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Development and validation of HPTLC method for determination of fimasartan in bulk and pharmaceutical dosage form

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ABSTRACT

A simple, rapid, and precise high-performance thin layer chromatographic method was developed and validated for the determination of Fimasartan in the pharmaceutical dosage form. The method employed TLC 20 cm × 10 cm aluminum-backed TLC plates coated with 200µm layers of silica gel $60F_{254}$ S as the stationary phase. Toluene: methanol: ethyl acetate: formic acid (8:1.2:0.9:0.3 v/v) are used as the solvent system. The system gave a compact spot for Fimasartan (0.6 ±0.02). The spectrodensitometric scanning-integration was performed at a wavelength of 265 nm. The regression equation data for calibration plot showed a good linear relationship with r^2 = 0.9997in the concentration range 800-2800 ng for Fimasartan. The method was subjected to validate for precision, accuracy, ruggedness, robustness with their forced degradation studies. The limit of quantification and limit of detection was found to be 52.81 and 160.03 respectively. This analysis proves that the given method is selective and reproducible for the determination of Fimasartan.

Keywords: Fimasatan, HPTLC, Validation.

INTRODUCTION

Fimasartan is a non-peptide angiotensin II receptor antagonist (ARB) used for the treatment of hypertension and heart failure. Through oral administration, Fimasartan blocks angiotensin II receptor type 1 (AT_1 receptors), reducing pro-hypertensive actions of angiotensin II, such as systemic vasoconstriction and water retention by the kidneys. [1] Fimasartan is chemically represented as 2-[2-butyl-4-methyl-6-oxo-1-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl]methyl] pyrimidin-5-yl]-N,N-dimethylethanethioamide.

Development of rapid and simple methods for determination of drugs in wide range of concentration in great interest.

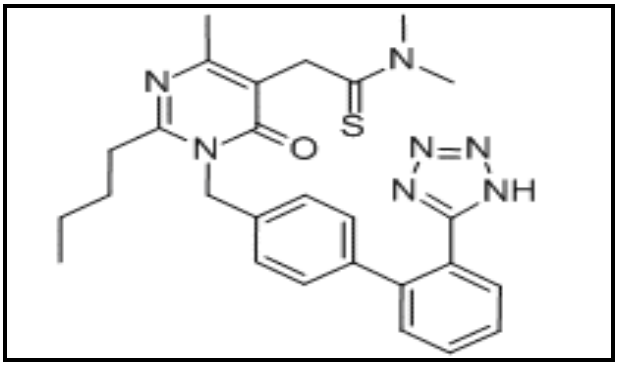

Fig. 1: Structure of Fimasartan

Literature review tells that various analytical methods have been reported for the determination of Fimasartan which include high performance liquid chromatography, UV spectroscopy, liquid chromatography-mass spectrometry method. [2, 3, 4, 5, 6, 7] The Korean Food and Drug Administration (KFDA) has approved Fimasartan as a drug in September 2010. It is marketed as Kanarb by Boryung Pharmaceuticals in Korea. The purpose of present work was to develop and validate HPTLC method for determination of Fimasartan in tablet dosage form.

MATERIAL AND METHODS

Materials

Fimasartan was gift sample from Alkem Laboratories, India. All chemicals and reagents were of analytical grade (MERCK Chem. Ltd., Mumbai). All reagents and chemicals used for analysis were of AR Grade.

HPTLC instrument [8, 9]

Chromatography was performed on 20 cm × 10 cm aluminum-backed TLC plates coated with 200 µm layers of silica gel $60F_{254}$ S (E. Merck, Darmstadt, Germany; supplied by Merck India, Mumbai, India). The plates were prewashed by methanol and activated at 100 – 110 °C for 10 min prior to chromatography. The samples were applied on the plates as 6 mm wide bands, by means of a CAMAG (Muttenz, Switzerland) Linomat-5 sample applicator fitted with a 100 µL sample syringe (Hamilton, Bonaduz, Switzerland). Plate was developed to a distance of 8 cm using toluene: methanol: ethyl acetate: formic acid (8:1.2:0.9:0.3) as mobile phase in a Camag twin-trough glass chamber previously saturated with mobile phase vapours for 15 min at ambient temperature. Densitometric scanning was performed at 265 nm using Camag TLC Scanner 3 equipped with winCATS software version 1.3.0.

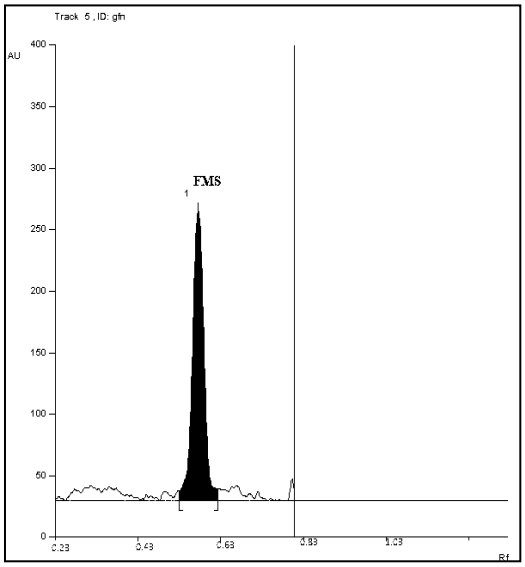

Fig. 2: A Typical HPTLC Chromatogram of Fimasartan

PREPARATION OF SOLUTION

Preparation of Stock Solution

For Calibration Plot, an accurately weighed Fimasartan (16 mg) was transferred to 10 mL volumetric flask; dissolved in methanol and the volume was made up to mark with the same solvent to give 1600 ng/µL solution. A series of standard curve was prepared over concentration range of 800 - 2800 ng. The data of spot area versus drug concentration was treated by linear least square regression analysis.

Preparation Sample Solution of Pharmaceutical Formulation

To determine the content of Fimasartan in the tablets, each tablet containing 60 mg of Fimasartan. Twenty tablets were accurately weighed and finely powdered. The powder equivalent to the 60 mg of the Fimasartan was weighed. Weighed powder was transferred to the 10 ml volumetric flask and diluted with methanol. Sonication is done for 10 minutes. The solution was diluted with the same solvent and filtered. The sample solution (1 µL, containing 1600 ng of Fimasartan) was applied on TLC plate, developed and scanned.

METHOD VALIDATION [10, 11]

The method was validated in accordance with ICH guidelines. The following parameters were validated.

Precision

The repeatability of sample and measurement of peak area were expressed in term of percentage RSD. Which shows intra-day and inter-day variation at three different concentration level 800, 1200 1600 ng per spot.

Robustness

By introducing small variation in composition of mobile phase, the effect on the results were examined. Robustness was studied at the concentration level of 1600 ng. The parameters like mobile phase composition, mobile phase volume, duration of saturation was studied.

Limit of detection and Limit of Quantification

Sensitivity of the given method was carried out in terms of Limit of Detection and Limit of Quantification. LOD = 3.3(SD/S) and LOQ = 10(SD/S). LOD and LOQ was based on the SD of the response and slope (S) of the calibration curve at levels approximating the LOD and LOQ. The amount of drug by spot versus average response (peak area) was plotted and equation was determined. The solution of Fimasartan was prepared and different concentration of stock solution range 800-1200 ng was applied in triplicate

Ruggedness

Ruggedness of the method was carried out by analysing 1600 ng (n = 6) of the Fimasartan, with the variation in the results and with the help of two analysts were checked.

Accuracy

Recovery study was carried out by spotting at 80%, 100%, 120% level that is known amount of standard Fimasartan was added to pre analysed sample and place them to the proposed TLC method.

RESULTS AND DISCUSSION

Optimization of chromatographic condition

Different ratios of methanol, toluene, and ethyl acetate were tried as mobile phase was tried but, tailing of spot, less persistent spots were observed in most of the attempts. In order to overcome the problems, toluene: methanol: ethyl acetate: formic acid (8:1.2:0.9:0.3 *v/v/v/v*) was tried and results is good resolution, sharp and symmetrical peak with R_f value of 0.65 for Fimasartan. Densitometric scanning was performed at 265 nm. The optimized method development of a selective and specific method of analysis.

Standard curve

The linear regression data for the calibration curve showed good linear relationship for the concentration range 800- 2800 ng. The linear regression was found to be Y = 2.2275x + 169.53, Slope = 2.2275, Intercept = 169.53, correlation coefficient = 0.9997

Table 1: Linear regression data for the calibration curve

Parameters	Results
Linearity range (ng per spot)	800 -1200
R^2 ± SD	0.9997 ± 0.0004
Slope	2.2275
Intercept	169.53

VALIDATION OF METHOD

Precision

Intra-day and Inter-day precision

The precision of sample application and measurement of peak area was expressed in the terms of the percentage RSD. Which include intra and inter-day variation of the drug at three different concentration levels 800, 1200, 1600 ng per spot.

Table 2: Intra-day and Inter-day precision

Precision	Conc. (ng/band) (n=3)	Mean ±SD	%RSD
Intra-day precision	800	1547.3 ±12.06	0.779
	1200	2515.3 ±35.00	1.391
	1600	3566.13 ±22.95	0.643
Inter-day precision	800	1642.1 ±3.48	0.212
	1200	2309.66 ±5.50	0.238
	1600	3616.33 ±9.07	0.25

Repeatability

Repeatability of sample application was assessed by applying 1 µL (1600 ng) of drug solution six times on a TLC plate followed by development of plate and recording the peak height and area for 6 bands. The results were listed in Table 3.

Table 3: Repeatability

Sr. No.	Conc. (ng/band)	Peak area ±SD
1	1600	4148.8
2	1600	4155.5
3	1600	4130.3
4	1600	4131.8
5	1600	4148.9
6	1600	4149.6
	Mean ±SD	4144.15 ±10.459
	% RSD	0.252

Robustness

The standard deviation of peak areas was calculated for parameter and % RSD was found to be less than 2%. The value of % RSD shown in Table 4 that indicates as robustness method.

Table 4: Robustness of Method

Parameters	± SD of peak area (n = 3)	% RSD
Mobile phase composition (± 0.5 mL)	25.552	0.615
Mobile phase volume (± 5ml)	21.273	0.529
Duration of saturation (± 5 min.)	59.915	1.501

Ruggedness

Ruggedness of the method was carried out by analysing 1600 ng (n=6) of the Fimasartan, with the variation in the results and with the help of two analysts were checked. The results are shown in the Table 5.

Table 5: Ruggedness

Analyst	Mean ±SD	%RSD
I	3510.86 ±10.63	0.302
II	3514.76 ±4.63	0.131

Recovery studies

The proposed method used for extraction and estimation of Fimasartan from pharmaceutical dosage formafter spotting with 80%, 100%, 120% of drug which afforded good recovery of the drug. The amount of drug added and amount determined and the percentage recovery are shown in Table 6.

Table 6: Recovery studies

Amount in %	Initial amount (ng) (n=3)	Amount added (ng/band)	% Recovered	% RSD
80	800	640	99.58	
100	800	800	100.34	0.669
120	800	960	99.01	

Analysis of Pharmaceutical Formulation

The spot was observed at R_f 0.62 was observed in the densitogram of the drug samples extracted from tablets. There was no interference from the excipients present in the tablets. The percentage drug content and percentage RSD were calculated. The low percentage RSD indicated the suitability of the method for routine analysis of the Fimasartan in pharmaceutical dosage form.

Table 6: Summary of the Validation Parameters

Parameters	Results
Linearity range	800 – 2800 ng/band
Slope	2.2275
Intercept	169.53
Correlation coefficient	0.9997
Precision	
Intra-day (n=3)	0.643-1.391
Inter-day (n=3)	0.212-0.25
Repeatability (n=6)	0.252
Accuracy (% Recovery)	99.58 ± 0.80
Limit of Detection	52.81
Limit of Quantification	160.03

CONCLUSION

The developed HPTLC technique is accurate, specific precise, and robust for the analysis of Fimasartan in tablets without the interference of any excipients. The statistical analysis proves that the method is reproducible and selective for the determination of Fimasartan as a bulk drug solution and in pharmaceutical formulations.

Acknowledgments

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