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Short communication

Rapid determination of valsartan in human plasma by protein precipitation and high-performance liquid chromatography

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Abstract

A high-performance liquid chromatographic (HPLC) method for the determination of valsartan in human plasma is reported. The assay is based on protein precipitation with methanol and reversed-phase chromatography with fluorimetric detection. The preparation of a batch of 24 samples takes 20 min. The liquid chromatography was performed on an octadecylsilica column (50 mm × 4 mm, 5 μm particles), the mobile phase consisted of acetonitrile −15 mM dihydrogenpotassium phosphate, pH 2.0 (45:55, v/v). The run time was 2.8 min. The fluorimetric detector was operated at 234/374 nm (excitation/emission wavelength). The limit of quantitation was 98 ng/ml using 0.2 ml of plasma. Within-day and between-day precision expressed by relative standard deviation was less than 5% and inaccuracy did not exceed 8%. The assay was applied to the analysis of samples from a pharmacokinetic study.

Keywords: Valsartan; Protein precipitation; Pharmacokinetics

1. Introduction

Valsartan (Fig. 1) is an orally active specific angiotensin II type 1 receptor blocker effective in lowering blood pressure in hypertensive patients [1]. Plasma levels peak 2 h after oral administration and then decline with a terminal half-life reported in various studies in the range 3–7 h [2–4]. The maximum plasma concentrations after single oral dose of valsartan (160 mg) reach 2–4 μg/ml. The drug is only minimally metabolized, valsartan is excreted largely (about 80%) as unchanged compound [5].

Several high-performance liquid chromatographic (HPLC) methods are available for determination of valsartan in plasma. All published assays employ native fluorescence of valsartan and use fluorimetric detection [2–4,6–9]. The sample preparation involves either liquid–liquid extraction with methyl-*tert*-butyl ether [3] and ethyl acetate [4] or solid-phase extraction using C_8 [6,7,9] and cyclohexyl [2,8] cartridges. The limit of quantitation in these procedures is 5–130 ng/ml, run times are typically 10–30 min.

The aim of this study was to develop a rapid HPLC method for valsartan determination in human plasma. The overall speed of analysis can be improved by the elimination of tedious extraction steps and optimization of chromatographic conditions. From the pharmacokinetic characteristics of valsartan it is evident that a limit of quantitation about 100 ng/ml is sufficient for pharmacokinetic studies with 160 and 320 mg capsules or tablets.

2. Experimental

2.1. Chemicals

Acetonitrile (for liquid chromatography) was Sigma–Aldrich (Prague, Czech Republic) product and methanol (for chromatography) was manufactured by Merck (Darmstadt, Germany). All other reagents and chemicals were of analytical grade. *o*-Phosphoric acid and potassium dihydrogenphosphate were manufactured by Fluka (Buchs, Switzerland). Valsartan was obtained from a local supplier, its purity was 99.87%.

2.2. Apparatus and conditions

The HPLC system consisted of the P1000 pump, AS3000 automatic sample injector, FL 2000 fluorimetric detector and

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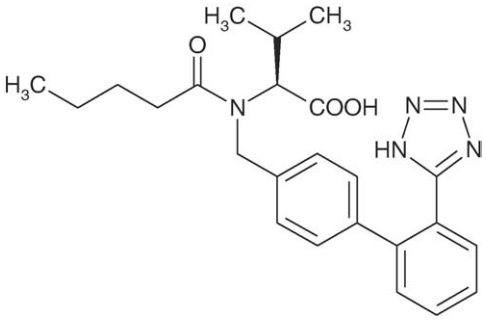

Fig. 1. Chemical structure of valsartan.

data station with PC1000 software, version 2.5 (Thermo Separation Products, Riviera Beach, FL, USA). The separation was performed on a Nucleosil C_{18} 120-5 50 mm × 4 mm column (Watrex, Prague, Czech Republic) protected with a C_{18} 4 mm × 3 mm I.D. precolumn (Phenomenex, Torrance, CA, USA); the temperature of the column oven was 40 °C.

The mobile phase consisted of acetonitrile −15 mM potassium dihydrogenphosphate, pH 2.0 adjusted with *o*-phosphoric acid (45:55, v/v), the flow-rate was 1 ml/min. The excitation and emission wavelengths were 234 and 374 nm, respectively and the time constant of the detector was set to 2 s.

2.3. Standards

Stock standard solutions of valsartan were made by dissolving of approximately 20 mg of accurately weighed substance in 25 ml of methanol. Separate solutions were prepared for the calibration curve samples and quality control ones. Further standard solutions were obtained by serial dilutions of stock solutions with methanol. The standard solutions were stored at −18 °C and were protected from light; they were stable for at least 6 weeks under these conditions.

The calibration and quality control plasma samples were prepared by addition of standard solutions to drug-free plasma in volumes not exceeding 2% of the plasma volume.

2.4. Preparation of the sample

The samples were stored in the freezer at −18 °C and allowed to thaw at room temperature before processing. Methanol (1 ml) was added to 0.2 ml of plasma, the tube was vortex-mixed for 30 s at 2000 rpm. The tube was centrifuged 3 min at 2500 × *g* and 500 μl of the supernatant was transferred to an autosampler vial. Five microlitres were injected into the chromatographic system. A batch of 24 samples can be prepared in 20 min.

2.5. Calibration curves

The calibration curve was constructed in the range 97.9–10,200 ng/ml to encompass the expected concentrations in measured samples. The calibration curves were obtained by weighted linear regression (weighing factor $1/x^2$): the valsartan peak area was plotted vs. the valsartan concentration in ng/ml. The suitability of the calibration model was confirmed by back-calculating the concentrations of the calibration standards.

3. Results and discussion

3.1. Sample preparation

Valsartan is a strongly fluorescent compound, under chromatographic conditions described in this paper the limit of detection is about 25 pg (injection volume 5 μl, signal-to-noise ratio 3:1). This response enabled us to reach the desired limit of quantitation 100 ng/ml using only protein precipitation as the sample preparation technique. Methanol was used as precipitation agent, because high concentration of acetonitrile in the sample would cause a distortion of valsartan peak due to higher eluting strength of this solvent. The recovery of valsartan was nearly 100%.

Acidic pH of the mobile phase must be used, because pH > 4 reduces significantly the intrinsic fluorescence of valsartan and other angiotensin II receptor antagonists [7]. Due to the simplicity of sample handling no internal standard was necessary which further speeds up the chromatographic analysis.

3.2. Chromatography

Typical chromatograms of drug-free plasma (a); spiked plasma at limit of quantitation (b) and plasma from a volunteer 16 h after the oral ingestion of 160 mg of valsartan (c) are shown in Fig. 2. The method selectivity was demonstrated on six blank plasma samples obtained from healthy volun-

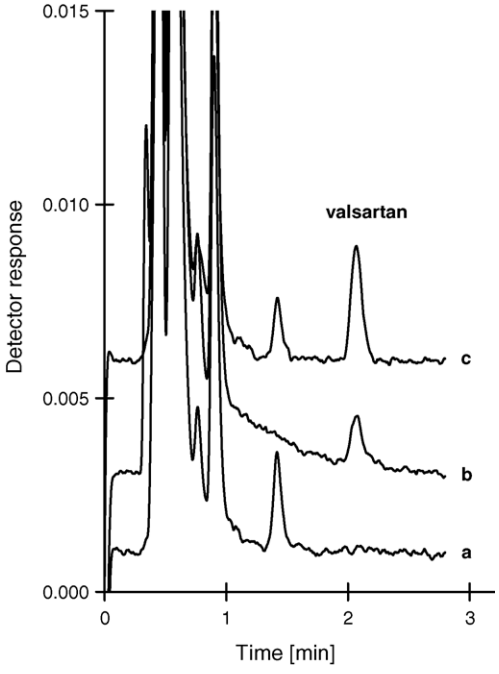

Fig. 2. Chromatograms of (a) a drug-free human plasma; (b) spiked plasma at limit of quantitation (97.9 ng/ml) and (c) a plasma sample from a volunteer 16 h after administration of 160 mg valsartan, the measured concentration of valsartan was 264.9 ng/ml.

teers: the chromatograms were found to be free of interfering peaks.

We aimed at optimizing the sensitivity and speed of the chromatography and therefore column dimensions 50 mm × 4 mm were selected. The benefit of this column is not only the increased sensitivity due to small peak volume, but also the possibility to obtain relatively large values of the capacity factor (4–6) in a short time. The retention times of valsartan was 2.05 min at a flow-rate 1.0 ml/min and the whole analysis was completed within 2.8 min. This is much faster than in the other published methods. The typical column efficiency expressed as the number of theoretical plates was about 2200 for valsartan. Acetonitrile should be selected as an organic modifier, because lower column efficiency was observed using methanol. More than 1000 samples were analyzed on the same column without any deterioration of its performance.

3.3. Linearity and limit of quantitation

The calibration curves were linear in the studied range. The calibration curve equation is $y = bx + c$, where y represents the area of valsartan peak and x represents concentration of valsartan in ng/ml. The mean equation (curve coefficients ± standard deviation) of the calibration curve ($N = 6$) obtained from six points was $y = 83.2\ (\pm 1.5)x - 205(\pm 572)$ (correlation coefficient $r = 0.9999$).

The limit of quantitation was 97.9 ng/ml ($N = 6$). At this concentration, the signal-to-noise ratio is approximately 10:1. The precision, characterised by the relative standard deviation, was 4.5% and accuracy, defined as the deviation between the true and the measured value expressed in percents, was −0.9% at this concentration ($N = 6$).

3.3.1. Intra-assay precision

Intra-assay precision of the method is illustrated in Table 1. It was estimated by assaying the quality control samples (low, medium and high concentration) six times in the same analytical run. The precision was better than 3% and the bias did not exceed 8% at all levels.

3.3.2. Inter-assay precision and accuracy

Inter-assay precision and accuracy was evaluated by processing a set of calibration and quality control samples (three levels analyzed twice, results averaged for statistical evaluation) on six separate runs. The samples were prepared in advance and stored at −18 °C. The respective data are given in Table 2. The precision was better than 5% and the inaccuracy did not exceed 2% at all levels.

Table 1
Intra-assay precision and accuracy

N	Concentration (ng/ml)			
	Added	Measured	Bias (%)	R.S.D. (%)
6	179.2	193.2	7.2	1.7
6	1065	1099	3.1	1.1
6	8891	9058	1.8	3.0

Table 2
Inter-assay precision and accuracy

N	Concentration (ng/ml)			
	Added	Measured	Bias (%)	R.S.D. (%)
6	179.2	179.3	0.1	4.9
6	1065	1080	1.4	1.3
6	8891	8819	−0.8	1.6

Table 3
Freeze and thaw stability

	N	Concentration (ng/ml)			
		357.8		8891	
		Measured	Bias (%)	Measured	Bias (%)
Cycle 1	3	366.6	2.5	8688	−2.3
Cycle 2	3	369.0	3.1	8464	−4.8
Cycle 3	3	363.9	1.7	8732	−1.8

3.3.3. Stability study

3.3.3.1. Freeze and thaw stability. Stock solutions of a low and high concentration were prepared. The solutions were stored at −18 °C and subjected for 3 thaw and freeze cycles. During each cycle triplicate 0.2 ml aliquots were processed, analyzed and the results averaged. The results are shown in Table 3. The concentration changes relatively to the nominal concentration are less than 5%, indicating no significant substance loss during repeated thawing and freezing.

3.3.3.2. Processed sample stability. Two sets of spiked samples with a low and a high concentration of valsartan were analyzed and left in the autosampler at ambient temperature. The samples were analyzed using a freshly prepared calibration samples 4 days later. The results are presented in Table 4. The processed samples are stable at room temperature for 4 days.

3.3.3.3. Stability of plasma samples. Two sets of plasma samples (with a low and high concentration) were stored in the freezer at −18 °C for 6 weeks. The samples were then analyzed using freshly prepared calibration samples. The results are presented in Table 5. The samples are stable at −18 °C for at least 6 weeks.

The stability of thawed plasma samples (with a low and high concentration) was studied for 24-h period at room temperature. The samples are stable under studied conditions (see Table 5 for results).

Table 4
Stability of processed samples

Sample	*C* (ng/ml)	*N*	*C* found (ng/ml)	R.S.D. (%)	Bias (%)
New	179.2	6	193.2	1.7	7.8
4-day-old	179.2	6	166.7	4.4	−7.0
New	8891	6	9058	3.0	1.9
4-day-old	8891	6	9194	2.4	3.4

Table 5
Stability of plasma samples

C (ng/ml)	Storage conditions	*N*	*C* found (ng/ml)	R.S.D. (%)	Bias (%)
179.2	24 h/20 °C	3	190.9	5.6	6.5
8891	24 h/20 °C	3	8824	0.4	−0.8
357.8	6 weeks/−18 °C	6	358.3	3.5	0.1
8891	6 weeks/−18 °C	6	9269	1.7	4.3

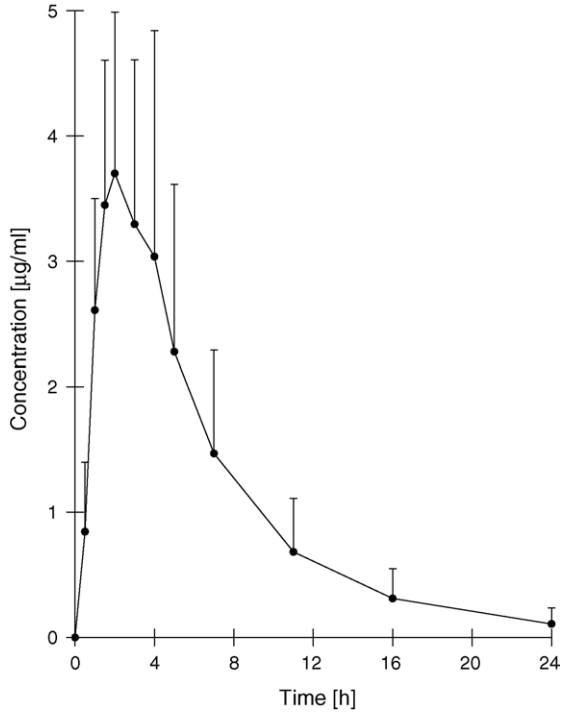

Fig. 3. Mean plasma concentrations (+S.D.) of valsartan after a single 160 mg oral dose of the drug to 26 healthy subjects.

3.4. Application to biological samples

The proposed method was applied to the determination of valsartan in plasma samples from a pharmacokinetic study, which was approved by the local ethics committee. The plasma samples were collected up to 24 h after a single oral dose of 160 mg valsartan (Diovan® 160 coated tablets, Novartis) to 26 healthy male volunteers: mean age of the group was 29 years (range 20–43), mean weight was 81 kg (range 61–97). Fig. 3 shows the mean plasma concentrations of valsartan. The plasma levels reached their maximum 2.5 h after the administration and thereafter the plasma level declined with an elimination half-time of 4.2 h. These values agree with previously published reports on the pharmacokinetics of valsartan [1–4]. The mean area under concentration–time curve (AUC) measured from 0 to the last non-zero sampling point was 96% of the value of AUC extrapolated from 0 to infinity. In all subjects, this value was higher than 90% which indicates a suitability of the analytical method for pharmacokinetic studies.

4. Conclusions

The validated method allows determination of valsartan in the 98–10200 ng/ml range. The assay is rapid, the analysis time is only 2.8 min. The preparation of a batch of 24 samples takes 20 min. The precision and accuracy of the method are well within the limits required for bioanalytical assays. The limit of quantification 98 ng/ml permits the use of the method for pharmacokinetic studies.

References

[1] A. Markham, K.L. Goa, Drugs 54 (1997) 299.
[2] G. Flesch, P. Müller, P. Lloyd, Eur. J. Clin. Pharmacol. 52 (1997) 115.
[3] N. Daneshtalab, R.Z. Lewanczuk, F. Jamali, J. Chromatogr. B 766 (2002) 345.
[4] E.K. Schmidt, K.-H. Antonin, G. Flesch, A. Racine-Poon, Eur. J. Clin. Pharmacol. 53 (1998) 451.
[5] F. Waldmeier, G. Flesch, P. Müller, T. Winkler, H.P. Kriemler, P. Bühlmayer, M. De Gasparo, Xenobiotica 27 (1997) 59.
[6] A. Sioufi, F. Marfil, J. Godbillon, J. Liq. Chromatogr. 17 (1994) 2179.
[7] L. González, J.A. López, R.M. Alonso, R.M. Jiménez, J. Chromatogr. A 949 (2002) 49.
[8] L.A. Brunner, M.L. Powell, P. Degen, Lab. Robot. Automat. 6 (1994) 171.
[9] R. Séchaud, P. Graf, H. Bigler, E. Gruendi, M. Letzkus, M. Merz, Int. J. Clin. Pharmacol. Ther. 40 (2002) 35.