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Simultaneous quantification of the enantiomers of verapamil and its N-demethylated metabolite in human plasma using liquid chromatography–tandem mass spectrometry

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Abstract

A stereoselective bioanalytical method for the simultaneous quantification of the enantiomers of verapamil and its active main metabolite norverapamil in human plasma has been developed and validated. The samples were analysed by liquid chromatography–electrospray-tandem mass spectrometry (LC–ESI-MS/MS) in the Selected Reaction Monitoring (SRM) mode using a deuterated internal standard. The stationary phase used for the chiral separation was a Chiral-AGP®. The enantiomers of verapamil were selectively detected from those of norverapamil by the mass spectrometer due to different molecular masses, although there was a chromatographic co-elution. Thus, time-consuming procedures like achiral preseparation or chemical derivatisation could be avoided. Higher detection sensitivity than earlier published methods based on fluorescence detection was obtained, although a mobile phase of high water-content and high flow-rate was introduced into the electrospray interface (85% aqueous ammonium acetate pH 7.4 + 15% acetonitrile at 0.6 ml/min). The enantiomers of verapamil and norverapamil could be quantified at levels down to 50 pg and 60 pg/500 μl plasma sample, respectively, with R.S.D. in the range of 3.6–7.8%. The presented method was successfully applied to an in vivo intestinal absorption and bioavailability study in humans, using the Loc-I-Gut® method.

Keywords: Enantiomer separation; Verapamil; Norverapamil

1. Introduction

Verapamil belongs to the pharmacological class of calcium channel blockers. It is used for the treatment of cardiovascular diseases like hypertension and angina pectoris [1]. The substance is a tertiary amine with one asymmetric carbon and it can thus exist in two enantiomeric forms (Fig. 1A). However, verapamil is administered as a racemate despite documentation of stereogenic differences in pharmacological potency as well as in pharmacokinetics [1–4]. It is extensively metabolised in the human body [5]. The main metabolite with pharmacological activity is the N-demethylated form norverapamil [2–4] (Fig. 1A). For the structures of the other main metabolites D617, D620 and PR23 see Fig. 1B.

Separation systems based on liquid chromatography with UV or fluorescence detection, have earlier been developed in order to individually determine the enantiomers of verapamil in biological samples [6–18]. Different types of chiral stationary phases (CSPs) have been used for this purpose. Both CSPs based on cellulose derivatives like Chiralpak AD® [6,7] and Chiralcel OD® [8,9], as well as protein-based ones like α_1-acid glycoprotein (Chiral-AGP®) [10–15] and ovomucoid [16–18] have in these studies demonstrated enantioselectivity towards verapamil. One obstacle, however, especially when using protein-based CSPs, has been the chromatographic interference with the metabolite norverapamil. This problem has been solved by increasing the selectivity using a combination of achiral and chiral chromatography [6,10,11,14–17] or by chemical derivatisation (acetylation) of norverapamil [12,13]. However, in one study, a chemometric approach has been used to optimise a chromatographic separation of both the enantiomers of

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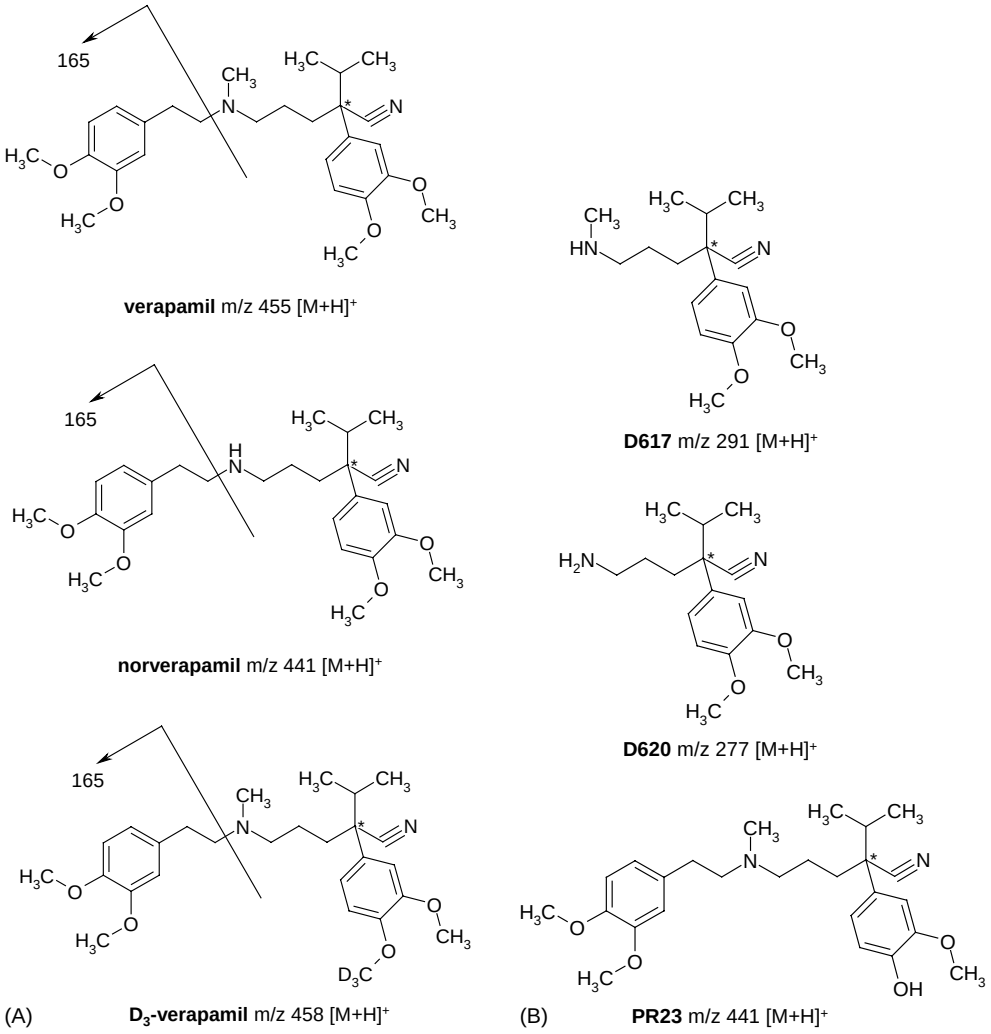

Fig. 1. (A) Structures of verapamil, norverapamil and D_3-verapamil (internal standard). The asymmetric carbons are marked with asterisks. The structures of the fragments corresponding to the daughter ions at *m/z* 165 are suggested. (B) Structures of the metabolites D617, D620 and PR23. The asymmetric carbons are marked with asterisks.

verapamil and norverapamil using a single column system (Chiral-AGP®) without derivatisation [18].

The use of a mass spectrometer as the detector for the chiral LC system would facilitate the situation, as there would be no need for chromatographic separation between the main component and its metabolites as long as they differ in molecular mass. Thus, coupled column systems or derivatisation steps can be omitted. One further advantage would be the possibility of using an isotopically labelled internal standard in order to improve the performance of the analytical method. There are a few examples in the literature of the coupling of protein-based CSPs to mass spectrometric detection using electrospray [19–23], thermospray [24] or atmospheric pressure chemical ionisation interfaces [25]. One major concern when developing these systems has been how the sensitivity of the ion source is affected by the high water content in the mobile phases necessary for obtaining the chiral resolution. However, a few different approaches have been tried to circumvent this problem, such as the use of a chiral narrow-bore column with a low volumetic flow-rate (0.25 ml/min) [21] or even a capillary column using 1–2 μl/min in combination with a sheath-liquid containing organic solvent [19].

The aim of the present study was to develop and validate a new enantioselective LC–MS/MS method for the simultaneous quantification of the enantiomers of verapamil and norverapamil in human plasma samples without the need for achiral preseparation or chemical derivatisation. The method was then applied to an in vivo intestinal pharmakokinetic study in humans.

2. Experimental

2.1. Chemicals

Rac-, (*R*)- and (*S*)-verapamil hydrochloride and *rac*-norverapamil hydrochloride were purchased from Sigma

(St. Louis, MO, USA). *Rac*-trideuterated [$^2H_3$] verapamil hydrochloride (denoted *rac*-D_3-verapamil) (internal standard), *rac*-D617 hydrochloride, *rac*-D620 hydrochloride and *rac*-PR23 hydrochloride were kind gifts from Knoll AG (Ludwigshafen, Germany). The water was purified using a Milli-Q Water Purification System (Millipore, Bedford, MA, USA). Blank human plasma was obtained from the University Hospital (Uppsala, Sweden). All other chemicals were of analytical reagent grade and used without further purification.

2.2. LC–UV and LC–MS/MS procedures

An Agilent 1100 series liquid chromatograph (Agilent Technologies, Waldbrunn, Germany) with a binary pump, degasser and an autosampler, was used. A Chiral-AGP® column (ChromTech, Hägersten, Sweden) with the dimensions 150 mm length × 4.0 mm inner-diameter, particle diameter 5 μm, was used for the chiral separations. In order to increase column life-time, a peek filter A-428X (UpChurch Scientific, Oak Harbor, WA, USA) was mounted between the injector and the column inlet. The flow-rate was 0.6 ml/min and the injection volume 50 μl. The mobile phase used for the method validation and analysis of plasma samples was 15% acetonitrile and 85% aqueous ammonium acetate buffer (0.020 M acetate) at pH 7.4. For mobile phase compositions tested during method development, see Table 1. When UV absorbance detection was used, it was performed with the Agilent 1100 diode-array G1315A, and the chromatograms were recorded at 272 nm. The retention factor, k' was calculated as $(t_R - t_0)/t_0$, where t_R is the retention time of the peak of interest and t_0 the dead-time of the system (time from injection to the frontal disturbance). N_R and N_S are the number of theoretical plates for the *R* and *S* enantiomers, respectively, and were calculated as $16(t_R{}^2/W_t{}^2)$, where W_t is the base width between the tangents in time units. The enantioresolution, Rs, was calculated as $2(t_{R2} - t_{R1})/(W_{t1} + W_{t2})$, where t_{R2} and t_{R1} are the retention times of the second and first eluted enantiomer, respectively, and W_{t1} and W_{t2} are the base widths between the tangents in time units for the first and second enantiomer.

For the LC–MS/MS experiments, the column outlet was connected to a Quattro LC (Micromass, Manchester, UK) quadrupole-hexapole-quadrupole mass spectrometer equipped with an electrospray interface (ESI). The instruments were controlled by a PC using the software MassLynx v. 3.3. The ESI parameters were optimised for sensitivity manually during direct infusion of a solution of verapamil standard with a syringe pump at 5 μl/min through a connecting T, where it was mixed with the LC mobile phase (flow-rate 0.6 ml/min). The final parameters for the ESI was set as follows: capillary voltage 1.00 kV, cone 57 V, extractor 9 V and RF lens 1.00 V. The desolvation temperature was 380 °C and the source block temperature 130 °C. The nebuliser gas flow was 100 l/h and the desolvation gas flow was 1000 l/h. For MS/MS analysis, the collision cell was filled with argon at a pressure of 1.0×10^{-3} mbar. The collision energy was set at 30 eV. For quantitative purposes, the instrument was run in Selected Reaction Monitoring (SRM) mode, recording the transitions from the respective $[M + H]^+$ ions to the following daughters: verapamil *m/z* 455 → 165, norverapamil *m/z* 441 → 165 and D_3-verapamil 458 → 165. For the selectivity test, the following SRM mode transitions were monitored for the other metabolites: D617 *m/z* 291 → 151, D620 *m/z* 277 → 151 and PR23 *m/z* 441 → 165.

Table 1
Effect of different mobile phase compositions on chromatographic parameters

		Mobile phase[a]	
		PB	AA
(A) UV absorbance detection			
Rac-verapamil	k'_S	6.71	5.33
	α	1.29	1.24
	N_R	2400	3100
	N_S	2500	2800
	Rs	2.65	2.39
Rac-norverapamil	k'_S	6.28	6.13
	α	1.17	1.15
	N_R	2900	3200
	N_S	2800	3000
	Rs	1.74	1.71
		Mobile phase[b]	
		AA pH 7.0	AA pH 7.4
(B) Mass spectrometric detection			
Rac-verapamil	N_R	2200	2200
	N_S	2200	2300
	Rs	2.20	2.36
Rac-norverapamil	N_R	2300	2900
	N_S	2100	2500
	Rs	1.52	1.67

[a] Mobile phase compositions: 15% acetonitrile + 85% of either sodium phosphate buffer (PB) 20 mM or ammonium acetate buffer (AA) 20 mM, both at pH 7.0.

[b] Mobile phase compositions: 15% acetonitrile + 85% ammonium acetate buffer (AA) either at pH 7.0 or pH 7.4 (20 mM acetate).

2.3. Drug administration and sampling

Eight healthy male volunteers, aged 23–35 years and weighing 60–95 kg, gave their informed consent to participate in this in vivo intestinal perfusion study by using the Loc-I-Gut® method [26,27]. All subjects underwent a full clinical examination prior to the study and all had normal clinical and laboratory values. None of the subjects received any medication other than the study drugs prior to or on the study day. Smoking and the consumption of alcohol and caffeine containing beverages were prohibited for at least 24 h prior to and during the study. The subjects also had to abstain from consuming grapefruit or its juices for at least 3 days before the perfusion experiments. The

study was performed at the Clinical Research Department, University Hospital, Uppsala, Sweden and was approved by both the Ethics Committee of the Medical Faculty, Uppsala University and the Swedish Medical Product Agency, Uppsala, Sweden. Each subject underwent one 100 min jejunal perfusion experiment with verapamil. The drug used was racemic verapamil hydrochloride (120 mg/l, 244 μM, Knoll AG, Darnstadt, Germany) and the total dose of verapamil given in each 100 min perfusion experiment was 24 mg. The intestinal perfusion solution consisted of potassium chloride 5.4 mM, 30 mM of sodium chloride, 35 mM of mannitol, 10 mM of D-glucose and 1.0 g/l of PEG 4000, all dissolved in a 70 mM phosphate buffer with a resultant pH of 6.5 and an osmolality of 290 mOsm/kg.

2.4. Preparation of standards, validation samples and stability testing procedure

Stock solutions of *rac*-verapamil hydrochloride and *rac*-norverapamil hydrochloride were prepared by weighing of the respective hydrochloride salt. These solutions were used to prepare standards at different concentrations for the calibration curve and the validation samples. Two different weighings for each analyte were used for the construction of the calibration curve and one separate weighing was carried out for the validation samples. To 500 μl of blank plasma, 100 μl of the respective standard solution of *rac*-verapamil hydrochloride and *rac*-norverapamil hydrochloride, were added to create standards or validation samples.

The calibration curves were constructed using linear regression with the peak area ratio (analyte/internal standard) as a function of analyte concentration. For the different calibration curve intervals used, see Table 2.

The method was validated for linearity, precision, accuracy and sensitivity by analysis of blank plasma samples spiked with *rac*-verapamil and *rac*-norverapamil (for concentrations see Table 2).

The selectivity towards endogenous compounds was evaluated by injection of blank plasma only spiked with internal standard. The effect of possibly interfering metabolites was studied by spiking blank plasma with the metabolites D617, D620 and PR23 together with verapamil and norverapamil.

The stability of the analytes in plasma were investigated by spiking verapamil and norverapamil at two levels to six portions of 500 μl blank plasma for each level (see Table 3). These plasma samples were then stored at room temperature for 4 days where after they were quantified using the described method calibrated with freshly prepared standards.

2.5. Sample preparation

To 500 μl of either the patient sample, standard or validation sample, 100 μl of internal standard solution was added (300 ng/ml).

The extraction method was slightly modified from Stagni et al. [13]. The plasma was made alkaline by addition of 100 μl of 2 M NaOH and was thereafter extracted with 6 ml hexane/2-butanol (98/2, v/v) for 20 min. After 10 min of centrifugation, the organic phase was transferred to a new tube. Fifty microliters of concentrated acetic acid was added and the organic phase was evaporated under a gentle stream

Table 2
Validation data

Analyte	Conc (ng/ml plasma)	*n*	Standard curve concentration interval (ng/ml)	Determination coefficient (r^2)	Precision (R.S.D.) (%)	Accuracy (%)
R-Verapamil	125	7	10.2–213 (10)[a]	0.9980	1.6	102
	25	6	10.2–213	0.9980	2.0	102
	2.5	15	0.10–10.2 (14)[a]	0.9983	3.8	90
	0.64	12	0.10–10.2	0.9983	1.4	96
	0.10	12	0.10–2.6 (10)[a]	0.9946	4.6	103
S-Verapamil	125	7	10.2–213 (10)[a]	0.9976	1.3	102
	25	6	10.2–213	0.9976	2.3	100
	2.5	15	0.10–10.2 (14)[a]	0.9984	5.9	89
	0.64	12	0.10–10.2	0.9984	2.1	95
	0.10	12	0.10–2.6 (10)[a]	0.9984	7.8	83
R-Norverapamil	121	7	9.6–191 (10)[a]	0.9909	3.5	101
	24	6	9.6–191	0.9909	4.0	97
	4.2	15	0.12–9.6 (14)[a]	0.9158	5.9	112
	0.62	12	0.12–2.4 (10)[a]	0.9918	3.1	74
	0.12	12	0.12–2.4	0.9918	4.3	107
S-Norverapamil	121	7	9.6–191 (10)[a]	0.9907	3.1	102
	24	6	9.6–191	0.9907	3.4	100
	4.2	15	0.12–9.6 (14)[a]	0.9635	6.4	136
	0.62	12	0.12–2.4 (10)[a]	0.9964	3.7	72
	0.12	12	0.12–2.4	0.9964	3.6	116

[a] Number in parenthesis: number of calibration standards in the interval.

Table 3
Stability testing in plasma

Analyte	Spiked concentration (ng/ml) Day 1	Determined concentration (ng/ml) Day 5 ± (R.S.D.)[a] (%)
R-Verapamil	25.0	25.2 ± 1.4
	2.50	2.32 ± 2.6
S-Verapamil	25.0	25.0 ± 1.5
	2.50	2.28 ± 1.9
R-Norverapamil	24.0	25.6 ± 14
	2.40	3.26 ± 11
S-Norverapamil	24.0	26.1 ± 14
	2.40	3.18 ± 9.8

[a] $n = 6$.

of nitrogen at 50 °C. The residue was then reconstituted in 100 ml of the mixture 0.01% aqueous acetic acid/acetonitrile (90/10, v/v).

3. Results and discussion

3.1. Method development

In the previous work by Sandström et al. [18], a mobile phase around pH 7 and acetonitrile as the organic modifier, were found to be optimal for the simultaneous chiral separation of verapamil and norverapamil using the Chiral-AGP® column and UV detection. This optimised separation method could however not be directly transferred to electrospray mass spectrometry (ESI-MS), as the mobile phase contained the poorly volatile phosphate buffer. The initial experiments in this study, were thus performed with UV detection, investigating the possibility of using the more ESI compatible ammonium acetate as the buffer component in the mobile phase (Table 1A, Fig. 2A–D). Although its buffer capacity was low at the tested pH, ammonium acetate proved to be a sufficiently good substitute for phosphate buffer concerning the chiral separation of both compounds. However, the second eluted enantiomer of verapamil still interfered with the first enantiomer of norverapamil. When coupling the system to MS detection, verapamil could be separately detected from norverapamil due to differing molecular masses, although there was a chromatographic co-elution (Fig. 2E). This feature provides a radical simplification compared to the earlier published methods [6,10–17], as no achiral preseparation or chemical derivatisation was necessary. The chiral resolution for both compounds decreased slightly using MS and was just on the limit of complete enantioseparation (Rs = 1.5) for *rac*-norverapamil (Table 1B). For that reason, the pH was raised slightly to 7.4, which gave

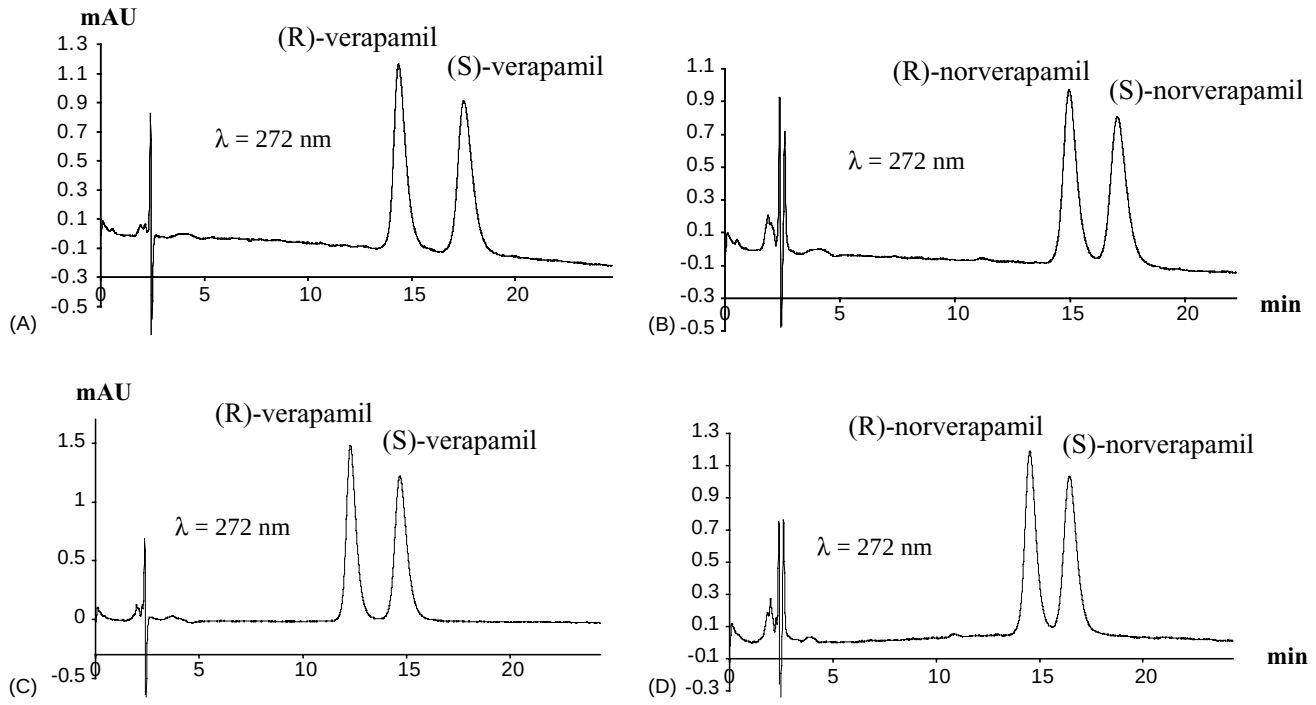

Fig. 2. Chiral separation of verapamil and norverapamil standards using UV absorbance detection at 272 nm. (A) *Rac*-verapamil (10^{-5} M): mobile phase: 15% acetonitrile + 85% aqueous phosphate buffer at pH 7.0. (B) *Rac*-norverapamil (10^{-5} M): mobile phase: 15% acetonitrile + 85% aqueous phosphate buffer at pH 7.0. (C) *Rac*-verapamil (10^{-5} M): mobile phase: 15% acetonitrile + 85% aqueous ammonium acetate buffer (20 mM) at pH 7.0. (D) *Rac*-norverapamil (10^{-5} M): mobile phase: 15% acetonitrile + 85% aqueous ammonium acetate buffer (20 mM) at pH 7.0. (E) Mixture of *rac*-verapamil (10^{-5} M) and *rac*-norverapamil (10^{-6} M) injected into the LC–MS system. Mobile phase: 15% acetonitrile + 85% aqueous ammonium acetate buffer (20 mM) at pH 7.0. Extracted ion chromatograms (MS scan mode) of *m/z* 455 (verapamil $[M + H]^+$) and *m/z* 441 (norverapamil $[M + H]^+$).

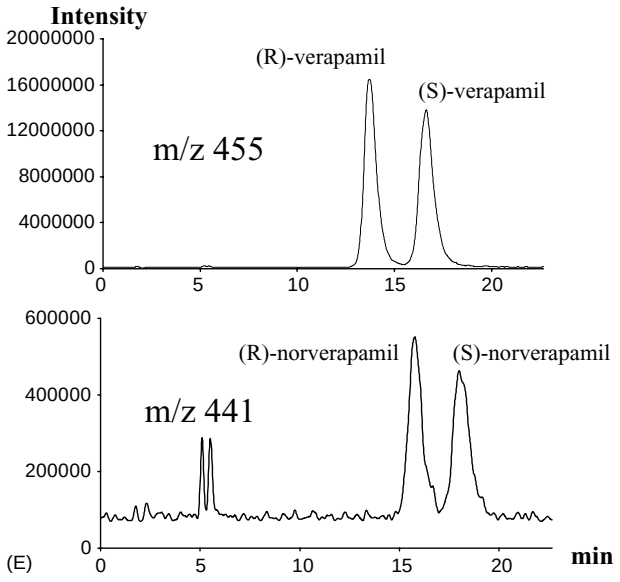

Fig. 2. (*Continued*).

an increase in Rs (Table 1B). All the subsequent plasma analyses were thus run with the mobile phase composition 85% ammonium acetate buffer (20 mM) pH 7.4 and 15% acetonitrile.

One problem when coupling protein-based columns to ESI-MS, is that the interface usually requires a high concentration of organic modifier to obtain a good spray. The protein-based stationary phases, however, works best in an aqueous environment and can only tolerate moderate amounts of organics [19,20]. As mentioned above in the present work, a mobile phase of 85% aqueous ammonium acetate buffer (20 mM) and only 15% acetonitrile had to be used to maintain chiral resolution for verapamil and norverapamil. In order to reduce the liquid flow into the electrospray interface, an attempt was made to use a column with a shorter inner-diameter (2.0 compared to 4.0 mm). However, the efficiency and thus the chiral resolution was too low using the narrower column, possibly due to greater influence of the extra column band broadening in the interface (results not shown). The wider column (4.0 mm inner-diamater) was thus used for the subsequent experiments. The use of a high desolvation gas flow-rate (1000 l/h) was necessary in order to avoid condensation of water in the atmospheric pressure region of the electrospray interface.

In order to obtain the highest possible detection sensitivity, SRM mode was chosen for data collection. Full scan daughter ion spectra were thus collected for verapamil, norverapamil and D_3-verapamil to select a fragment ion to monitor. The spectra of both analytes and the internal standard had their most intense peak at *m*/*z* 165, which was chosen for SRM, Fig. 3. These fragments were probably a result of cleavage of one of the nitrogen–carbon bonds (cf. Fig. 1).

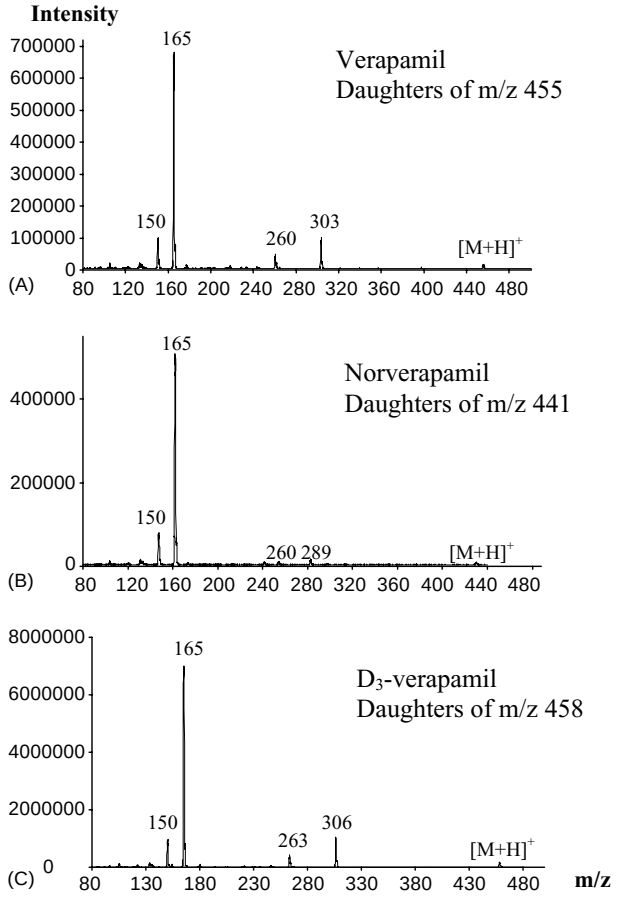

Fig. 3. Daughter ion spectra of (A) verapamil, (B) norverapamil and (C) D_3-verapamil (internal standard) acquired during constant infusion at 30 eV collision energy.

3.2. Validation of the quantitative method

D_3-verapamil was used as internal standard for both verapamil and its metabolite, as no deuterated norverapamil was available. The possible occurrence of endogenous compounds or isotopic impurities in the internal standard that could interfere with the data collection, was investigated. In Fig. 4, a chromatogram obtained from an extract of blank plasma spiked with only the internal standard is shown. Obviously, there were no significant peaks in the SRM channels of any of the analytes.

The selectivity of the method towards the metabolites D617, D620 and PR23 was also tested (for structures see Fig. 1B). The N-dealkylated D617 and 620 could be separated chromatographically from the enantiomers of verapamil and norverapamil (chromatogram not shown). Furthermore, these metabolites have lower molecular weights than the analytes and would thus not be detected in the present quantitative SRM method for verapamil and norverapamil (Fig. 5). The O-dealkylated PR23 has the same molecular weight as norverapamil and might thus be a possible interferent. However, the retention of the enantiomers of PR23 was slightly higher than the corresponding enantiomers of

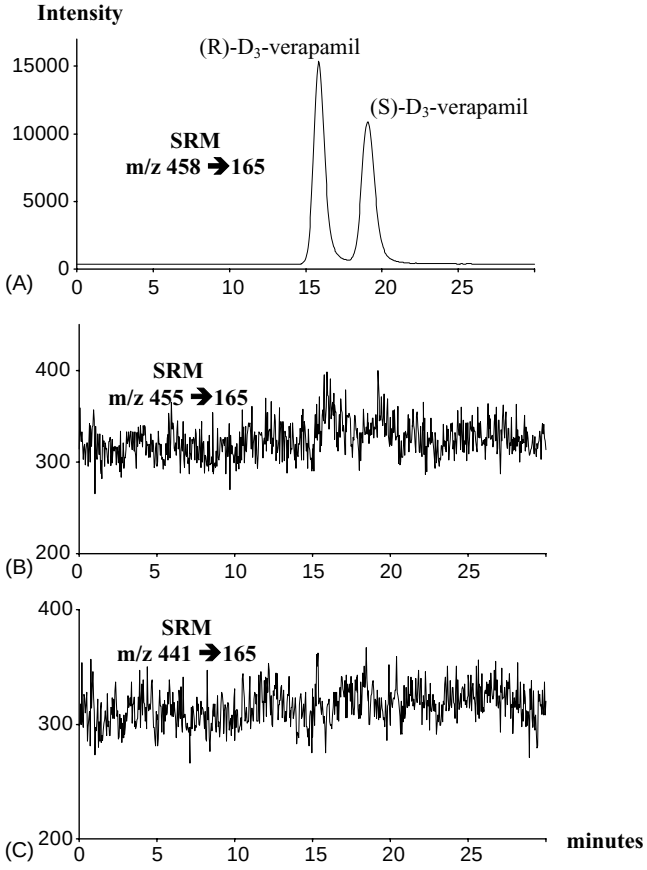

Fig. 4. LC–MSMS chromatogram of an extract from blank plasma spiked with internal standard only. SRM channels: (A) D_3-verapamil; (B) verapamil; (C) norverapamil.

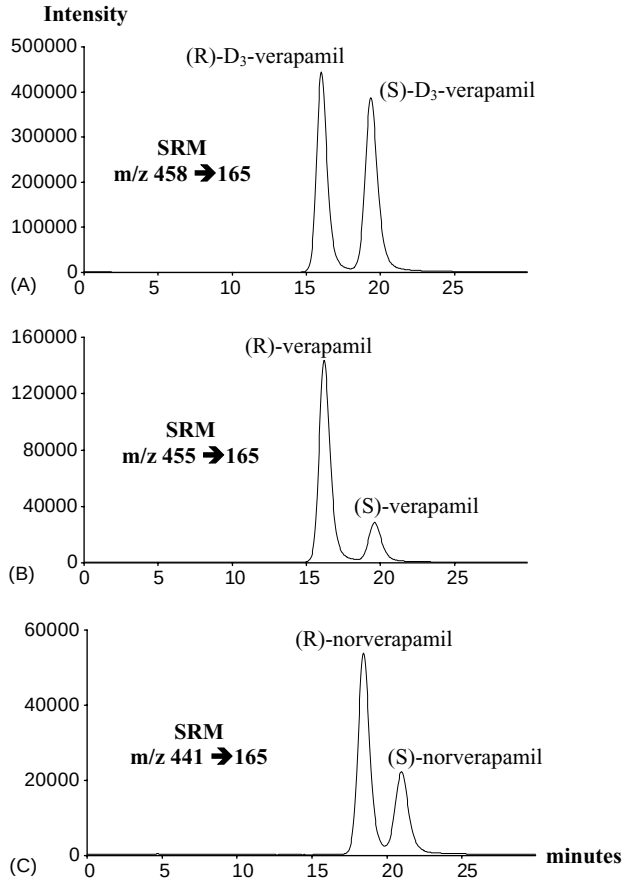

Fig. 5. LC–MSMS chromatogram of a typical patient sample. SRM channels: (A) D_3-verapamil; (B) verapamil; (C) norverapamil.

norverapamil. Only D617 could be detected when SRM channels for these three metabolites were set up in order to evaluate their detectability in patient plasma after administration of verapamil. The failure to detect PR23 could be due to its low extraction recovery at high pH caused by its phenolic properties or its high degree of conjugation [5].

The possible suppression of ionisation that might be caused by the co-elution of (*R*)-norverapamil, (*S*)-verapamil and (*S*)-D_3-verapamil, was studied. The ratio between the peak areas of the enantiomers of the racemic internal standard at a constant concentration [$area_{(S)}/area_{(R)}$] was plotted as a function of increasing (*R*)-norverapamil concentration in spiked blank plasma, Fig. 6. This ratio only varied within ±2% from the theoretical value of one and there was no visible trend at increasing (*R*)-norverapamil concentration. Any significant suppression of ionisation by the co-elution of (*S*)-D_3-verapamil and (*R*)-norverapamil would have resulted in a decrease in the $area_{(S)}/area_{(R)}$ ratio at increasing concentration of (*R*)-norverapamil.

The results from the validation of the quantitative method are presented in Table 2. The standard curves were linear with $r^2 > 0.99$ in all intervals except one. The enantiomers of verapamil could be quantified with acceptable precision and accuracy at least down to a level of 50 pg/sample (110 fmol/sample or 100 pg/ml plasma). As expected, D_3-verapamil worked better as internal standard for verapamil than for norverapamil. Thus, the accuracies were not as good for some of the lower validation levels of norverapamil. However, at 60 pg/sample (140 fmol/sample or 120 pg/ml plasma), both the accuracy and precision were acceptable for both enantiomers. This means that the method described in this paper was more sensitive than the earlier published ones using chiral LC with fluorescence detection for the determination of the enantiomers of verapamil (earlier published LOQ: 2 ng/ml [11], 2.5 ng/ml [6], 3 ng/ml [13], 10 ng/ml [9], 20 ng/ml [12], 25 ng/ml [10]) and/or norverapamil (earlier published LOQ: 2 ng/ml [13], 5 ng/ml [6], 10 ng/ml [9], 30 ng/ml [10], 50 ng/ml [12]) in human plasma. In a previous chiral LC–off line GC–MS study, the LOQ for the enantiomers of verapamil was 0.25 ng/ml [28]. The present method was simpler to perform, as neither a preseparation nor chemical derivatisation was necessary, as the tandem mass spectrometric detection offered a greater deal of selectivity compared to fluorescence or UV absorbance.

The stability testing of verapamil and norverapamil in plasma at room temperature demonstrated that no substantial amount of the analytes were degraded at these conditions for 4 days (Table 3).

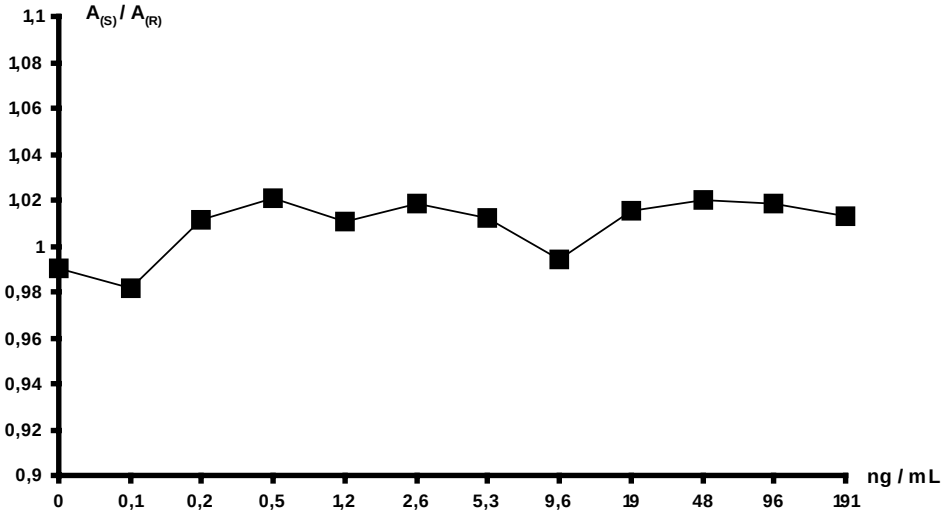

Fig. 6. Peak area ratio of racemic D_3-verapamil $[A_{(S)}/A_{(R)}]$ at a constant concentration (60 ng/ml plasma) as a function of increasing (*R*)-norverapamil concentration.

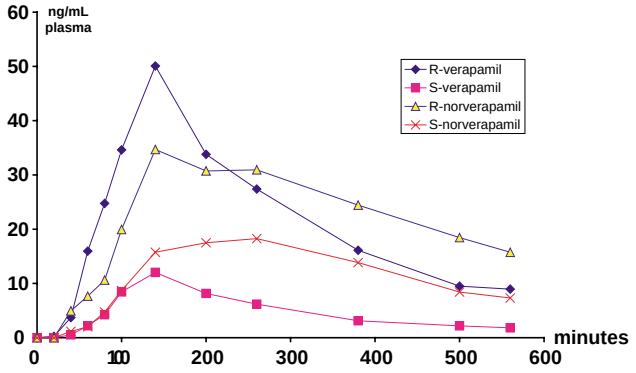

Fig. 7. Example of plasma profiles of the enantiomers of verapamil and norverapamil.

3.3. Bioanalytical application

A typical plasma profile of the enantiomers of verapamil and norverapamil during the single-pass perfusion of the jejunum using the Loc-I-Gut® device, is shown in Fig. 7. The plasma concentration-time profiles also confirm that (*S*)-verapamil is significantly more rapidly metabolised than (*R*)-verapamil. It is also interesting to see that the main metabolite norverapamil seems to follow the same pattern, despite more (*S*)-norverapamil was formed it has a lower AUC than (*R*)-norverapamil. This was most likely due to a significantly higher degree of metabolism of (*S*)-norverapamil as well.

4. Conclusions

A new stereoselective LC–MS/MS method has been developed and validated for the simultaneous quantification of the enantiomers of verapamil and its active metabolite norverapamil in human plasma. A higher sensitivity was obtained compared to earlier published methods for the same purpose. Furthermore, the use of mass spectrometric detection provided a substantial simplification in method operation, as detection selectivity could be obtained between verapamil and norverapamil without achiral preseparation or chemical derivatisation. Both verapamil and norverapamil proved to be stable in human plasma at room temperature for at least 4 days. The method was successfully applied to a biopharmaceutical in vivo absorption study, where the plasma levels of the separate enantiomers of verapamil and norverapamil could be determined as a function of time, after single-pass perfusion of the jejunum using the Loc-I-Gut® device.

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