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Short communication

Comparison of UV- and second derivative-spectrophotometric and LC methods for the determination of valsartan in pharmaceutical formulation

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Abstract

UV- and second derivative-spectrophotometric and high-performance liquid chromatographic methods for the determination of valsartan in pharmaceutical formulation have been developed. For the first method, UV-spectrophotometry, standard solutions were measured at 205.6 nm. The linearity changes were found to be 2.0–10.0 μg ml^{-1} in ethanol and the regression equation was $A = 1.05 \times 10^{-1}C + 4.26 \times 10^{-2}$ ($r = 0.9997$). For the second method, the distances between two extremum values (peak-to-peak amplitudes), 221.6 and 231.2 nm were measured in the second order derivative-spectra of standard solutions. Calibration curves were constructed by plotting $d^2A/d\lambda^2$ values against concentrations, 0.5–4.0 μg ml^{-1} of valsartan standards in ethanol. Regression equation of linear calibration graph was calculated as $D = 2.9 \times 10^{-2}C - 2.37 \times 10^{-3}$ ($r = 0.9996$). The third method was based on high-performance liquid chromatography on C18 column using acetonitrile, phosphate buffer as a mobile phase and losartan as internal standard. Detection was carried out at 265 nm with a UV-detector. The assay was linear over the concentration range at 1.0–5.0 μg ml^{-1} and regression equation was found to be $A = 2.74 \times 10^{-1}C + 2.06 \times 10^{-2}$ ($r = 0.9991$). Commercial capsules containing 160 mg valsartan were analysed by the developed methods and the results obtained were compared statistically at 95% confidence level.

Keywords: Valsartan; UV-spectrophotometry; Derivative-spectrophotometry; High-performance liquid chromatography

1. Introduction

The chemical formula of valsartan is *N*-(1-oxopentyl)-*N*-[[2′-(1H-tetrazol-5-yl) [1,1′-biphenyl]-4-yl]methyl]-L-valine (Fig. 1). Valsartan is a potent, highly selective, and orally active antagonist at the angiotensin II AT1-receptor that is used for the treatment of hypertension [1–3]. The pharmacokinetic properties of valsartan have been investigated in healthy volunteers after oral administration of the sample [4].

High performance liquid chromatographic (HPLC) determination of valsartan in biological fluids was studied [5–7] and also a chiral HPLC method was developed [8]. Valsartan and hydrochlorothiazide were determined in tablets simultaneously by HPLC [9,10] and first deriva-

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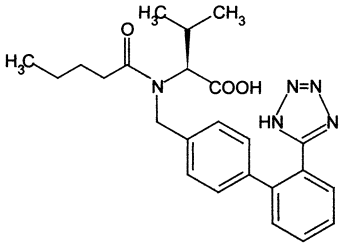

Fig. 1. Chemical structure of valsartan.

tive UV-spectrophotometry [10]. Spectrofluorimetric method was developed for determination of losartan and valsartan in human urine [11].

No UV- and derivative-spectrophotometric and HPLC studies on valsartan, individually in pharmaceutical preparations have been found in the literature.

In this study, UV- and second derivative-spectrophotometric and HPLC methods have been developed for the determination of valsartan and applied to commercial capsules and the results obtained these methods were statistically compared. Because these methods are generally used for the drug assays in pharmaceutical sciences. The proposed methods are recommended for the routine analysis since they are rapid, simple, accurate and also sensitive and specific by no heating and no organic solvent extraction.

2. Experimental

2.1. Materials

Pharmaceutical grade valsartan and losartan potassium, internal standard were generous gifts from Novartis and Merck Sharp and Dohme, respectively. All analytical and HPLC grade chemicals were supplied from Merck (Darmstadt). A commercial preparation, Diovan® capsules (produced by Novartis, Turkey, containing 160 mg valsartan per capsule) was assayed.

2.2. Apparatus

A Shimadzu UV-160 A UV–VIS spectrophotometer with data processing system was used. UV- and derivative-spectra of the solutions were recorded in 1 cm quartz cells at a scan speed of 2400 nm min^{-1}, a scan range of 200–400 nm, fixed slit width of 2 nm and derivation interval ($\Delta\lambda$) 3.5 nm.

A Shimadzu LC10 high performance liquid chromatograph with SPD-10 A spectrophotometric detector and the automation system software was used for the chromatographic analysis of valsartan.

2.3. HPLC conditions

A Shim-pack C18 column (250 × 4.6 mm i.d., 10 μm) (Shimadzu) was used at room temperature. Isocratic elution was carried out at a flow rate 1.3 ml min^{-1} with the mobile phase containing 45% acetonitrile and 55% phosphate buffer solution (pH 2.7). Losartan was used as internal standard and the substances were detected at 265 nm.

2.4. Solutions

For calibration, two series of valsartan solutions containing 2.0, 4.0, 6.0, 8.0, 10.0 and 0.5, 1.0, 2.0, 3.0, 4.0 μg ml^{-1} were prepared by diluting the stock valsartan standard (0.1 mg ml^{-1} in ethanol) with ethanol in volumetric flasks (10 ml) for UV- and derivative-spectrophotometric methods, respectively.

For HPLC analysis, a series of valsartan solutions (1.0, 2.0, 3.0, 4.0 and 5.0 μg ml^{-1}) was also prepared from the stock valsartan solution (0.1 mg ml^{-1} in acetonitrile) and diluted by the mobile phase. These solutions contained losartan as internal standard at 3.0 μg ml^{-1}.

2.5. Assay procedure

A total of ten capsules content were weighed and finely powdered. A portion of the powder, equivalent to about 10 mg valsartan, was weighed accurately and transferred into 100-ml volumetric flask and 50 ml ethanol was added. After ultrasonic vibration for 30 min, the mixture was diluted to volume with ethanol and filtered. For HPLC analysis, acetonitrile was used instead of ethanol.

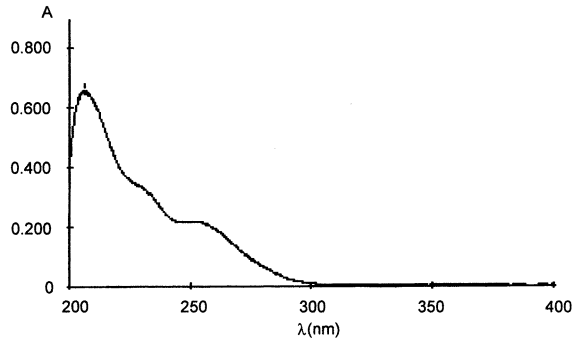

Fig. 2. Absorption spectrum of valsartan (6 μg ml^{-1} in ethanol).

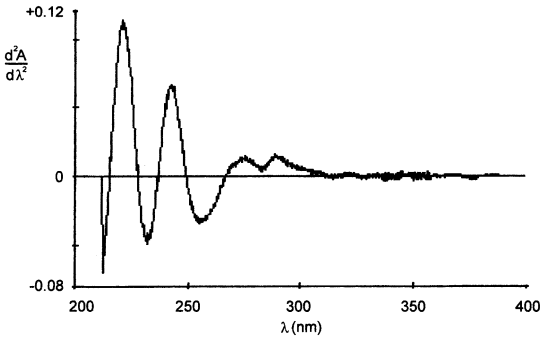

Fig. 3. Second derivative spectrum of valsartan (6 μg ml^{-1} in ethanol).

Appropriate dilutions were made into 6.0 and 2.0 μg ml^{-1} with ethanol from the stock solution for UV- and second derivative-spectrophotometric analysis, respectively. Meanwhile the final concentration was 3.0 μg ml^{-1} for HPLC method, internal standard at 3.0 μg ml^{-1} was added and dilution was made with mobile phase.

Absorption spectra were recorded in the wavelength range 200–400 nm against ethanol as a blank at 205.6 nm. The distances between two extremum values (peak-to-peak amplitudes), 221.6 and 231.2 nm were measured in the second order derivative-spectra of the solutions for the determination of valsartan.

3. Results and discussion

The solubility of valsartan in water is lower than in ethanol and methanol. Valsartan solutions were prepared with ethanol, nontoxic and cheaper instead of methanol and they were stored at 4 °C and stable for a month.

UV absorption spectrum of valsartan showed a maximum absorbance at 205.6 nm (Fig. 2). The linearity ranges were found to be 2.0–10.0 μg ml^{-1} in ethanol. Limit of detection and limit of quantitation were 0.5 and 2 μg ml^{-1}, respectively. The regression equation was $A = 1.05 \times 10^{-1}C + 4.26 \times 10^{-2}$ ($r = 0.9997$). In Table 1, the statistical parameters are given; the regression equations calculated from the calibration graphs, along with the standard deviations of the slope (S_b) and intercept (S_a) on the ordinate.

The optimization of the derivative spectrum was based on the influence of the $\Delta\lambda$ with the object of optimized the relation signal to noise ratio. The second order derivative spectrum of valsartan gave sharper and better-defined peaks when compared with the original UV absorption and other order derivative spectrums and it had the better signal to noise ratio (Fig. 3). Valsartan was determined by using peak-to-peak method be

Table 1
Analytical data for the calibration graphs ($n = 6$) for the determination of valsartan by the proposed methods

Parameters	UV-spectrophotometric method	Second derivative method	HPLC method
Range (μg ml^{-1})	2.0–10.0	0.5–4.0	1.0–5.0
Regression equation (Y)			
Slope (b)	1.05×10^{-1}	2.90×10^{-2}	2.74×10^{-1}
Std. Dev. on slope (S_b)	8.50×10^{-4}	1.50×10^{-2}	1.60×10^{-2}
Intercept (a)	4.26×10^{-2}	-2.37×10^{-3}	2.06×10^{-2}
Std. Dev. on intercept (S_a)	1.05×10^{-1}	6.85×10^{-4}	2.17×10^{-1}
Correlation coefficient (r)	0.9997	0.9996	0.9991

measured the distances between two extremum wavelengths, 221.6 and 231.2 nm. These wavelengths were selected depend on obtained the maximum values. Calibration curves were constructed by plotting $d^2A/d\lambda^2$ values against concentrations (0.5–4.0 μg ml^{-1}) of valsartan standards in ethanols. Limit of detection and limit of quantitation were 0.125 and 0.5 μg ml^{-1}, respectively. Regression equation of linear calibration graph was calculated as $D = 2.9 \times 10^{-2}C - 2.37 \times 10^{-3}$ ($r = 0.9996$).

For HPLC analysis, initially various mobile phase compositions were tried to separate valsartan and losartan as internal standard on C18 column by isocratic system. Then the mixture of 45% acetonitrile and 55% phosphate buffer solution (pH 2.7) was selected as mobile phase for a good separation and the short run time. Detection was at 265 nm and retention times were 6.14 and 4.08 min for valsartan and losartan, respectively (Fig. 4). Limit of detection and limit of quantitation were 0.2 and 1 μg ml^{-1}, respectively. Peak area ratios were plotted against corresponding concentrations 1.0–5.0 μg ml^{-1} and the regression equation was found to be $A = 2.74 \times 10^{-1}C + 2.06 \times 10^{-2}$ ($r = 0.9991$).

Valsartan was determined in capsules by the developed methods and the results obtained are presented in Table 2 where excellent agreement between the three procedures can be observed. According to Table 3 shows the statistical comparison of the results, there is no significant difference between UV-spectrophotometric and HPLC; second derivative-spectrophotometric and HPLC methods since the calculated t- and F-tests did not exceed the theoretical values at the 95% confidence level.

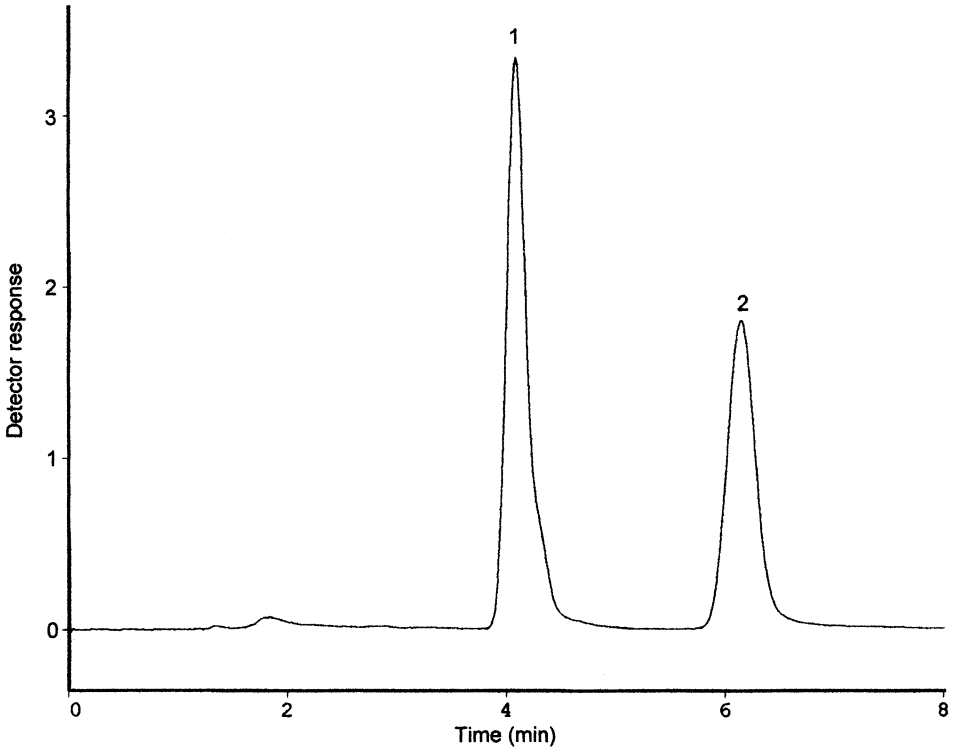

Fig. 4. HPLC chromatogram of losartan (1) and valsartan (2) (3 μg ml^{-1} of 1 and 2 in mobile phase, sample volume is 20 μl).

Table 2
Assay results of capsules containing 160 mg valsartan ($n = 6$)

Statistical value	UV-spectrophotometric method	Second derivative method	HPLC method
$\bar{x}$	159.50	160.10	159.85
S.D.	0.41	0.33	0.38
S.D. (%)	0.26	0.21	0.24
% Recovery	99.69	100.08	99.91

Table 3
Statistical comparison of the results obtained by proposed methods

Methods	t	F
UV-spectrophotometric HPLC	1.49	1.16
Second derivative HPLC	1.18	1.33

$n = 6$; $P = 0.05$; $t = 2.23$; $F = 5.05$.

4. Conclusion

The proposed second derivative-spectrophotometric method assured a better precision and accuracy, and also determining lower concentration of valsartan in capsules than UV-spectrophotometric method. This method was more sensitive than the literature findings in terms of the limit of quantitation and the range of linearity [9–11]. The main advantage of this method is significantly shortening of analyse time, low cost of analyse, and widespread access to apparatus while the HPLC procedure is rather time-consuming for routine assays and require too much solvents and also an expensive apparatus.

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