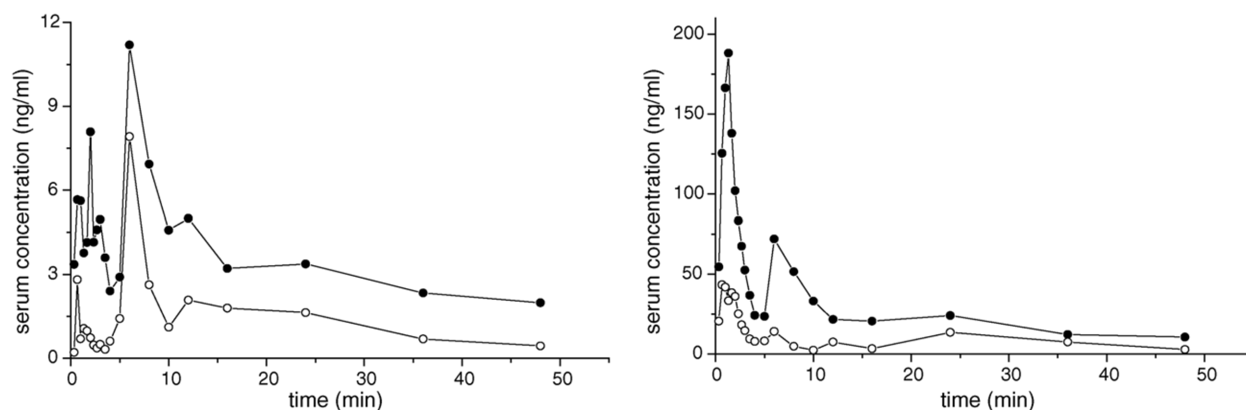

Fig. 5. Serum concentration–time profiles of ezetimibe (left panel) and its glucuronide (right panel) of two subjects after oral single dose of 20 mg ezetimibe. *15 carrier of SCLO1B1 gene (polymorphism): filled circles, *1a carrier of SCLO1B1 gene (wild type): open circle.

Table 4
Pharmacokinetic characteristics of ezetimibe and its glucuronide in one carrier of the *SLCO1B1* wild-type allele *1a and one carrier of the *15 allele

	Ezetimibe		Ezetimibe glucuronide	
	SLCO1B1 *1a	*SLCO1B1* *15	*SLCO1B1* *1a	*SLCO1B1* *15
$AUC_{0–48h}$ (ng*h/ml)	68.2	169	427	1303
C_{max} (ng/ml)	7.92	11.2	43.3	188
$t_{1/2}$ (h)	15.3	31.6	10.2	20.1
$CL_{intestinal}$ (ml/min)	3665	960	358	1.86
CL_R (ml/min)	2.74	2.06	38.1	28.9
$CL_{M(feces)}$ (ml/min)	–	–	2244	14.3
$CL_{M(urine)}$ (ml/min)	–	–	238	222
Ae_{feces} (mg)	15.0	9.76	9.18	0.15
Ae_{urine} (mg)	0.01	0.02	0.98	2.26

higher in the carrier of the *15 allele compared to the wild-type subject. This was associated with dramatically decreased intestinal clearance values, lower fecal excretion but increased amount excreted into the urine. Consistently, the half-life was markedly elevated in our subject with the *15 allele. We concluded from these observations in one carrier of the *15 allele, that ezetimibe and/or is glucuronide might be substrates of the liver specific uptake transporter protein SLCO1B1 as shown for pravastatin and fexofenadine [17,18]. However, binding studies with hepatic SLCO1B1 and pharmacokinetic data from a sufficient number of subjects with *SLCO1B1* polymorphisms is required to draw reliable conclusions.

4. Conclusions

The described analytical method was selective and sensitive for the quantification of unchanged ezetimibe in human serum, urine and feces sampled in pharmacokinetic studies. Concentrations of the major metabolite ezetimibe glucuronide were assessed by determination of unchanged ezetimibe after hydrolysis of the samples using β-glucuronidase. The developed assay enables quantitative determination of ezetimibe and its major metabolite in man without using radioactive analogues. Our experimental study suggests that the hepatic uptake transporter SLCO1B1 is involved in disposition of ezetimibe.

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