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Research Article

Liquid Chromatographic Method Development and Validation for Estimation of Amisulpride in Pharmaceutical Dosage Form

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Abstract

Amisulpride was quantified using a simple and reliable HPLC technique developed and validated. Acquity BEH C18 (150 x 3.0mm, 1.7µm) column as stationary phase was used to achieve chromatographic segregation. The mobile phase was a phosphate buffer pH 6.5, and acetonitrile (60:40, v/v) was pumped through the column. The wavelength of the ideal choice was 248 nm. The amisulpride retention time was discovered to be 4.25 minutes. The amisulpride regression equation yielded LOD and LOQ values of 0.15µg/ml and 0.47µg/ml, respectively. The percentage of recovery of amisulpride was found to be between 99.89 and 102.32 %, respectively. The regression equation was found to be y = 44556x + 22947. Since run time and retention time were minimized, the system developed was simple and cost-effective, and it could be used for routine analysis in quality control.

Keywords: RP-HPLC, Amisulpride, Peak area, Retention time, Accuracy, Validation.

INTRODUCTION

Amisulpride is chemically known as 4-amino-N-[(1-ethylpyrrolidin-2-yl)methyl]-5-ethylsulfonyl-2-methoxybenzamide with molecular formula $C_{24}H_{28}N_2O_3$ and molecular weight 369.48 g·mol^{-1}. The treatment of schizophrenia has progressed because the therapeutic efficacy, tolerability, and security profiles of abnormal antipsychotics appear to be better than those of traditional neuroleptics with the unique pharmacological profile of a pure D2/D3 receptor blocker. It is an antipsychotic agent with effective positive and negative side effects, which can improve the functioning and personal satisfaction of patients suffering from schizophrenia. It exhibits antidepressant properties in patients with psychiatric disorders, dysthymia, and major depression [1]. Amisulpride is also an antiemetic agent that prevents and alleviates postoperative nausea and vomiting. It primarily works by blocking dopamine signaling in the chemoreceptor trigger zone, a brain area that relays stimuli to the vomiting center -amisulpride prolactinoma, leading to an association with benign pituitary tumors such as prolactinoma [2]. The chemical structure of amisulpride is shown in (Figure1). The literature survey summarizes that few analytical methods have been reported for the estimation of Amisulpride in pharmaceutical preparations: spectrofluorimetric, High-Performance liquid chromatography [3], capillary electrophoresis [4], Liquid Chromatography–tandem mass spectrometry [5, 6], GC-MS [7]. These highly sophisticated methods have minor drawbacks, such as a lack of sensitivity and time-consuming, tedious, and expensive instruments. The proposed method aims for a simple, rapid, and effective liquid chromatographic method for amisulpride development and validation studies.

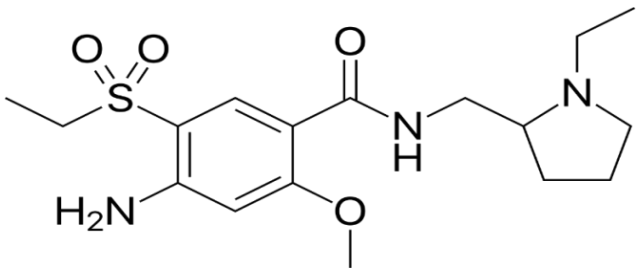

Figure 1: Chemical structure of Amisulpride

EXPERIMENTAL

Chemicals and Reagents: Active Pharmaceutical Ingredient of amisulpride were acquired from Bio-Leo labs, Hyderabad. Tablets (Amispeed) were purchase from local pharmacy which were manufactured by S. P Pharmaceuticals, India. Obtained Ultra-pure water from a Millipore system. Methanol (HPLC grade) was obtained from Merck, India. All other chemical utilized in the analysis were of Analytical grade.

Instrumental and analytical conditions: Reverse phase High-Performance liquid chromatographic analyses were carried out on Shimadzu (LC 2010 CHT) equipped with an Autosampler. The absorbances of amisulpride solution were measured using a Nicolet evolution 100 UV-VIS spectrophotometer and Vision pro Software. Chromatographic separation was achieved by using Phenomenex C_{18} (150 X 4.6mm,5 µm) column. UV detection was performed at 248 nm. UV spectra scanning from 190-400 nm was recorded online for peak identification. Phosphate buffer pH 6.5 and Acetonitrile (60:40, v/v) was used mobile phase. The flow rate was 1.0 mL/min.

Phosphate buffer: To prepare Phosphate buffer pH 5.5 solution 6.8gm of Potassium di hydrogen orthophosphate was included in 1000 ml distilled water. The solution to pH 5.5 was adjusted by using sodium hydroxide.

Preparation of primary and secondary standard solutions: Weighed about 100 mg of amisulpride active pharmaceutical ingredient and transferred into a 100 ml volumetric flask. Initially, added 20 mL of methanol to confirm complete solubilization, and adjusted the volume with the same solvent as a primary standard solution. Further dilutions were prepared to get a final concentration of 100 µg/mL as the secondary standard solution.

Analysis of Marketed formulation: Tablets (each tablet containing 100 mg) were analyzed using the validated method. The average weight of the tablets was determined. Tablets were finely powdered and triturated well. A portion of the powder, equivalent to about 100 mg of amisulpride was carefully weighed and transferred into a 100 mL volumetric flask followed by the addition of 100 mL of methanol. The solution was sonicated for 35 min and dilute with it. Moreover, the dilutions were made to get the final concentration to 100 µg/mL. The obtained solution was filtered using a membrane filtered (0.45 µm).

Method validation [8]

Calibration Curve: Standard stock solutions containing 1 mg/ml of amisulpride were prepared. Aliquots of these solutions were diluted with the diluent to five different concentrations, corresponding to 50–150 µg/ml of amisulpride. The calibration curve for different concentrations versus peak area was plotted for amisulpride. The obtained data were subjected to regression analysis using the least squares method with a weighting factor of $1/x$.

Precision: The precision was determined for intermediate precision i.e. intraday and interday. The intraday precision was evaluated by analyzing six samples at 100 % of the test concentration (1.0 mg/mL) at a final analyses concentration of 100 µg/mL. Similarly, the interday precision was evaluated on three consecutive days. Finally, the relative standard deviation was calculated for each study.

Accuracy: The standard addition method determines the accuracy of the proposed method by taking amisulpride reference standards that were accurately weighed and added to a commercial formulation of tablet powder at three different concentration levels (50, 100, and 150 µg/mL), respectively. Samples were prepared in triplicate at each level, and the recovery percentage was determined.

Specificity: Spectral purities of amisulpride and chromatographic peaks were evaluated using the UV spectra recorded by the UV detector. Additionally, a solution containing a mixture of the tablet excipients was prepared using the sample preparation procedure and injected into the chromatographic instrument to evaluate possible interfering peaks.

Robustness: Method robustness was assessed by determining system suitability parameters in six solutions at stock concentration. The samples were assayed under nominal conditions and after variations of the following analytical parameters: proportion of organic solvent in the mobile phase (±1%), oven temperature (± ° C) and flow rate (± 0.1 mL/min). The results were evaluated regarding the content of amisulpride (% RSD) and critical responses of the chromatographic systems.

Limit of detection and limit of quantification: Limits were measured based on the standard deviation method. The detection limit is the lowest concentration of the measurement that can be detected at a specified level of confidence. LOQ is the lowest concentration at which the performance of a method or measurement system is the acceptable level of repeatability, precision, and trueness. These two parameters were calculated using the formula LOD = 3.3 ×SD/S and LOQ = 10 × SD/S, where SD standard deviation of response (peak area) and S = slope of the calibration curve.

RESULTS AND DISCUSSION

It is essential to develop reliable separation methods for the quality control of drug molecules, which will allow the separation, identification, and determination of content and its impurities. At present work, the reverse phase high-performance liquid chromatographic method for the quality control of amisulpride was developed and validated. In Figure 2, the chromatogram represents amisulpride with an average retention time of 4.25 ±0.3 min.

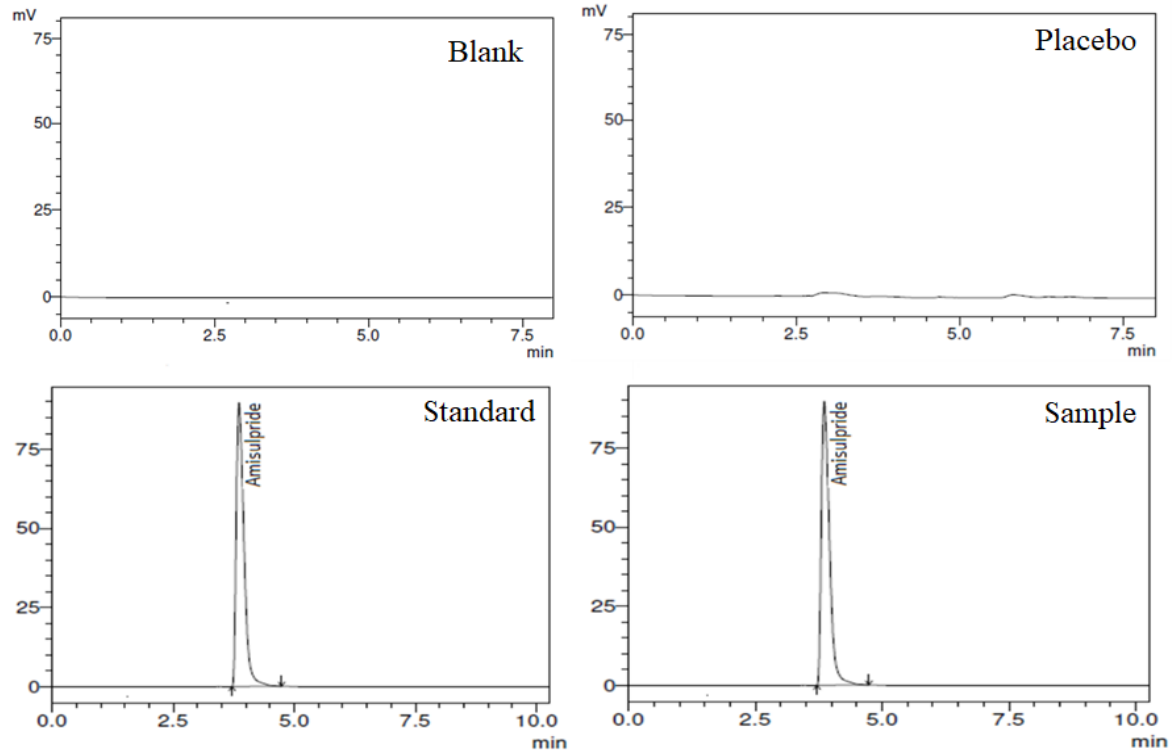

Figure 2: Chromatograms of blank, placebo, standard and sample for amisulpride

Specificity: The specificity of the method was monitored by analyzing the placebo and standard solution. No peak was detected close to the retention time of amisulpride which proved the high degree of specificity of the method.

Linearity, quantification limit, and detection limit: A calibration curve was observed between the detector response of amisulpride and its corresponding concentration over 50-150 µg/mL (Figure 3). The obtained data were found to be linear (r > 0.999). The mean linear regression equation of the peak area versus drug concentrations of amisulpride was typically Y = mx + C. The LOQ of this assay method was 0.15 µg/mL with a corresponding relative standard deviation method. The LOD was found to be 0.47 µg/mL.

Table 2 Analytical Performance data for the determination of the amisulpride

Parameters	Result
Working range (µg/mL)	50-150 (µg/mL)
Intercept	22947
Slope	44556
Correlation coefficient (r)	0.9995
LOD (µg/mL)	0.15 µg/mL
LOQ (µg/mL)	0.47 µg/mL

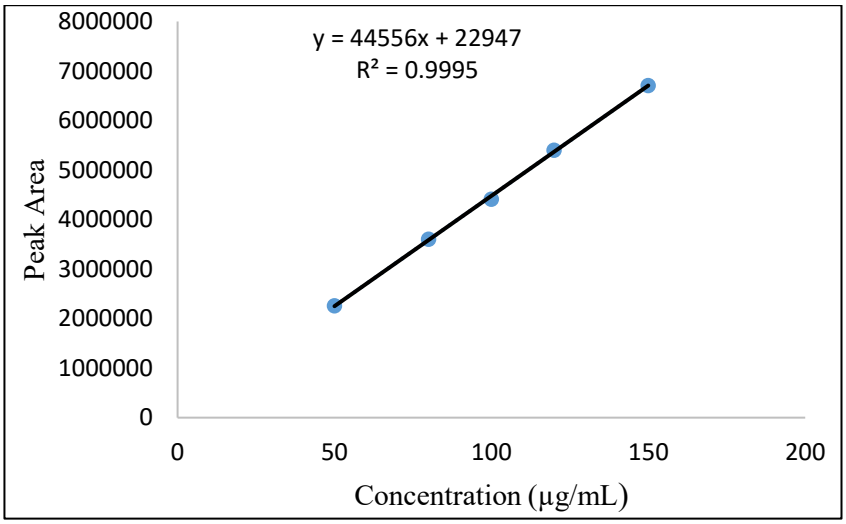

Figure 3: Linearity graph for amisulpride

Accuracy: The solutions were reanalyzed by proposed method, results of recovery studies are reported in table 2 which showed that % amount found was between 99.89 and 102.32 % with % RSD <2, indicating that the developed method is an accurate method for determination of amisulpride.

Table 2: Statistical analysis for Accuracy of the proposed method

Component level	Amount of drug added (µg/mL)		Amount recovered (µg/mL)	% Recovery	% RSD
	Pure	Formulation			
50 %	50	25	75.46	100.61	0.37
	50	25	74.92	99.89	
	50	25	75.31	100.42	
100 %	50	50	100.79	100.78	0.77
	50	50	101.23	101.23	
	50	50	102.32	102.32	
150 %	50	75	125.20	100.16	0.26
	50	75	125.43	100.34	
	50	75	125.85	100.74	

Precision: The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). These results show the reproducibility of the assay. The % RSD values found to be less than two indicate this method was precise for determining amisulpride in the marketed formulation. The results of precision (intra and inter-day precision) are shown in Table 3.

Table 3: Precision studies

Component	Concentration (µg/mL)	Intraday precision[a]	Interday precision[a]
Amisulpride	100	100.96	102.10
	100	100.98	102.48
	100	101.11	102.10
	100	102.20	102.10
	100	102.34	102.12
	100	102.32	102.83
	% RSD	0.68	0.30

[a]Average of three estimates

Analysis of amisulpride in the dosage form: Experimental results of the amount of amisulpride in the selected commercial tablets, expressed as a percentage of label claim were in good agreement with the label claims hereby suggesting that there is no interference from any of the excipients which are typically present. The drug content was found to be 98.06% for amisulpride.

Table 4: Results of table assay (n =3)

Drug	Label Claim	Amount of Drug estimated (mg/tab)	Assay
Amisulpride (Amispeed)	100 mg	99.6 ± 0.528	98.06 ±1.29

Robustness: Robustness was evaluated to ensure that the HPLC method is insensitive to small changes in the experimental conditions. In this study, the wavelength and flow rate were changed. None of the modifications caused any significant change in the response of amisulpride peaks.

Table 5: Robustness study

Parameter	Variation	Observed value		
		RSD % of area	Tailing factor (T)	Theoretical plate (N)
Flow rate	0.4 mL/min	0.37	1.74	5550
	0.6 mL/min	0.77	1.23	5869
Wavelength	246 nm	0.25	1.53	5852
	250 nm	0.67	1.59	5436

CONCLUSION

The developed method is rapid, robust, precise, and accurate. Statistical analysis proves that the method is suitable for the analysis of tablet dosage form shows that excipients are not interfere with the analytical determination. Thus, the method can be applied for its estimation in biologicals fluids

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The authors do not have any competing interest in this research work.

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