A VALIDATED HPTLC DETERMINATION OF AN ANGIOTENSIN RECEPTOR BLOCKER OLMESARTAN MEDOXOMIL FROM TABLET DOSAGE FORM.

***Della Grace Thomas Parambi[1], Dr. Sr. Molly Mathew[2], Dr.V.Ganesan[3], Anila Jose[4] , Dr. K. G.Revikumar[5]**

[1] Dept.of Pharmaceutical Analysis, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham University, AIMS Health Care Campus, AIMS, Ponekkara (P.O.), Kochi, Kerala, India – 682041.

[2] Principal, Malik Deenar College of Pharmacy, Kasaragod, India - 671321.

[3]Principal, Erode college of pharmacy, Erode, India -638112.

[4]Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham University, AIMS Health Care Campus, AIMS, Ponekkara (P.O.), Kochi, Kerala, India – 682041.

[5] Principal, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham University, AIMS Health Care Campus, AIMS, Ponekkara (P.O.), Kochi, Kerala, India – 682041.

***Email:** dellajesto@gmail.com

ABSTRACT

A quantitative HPTLC method for determination of Olmesartan Medoxomil in tablet dosage form has been established and validated. Olmesartan from formulations was separated and identified on silica gel 60 F_{254} .HPTLC plates with chloroform: acetonitrile: toluene: glacial acetic acid, in the ratio 1:8:1:0.1(v/v) was used as mobile phase. The plates were developed to a distance of 8 cm. Quantitation was performed at λ=301nm by reflectance scanning and well resolved results were obtained for Olmesartan medoxomil. The method was validated for precision, recovery, robustness, specificity and ruggedness. The calibration plot for olmesartan standard was linear with r =0.9991, slope = 5.328 and intercept=356.9. The limit of detection and limit of quantitation of Olmesartan were found to be 4.79 and 15.97 ng per spot respectively. The percentage recovery was found to be 99.37% for Olmesartan. The method showed good repeatability and recovery with relative standard deviation less than 2. The method is selective and specific with potential application in pharmaceutical analysis.

Keywords: Olmesartan medoxomil, HPTLC method, Method validation.

INTRODUCTION

Olmesartan medoxomil is the most recent member of Angiotensin receptor blocker[1,2,3] which is chemically, (5-methyl-2-oxo-2H-1,3-dioxol-4-yl) methyl 4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1H-imidazole-5-carboxylate. Key structural elements of Olmesartan medoxomil include a hydroxy alkyl substituent at the imidazole 4- position and a hydrolysable ester at the imidazole 5- position. Inter and Intra molecular hydrogen bonding involving these groups may contribute to the potentiation of antagonistic activity. After the oral administration, Olmesartan medoxomil is de-esterified in the intestinal tract to produce the active metabolite Olmesartan, which undergoes no additional metabolic change[4]. The marked anti-hypertensive efficacy of Olmesartan medoxomil may result from a unique pharmacological interaction of the drug with the AT_1 receptor, resulting in a potent, long lasting, dose dependent blockade of A_2. This characterizes the structural features of Olmesartan that may be responsible for its clinical efficacy. Literature survey reveals that Olmesartan medoxomil can be estimated by RP-LC[5] UV spectrophotometric methods individually or in combination with other drugs[6] and HPLC[7]. However, there is no HPTLC method reported for the estimation of Olmesartan from pharmaceutical dosage forms. Present work describes a simple, economical, accurate and precise method for the estimation of Olmesartan medoxomil in tablet formulations.

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 0.2cm (E.Merck, Mumbai) were used as a stationary phase.

Materials: Pure drug, Olmesartan was supplied as a gift sample by RANBAXY Laboratories Limited, Delhi. Tablet formulations containing Olmesartan of the brand names OLMESAR of MACLEDDS Pharmaceuticals, Mumbai and OLMECIP of CIPLA, Gujarat 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.

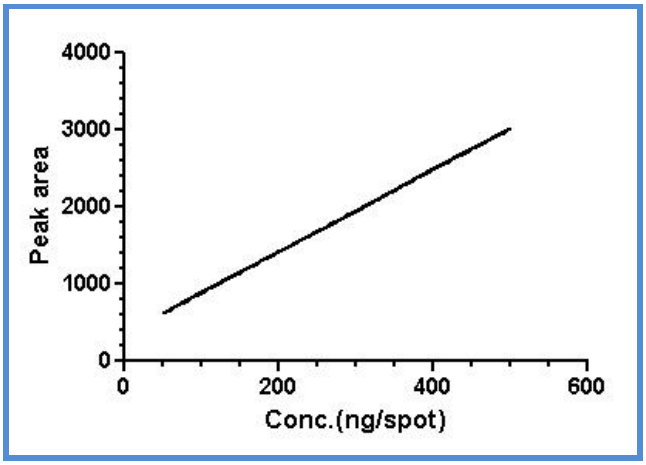
Stock solution: Standard stock solutions of Olmesartan was prepared by dissolving 10mg of drug in 10ml of methanol to get a concentration of 1mg/ml. 0.5ml of the above solution was diluted to 10ml to get a concentration of 50µg/ml. From the above stock solution, different volumes like 1, 2, 4, 6, 8,10µl 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.

Table 1: Linear regression data for calibration plots (n=3)

Linear range	50-500ng/band
Correlation coefficient	0.999
Slope	5.328
95% confidence limit of slope	5.249-5.408
Intercept	356.9
95% confidence limit of intercept	332.7-381.0

Figure 1: Calibration curve of Olmesartan



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^{0}C). 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.

RESULTS AND DISCUSSION

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 olmesartan peaks of R_F= 0.67 ± 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 [8, 9]

Precision

The intra-day and inter day precision of the method were estimated by performing six determinations of drug solution at two different concentrations 200 and 400ng /spot for four times. Results are shown in **Table 2.**

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 olmesartan, 200 and 400ng per band. The low values of %RSD obtained after introduction of these small changes (Table 3) were indicative of robustness of the method.

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 were 4.79 and 15.97ng/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 Olmesartan medoxomil from pharmaceutical formulations were confirmed by comparing the R_f and UV spectra of the separated bands with those from the standard. (**Figure 2**) The peak purity of olmesartan 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 olmesartan.

Recovery

The analyzed sample was spiked with an additional 200, 400ng of Olmesartan standard and mixtures were analyzed again, in triplicate by the proposed method, to check different amounts of the drug from the formulation. Recovery was 99.34-101.34 % which was shown in **Table no. 4.**

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 200 and 400 ng per band and the results were listed in **Table no. 5**

Table 2: Inter day and intraday precision of the HPTLC method (n=6)

Amount[ng/band]	Intra-day precision		Inter-day precision	
	Mean area [AU]	RSD [%]	Mean area [AU]	RSD [%]
200	1806	0.38%	1854	1.51%
400	2869	0.635%	2855	1.29%

Table 3: Robustness of the method (n=6)

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

[b)] Average for two amounts: 200 and 400ng/band.

Figure 2: UV Spectra of Olmesartan medoxomil

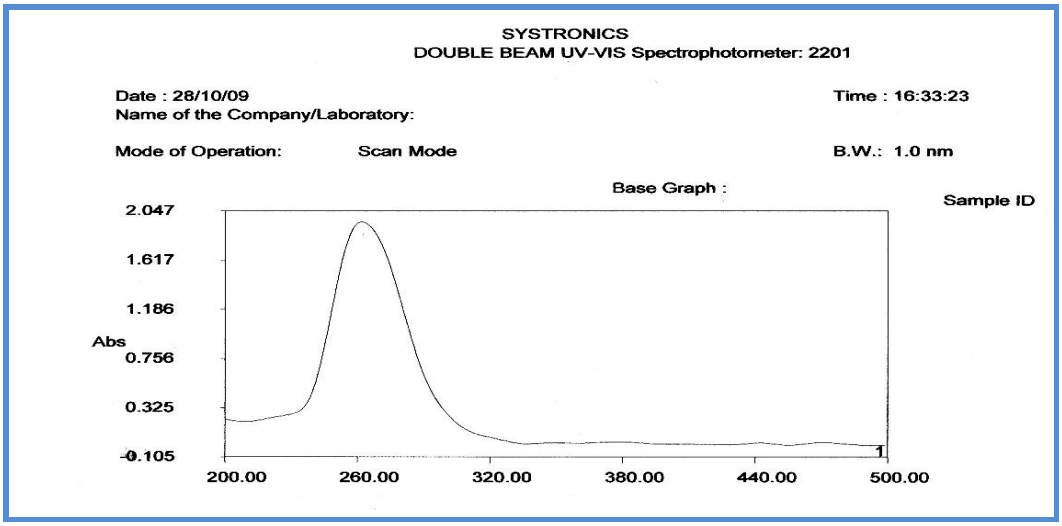


Table 4: Results from studies of recovery of Olmesartan medoxomil (n=3)

Amount of drug added (%)	Theoretical content (ng)	Recovery (%)	RSD (%)
25	200	100.80	1.07
50	400	101.34	1.47
75	600	99.34	1.34

Table 5: Ruggedness of the method (n=6)

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

[b)] Average for two amounts: 200 and 400ng/band.

Assay of Olmesartan in tablets:

The suitability of the method was examined by assay of olmesartan in tablets, by applying 400ng/band. Bands of R_f = 0.67±0.02 for Olmesartan were observed in the chromatogram obtained from the drug extracted from tablets.(Figure:3) It is evident that there was no interference from excipients commonly present in tablets. The drug content was found to be 99.34% (19.35mg Olmesartan), %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.

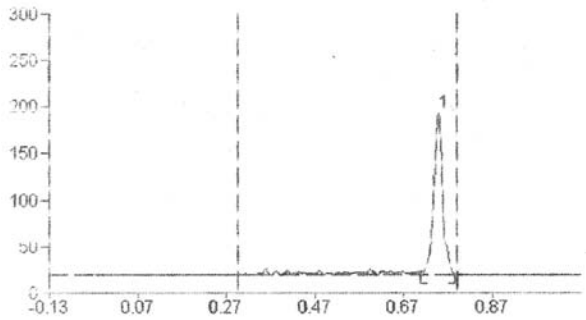


figure:3 chromatogram of 500ng olmesartan

CONCLUSION

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

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