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Research Article

Development and Validation of High Performance Thin Layer Chromatography for the Determination of Ambrisentan in Bulk and Pharmaceutical Dosage Form

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Abstract

A simple, precise, sensitive and accurate high performance thin layer chromatography method for the determination of ambrisentan in bulk and in pharmaceutical dosage form was developed and validated. HPTLC separation was performed on aluminum plates precoated with silica gel 60 F254 (10 × 10) as the stationary phase and mobile phase optimized as toluene: methanol (3.5: 1.5 v/v). The method was found to give a compact spot for ambrisentan at retention factor (RF) value 0.48 ± 0.02. Densitometric scanning was Performed at 263 nm respectively. Accuracy for the marketed formulation endobloc was found to be 99.75-101.69% the proposed HPTLC method can routinely be used for determination of ambrisentan in bulk and Pharmaceutical dosage form.

Keywords: Ambrisentan; HPTLC; Development and Validation

Introduction

Ambrisentan chemically is (+)-(2S)-2-[(4,6-dimethylpyrimidin-2-yl) Oxy] -3-methoxy-3, 3-diphenylpropanoic acid (figure 1), having molecular formula: $C_{22}H_{22}N_2O_4$, with molecular weight 378.421 g/mol [1]. Ambrisentan is an orally active selective type an endothelin receptor antagonist. It is used in the treatment of pulmonary hypertension [2]. Various analytical methods reported in literature survey for the estimation of ambrisentan. Such as, extractive spectrophotometric determination of ambrisentan [3]. Spectrophotometric Methods for the determination of ambrisentan using charge transfers reagents [4]. UV-visible spectrophotometric and RP-HPLC method development [5]. LC-ESI-MS/MS method for quantification of ambrisentan in plasma [6]. Simple validated RP-HPLC method for estimation of ambrisentan [7,8]. Stability indicating RP-HPLC methods for determination of ambrisentan and tadalafil [9]. Validated stability indicating high performance liquid chromatographic methods for the determination of ambrisentan [10,11], has been found in literature. However, HPTLC method is not reported for the determination of ambrisentan in bulk and pharmaceutical dosage form. So, the objective of the present study is to develop simple, precise, sensitive and accurate HPTLC method for the determination of ambrisentan in bulk and pharmaceutical dosage form.

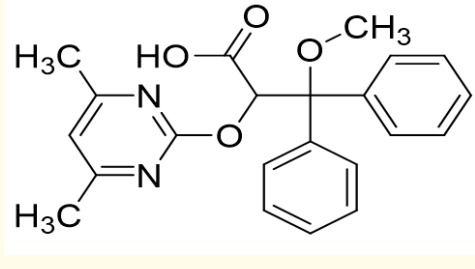

Figure 1: Structure of Ambrisentan.

Experimental

Chemical, reagent and solutions

Ambrisentan was obtained as a gift sample from Cipla pharmaceutical Ltd., Patalganga Mumbai. The commercial tablet dosage form of endobloc containing 10 mg of ambrisentan manufactured by Cipla Ltd., Mumbai was procured from local market. Methanol purchased from Merck Ltd., Worli, Mumbai, India.

Chromatographic Condition and instrumentation

The sample was spotted in the form of band 6 mm with a camag micro liter syringe on precoated silica gel aluminum plate 60F254 (20 cm × 10 cm with 0.2 mm thickness, E. Merck, Germany) using a Camag Linomat 5 (Switzerland). The plates were brainwashed with methanol and activated at 110° for 5 min, prior to chromatography. The mobile phase consists of toluene: methanol (3.5:1.5 v/v). Linear ascends development was carried out in 10×10 cm twin trough glass chamber. The optimized mobile phase chamber saturation time was 20 min, at room temperature (25 ± 2°) and relative humidity (60 ± 5%) and development distance was 80mm; the TLC plate was dried in a current of air dryer. Densitometric scanning was performed on a camag TLC scanner 3 equipped to win a CATS software version 1.3.0. At 263 nm. The source of radiation utilized was deuterium lamp.

Preparation of standard solution and linearity study

Standard solution was prepared by accurately weighed 10 mg of ambrisentan powder and transferred in 10 ml volumetric flask containing 5 methanol and volume was adjusted to mark with same solvent to get a concentration of 1000 µg/ml. From this 0.6-3.6 µl of the solution were applied on TLC plate to obtain concentration of 300, 600, 900, 1200, 1500 and 1800 ng per spot of ambrisentan, respectively. The calibration curve was plotting by area versus drug quantity per band. Calibration equations were determined by use of linear regression analysis and correlation coefficients (r^2) were calculated (Figure 2). Linearity studies results reported in Table 1.

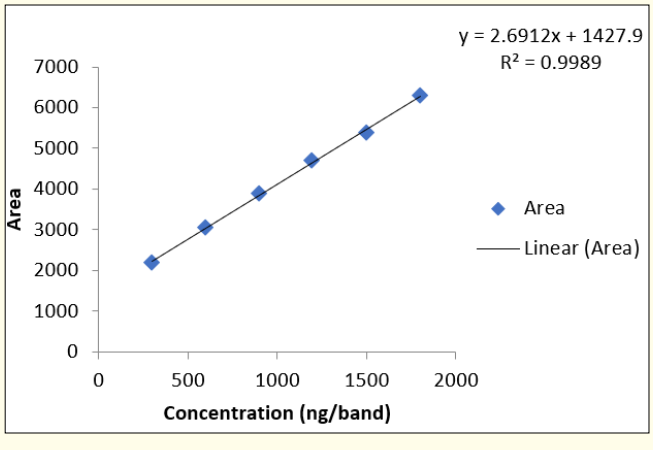

Figure 2: Calibration curve of Ambrisentan.

Concentration (ng/band)	Peak area mean ± SD	%RSD
300	2194.4 ± 7.54	0.34
600	3058.4 ± 20.49	0.67
900	3888.3 ± 69.65	1.79
1200	4716.2 ± 35.33	0.74
1500	5388 ± 25.05	0.46
1800	6279.5± 51.42	0.81

Table 1: Result of Linearity Study.

Analysis of tablet formulation

Twenty tablets were accurately weighed and powdered. An amount of powdered drug equivalent to 10mg of ambrisentan was transferred to 10 ml volumetric flask containing 7 ml methanol and sonicated for 20 min. volumetric flask shakes manually for 10 min; volume was made up to the mark using same solvent. The standard solution was then filtered through Whatman filter paper no. 41 giving concentration of stock solution 1000 µg/ml. Concentrations of 900 ng/band of ambrisentan were spotted on HPTLC plates. The plates were developed and scanned. The concentrations of the drug were assessed from the linearity curves (Figure 3). The results are depicted in Table 2.

Concentration (ng/band)	Amount found (ng/band)	% Amount found
900	903.64	100.40
900	916.16	101.79
900	880.63	97.84
900	890.82	98.98
900	906.39	100.71
900	909.62	101.06

Table 2: Result of Analysis of Tablet formulation.

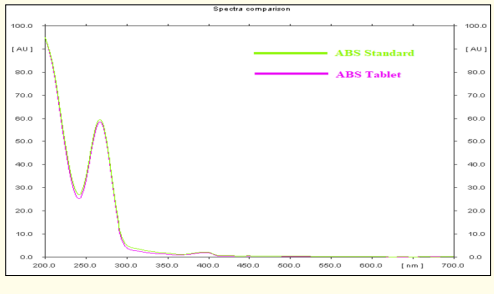

Figure 3: The peak purity spectra of Ambrisentan standard and Ambrisentan extracted from tablet, scanned at peak-start, peak- apex and peak- end position of the band.

Method Validation

Precision

The precision of the proposed method was ascertained by actual determination of three replicates of 600, 900 and 1200 ng/band concentration of ambrisentan was applied precoated silica gel aluminum plate. It was studied in terms of intra and inter-day variations.

Recovery Studies

Recovery studies was executed by applying the method to drug samples, in which known amount of ambrisentan at 80%, 100% and 120% was added to a pre-quantified sample solution.

Robustness

To determine the robustness of the purposed method, the experimental condition was deliberately changed such as mobile phase composition, duration of chamber saturation, development distance.

Ruggedness

This parameter of the method was evaluated by two analysts using same environmental and experimental conditions.

Detection of limit and quantification of limit

The detection of limit (DL) and quantification of limit (QL) of the drug (ambrisentan) were calculated by using the equations.

$DL = 3.3 \times \sigma / S$

$QL = 10 \times \sigma / S$

Specificity

The peak purity of ambrisentan was assessed by correlating the spectra of ambrisentan extracted from tablets and ambrisentan standard.

Result and Discussion

HPTLC method development and optimization

In this study, determination of ambrisentan in bulk and pharmaceutical dosage form was performed by HPLTC method. TLC procedure was first optimized normal-phase HPTLC on silica gel 60 F254 with various ratios of methanol, toluene were used as mobile phase, but, tailing observed to overcome the problem, methanol: toluene (1.5:3.5 *v/v*) was used and the result is a good resolution and sharp peak with Rf (retention factor) value of 0.48 ± 0.02 for ambrisentan (Figure 4). 3-D linearity chromatogram of Ambrisentan (Figure 5).

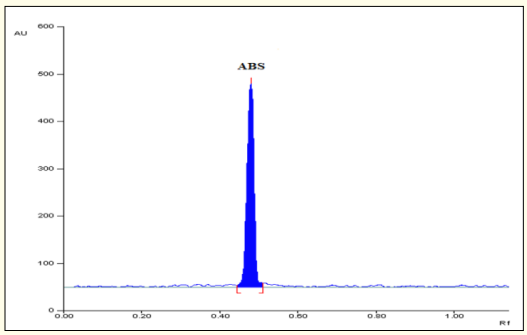

Figure 4: Typical HPTLC chromatogram of ambrisentan (Rf = 0.48).

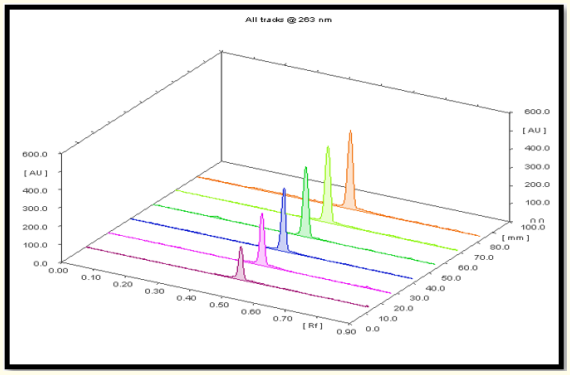

Figure 5: 3-D linearity chromatogram of Ambrisentan.

Validation of method

The method was validated with respect to various parameters including linearity, limit of detection, and quantification of limit, precision, ruggedness and accuracy according to ICH guidelines.

Precision

Precision of the method was studied as repeatability and intra-day and an inter-day variation was found to be less than 2 shown in table 3.

Concentration (ng/band)	Amount found (ng/band) ± SD (n=3)	%RSD
Intra-day precision		
600	602.99 ± 4.91	0.81
900	891.59 ± 9.30	1.04
1200	1202.98 ± 9.60	0.79
Inter-day precision		
600	599.52 ± 3.80	0.63
900	890.90 ± 7.37	0.82
1200	1204 ± 6.29	0.52

Table 3: Result of Precision study.

n-number of determinations

Recovery

Recovery experimental was performed at three different levels i.e. 80, 100 and 120%. To the pre-analyzed sample solutions, a known amount of mixed drug standard solution The purposed method when used for determination of ambrisentan from a known amount of mixed drug standard solution of were spotted at three different levels The chromatogram was developed and scanned as described above; the results of % recovery are shown in table 4.

Drug	Initial Amount (ng/band)	Amount added (ng/band)	Amount Recovered (ng/band)	% Recovery (n=3)	% RSD
	600	480	1082.53	100.52	1.36
ABS	600	600	1198.53	99.75	0.38
	600	720	1332.21	101.69	1.45

Table 4: Result of Recovery study.

n-number of determinations

Robustness

Robustness was studied in six replicate at the concentration 900 ng/band of ambrisentan. In this experiment, three parameters (mobile phase composition, mobile phase, development distance, duration of saturation) were studied; the results are shown in table 5.

Ruggedness

Ruggedness of the proposed method was studied by two different analysts using the same experiment and environmental conditions. The band 900 ng/band of ambrisentan were applied on RP-HPTLC plates. Results shown in table 6.

Sr. no.	Parameters	± S.D. of peak Area	% R.S.D. [n=6]
1	Mobile phase composition		
	3: 2 *v/v*	39.16	1.01
	4: 1 *v/v*	48.31	1.26
2	Development distance		
	75 mm	41.60	1.71
	85 mm	62.64	1.62
3	Duration of saturation		
	15 min	36.11	0.94
	25 min	43.78	1.64

Table 5: Result of Robustness study.

n-number of determinations

Drug	Concentration (ng/band)	Amount Found (%) ± S.D.	
		Analysts- I (n=6)	Analysts- II (n=6)
Ambrisentan	900	99.19 ± 0.90	99.66 ± 0.20

Table 6: Result of Ruggedness study.

n-number of determinations

Sensitivity

The sensitivity of proposed methods was estimated in terms of Detection of Limit (LOD) and Quantification of Limit (LOQ). The LOD and LOQ were calculated using equation LOD = 3.3 X N/B and LOQ = 10 X N/B; where, 'N' is standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve. Ambrisentan solutions of 300, 350, 400,450, 500 and 600 ng/band were applied on HPTLC plates developed and scanned. The LOD and LOQ values found were 13.63 ng and 41.30 ng, respectively.

Specificity

The peak purity of ambrisentan was determined by comparison with the spectra at peak start, peak apex and peak end position of the spot. r^2 [S, M] = 0.9998, and r^2 [M, E] = 0.9987. Good correlation (r^2 = 0.9989). The peak purity spectra were shown in Figure.

Conclusion

The proposed HPTLC method has been developed successfully and validated for the determination of drug (ambrisentan) in pharmaceutical dosage form. The results indicated that the developed methods were precise, accurate, sensitive and robust. Hence, the developed HPTLC method is suitable for routine determination in commercial tablet.

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