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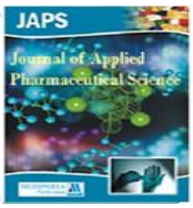

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Quantitative analysis of valsartan in tablets formulations by High Performance Thin-Layer Chromatography

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ABSTRACT

A new, simple, accurate, and precise high-performance thin-layer chromatographic (HPTLC) method has been established for quantitative analysis of valsartan in tablet formulations. Standard and sample solutions of valsartan were applied to precoated silica gel G 60 F254 HPTLC plates and the plates were developed with chloroform: acetonitrile: toluene: glacial acetic acid, in the ratio 1:8:1:0.1 (v/v) (v/v), as mobile phase. UV detection was performed at 254 nm. The retention factors of valsartan was 0.65. The calibration plot for Valsartan standard was linear with r =0.9999, slope = 5.328 and intercept=356.9. The limit of detection and limit of quantitation of Valsartan were found to be 5and 16 ng per spot respectively. The percentage recovery was found to be 99.37% for Valsartan. The method showed good repeatability and recovery with relative standard deviation less than 2. Method was validated in accordance with the requirements of ICH guidelines and was shown to be suitable for purpose. The method is selective and specific can be used for determination of the routine analysis of valsartan in tablets. Tablet excipients did not interfere with the chromatography.

Key words: Valsartan, HPTLC method, Method validation,mobile phase.

INTRODUCTION

Valsartan,(S)-N-valeryl-N-[2′-(1H-tetrazol-5-yl)biphenyl-4-yl) methyl] valine (B.P,2005; E.P, 2005; Martindale,2002; USP, 2004), is a potent, highly selective, orally active, specific angiotensin II receptor antagonist used as a hypotensive drug(Hardman et al 2001; The Merck Index, 2001; Moffat et al, 2004). Very few methods for determination of valsartan individually have appeared in the literature. Methods used include HPLC analysis after liquid extraction (Daneshtalab et al, 2002; Macek et al, 2001), and UV, second derivative spectrophotometric, and LC methods(Satana et al, 2001; Tatar et al, 2002) have been compared. A literature survey has revealed there is no HPTLC method for analysis of valsartan 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 (Cazes et al 2002;Heftman et al 2004; Scott et al 2001;Sethi 1996;Sherma 2002), to use the method for analysis of the drug content of tablets.

MATERIALS AND METHODS

Instrumentation

Analysis was performed on a CAMAG Linomat 5" model instrument. Hamilton syringe, Camag TLC scanner3, Camag WinCAT software, Camag Twin-trough chamber (10x10cm), and ultrasonicators were used for the study. Silica gel60 F_{254} TLC plates 10x10cm with layer thickness

Pure drug, Valsartan was supplied as a gift sample by TORRENT PHARMACEUTICALS LTD, Ahmedabad. Tablet formulations containing Valsartan of the brand names DIOVAN of NOVARTIS INDIA LTD and VALZAAR of TORRENT PHARMACEUTICALS LTD, Ahmedabad , were purchased from local pharmacy shop.

Solvent

Method development started with the selection of solvent and methanol was the best choice of solvent, which was followed by the optimization of mobile phase for the study.

Stock solution

Standard stock solutions of Valsartan was prepared by dissolving 10mg of drug in 10ml of methanol to get a concentration of 1mg/ml. 0.5ml of the above solution was diluted to 10ml to get a concentration of 50µg/ml. From the above stock solution, different volumes like 1, 2, 4, 6, 8,10µl were taken and spotted on to the plate, followed by development and scanning. Peak areas were recorded. Calibration graph was plotted against concentration of the standard and peak areas. Linear regression data showed a good linear relationship over a concentration range of 50-500ng/spot. Linear regression data for the calibration plots (n=3) are listed in table no. 1.

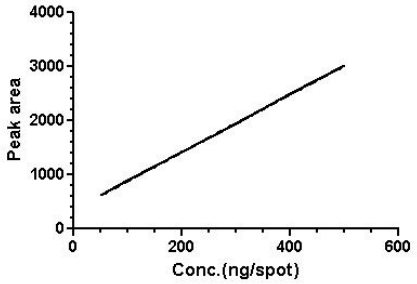

Fig 1: Calibration curve of Valsartan.

Table no.1 Linear regression data for calibration plots (n=3)

Linear range	50-500ng/band
Correlation coefficient	0.9999
Slope	5.328±0.02866
95% confidence limit of slope	5.249-5.408
Intercept	356.9±8.697
95% confidence limit of intercept	332.7-381.0

Sample preparation

Tablets containing 10mg was taken and dissolved in 10ml of methanol to get a concentration of 1mg/ml. To ensure complete extraction of the drug the flask was sonicated. (Fast clean ultra sonic cleaner, Enertech Electronics, Mumbai, India) for 30min at room temperature (25 ± 2^0C). 0.5ml of the above solution was diluted to 10ml to get a concentration of 50µg/ml. From the above stock solution, different volumes like 1, 2, 4, 6, 8,10µl were taken and spotted on to the plate, followed by development and scanning. Peak areas were recorded. Concentration of the drug was calculated from peak area obtained from the calibration graph.

Selection of the optimum mobile phase

In attempts to optimize the mobile phase, various mobile systems were tried for the study and finally chloroform: acetonitrile: toluene: glacial acetic acid, in the ratio 1:8:1:0.1 (v/v) were selected. Use of this mobile phase resulted in sharp, well defined Valsartan peaks of R_F= 0.65 ± 0.02. Well defined bands were obtained only when the chamber was saturated with the mobile phase for 30 min at room temperature before plate development.

VALIDATION OF THE METHOD

(ICH Guidelines1996;reviewer guidance1994)

Precision

The intra-day and inter day precision of the method were estimated by performing six determinations of drug solution at two different concentrations 300 and 500ng /spot for four times. Results are shown in Table 2.

Table 2 Inter day and intraday precision of the HPTLC method [a)]

Amount[ng/band]	Intra-day precision		Inter-day precision	
	Mean area[AU]	RSD[%]	Mean area[AU]	RSD[%]
300	1735	0.38%	1740	1.83%
500	2930	1.388%	2927	1.29%

[a)] n = 6

Robustness

Robustness was checked by analysis of the sample solutions after making small changes to mobile phase composition. Chloroform: acetonitrile: toluene: glacial acetic acid, in the ratio 1:8:1:0.1 and 0.5:8.5:1:0.1 were selected with different distances 8 and 9 cm for different amounts of Valsartan 300 and 500ng per band. The low values of %RSD obtained after introduction of these small changes (Table 3) were indicative of robustness of the method.

Table 3 Robustness of the method [a)]

Condition	Recovery[%] [b)]	RSD [%] [b)]
Mobile phase composition		
Chloroform: acetonitrile: toluene: glacial acetic acid, in the ratio 1:8:1:0.1	100.5	0.78
Chloroform : acetonitrile: toluene: glacial acetic acid, in the ratio 1:8:1:0.1 and 0.5:8.5:1:0.1	99.16	1.21
Development distance		
8cm	101.24	0.84
9cm	99.89	1.16

[a)] n = 6, [b)] Average for two amounts: 300 and 500ng/band.

LOD and LOQ

The limits of detection and limit of quantitation were calculated from the slope(s) of the calibration plot and the standard

deviation (SD) of the response by use of the equations LOD = 3.0 x SD/S and LOQ = 10 x SD/S.The limit of detection and limit of quantitation obtained by this method were5 and 16 ng/spot respectively,which indicates the sensitivity of the method is adequate.

Specificity

Specificity of the method is ascertained by analyzing reference standard and samples. The bands for Valsartan from pharmaceutical formulations were confirmed by comparing the R_f and UV spectra of the separated bands with those from the standard. The peak purity of Valsartan was assessed by comparing the spectra acquired at the peak start(S), peak apex (M), and peak end (E) of a band. It was found that *r*(S, M) = 0.9998 and *r* (M, E) = 0.9999. Good correlation (*r*=0.9997) was also obtained between standard and sample spectra of 1Valsartan.

Ruggedness

Ruggedness is a measure of reproducibility of a test result under normal, expected operating conditions from instrument to instrument and from analyst to analyst. Ruggedness was tested by analysis of 300 and 500 ng per band and the results were listed in Table no. 4

Table 4 Ruggedness of the method [a)]

Variable	Recovery[%][b)]	RSD [%][b)]
Analyst I	99.58	1.21
Analyst II	100.45	1.18

[a)] n = 6, [b)] Average for two amounts: 300 and 500ng/band.

Assay of Valsartan in tablets

The suitability of the method was examined by assay of Valsartan in tablets, by applying 500ng/band. Bands of R_f = 0.65±0.02 for Valsartan were observed in the chromatogram obtained from the drug extracted from tablets. It is evident that there was no interference from excipients commonly present in tablets. The drug content was found to be 99.34% (39.35mg Valsartan), %RSD1.41, n = 6). The low % RSD value indicated the method was suitable for analysis of this drug in pharmaceutical dosage forms, because it could be validated in accordance with the specifications stipulated by regulatory standards for pharmaceutical products.

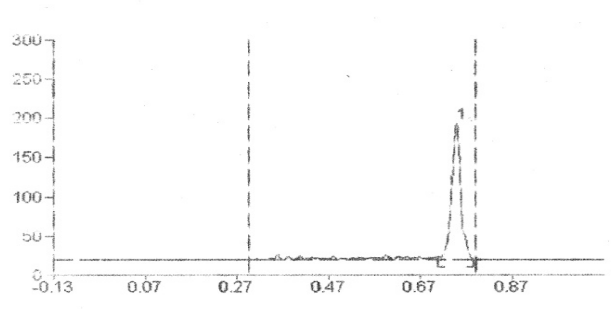

Fig 2 Chromatogram of 500ng/spot of valsartan

RESULTS AND DISCUSSION

This HPTLC technique is precise, robust, and accurate and could find application in routine quality control analysis of pharmaceutical formulations.

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