Short communication

SPECTROPHOTOMETRIC DETERMINATION OF AMISULPRIDE

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Abstract

Two new simple, sensitive and rapid spectrophotometric methods were developed for the quantitative estimation of amisulpride (AMS) in bulk drug and pharmaceutical formulations (tablets). The methods are based on the reaction of amisulpride with sodium nitrite in acid medium to form diazonium salt, which is coupled with phloroglucinol (Method A) and resorcinol (Method B) to form azo dyes showing absorption maxima at 418.5 nm and 403.8 nm respectively. Beer's law is obeyed in the concentration range of 2-10 μg/mL of amisulpride for method A and 4-20 μg/mL of amisulpride for method B. The molar absorptivity and sandell's sensitivity of AMS-phloroglucinol and AMS-resorcinol are 1.8 x 10⁴, 0.25 and 1.1 x 10⁴, 0.0625 respectively. The optimum reaction conditions and other analytical parameters were evaluated. The methods were successfully extended to pharmaceutical preparations (tablets) containing amisulpride.

Key words: *Amisulpride, Spectrophotometric Determination, Phloroglucinol, Resorcinol*

Amisulprid'in Spektrofotometrik Miktar Tayini

Hem saf halinde hem de farmasötik preparatlarda (tablet) amisulprid'in miktar tayini için iki yeni basit, hassas ve hızlı spektrofotometrik yöntem geliştirilmiştir. Yöntemler, amisulprid'in asidik ortamda sodium nitrit ile dizonyum tuzu meydana getirmesi ve bunun floroglusinol (metod A) ve resorsinol (metod B) ile kuplajı sonucunda sırasıyla 418.5 nm ve 403.8 nm lerde maksimum absorpsiyona sahip azo boyaları meydana getirmesine dayanır. Amisulprid için Beer kanunu A metodunda 2-10 μg/mL, B metodunda 4-20 μg/mL aralığında geçerlidir. AMS-floroglusinol ve AMS-resorsinol için molar absorptivite ve Sandall değerleri sırasıyla 1.8 x 10⁴, 0.25 ve 1.1 x 10⁴, 0.0625 olarak bulunmuştur. Optimal reaksiyon şartları ve diğer analitik parametreler tayin edilmiştir. Yöntemler, amisulprid içeren farmasötik preparatlara başarıyla uygulanmıştır.

Anahtar kelimeler: *Amisulprid, Spektrofotometrik tayin, Floroglusinol, Resorsinol*

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INTRODUCTION

Amisulpride (Figure 1)(**AMS**), is chemically, 4-amino-N- {[(2RS)-1-ethyl pyrolidin-2-yl] methyl}-5(ethyl Sulphonyl)-2-methoxy benzamide (1-3) and is used in treatment of Schizophrenia. It has high affinity for dopamine D_2/D_3-receptor antagonist. Chemical structure of Amisulpride is given in Figure 1.
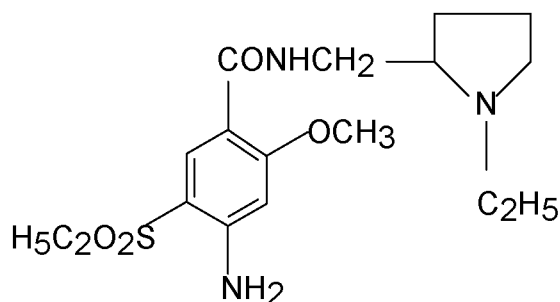

Figure 1. Amisulpride (I)

Literature survey reveals different analytical methods for the estimation of Amisulpride in biological systems like HPLC using either UV (4-5) or fluorescence (6-7) detection, potentiometric analysis (8), an IR, UV spectrophotometric and a HPLC (9) method was reported. A UV spectrophotometric, chromatographic and an electrophoretic method was also reported for the quantitative estimation of amisulpride in pharmaceutical formulations (10).

In the present investigation two new visible spectrophotometric methods were developed for the determination of amisulpride in bulk drug and pharmaceutical formulations. The methods are based on the reaction of amisulpride with sodium nitrite in acid medium at 0 ^{0}C to form diazonium salt, which is coupled with phloroglucinol (II) (Method A) and resorcinol (III) (Method B) to form azo dyes (IV,V) (Scheme 1) (11), showing absorption maxima at 418.5 nm and 403.8 nm respectively. The results obtained were compared with those obtained by literature method (12) and a good agreement was observed from the comparison.

MATERIALS AND METHODS

All spectral measurements were done on shimadzu 1700 UV/Visible spectrophotometer with 1 cm matched glass cells.

Materials and reagents

All chemicals used were of analytical grade and double distilled water was used for preparing the reagent solution. Amisulpride was obtained from Sun Pharma India Ltd, Mumbai.

0.5 % (w/v) aqueous solution of phloroglucinol
0.3 % (w/v) aqueous solution of resorcinol
0.2 % (w/v) aqueous solution of sodium nitrite
0.5 % (w/v) and 5 % (w/v) solution of ammonium sulphamate and 5N HCl were used.

Standard stock solution of amisulpride was freshly prepared by dissolving 100 mg of amisulpride in 20 mL methanol in a 100 mL volumetric flask and diluted upto the mark with water (1 mg/mL). The final concentration of amisulpride was brought to 100 μg/mL with double distilled water.

General Procedure
Method-A
   Aliquots of AMS ranging from 0.2-1.0 mL (1 mL = 100 µg) were transferred into a series of 10 mL volumetric flasks. To this 1 mL of 0.2 % sodium nitrite and 1.5 mL of 5N HCl were added and kept in ice at 0^0C for 10 minutes to allow the diazotisation reaction to complete. Add 1 mL of 5 % of ammonium sulphamate. After 2 minutes add 1 mL of 0.1 % of phloroglucinol. Make up the volume upto 10 mL with double distilled water. After 5 minutes, the absorbance was measured at 418.5 nm against reagent blank.

Method-B
   Aliquots of AMS ranging from 0.4-2.0 mL (1 mL = 100 µg) were transferred into a series of 10 mL volumetric flasks. To this 1 mL of 0.1 % sodium nitrite and 2 mL of 5N HCl were added and kept in ice at $0\ ^0C$ for 10 minutes to allow the diazotisation reaction to complete. Add 1 mL of 0.5 % of ammonium sulphamate. After 2 minutes add 1mL of 0.3 % of resorcinol. Make up the volume upto 10 mL with double distilled water. After 5 minutes, the absorbance was measured at 403.8 nm against reagent blank.

Assay procedure for tablets:
   Accurately weighed quantities of powdered tablets equivalent to 100 mg of drug was dissolved in 20 ml methanol and filtered. The filtrate was made upto 100 mL with double distilled water. Assays were performed as described under general procedure.

RESULTS AND DISCUSSION

   In the present study, two visible spectrophotometric methods were developed for the determination of amisulpride in bulk drug and formulations. These two methods involve the diazotisation of AMS, followed by the coupling of diazonium salt with phloroglucinol (Method A) and resorcinol (Method B) (Scheme 1). The colored azo dyes formed in method A and method B showed absorption maxima at 418.5 nm and 403.8 nm respectively and obeyed Beer's law in the concentration range of 2-10 µg/mL and 4-20 µg/mL respectively.

Optimization of conditions
   Relevant influences of various reaction variables on the color development were tested to establish the most favorable conditions.
   Effect of temperature: The rate of reaction were slow at room temperature, increased gradually upto a maximum when the temperature was decreased to 0 ^0C. The colored azo dyes showed constant maximum absorption at 0 ^0C.
   Effect of essential parameters: The effect of essential parameters for diazotisation like concentration of HCl and sodium nitrite, waiting period, concentration of ammonium sulphamate, waiting period, volume and concentration of phloroglucinol and resorcinol to ascertain optimum conditions were studied by means of control experiments by varying one parameter at a time. The obtained optimum conditions were applied for the assay.
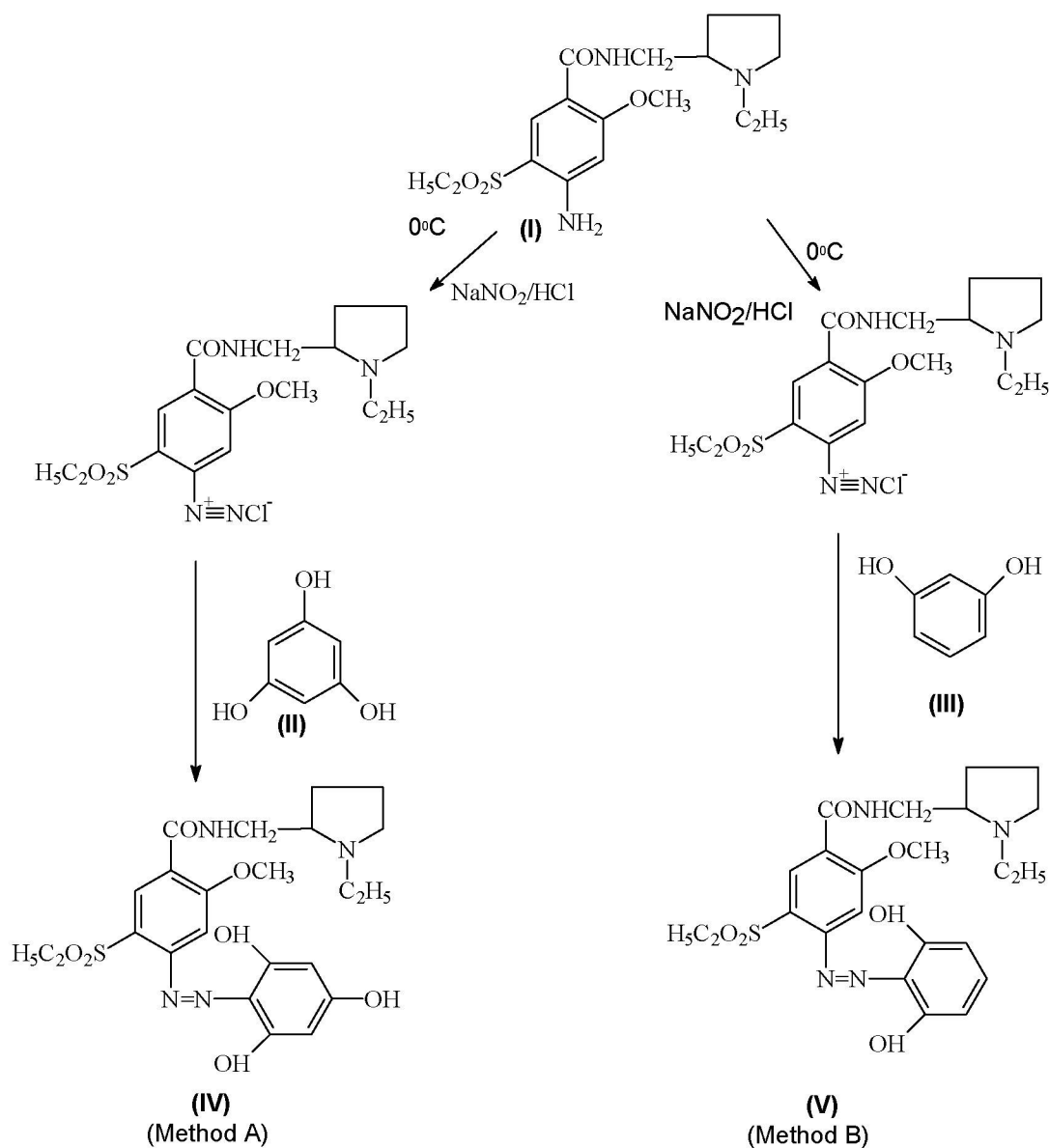
Scheme 1. Reaction mechanism

Under the optimum conditions described as above, the calibration graph for AMS were obtained by using the relationship between concentration and its corresponding absorbance.

The optical characteristics such as absorption maxima, Beer's law limit, molar absorptivity and Sandell's sensitivity are presented in Table 1. The regression analysis using the method ofleast squares was made for the slope (b), intercept (a) and correlation coefficient (γ) for each method are obtained for different concentrations and the results are summarized in Table 1.

The percent relative standard deviation and percent range of error (0.05 and 0.01 level of confidence limits) calculated from the eight measurements, $3/4^{th}$ of the upper Beer's law limits of AMS are given in Table 1. The molar absorptivities of the resulting colored chromogens

indicated good sensitivity of methods. The methods were found to be sensitive with values.The LOD and LOQ values were calculated from the calibration graph using the equation:

$$LOD = 3 \times SD/b$$
$$LOQ = 10 \times SD/b$$

Where SD is the standard deviation of the intercept and b is the slope of the calibration graph. The results are as shown in Table-1.

The proposed methods were compared with UV method (12) and the results obtained were statistically evaluated (Table 2).

For the proposed method, calculated t and F values are lower than theoretical values.
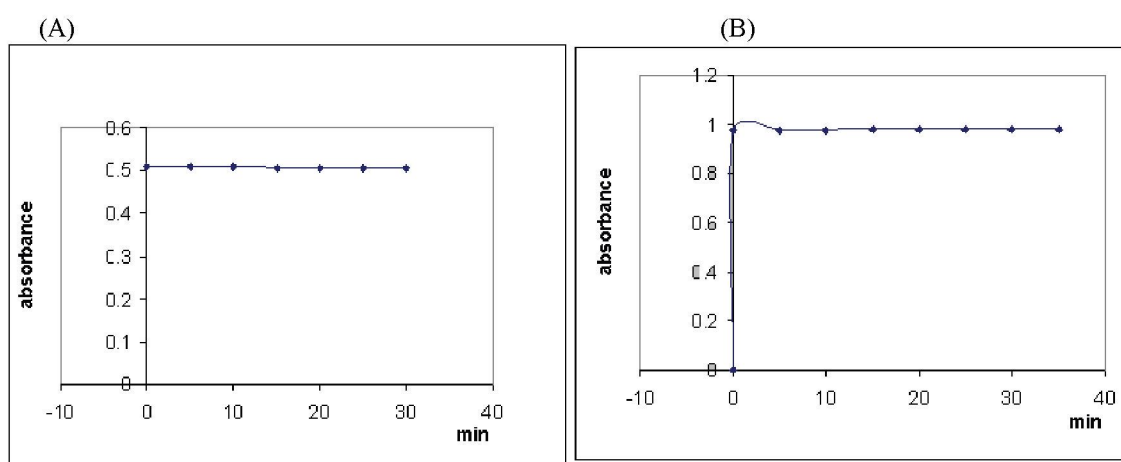
(A)

(B)

Figure 2.The effect of duration on reaction of AMS with phloroglucinol (A) and resorcinol (B)

To test the validity of the method, results for the proposed methods and comparison method were compared and recovery experiments were conducted by adding known quantities of standard drug (100 % purity) to various pre-analysed sample formulations of AMS and then analyzing the mixtures by the proposed method (Table 2).

Precision

The precision for the proposed methods were investigated by intra-day and inter-day determination of AMS with phloroglucinol and resorcinol at three different concentrations (2.6 and 10 µg/mL) and (4.12 and 20 µg/mL) respectively. The intra-day studies performed in one day (for each level n=5) and inter-day studies in five days over a period of two weeks. The intra-day and inter-day precisions are expressed as relative standard deviation (RSD %) and the data obtained (Table 3) proved good precision for the developed method.

Table 1. Optical characteristics and statistical data of the regression equation for AMS reactions with phloroglucinol and resorcinol.

Parameters	Method A	Method B
λ_{max} (nm)	418.5	403.2
Beer's law limits (μg/mL)	2-10	4-20
Molar Absorptivity (L mole^{-1} cm^{-1})	1.8×10^4	1.5×10^4
Sandell's sensitivity (μg cm^{-2} per 0.001 absorbance unit)	0.25	0.0625
Regression equation[b]		
Slope	0.50×10^{-1}	0.45×10^{-1}
Intercept	0.02×10^{-1}	0.05×10^{-1}
Correlation coefficient,	1.0004	0.9931
LOD (μg/mL)	0.066	0.135
LOQ (μg/mL)	0.200	0.411
% RSD	0.32	0.17
Range of Errors**		
Confidence limits with 0.05 level	$\pm$ 0.0008	$\pm$ 0.0008
Confidence limits with 0.01 level	$\pm$ 0.0012	$\pm$ 0.0012

[a]Average of six determinations
[b]A= a+bC (where C is the concentration of AMS in μg/mL

Table 2. Analysis of AMS with resorcinol and phloroglucinol in tablets by the proposed methods and comparison method (UV).

Preparation	Label claim mg/tablet	mg/tablet $\pm$ standard deviation,% (n=6)			t-test[*]	F-test[*]
		Proposed methods		Reference method (12)		
		A	B	UV		
T_1	50 mg	49.95 $\pm$ 0.02	49.45 $\pm$ 0.03	49.56 $\pm$ 0.01	2.02	2.65
T_2	50 mg	49.56 $\pm$ 0.05	49.23 $\pm$ 0.05	49.32 $\pm$ 0.04	1.86	1.13

[a]Theoretical values at 95% confidence limit; t=2.23 and F=5.02

Table 3. Results from intra-day and inter-day precision experiments.

	Actual Concentration µg/mL	Intra-day			Inter-day		
		Xort±SE	RSD (%)	(%) Bias	Xort±SE	RSD (%)	(%) Bias
Method A	2	2.01±4.42	1.99	-0.15	2.12±4.78	2.03	-0.56
	6	6.03±3.46	0.87	-0.17	6.21±3.62	1.99	-1.08
	10	10.03±3.34	1.02	-0.34	10.12±3.72	1.06	-0.45
Method B	4	4.03±1.13	1.78	-0.08	3.39±0.96	1.38	-0.06
	12	12.02±1.14	0.99	-0.12	12.22±1.23	1.13	-1.22
	20	20.13±2.23	0.57	0.16	19.87±0.34	0.33	-0.08

SE: Standard Error

Robustness and Ruggedness

The robustness of the proposed methods were examined by evaluating the influence of small variations of the procedure, variables such as temperature and added reagent volume. For the ruggedness of the method, the proposed methods were carried out by two analysts and no considerable difference was observed (100.66 ± 0.65 Vs 100.81 ± 0.71%).

The obtained reproducible results (Table 4, Figure 2) showed that none of these variables and changes significantly affected the assay of drugs. The developed methods produced are accurate and reproducible results and were applicable to the available dosage forms, tablets.

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Accuracy

In order to determine the accuracy of the proposed methods, recovery measurements were performed on synthetic samples. The tablet excipients used were found not to interfere with the measurements. The results are shown in Table 5.

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Table 4.Results from robustness experiments.

		Average of found concentration	SD	RSD (%)	Recovery (%)
Method A	Added reagent volume(1+0.05mL)	5.08	0.025	0.439	101.2
	Added reagent volume(1-0.05mL)	5.09	0.032	0.576	101.5
	Time of diazotisation reaction(10+0.5min)	5.04	0.019	0.401	101.2
	Time of diazotisation reaction(10-0.5min)	5.07	0.021	0.411	100.8
	Time of coupling reaction(5+0.5min)	5.02	0.016	0.395	101.2
	Time of coupling reaction (10-0.5min)	5.09	0.032	0.576	100.8
Method B	Added reagent volume(1+0.05mL	5.10	0.034	0.584	101.6
	Added reagent volume(1-0.05mL)	5.03	0.019	0.401	100.2
	Time of diazotisation reaction(10+0.5min)	5.09	0.032	0.576	100.2
	Time of diazotisation reaction(10-0.5min)	5.01	0.013	0.375	101.2
	Time of coupling reaction(5+0.5min)	5.07	0.021	0.411	100.6
	Time of coupling reaction (10-0.5min)	5.05	0.021	0.412	100.4

Table 5. Analysis of AMS from various excipients by the proposed method.

AMS	Amount present (mg)	Excipients Talc	Dextrose	Starch	Sodium alignate	Gelatin	Gum acacia	%Recovery±S.D
Method A	50 mg	10	10	10	5	5	5	99.8 ± 0.9
Method B	50 mg	20	30	20	20	10	20	99.5 ± 1.0

^{a}Average recovery from five experiments

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