



SPECTROPHOTOMETRIC DETERMINATION OF AMISULPRIDE

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ABSTRACT

Three simple and sensitive spectrophotometric methods (A, B and C) have been developed for the quantitative estimation of amisulpride in bulk drug and pharmaceutical dosage forms. Method A is based on the diazotisation of amisulpride with nitrous acid followed by its coupling in situ with N-(1-naphthyl) ethylenediamine dihydrochloride to form pink coloured chromogen exhibiting absorption maximum at 523.5 nm and Beer's law is obeyed in the concentration range of 2-10 µg/mL. Method B is also based on the diazotisation of drug with nitrous acid followed by its coupling insitu with β-naphthol to form orange coloured chromogen exhibiting absorption maximum at 482.5 nm and Beer's law is obeyed in the concentration range of 2-10 µg/mL. Method C is based on the reaction of amisulpride with Folin-Ciocalteau (FC) reagent in alkaline condition to form stable blue coloured chromogen with absorption maximum at 736 nm and Beer's law is obeyed in the concentration range of 10-50 µg/mL. The results are compared with those obtained using UV spectrophotometric method in 0.1N HCl at 226.5 nm

Key words : Amisulpride, Spectrophotometric, Diazotisation

INTRODUCTION

Amisulpride (**1**)(AMS)¹⁻³, is chemically, 4-amino-N- {[(2RS)-1-ethyl pyrrolidin-2-yl] methyl}-5(ethyl sulphonyl)-2-methoxy benzamide and it is used in treatment of Schizophrenia. It has high affinity for dopamine D₂/D₃-receptor antagonist. 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 and an I. R, UV spectrophotometric and HPLC method⁸ are also reported. A UV spectrophotometric method, a chromatographic method and few electrophoretic methods are also reported for the quantitative estimation of amisulpride in pharmaceutical formulations⁹. In the present

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investigation, three new visible spectrophotometric methods (A, B and C) have been developed for the quantitative estimation of amisulpride in bulk drug and pharmaceutical dosage forms¹⁰.

The amino group in amisulpride (**1**) is diazotised with nitrous acid (NaNO₂/HCl) at 0°C and diazonium salt thus formed, is coupled with N- (1-naphthyl) ethylenediamine dihydrochloride (**2**) (Method A) and β-naphthol (**3**) (Method B). The coloured chromogens formed (**4**, **5**) for method A and B are stable for more than 4h having absorption maximum (λ_{max}) at 523.5 nm and 482.5nm. Beer's law is obeyed in the concentration range of 2-10 µg/mL in both the cases. Method C is based on the reaction of amisulpride (**1**) with folin-ciocalteau (FC) reagent (**6**) in alkaline condition to form blue coloured chromogen (**7**) with absorption maximum at 736 nm and Beer's law is obeyed in the concentration range of 10-50 µg/mL. The blue coloured complex is due to reduction of 1, 2 and 3 oxygen atoms of FC reagent and the formation of molybdenum blue or tungsten blue. Spectrophotometric parameters are established for standardization of the methods including statistical analysis of data. These methods have been successfully extended to the pharmaceutical dosage forms (tablets) containing amisulpride.

EXPERIMENTAL

All spectral measurements were done on Shimadzu 1700 UV/Visible spectrophotometer.

Reagents

Analytical grade reagents were used. Commercially available samples were purified.

- (i) Methyl alcohol
- (ii) Methanolic solution of N- (1-naphthyl) ethylene diamine dihydrochloride (0.4%w/v)
- (iii) Methanolic solution of β-naphthol (2%w/v)
- (iv) 5N Hydrochloric acid
- (v) Aqueous solution of sodium nitrite (0.2%w/v)
- (vi) Aqueous solution of ammonium sulphamate (1%w/v)
- (vii) FC Reagent (1N, Loba Chemie)
- (viii) Double distilled water.

Working standard of drug solution

About 100 mg of amisulpride was accurately weighed and dissolved in 20.0 mL of methyl alcohol in 100.0 mL of volumetric flask and diluted upto the mark with methyl alcohol (1 mg/mL). The final concentration of amisulpride was brought upto 100.0 $\mu\text{g/mL}$ with methyl alcohol.

Sample preparation

Two commercial tablets from different batches of a brand were analysed by the proposed methods. Five tablets of formulation each containing 50 mg of amisulpride were accurately weighed and powdered. Weight of tablet powder equivalent to 100 mg of drug was taken in 40 mL of methanol and shaken for 15 min, filtered into 100 mL volumetric flask through cotton wool and the remaining amount of methyl alcohol was added through tablet powder to make upto 100.0 mL. Final concentration of was brought upto 100 $\mu\text{g/mL}$ with methyl alcohol.

Assay

Method A : Aliquots of amisulpride ranging from 0.2-1.0 mL (1 mL = 100 $\mu\text{g/mL}$) were transferred into a series of 10.0 mL volumetric flasks. To each flask 1.5 mL of hydrochloric acid (5N) and 1 mL of sodium nitrite were added and kept in ice for 10 min. Then 1ml of aqueous solution of ammonium sulphamate (1%) was added and solution was shaken thoroughly. After 2 min, 1 mL of coupling reagent, N-1- (naphthyl) ethylene diamine dihydrochloride was added and diluted to mark with distilled water. The absorbance was measured at 523.5nm against reagent blank. The pink coloured chromogen was stable for more than 4hrs. The amount of amisulpride present in the sample was computed from calibration curve.

Method B: Aliquots of amisulpride ranging from 0.2-1.0 mL (1 mL = 100 $\mu\text{g/mL}$) were transferred into a series of 10.0 mL volumetric flasks. To each flask 1.5 mL of hydrochloric acid (5N) and 1 mL of sodium nitrite were added and kept in ice for 10 min. Then 1 mL of aqueous solution of ammonium sulphamate (2% w/v) was added and solution was shaken thoroughly. After 2 min. 1 mL of coupling reagent, β -naphthol was added and diluted to mark with distilled water. The absorbance was measured at 482.5 nm against reagent blank. The pink coloured chromogen was stable for more than 3 hrs. The amount of amisulpride present in the sample was computed from calibration curve.

Method C: Aliquots of amisulpride ranging from 1.0-5.0 mL (1 mL = 100 $\mu\text{g/mL}$)

were transferred into a series of 10.0 mL volumetric flasks. To each flask, 1 mL of Na_2CO_3 solution (20% w/v) and 1 mL of FC reagent (1N) were added. The volumes were made upto the mark with water. The absorbance of the blue coloured solution was measured at 736 nm against reagent blank. The amount of amisulpride present in the sample was computed from calibration curve.

The results of the above methods are compared with results obtained with UV spectrophotometric method¹¹. In UV method, solution of amisulpride in 0.1N hydrochloric acid, either pure or formulation (100 $\mu\text{g}/\text{mL}$) was prepared. Aliquots of amisulpride ranging from 0.2-1.0 mL (1.0mL = 100.0 $\mu\text{g}/\text{mL}$) were transferred into series of 10.0 mL volumetric flasks. The volumes were made upto the mark with 0.1N hydrochloric acid and the absorbance of the solution was measured at 226.5 nm against solvent blank. The amount of amisulpride was computed from calibration curve.

RESULTS AND DISCUSSION

The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity are presented in Table 1. The regression analysis using method of least squares was made for the slope (b), intercept (a) and correlation coefficient (r) from 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 eight measurements, 3/4 of of the upper Beer's law limits of amisulpride are given in Table 1. The results showed that these methods have reasonable precision. Comparison of the results obtained with the proposed and UV methods for dosage forms (Table 2) confirm the suitability of these methods for pharmaceutical dosage forms. The optimum conditions for the colour development for methods A, B and C were established by varying the parameters one at a time and keeping the other parameter fixed and observing the effects of the product on the absorbance of the coloured species and incorporated in the procedures.

Table 1 : Optical characteristics and precision

	Method A	Method B	Method C
λ_{max} (nm)	523.5	482.5	736
Beer's law limits ($\mu\text{g}/\text{mL}$) (C)	2-10	2-10	10-50
			Cont...

	Method A	Method B	Method C
Molar absorptivity (lit. mol ⁻¹ cm ⁻¹)	2.1 x 10 ⁴	2.2 x 10 ⁴	0.819 x 10 ⁵
Sandell's sensitivity(μg/cm ² 0.001 absorption units)	0.031	0.25	0.0625
Regression equation(Y*)			
Slope(b)	0.0498	0.050	0.017
Intercept(a)	0.0436	0.061	0.013
Correlation co-efficient(r)	0.9996	0.9992	0.9200
% RSD	0.3790	0.2635	0.1490
Range of errors**			
Confidence limits with 0.05 level	±0.0010	± 0.0007	± 0.0008
Confidence limits with 0.01 level	±0.0016	± 0.0011	± 0.0012

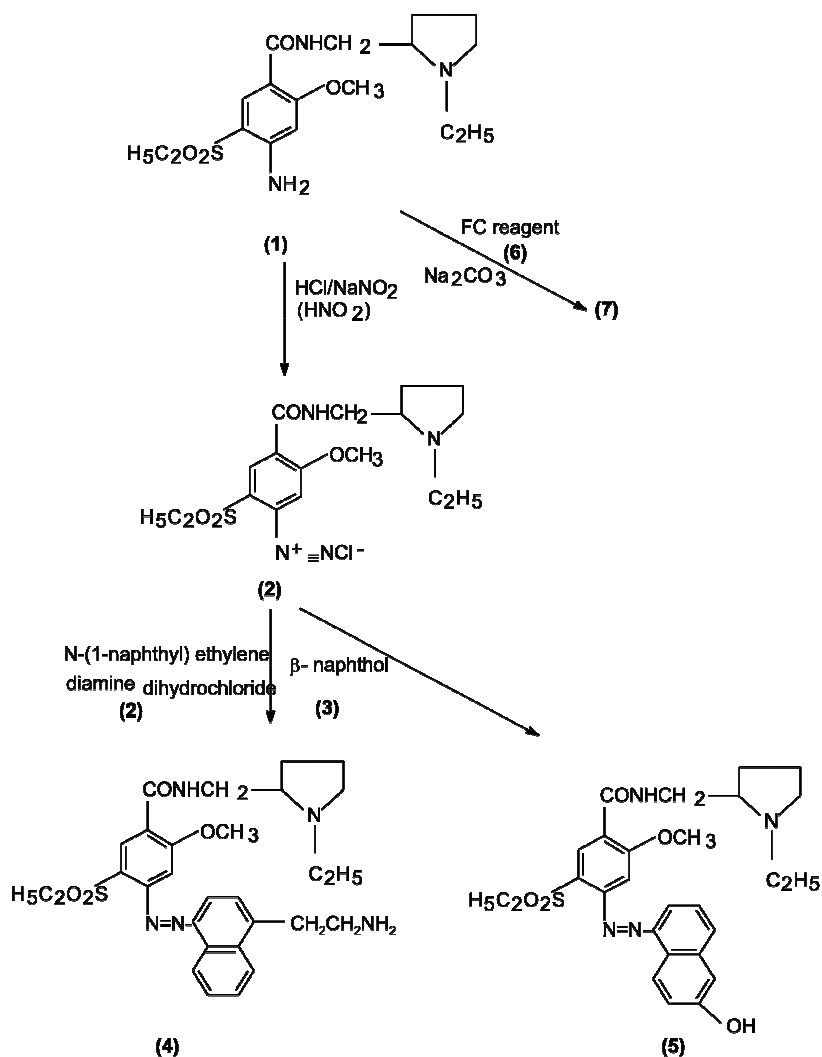
*Y = bC + a where C is the concentration of amisulpride in (μg/mL and Y is the absorbance at the respective (λ_{max}). **For eight measurements.

In order to justify the reliability and suitability of the proposed methods, known quantities of pure amisulpride was added to its various preanalysed formulations and the mixtures were analysed by the proposed methods. The results of recovery experiments are also summarized in Table 2. The other active ingredients and excipients usually present in pharmaceutical dosage forms did not interfere.

Table 2 : Evaluation of amisulpride in pharmaceutical dosage forms

Sample*	Labelled amount (mg)	Amount obtained(mg)						
		Proposed method			Reference method UV	Percentage** recovery		
		A	B	C		A	B	C
T ₁	50	49.56 ± 0.02	49.72 ± 0.03	49.62 ± 0.02	49.56 ± 0.03	99.87	99.65	99.75
T ₂	50	49.03 ± 0.02	49.65 ± 0.02	49.45 ± 0.03	49.32 ± 0.02	99.54	99.85	99.47

*T₁ and T₂ are tablets from different batches. ** Average of 8 determinations.



Scheme

The proposed methods are found to be simple, sensitive, selective, economical, accurate and precise and can be used in the determination of amisulpride in bulk drug and its pharmaceutical dosage forms in a routine manner.

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