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Novel RP-HPLC method for the determination of Amisulpride in pure and pharmaceutical formulations

P. Ravisankar[*1,2] and G. Devala Rao[3]

[1]*Department of Pharmaceutical Analysis and Quality Assurance, Vignan Pharmacy College, Vadlamudi, Guntur (Dist.), Andhra Pradesh, India*
[2]*Faculty of Science, Sri Chandrasekharendra Saraswathi Viswa Maha Vidyalaya (SCSVMV University), Enathur, Kanchipuram, T.N., India*
[3]*Department of Pharmaceutical Analysis, KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada, Andhra Pradesh, India*

ABSTRACT

The core aim of present work was to develop a simple, precise, rapid and reproducible isocratic reverse phase high performance liquid chromatographic (RP-HPLC) method for the estimation of Amisulpride (AMS) in pure and in tablet dosage form. An isocratic RP-HPLC was performed on a Phenomenex C_{18} column (250 x 4.6 mm, 5 micron size column, ambient temperature with mobile phase containing phosphate buffer duly adjusted to pH 4.1 with ortho phosphoric acid and acetonitrile in the ratio of 80:20 v/v. The flow rate was 1 mL/min and eluent was monitored at 227 nm. The chromatogram of AMS was found that the retention time is 5.601 min. The proposed method was linear to the concentration verses peak area responses which was found in the range of 2-10 µg/mL with R^2 value of 0.9999. The reproducibility results of AMS were observed to be % 0.039 (% RSD) which speaks that this method can be reproducible for different tablets. The % RSD values were found to be less than 2 within the limit The percentage recoveries of active pharmaceutical ingredient from dosage forms ranged from 99.53 to 99.72 which indicate that the proposed method to be an accurate. The LOD and LOQ were found to be 0.0175 and 0.0577 respectively. The positive results of robustness of this method permits for extensive application for analysis of drugs. The % assay values for Sulpitac and Solian are 99.16±0.21and 99.85±0.11respectively. This method was specific as no interferences were detected at its retention time of peak drug. Therefore this RP-HPLC method is highly convenient, accurate, precise and economical with less retention time and can be used for the routine determination of AMS in pharmaceutical dosage form.

Key words: Amisulpride, RP - HPLC, Validation, Quantitative determination, ICH guidelines.

INTRODUCTION

AMS [1] belongs to a group of medicines called substituted benzamide antipsychotics. AMS used for easing the symptoms of severe or sudden (acute) and ongoing or long term (chronic) schizophrenia, a sort of mental health condition which affects the way of thinking, feeling or action. Symptoms of schizophrenia such as hallucinations seeing, hearing or sensing which are not apparent in the patient, delusions, unusual suspiciousness, emotional or social withdrawal. People with schizophrenia may also feel into depression, tension, anxiety or uneasiness. AMS can help to get rid of these symptoms. At higher doses it inhibits postsynaptic dopamine D2 and D3 receptors, which results in reducing dopaminergic transmission. The drug is available from branded companies in four different strengths ranging from 50 to 400 mg with terminal half life of 12 hours. The chemical nomenclature of AMS is 4-Amino-N-[(1-ethyl-2-pyrrolidinyl) methyl]-5-(ethylsulphonyl)-2-methoxy benzamide and its molecular formula is $C_{17}H_{27}N_3O_4$ and molecular weight is 369.5 g/mol. The commercial formulations of AMS in tablet form with 50 mg, 100 mg and 200 mg manufactured on the brand name of Amisyt by East West pharma were obtained locally and

utilized in the analysis of the drug. Amisulpride (AMS) is not official drug in BP and USP. Extensive literature studies on the developed analytical methods on Amisulpride revealed that very few analytical methods have been forthcoming to determine this drug in pharmaceutical dosage form. Some of them are non-aqueous titration [2], chromatographic, electrophoretic and spectrophotometric methods [2-7], HPLC [8], LC-MS [9] for the estimation of AMS were reported. But very few RP-HPLC methods have been developed for determination of AMS in the tablet form. However, the requirement of very simple, fast, efficient, accurate, precise, time-saving and highly reliable analytical RP-HPLC method for routine quality control purpose always necessities to see a new and better method. Thus the author has aimed to develop an efficient method that could determine AMS in its formulations. Therefore keeping all these in view, there is ever imperative need to develop a method which is simple and fast and economical. The structural formula of the AMS drug is shown in Figure 1.

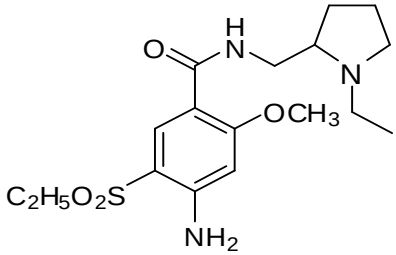

Figure 1. Chemical structure of Amisulpride

EXPERIMENTAL SECTION

All the chemicals and reagents used in the present study were of Anal R grade and solvents were of HPLC grade. To attain high accuracy and reliability of the results of the research work, calibrated glassware (Borosil, India) was used. Almost all the glass ware employed for volumetric and general procedure in the entire research study were washed thoroughly with triple distilled water and then rinsed with methanol and dried well before use. The commercial tablets of Amisulpride are procured from local market. The details of procured materials are shown in Table 1 and the details of instruments used are shown in Table 2.

Table 1. Drugs and chemicals procured and used for the present study.

S.No.	Materials	Procured from
1.	Amisulpride reference standard	Hetero Labs Ltd., Hyderabad
2.	Acetonitrile HPLC grade	Merck pharmaceutical pvt. Ltd., Mumbai.
3.	Water HPLC grade	Merck Specialties Pvt. Ltd., Mumbai.
4.	Methanol HPLC grade	Merck Specialties Pvt. Ltd., Mumbai.
6.	Potassium dihydrogen phosphate	S.D. Fine Chem. Ltd., Mumbai, India
7.	O-Phosphoric acid	S.D. Fine Chem. Ltd., Mumbai, India
8.	Triethylamine	Merck Pharmaceuticals Private Limited, Mumbai.

Table 2. Instruments used for the present study.

S. No.	Name of Instrument	Model	Manufacturer
1	Digital balance	Essae vibra AJ (0.001g)	Essae-Teraoka Ltd.,
2	Ultrasonic bath sonicator	Model no-91250 mode	PCI Ltd., Mumbai.
3	pH meter	Elico LI120 pH meter	Elico India Ltd.,
4	Spectrophotometer	Elico SL159 UV-Vis Spectrophotometer	Elico India Ltd.,
5	HPLC	Shimadzu HPLC, Class VP series with two LC-10AT VP pumps.	Shimazdu

Preparation of standards

Preparation of mobile phase

By dissolving potassium dihydrogen orthophosphate of 1.3609 g in 1000 mL of HPLC grade water, 10 mM phosphate buffer was made ready by adding 1 mL of trietyl amine duly adjusting pH to 4.1 with orthophosphoric acid. To the said phosphate buffer acetonitrile was thoroughly mixed in the ratio of 80:20 v/v and the mobile phase so prepared was filtered through 0.22 µm nylon membrane filter and properly degassed by way of sonication.

Preparation of standard AMS drug solution

100 mg of pure AMS was accurately weighed and allowed to dissolve in mobile phase of 100 mL in a standard 100 mL volumetric flask and obtained 1mg /mL stock solution. A series of five standard solutions in the separate concentration range of 2 mg /mL, 4 µg/mL, 6 µg/mL, 8 µg/mL and 10 µg/mL were prepared by suitable dilution of the stock solutions with the mobile phase.

Preparation of sample

20 tablets of AMS was poured into a mortar and thoroughly ground into smooth powder. Out of this tablet powder equivalent to 100 mg of AMS was taken and extracted in 100 mL quantity of mobile phase and the resulting solution so obtained was filtered through 0.22 µm nylon membrane filter duly degassed by way of sonication. The same solution was further diluted stepwise with mobile phase as prepared under standard solution to get required distinct concentrations.

RESULTS

Selection of detection wavelength

The chief goal of this study was to develop a rapid RP-HPLC method for the determination of AMS in bulk drug and tablet formulation by utilizing most commonly used column C_{18} with Ultra-violet detection at appropriate wavelength (227 nm). By using UV spectrophotometer the AMS solution was scanned in the region of 200 - 400 nm in spectrum mode. The outcome of result showed that the maximum absorption of Amisulpride was noticed at 227 nm and this maximum absorbance was used as detection wave length. The maximum absorbance curve for AMS is noted in Figure 2.

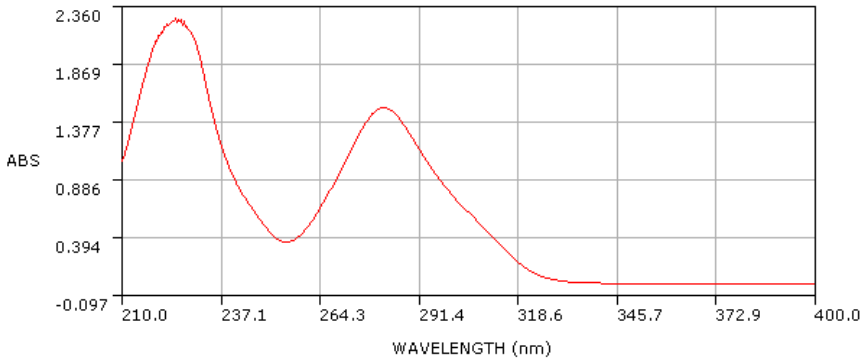

Figure 2. UV spectrum of Amisulpride

Optimization and method development

The main intention of method development is that all the required chromatographic conditions are inevitably optimized. To get appropriate optimized HPLC conditions different analytical columns with several mobile and stationary phases, flow rate and buffers of pH were keenly observed in the present investigation. Finally a mobile phase consisting of phosphate buffer duly adjusted to pH 4.1 with ortho phosphoric acid, acetonitrile in the ratio of 80:20 v/v and stationary phase made up of Phenomenex C_{18} column with 4.6 X 250 mm, 5 µm particle size was noted and the mobile phase as well as the detection wave length was correctly adjusted to 1mL/min and 227 nm respectively at ambient column temperature and found to be aptly suitable to analyze the AMS. As a result of which peaks of good shape were observed and the obtained peak was symmetrical and tailing factor was within the specified limits. Summary pertaining to optimized chromatographic conditions of the proposed method is mentioned Table 3.

Table 3. Optimized chromatographic conditions for the proposed method M_{11}

Parameter	Chromatographic conditions
Instrument	Shimadzu HPLC, Class VP series with two LC-10AT VP pumps.
Column	Phenomenex C_{18} Column (4.6 X 250 mm, 5 µm)
Detector	SPD - 10A VP UV-Vis detector
Diluents	10 mM Phosphate Buffer(pH-4.1) : Acetonitrile (80:20 v/v)
Mobile phase	10 mM Phosphate Buffer(pH-4.1) : Acetonitrile (80:20 v/v)
Flow rate	1mL/min.
Detection wave length	By UV at 227 nm.
Run time	8 minutes.
Temperature	Ambient temperature (25 ^{o}C).
Volume of injection loop	20 µL.
Retention time (R_t)	5.601 minutes.

Recommended procedures
After close observation of the concerned parameters based on the detailed results obtained and discussions of this part the following procedures were recommended for deciding AMS in bulk samples as well as pharmaceutical formulations.

For bulk samples
To get a stable base line the HPLC system was stabilized for 40 minutes subject to the chromatographic conditions described in Table 3. One blank followed by 6 replicates of a single standard solution was injected to check the system suitability. The standard solutions with five replicates of 2 µg/mL, 4 µg/mL, 6 µg/mL, 8 µg/mL and 10 µg/mL were injected. The retention time and average peak areas were noted. By putting concentrations of AMS on X - axis and peak areas on Y-axis, calibration graph was drawn duly counting the quantity of AMS drug existed in the sample drug with the aid of calibration graph.

For pharmaceutical formulations
Twenty tablets of the drug was put into a mortar and crushed to a smooth powder. From this grounded tablet powder equivalent to 50 mg of AMS was correctly taken and allowed to extract in 100 mL of mobile phase. The solution so obtained was filtered through 0.22 µg nylon membrane filter and degassed by way of sonication. The said solution was again diluted suitably for chromatography. The results of assay in respect of the proposed method are mentioned in Table 12.

Method validation
According to the guidelines of ICH Q2 (R1) all the parameters as discussed bellow were analyzed and validated accurately following the procedure of the proposed method.

System suitability
During analytical method development system suitability test (SST) gives an added level of confidence that the accurate mobile phase, flow rate, temperature, and column were used which ensures the system performance (pump and detector) where in parameters of System suitability such as retention time, resolution, efficiency (number of theoretical plates) and tailing factor are involved and they should be within the defined limits. The results of system suitability in respect of the proposed method are mentioned in Table 4.

Table 4. System suitability results for the proposed method

S.No.	Parameters	Results*
1	Retention time	5.601 minutes
2	Theoretical plates	9,503
3	Theoretical plates per meter[t.p/m]	1,90,060
4	Tailing factor	1.163
5	Resolution	-

**Results of triplicate values (n=3)*

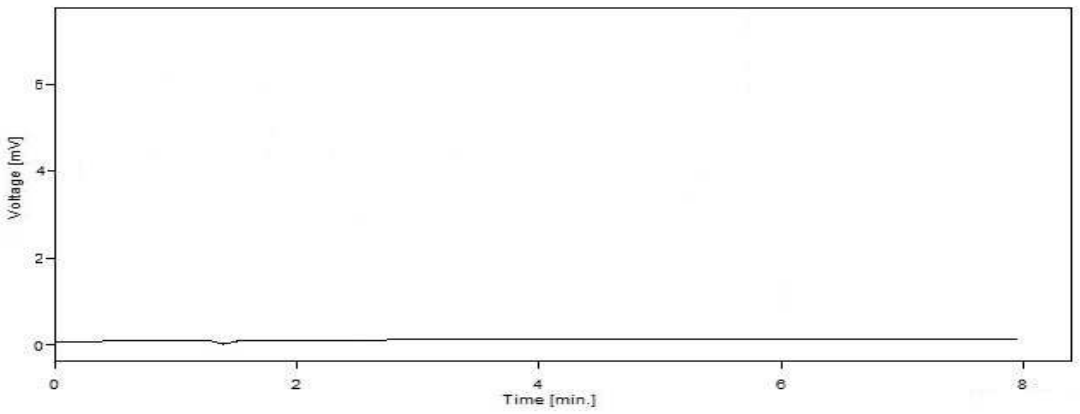

Figure 3. Chromatogram of the blank solution

Specificity
The specificity test of this new method demonstrated that the common excipients like lactose anhydrous, microcrystalline cellulose and magnesium sterate have been added to a sample solution by way of injection into the HPLC system. It was clearly observed that the said excipients from the sample did not meddle with the peak drug which speaks that they did not disturb the elution of the Amisulpride. It was observed that there was no interference

from placebo solution at the retention time of an Amisulpride peak. Figure 3 and Figure 4 shows the chromatogram of blank and chromatogram of AMS synthetic drug.

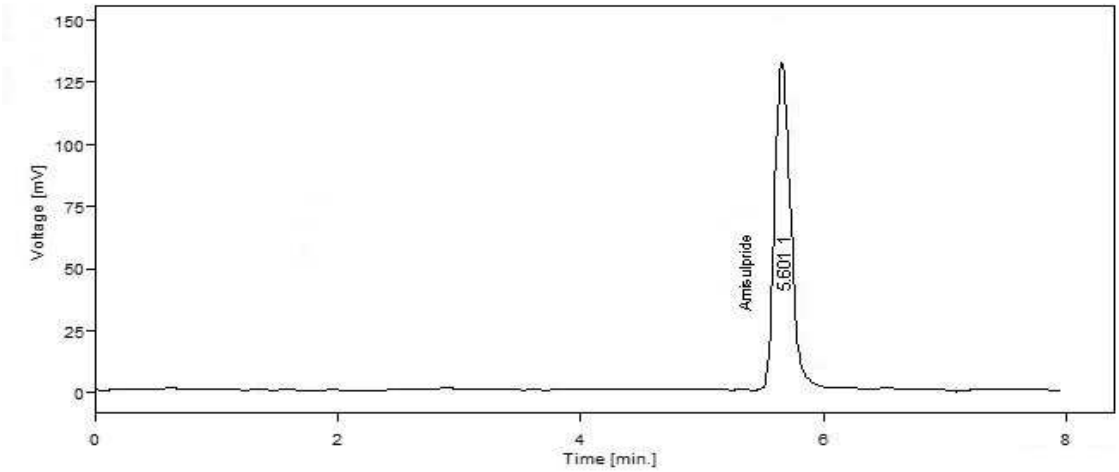

Figure 4. Chromatogram of Amisulpride synthetic drug

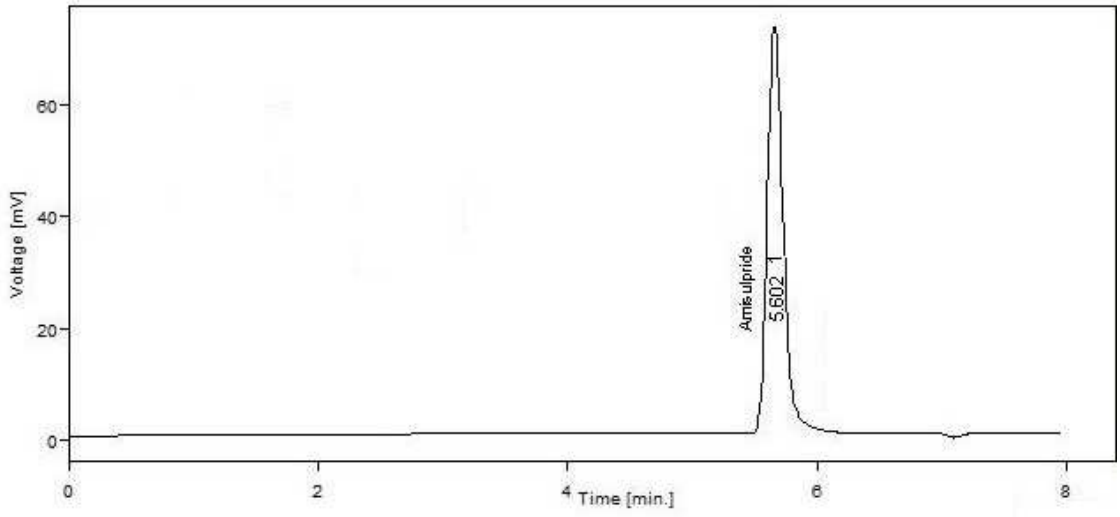

Figure 5. Standard chromatogram of Amisulpride (2 µg/mL)

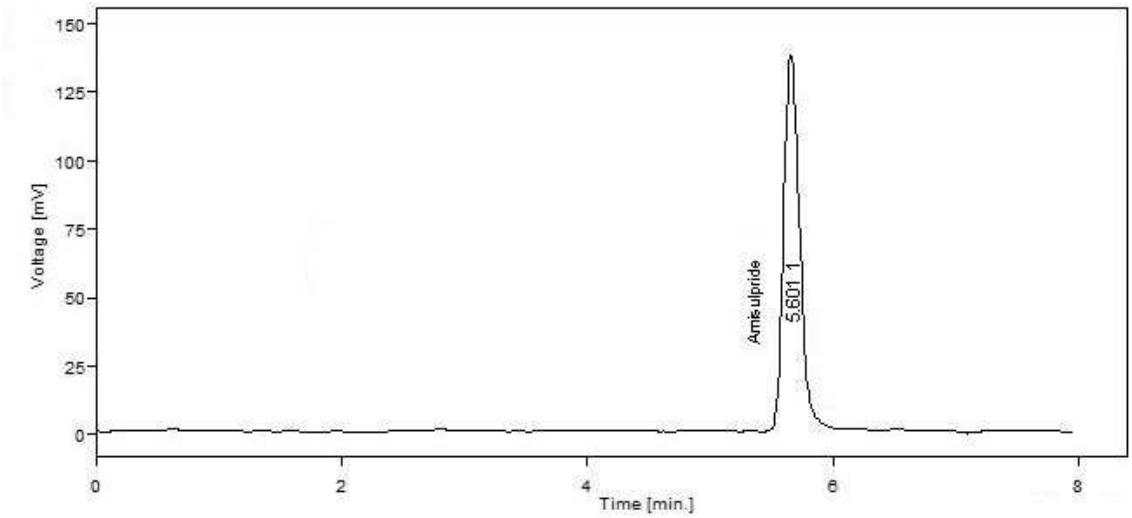

Figure 6. Standard chromatogram of Amisulpride (4 µg/mL)

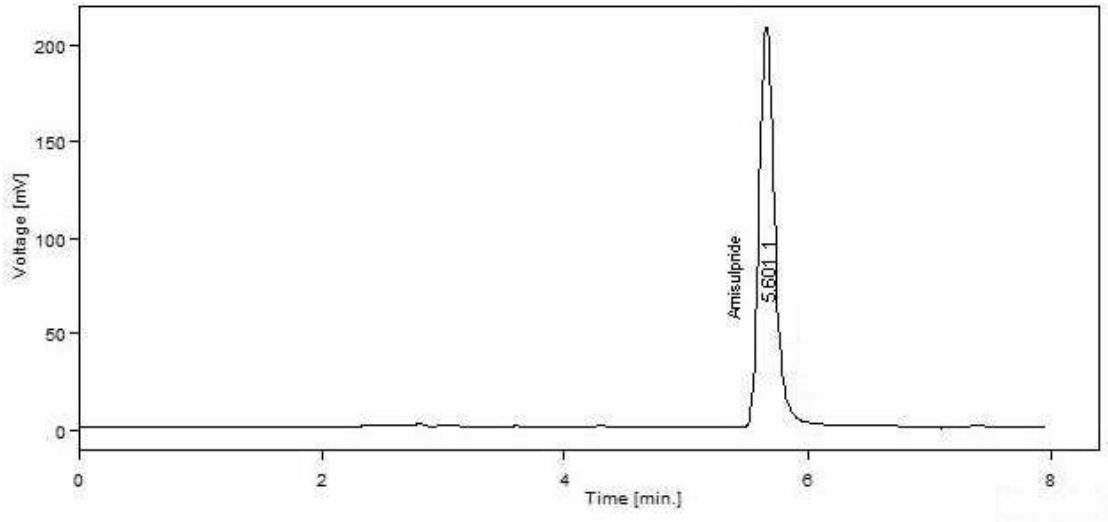

Figure 7. Standard chromatogram of Amisulpride (6 µg/mL)

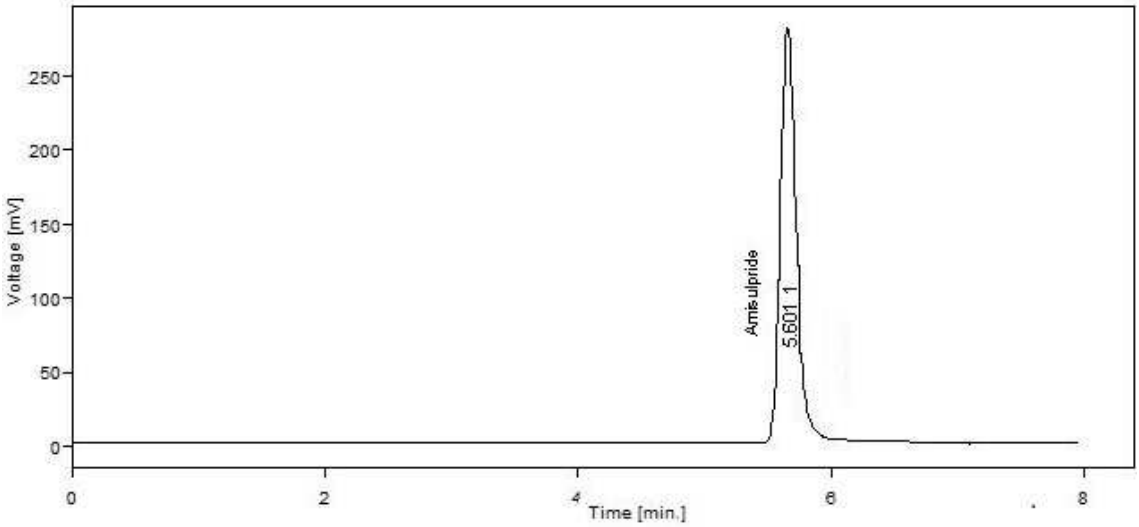

Figure 8. Standard chromatogram of Amisulpride (8 µg/mL)

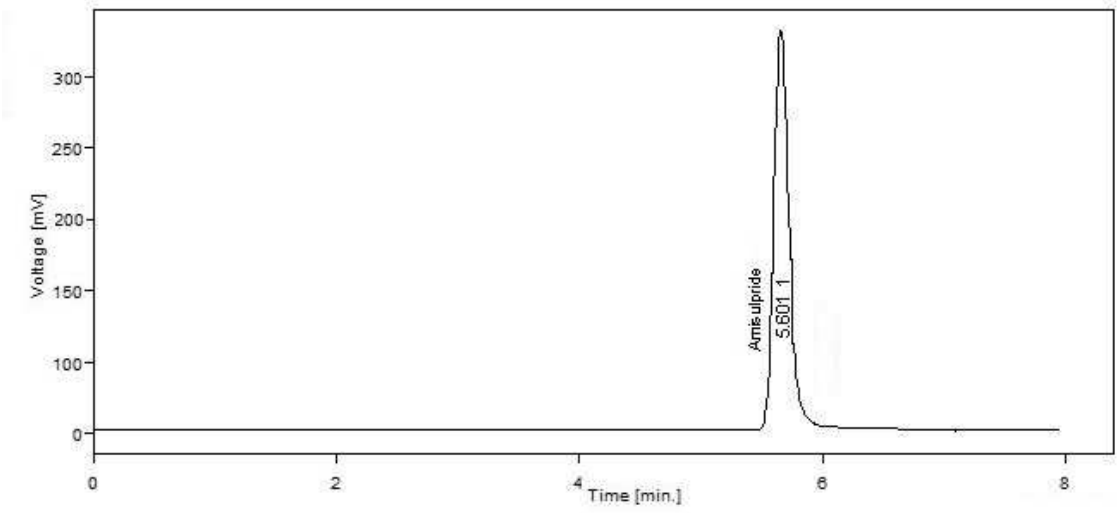

Figure 9. Standard chromatogram of Amisulpride (10 µg/mL)

Linearity

To determine the linearity for proposed AMS drug, standard solutions at separate concentrations over the concentration range of 2 - 10 µg/mL AMS was taken and the method of least square analysis was performed to obtain slope, intercept and correlation coefficient values. Figure 5 to 9 shows the representative chromatograms and

the calibration curve of AMS is shown in Figure 10. Linearity data and regression analysis data of the AMS are shown in Table 5 and 6 respectively.

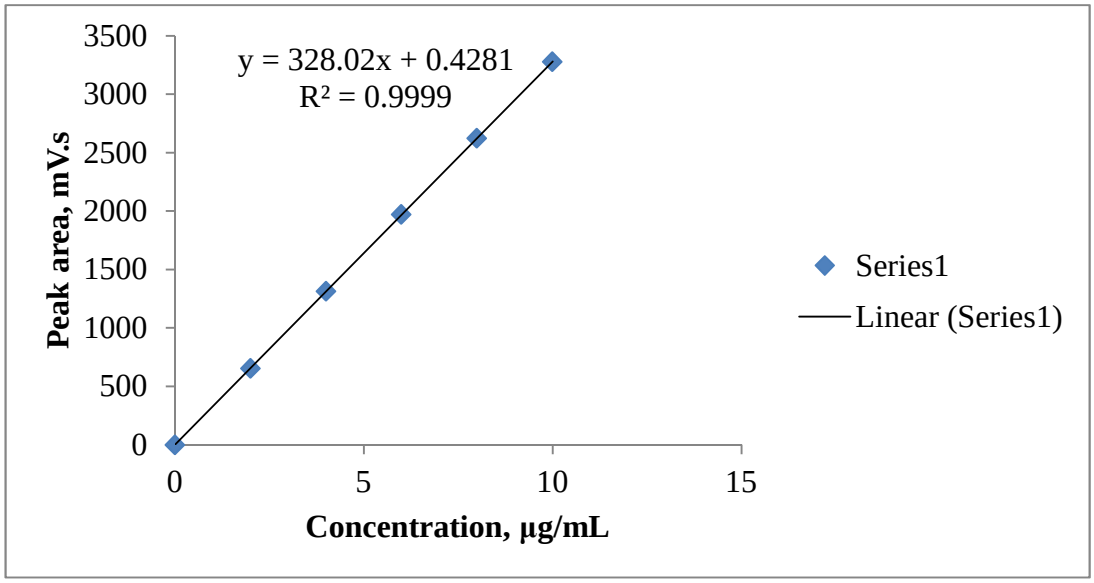

Figure 10. Linearity plot for Amisulpride

Table 5. Linearity data of Amisulpride

S. No.	Concentration(µg/mL)	Peak area, mV.s.
1	0	0
2	2	654.12
3	4	1314.28
4	6	1971.42
5	8	2625.21
6	10	3278.25

Table 6. Regression analysis data of the Amisulpride

Parameter	Method
Detection wavelength (λ max)	By UV at 227 nm
Linearity range (µg/mL)	2-10 µg/mL
Regression equation (Y=a+bx)	Y=328.023x +0.4281
Slope (b)	328.023
Intercept (a)	0.4281
Standard deviation of slope (S_b)	0.28737
Standard deviation of intercept (S_a)	1.74013
Standard error of estimation (Se)	2.40433
Correlation coefficient (R^2)	0.9999
Percentage range of errors* (Confidence limits) 0.05 significance level 0.01 significance level	 0.0627 0.0816

**Average of six determinations*

Precision

The main reason of the study of precision is to establish that the promoted RP - HPLC is accurate for analyzing AMS in pharmaceutical formulations as well as bulk forms. The following precision analysis was carried out.

To determine the intra-day precision, sample drug solution AMS of 10 µg/mL was prepared with mobile phase separately for six times and same process of analysis was held six times in the same day. The intermediate precision (inter-day precision) was determined by analyzing fresh sample solutions of AMS continuously for six successive days. The mean, SD and % RSD were calculated and it was noticed that it was lying within the agreeable criteria of 2.0. The results of intra as well as inter-day precision study are shown in Table 7 and 8.

Table 7. Intra-day precision results of Amisulpride

Repeatability		Injection no.	Peak area (mV.s)	% Assay	% RSD n=6
Sample	Concentration (µg/mL)				
Amisulpride	6 µg/mL	1	1970.32	100.090	0.024
		2	1971.42	100.145	
		3	1970.54	100.101	
		4	1971.12	100.130	
		5	1970.13	100.080	
		6	1970.65	100.106	

Table 8. Inter-day precision results of Amisulpride

Intermediate precision		Injection no.	Peak area (mV.s)	% Assay	% RSD n=6
Sample	Concentration (µg/mL)				
Amisulpride	6 µg/mL	1	1971.32	100.140	0.027
		2	1970.16	100.081	
		3	1970.76	100.112	
		4	1970.12	100.079	
		5	1970.32	100.090	
		6	1971.23	100.136	

Accuracy (Recovery studies)

Known quantity of pure AMS drug was added to the already analyzed formulation and the accuracy of the method was evaluated three times with three separate concentrations equal to 50 %, 100 % and 150 % of the active ingredient. Accuracy was determined by calculating the concentration of recovered amount of ASP % RSD of recovery and % recovery of each concentration. The Table 9 shows the recoveries of the drugs from the spiked concentrations.

Table 9. Recovery results of Amisulpride

S. No.	Level of addition (%)	Amount added (µg/mL)	Amount recovered (µg/mL)	Mean % Recovery ± SD	% RSD #
1	50 %	5	4.98	99.53 ± 0.305	0.265
		5	4.96		
		5	4.98		
2	100 %	10	9.98	99.63 ± 0.208	
		10	9.94		
		10	9.97		
3	150 %	15	14.98	99.72 ± 0.279	
		15	14.91		
		15	14.98		

SD = Standard deviation # Average of three levels

Robustness

To establish robustness of the proposed new method analysis of AMS was held under separate experimental conditions by effecting few changes in chromatographic conditions like buffer concentration, pH, flow rate, detection wave length and column temperature but it was noticed that the results obtained were not affected by such small changes. The results achieved are explained in Table 10.

Table 10. Robustness results of Amisulpride

S. No.	Parameter	Optimized condition	Used condition	Peak area (mv.s)	Retention Time (R_t), Minutes	Plate count	Peak asymmetry
1.	Flow rate (± 0.2 mL/min)	1.0 mL	0.8	3284	5.605	9510	1.065
			1.0	3278	5.601	9503	1.063
			1.2	3270	5.598	9498	1.064
2.	Detection wavelength (± 5 nm)	215 nm	210	3278	5.601	9504	1.064
			215	3278	5.601	9503	1.063
			220	3279	5.602	9503	1.163
3.	Mobile phase composition (± 5 v/v)	80:20	85:15	3280	5.603	9502	1.063
			80:20	3278	5.601	9503	1.063
			75:25	3274	5.596	9501	1.162

LOD and LOQ

LOD and LOQ for AMS were evaluated by injecting a series of solutions duly diluted with known concentrations. Based on the peak response and slope of the regression equation of the parameters of LOD and LOQ were decided.

The LOD of drug noticed as 0.0175 µg/mL and LOQ was found to be 0.0577 µg/mL. By adopting the following formula LOD = 3.3 (SD)/S and LOQ= 10 (SD)/S, where SD = standard deviation of response and S = slope of the calibration curve were computed. Table 11 represents the LOD and LOQ results.

Table 11. LOD and LOQ results of Amisulpride

Limit of Detection (LOD)	0.0175 µg/mL.
Limit of Quantitation (LOQ)	0.0577 µg/mL.

Table 12. Assay results of Amisulpride.

S. No.	Pharmaceutical Formulations	Labeled amount	Amount found* (mg) (mean ± SD)	% Assay ± SD
1	Amysyt	50 mg	49.58 ± 0.21	99.16 ± 0.21
2	Solian	100 mg	99.85 ± 0.12	99.85 ± 0.11

** = Mean of six determinations*

DISCUSSION

Soon after completion of thorough validation of the developed method it is keenly noted that the data obtained for all the parameters are quite satisfactory as the % RSD was detected to be less than 2 in all parameters. The proposed method was linear to the concentration verses peak area responses which was found in the range of 2 - 10 µg/mL with r^2 value of 0.9999. It was observed that the % RSD values of Precision for intra-day and inter-day precision was 0.024 and 0.027 respectively pertaining to AMS and the values of % RSD were found to be within the limit which clearly shows that the proposed method was fairly precise and reproducible. The percentage recoveries of active pharmaceutical ingredient from dosage forms ranged from 99.53 % to 99.72 % indicate that the proposed method to be fairly an accurate one. This method was said to be specific to AMS drug as no interferences of excipients and other additives were detected with the peaks of analytes. The positive results of robustness of this method permits for extensive application for analysis of drug. The LOD and LOQ were found to be 0.0175 and 0.0577 respectively. From the above observation it was noticed that the proposed method is sensitive. The assay results of different pharmaceutical formulations reveal that the proposed method is suitable for their analysis with virtually no interference of the usual additives presented in pharmaceutical formulations.

CONCLUSION

The present proposed developed RP - HPLC method for the quantification of AMS has numerous advantages like less retention time, excellent peak symmetry and good linearity, highly sensitive, simple, precise, accurate and robust and less retention time which were found with this analysis. The mobile phase can be easily prepared and diluent is economical and readily available. With this RP - HPLC method though mobile phase is necessary there is no need of modern techniques or instruments are necessary for the preparation of mobile phase in this regard. The proposed method can be used for the routine analysis of AMS in bulk preparations of the drug and in pharmaceutical dosage forms for routine application in quality control laboratories without interference of excipients existed in the pharmaceutical preparations. This method is practically proved to be validated in all respects according to ICH Q2 (R1) guide lines and results clearly states the good quality of the method and it is useful for the quality control analysis of AMS in routine manner in active pharmaceutical ingredient (API) and pharmaceutical preparations.

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REFERENCES

[1] Current Index of Medical Specialities (CIMS-129, Apr-July [update 2]) UBM Medica India Private Limited, Bangalore, **2015**; 185.
[2] British Pharmacopoeia (2012) Her Majesty's Stationary Office British Pharmacopoeia Commission. London; Vol. I, *Ph Eur monograph* 1440 online.
[3] R. Skibinski; L. Komsta Hopkala; I. Suchodolska. *Anal. Chim. Acta*. **2007**, 590 (2), 195-202.
[4] Ch. Devadasu; P. Ravisankar; P. Srinivasa Babu; Devala Rao G. *The Indian pharmacist*, **2011**, 9 (7), 70-74.
[5] P. Ravi Sankar; Ch. Devadasu; S. Sowjanya; P. Srinivasa Babu; G. Devala Rao. *Int. J. Chem. Sci.*, **2012**, 10 (1), 203-212.

[6] S. Sharma; M. Neog. *Anal. Sci.*, **2010**, 26 (4), 485-489.
[7] Syeda Humaira; AK. Day; SA. Raju. *Int. J. Chem. Sci.*, **2008**, 6 (1), 437-440.
[8] Ch. Devadasu and P. Ravisankar. *Int. J Res Pharm Biomed Sci.*, **2011**, 2 (1), 202-211.
[9] CH. Balasekhara Reddy; Babu Rao. *Sci*.*Pharm*.**2011**, 79 (3), 583-599.
[10] International Conference on Harmonization. Validation of Analytical Procedures: Text and Methodology Q2 (R1), **2005**.