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QUANTITATIVE ANALYSIS OF VALSARTAN AND HYDROCHLOROTHIAZIDE IN TABLETS BY HIGH PERFORMANCE THIN-LAYER CHROMATOGRAPHY WITH ULTRAVIOLET ABSORPTION DENSITOMETRY

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SUMMARY

A new, simple, accurate, and precise high-performance thin-layer chromatographic (HPTLC) method has been established for simultaneous analysis of valsartan and hydrochlorothiazide in tablet formulations. Standard and sample solutions of valsartan and hydrochlorothiazide were applied to precoated silica gel G 60 F_{254} HPTLC plates and the plates were developed with chloroform–ethyl acetate–acetic acid, 5:5:0.2 (*v*/*v*), as mobile phase. UV detection was performed densitometrically at 248 nm. The retention factors of valsartan and hydrochlorothiazide were 0.27 and 0.56, respectively. The linear range was 800–5600 ng per spot for valsartan and 125–875 ng per spot for hydrochlorothiazide; the correlation coefficients, *r*, were 0.9998 and 0.9988, respectively. The method was validated in accordance with the requirements of ICH guidelines and was shown to be suitable for purpose. The method was successfully used for determination of the drugs in tablets. Tablet excipients did not interfere with the chromatography.

INTRODUCTION

Valsartan, (*S*)-*N*-valeryl-*N*-[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl]valine, is a potent, highly selective, orally active, specific angiotensin II receptor antagonist used as a hypotensive drug. Hydrochlorothiazide, 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide, is a diuretic drug [1–8]. The rationale behind this drug combination is that in treatment of hypertension in patients whose blood pressure is not adequately controlled by monotherapy, oral administration of valsartan with

hydrochlorothiazide has been found more effective than use of either drug alone.

Very few methods for determination of valsartan individually have appeared in the literature. Methods used include HPLC analysis after liquid extraction, and UV, second derivative spectrophotometric, and LC methods have been compared [9–12]. There are several reports of the determination of hydrochlorothiazide individually or in combination with other drugs, including use of HPLC, HPTLC, spectrophotometry, and non-aqueous potentiometric titration [13–42]. A literature survey has revealed there is no HPTLC method for analysis of valsartan and hydrochlorothiazide in pharmaceutical preparations. The purpose of this research was to establish such a method and, after validation in accordance with International Conference on Harmonization (ICH) guidelines and the directives for good laboratory practice [43-49], to use the method for analysis of the drug content of tablets.

EXPERIMENTAL

Chemicals and Reagents

Pharmaceutically pure samples of valsartan and hydrochlorothiazide were generous gifts from Torrent and Sun Pharmaceuticals, respectively. All chemicals were of analytical or HPLC grade and were supplied by Merck and Qualigens Fine Chemicals, Mumbai, India. A commercial preparation, Valzaar-H tablets (Torrent Pharmaceuticals, Baddi, India) containing 80 mg valsartan and 12.5 mg hydrochlorothiazide per tablet was assayed.

Preparation of Standard Solutions

A combined stock solution containing 8 mg mL^{-1} valsartan and 1.25 mg mL^{-1} hydrochlorothiazide was prepared in 10 mL ethanol. A calibration solution containing 800 ng μL^{-1} valsartan and 125 ng μL^{-1} hydrochlorothiazide, were prepared by dilution of the stock solution. This solution was used to apply 800–5600 ng valsartan and 125–875 ng hydrochlorothiazide to the plates.

Preparation of Sample Solutions

Twenty Valzaar-H tablets were weighed and finely powdered. Powder equivalent to approximately 80 mg valsartan and 12.5 mg hydrochlorothiazide was weighed accurately, dissolved in ethanol, and diluted to 10

mL with the same solvent. The sample solution was then filtered through a 0.45-μm filter (Millipore, Milford, MA, USA).

Thin-Layer Chromatography

HPTLC was performed on 10 cm × 20 cm aluminium-backed HPTLC plates coated with 0.2 mm layers of silica gel 60 F_{254} (E. Merck, Darmstadt, Germany). Before use the plates were washed with methanol and stored in a desiccator. Samples were applied to the plates as 6-mm bands, 11.6 mm apart and 10 mm from the lower edge, by means of a Camag (Muttenz Switzerland) Linomat V applicator equipped with a Hamilton (Reno, Nevada, USA) 100-μL microsyringe. The rate of application was 15 s μL^{-1}. Ascending development of the plates, with chloroform–ethyl acetate–acetic acid, 5:5:0.2 (*v*/*v*), as mobile phase was performed at 25 ± 2°C in a Camag twin-trough TLC chamber previously saturated with the mobile phase for 30 min. The development distance was 70 mm. After development the plates were dried for 5 min in an oven at 50°C. Densitometric scanning was then performed at 248 nm (λ_{max} for the compounds) with a Camag TLC scanner 3 equipped with Wincats Software Version 1.3.0, using the deuterium light source. The slit dimensions were 6.00 mm × 0.45 mm.

Validation of the Method

The method was validated in accordance with ICH guidelines.

Linearity

Amounts of standard solutions equivalent to 800, 1600, 2400, 3200, 4000, 4800, and 5600 ng per spot valsartan and 125, 250, 375, 500, 625, 750, and 875 ng per spot hydrochlorothiazide were applied to the plates and the plates were developed, dried, and scanned as described above. Calibration graphs for both drugs were constructed by plotting peak areas against the corresponding amounts (ng $spot^{-1}$). Each amount was chromatographed six times.

Sensitivity

The sensitivity of measurement of valsartan and hydrochlorothiazide was estimated as the limits of quantification (LOQ) and detection (LOD), which were calculated by the use of the equations $LOD = 3 \times N/B$ and $LOQ = 10 \times N/B$, where N is the standard deviation of the peak areas of the drugs ($n = 3$), taken as a measure of the noise, and B is the slope of the corresponding calibration plot.

Precision

The precision of the method was assessed by replicate ($n = 5$) analysis of both pharmaceutical preparations.

Precision was also studied by analysis of standard solutions containing both the drugs at concentrations covering the entire calibration range. Intra-day precision (% CV) was determined by analysis of the solutions three times on the same day. Inter-day precision (% CV) was assessed by analysis of the solutions on three different days over a period of one week.

Accuracy

The accuracy of the method was determined by the method of standard additions at three different levels, i.e. by multiple level recovery studies. Sample stock solution containing 3200 ng mL^{-1} of valsartan and 500 ng mL^{-1} of hydrochlorothiazide was prepared from the tablet formulation and spiked with amounts of the drugs equivalent to 80, 100, and 120% of the amounts present in the original solution. These solutions were then analysed as described above.

Specificity

Peak purity for valsartan and hydrochlorothiazide was tested by comparing spectra acquired of at the start (S), apex (A), and end (E) of the peaks.

Repeatability

Repeatability of sample application was assessed by spotting 1 μL of drug solution seven times on an HPTLC plate, developing the plate, and recording peak height and the area for the spots.

Repeatability of measurement of peak height and area were determined by spotting 1 μL of standard drug solution on an HPTLC plate, developing the plate, and scanning the developed spot seven times without changing the position of the plate.

RESULTS AND DISCUSSION

Validation of the Method

Linearity

Response to valsartan and hydrochlorothiazide was linear in the concentration ranges 800–5600 and 125–875 ng $spot^{-1}$, respectively. The

regression equations for valsartan and hydrochlorothiazide (n = 6) were $y = 2.4135x + 2664.8$ and $y = 7.6297x + 542.76$, respectively, where y is response and x the amount chromatographed. The correlation coefficients, r, were 0.9998 and 0.9988 respectively, over these concentration ranges.

Sensitivity

The limits of quantification (LOQ) and detection (LOD) for valsartan were 377.91 and 124.71 ng, respectively. For hydrochlorothiazide the values were 114.46 and 47.67 ng, respectively.

Precision and Accuracy

Results and statistical data from replicate (n = 5) analysis of both drugs in Valzaar-H tablets are reported in Table I. Results from determination of intra-day and inter-day precision, by analysis of standard solutions covering the entire calibration range, are listed in Table II. Results from determination of recovery of valsartan and hydrochlorothiazide from

Table I

Results from assay of valsartan and hydrochlorothiazide in tablets

Tablet	Component	Label claim (mg)	Amount found (mg ± *SD*; n = 6)	% Label claim	*RSD* (%)
Valzaar-H	Valsartan	80	79.97 ± 0.08	99.75	0.78
	Hydrochlorothiazide	12.5	12.45 ± 0.14	99.67	1.12

n is number of replicates

Table II

Results from precision studies

Component	Amount applied (ng)	Intra-day precision (*RSD* (%), n = 3)	Inter-day precision (*RSD* (%), n = 3)
Valsartan	800	0.380	0.416
	1200	0.226	0.217
	1600	0.212	0.343
Hydrochlorothiazide	1000	0.642	1.162
	1500	0.407	0.508
	2000	0.325	0.434

tablet formulation solution spiked with amounts of the drugs equivalent to 80, 100, and 120% of the original amounts are listed in Table III. All the results obtained are within acceptable limits.

Table III

Results from of recovery studies

Brand name	Component	Amount spiked (%)	Recovery (%)	*RSD* (%)	Recovery (%)	*RSD* (%)
Valzaar-H	Valsartan	80	100.79	0.754	99.59	0.508
		100	100.61	0.567	99.78	0.352
		120	100.36	0.463	100.18	1.127
	Hydrochlorothiazide	80	100.89	0.451	99.75	1.366
		100	101.46	0.397	99.89	1.210
		120	100.41	0.388	98.56	0.916

Specificity

The mobile phase used resolved the drugs very efficiently, as shown in the Fig. 1. The R_F values of valsartan and hydrochlorothiazide were 0.27 and 0.44, respectively. Typical overlaid absorption spectra of valsartan and

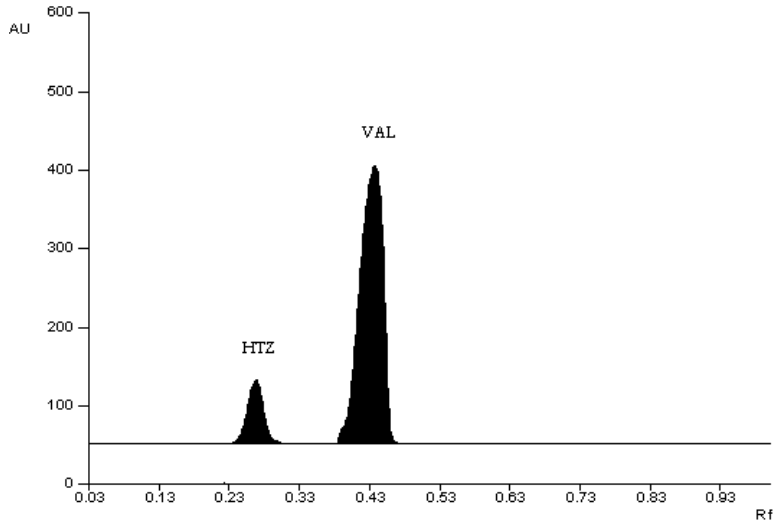

Fig. 1

Chromatogram obtained from analysis of a laboratory mixture of the drugs: HTZ, hydrochlorothiazide; VAL, valsartan

hydrochlorothiazide are shown in Fig. 2. The correlation among spectra acquired at the start (S), apex (A), and end (E) of the peaks were indicative of peak purity for both valsartan (correlation $r_{(S,M)} = 0.9999$, $r_{(M,E)} = 0.9995$) and hydrochlorothiazide (correlation $r_{(S,M)} = 0.9998$, $r_{(M,E)} = 0.9997$). It can thus be concluded that no impurities or degradation products migrated with the peaks obtained from standard solutions of the drugs.

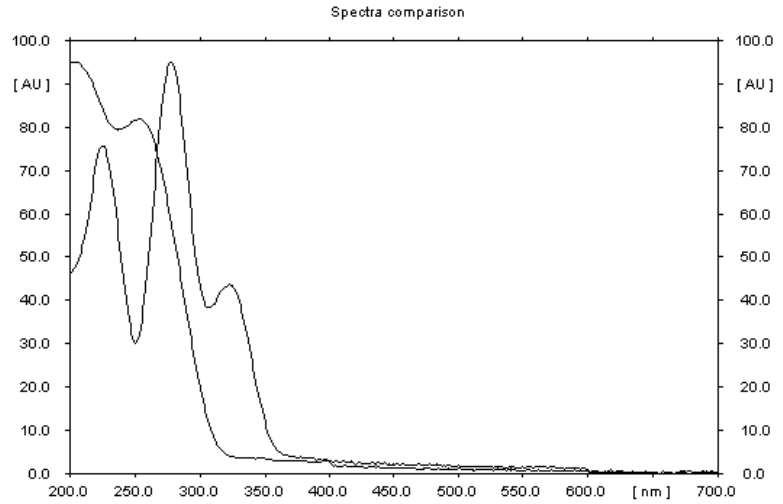

Fig. 2

Overlaid absorption spectra of valsartan and hydrochlorothiazide

Repeatability

Measurement of the repeatability of sample application showed *RSD* for peak height and peak area were 0.48% and 0.49% respectively. For hydrochlorothiazide the values were 0.69% and 0.71% respectively.

When the same spot was scanned seven times without changing the position of the plate *RSD* for measurement of peak height and peak area were 0.26% and 0.09%, respectively, for valsartan and 0.60% and 0.13%, respectively, for hydrochlorothiazide.

CONCLUSION

This method for simultaneous determination of valsartan and hydrochlorothiazide in antihypertensive pharmaceutical preparations is very

simple, rapid, and furnishes accurate and precise results. Among its advantages are short run time and large sample capacity both of which significantly reduce the duration of the analysis.

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