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Journal of Chromatography B, 766 (2002) 345–349

JOURNAL OF CHROMATOGRAPHY B

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Short communication

High-performance liquid chromatographic analysis of angiotensin II receptor antagonist valsartan using a liquid extraction method

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Received 2 July 2001; received in revised form 15 October 2001; accepted 15 October 2001

Abstract

Purpose: To develop an easy assay for the quantitation of the angiotensin II receptor antagonist valsartan in human plasma using a liquid extraction procedure. *Method:* The method involves acid extraction from 1 ml human plasma with methyl-*tert*.-butyl ether followed by back-extraction into a basic medium. An isocratic HPLC equipped with reverse phase column and a fluorescence detector was used at room temperature. *Results:* The response to 10–2000 ng/ml valsartan was linear. In plasma of three human subjects given 160 mg valsartan orally, concentrations of 25–1540 ng/ml were observed. *Conclusion:* This convenient method is suitable for pharmacokinetic studies of valsartan. © 2002 Published by Elsevier Science B.V.

Keywords: Valsartan

1. Introduction

Valsartan ((*S*)-*N*-valeryl-*N*-[2′-(1*H*-tetrazol-5-yl) biphenyl-4-yl)-methyl]-valine; Fig. 1) is an orally active specific angiotensin II receptor antagonist used as a hypotensive drug [1–3]. A gas chromatographic, mass spectroscopic [4], and a number of high-performance liquid chromatographic (HPLC) methods are available for separation and quantitation of valsartan from biological fluids [5–7]. The available

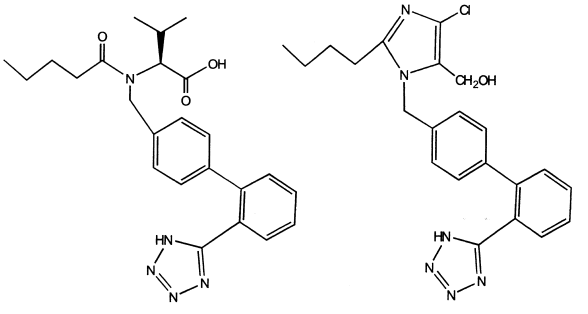

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Fig. 1. Structures of valsartan (CGP 48933, I) and the internal standard, losartan (DuP 753, 11).

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1570-0232/02/$ – see front matter
PII: S0378-4347(01)00507-2

HPLC assays require relatively lengthy sample preparation involving solid-phase extraction, and the use of commercially unavailable internal standards [5–7]. We report an easy valsartan assay using a liquid extraction method and a commercially available internal standard.

2. Experimental methods

2.1. Chemicals

Valsartan was obtained as a gift from Novartis Pharma (Basel, Switzerland). Losartan (DUP 532), the internal standard, was a gift from Merck-Frosst Pharmaceuticals (Rahway, NJ, USA). Sodium hydroxide, potassium dihydrogen orthophosphate (minimum assay 99%) and orthophosphoric acid (minimum assay 85%) were all purchased from BDH Chemicals Canada (Edmonton, Canada). HPLC grade methyl-*tert*.-butyl ether (MTBE) and acetonitrile were obtained from Fisher Scientific (Edmonton, Canada).

2.2. Apparatus and chromatographic conditions

The HPLC apparatus consisted of a model 590 pump, a 712 Wisp autosampler, and a scanning fluorescence detector model 470 (Waters, Millipore, Mississauga, Canada). The excitation and emission wavelengths were set at 265 and 378 nm, respectively. The apparatus also included a model 3390 A recorder integrator (Hewlett-Packard, Palo Alto, CA, USA). A pre-packed ODS 10 cm×4.6 mm I.D. C_{18}analytical column packed with 5-μm particles (Phenomenex, Torrance, CA, USA) attached to a NovaPak C_8 Guard-Pak HPLC Precolumn insert (Waters, Millipore, Mississauga, Canada) was used. The columns were operated at ambient temperature. The mobile phase consisted of 70% pH 2.8 phosphate buffer and 30% acetonitrile, which was pumped at a flow-rate of 1.3 ml/min. The phosphate buffer for the mobile phase was prepared by dissolving 1.36 g of potassium dihydrogen phosphate in 1 l of distilled water. The final pH was adjusted to 2.8 with ~17 drops of 85% orthophosphoric acid.

2.3. Standard solutions

A stock standard solution of valsartan (50 μg/ml) was prepared by dissolving the drug in a solution of 0.1 *M* KOH, and adjusting the pH to 8 with 1 *M* solution of HCl. A stock solution of the internal standard (2 μg/ml) was prepared in 70:30 (v/v) methanol-deionized water. Both stock solutions were stored at 4°C. The same solvents were used to make further dilutions in order to prepare working standard solutions of valsartan (0.25, 0.5, 1, 5, and 10 μg/ml) and losartan (5 μg/ml).

2.4. Sample preparation

Concentrations of 10, 70, 100, 500, 1000 and 2000 ng/ml of valsartan were prepared in flint glass disposable culture tubes (Fisher Scientific, Edmonton, Canada) using varying volumes of the working stock solutions of the drug. A 100-μl aliquot of the 5-μg/ml internal standard and 1 ml of blank human plasma were added. Solutions were then acidified to pH 2.5 with 125 μl of 1 *M* phosphoric acid. To the analytes were added 10 ml of MTBE. The solutions were vortex-mixed for 3 min and centrifuged at 1800 *g* for 5 min. The organic solvent was transferred to clean tubes containing 200 μl of 0.05 *M* NaOH (pH>10). The analytes were back-extracted into this aqueous layer by 2-min vortex mixing and centrifugation for 5 min at 1800 *g*. The aqueous layer was frozen by immersing the tubes in a dry ice-acetone bath. The organic layer was discarded and the aqueous layer thawed and neutralized with 75 μl of 0.2 *M* phosphoric acid. Aliquots of 125 μl were injected into the HPLC.

2.5. Quantification

Area under the HPLC response peak was recorded for the analytes and the drug/internal standard peak ratios were plotted versus concentrations. Linear regression was used to estimate the best fit.

2.6. Extraction efficiency, accuracy, and precision

To calculate extraction efficiency, solutions for various valsartan concentrations were made using valsartan stock concentrations of 0.25, 1, and 10

μg/ml and 1 ml blank human plasma ($n=3$) as described above. The samples were then extracted as described above and aliquots of 125 μl were injected into the HPLC. The peak areas of samples were compared with that obtained after direct injection of unextracted valsartan solutions.

To determine the variability of the intra-day and inter-day extraction, aliquots of blank plasma were spiked with valsartan and the internal standard to make final concentrations of 10, 100, 500, 1000, 1500, and 2000 ng/ml. The samples were then extracted according to the developed method ($n=6$ for intra-day variability and $n=4$ for inter-day variability). The accuracy and precision for the intra-day and inter-day samples were determined as the % recovery (found concentration/given concentration× 100%) and the % coefficient of variation (C.V.), respectively.

2.7. Human valsartan plasma concentration

Plasma valsartan concentration was measured in three subjects who volunteered in a clinical study. The study was approved by the University of Alberta Human Ethics Committee, and signed consent forms were obtained. Following an overnight fast, a single 160-mg oral dose of valsartan was administered with 250 ml water and venous blood samples were taken at 0.15, 0.30, 0.45, 1, 2, 3, 4, 5, 8, and 12 h post-dose. Plasma was separated and stored at −70° until analyzed for valsartan. Valsartan is stable for at least 6 months under these conditions.

3. Results and discussion

Valsartan and internal standard, losartan, share structural similarities since both possess biphenyl tetrazol rings (Fig. 1). Since both drugs are weak acids [8], they can be extracted from plasma into a suitable organic solvent at low pH. In addition, losartan is commercially available, is well extracted with MTBE, and under the conditions described here, the resultant peak does not interfere with that of valsartan. MTBE was tested and chosen due to its ability to efficiently extract both valsartan and internal standard from human plasma. To further concentrate and clean the extract, the analytes were back-extracted into basic medium (pH>10) and neutralized before injection to prevent damage to the column. Under the conditions used, peaks representing losartan and valsartan appeared at ~11 and ~26 min following injection into the system, respectively (Fig. 2). Chromatograms of prepared blank human plasma containing as low as 10 ng/ml of valsartan indicate good baseline resolution from endogenous substances. The assay allowed a run time of 30 min.

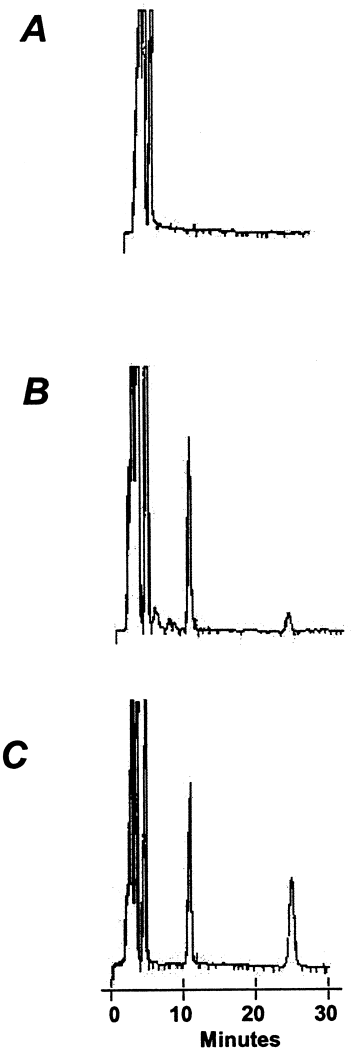

Fig. 2. Examples of chromatograms obtained from an extract of 1 ml blank human plasma (A), a sample plasma spiked with 10 ng/ml of valsartan (B), and a sample from a patient 5 h post-dose (C). Losartan and valsartan peaks are depicted at ~11 and ~26 min after injection into the HPLC system.

An excellent linearity was observed ($r^2>99$) between response (peak-area ratios of the analyte to the internal standard) and concentration within the examined range of 10–2000 ng/ml. Equations describing typical standard curves of the low (10–500 ng/ml) and high ranges (500–2000 ng/ml) were $y=0.0199x-0.0071$ and $y=0.0209x-0.3172$, respectively. The limit of quantitation of the assay was set at 10 ng/ml (C.V.<11%) with a percent accuracy of 96±4% based on 1-ml plasma sample, which is comparable to those previous reported. However, solutions containing as low as 2.5 ng/ml exhibited a signal-to-noise ratio of 5. The average extraction efficiency for concentrations of 10, 100 and 1000 ng/ml valsartan was 69±4.3%. In addition, the observed accuracy and precision (Table 1) were well above the acceptable limit and comparable with other reported methods [6].

The present method is superior to those reported earlier due to its simplicity and convenience. The method of Waldmeier et al. [5] is non-specific since it is based on determination of radioactivity of ^{14}C compounds. The method published by Francotte et al. [7] is a chiral assay developed to separate valsartan from its enantiomers, CGP 49309. The separation was carried out in the absence of an internal standard. It also requires a chiral α1-acid glycoprotein column. The method of Sioufi et al. [6] involved a detailed sample preparation using a liquid–solid extraction procedure. It was also carried out using a commercially unavailable internal standard [6].

Fig. 3 depicts the mean plasma valsartan concentration versus time profile following a single 160-mg dose to three patients over a 12-h post-dose period. The peak plasma concentration (C_{max}) was 1.54±0.45 μg/ml and was achieved within 2 h. The area under the concentration time curve (AUC) was 8.17±0.61 mg/l h and the terminal half life ($t_{1/2}$) was 3.15±0.21 h. The oral clearance for valsartan was determined to be 9.83±0.75 l/h. The drug concentration was well within the minimum detectable concentration at least up to 12 h post-dose. Our calculated pharmacokinetic indices are in agreement with earlier reported values [5,6].

4. Conclusion

The present HPLC assay of valsartan offers advantages over those previously reported in terms of convenience of using liquid–liquid extraction and the use of a commercially available internal standard, losartan. The use of losartan as the internal standard indicates that the assay may be suitable for determination of the latter as well. The method has sufficient sensitivity for pharmacokinetic studies following single oral doses of 160 mg valsartan.

Acknowledgements

This work was supported by a grant from Novartis Pharma, Basel, Switzerland and grant # 983587 from the Canadian Institute of Health Research.

Table 1
Accuracy (% error) and precision (coefficient of variation; % C.V.) of the valsartan spiked solutions

Added	Inter-day variability ($n=4$)			Intra-day variability ($n=6$)		
	Concentration (ng/ml)		C.V. (%)	Concentration (ng/ml)		C.V. (%)
	Observed	% Error		Observed	% Error	
10	9.9	99.3±10.8	10.8	10.0	99.8±8.1	8.9
100	96.5	96.5±6.8	7.1	95.3	95.3±7.3	8.4
500	465.8	93.2±8.7	9.4	456.1	91.2±7.3	8.8
1000	917.8	91.8±2.4	2.6	883.1	88.3±5.2	6.6
1500	1470.1	98.0±2.2	2.2	1472.7	98.2±1.9	2.1
2000	2012.9	100.6±0.7	0.7	2011.3	100.6±0.9	0.8

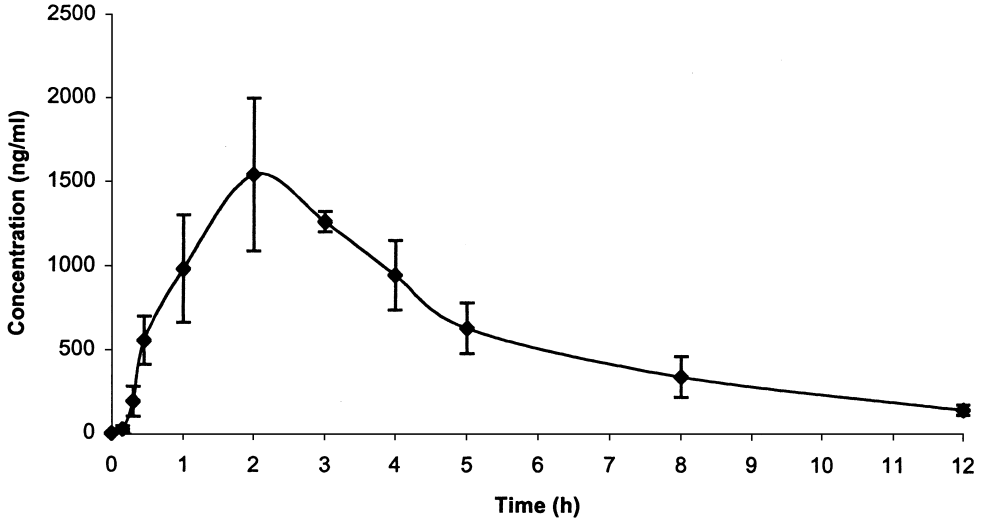

Fig. 3. Plasma concentration–time curve of valsartan following a single 160-mg oral dose of the drug to three subjects. Error bars represent standard deviation.

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