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RESEARCH ARTICLE

NOVEL SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF AMISULPRIDE IN PURE AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

Three simple, sensitive and rapid visible spectrophotometric methods have been developed for the estimation of Amisulpride in bulk and pharmaceutical formulation. Method M_1 is based on the diazotization of primary aromatic amine of AMS coupling with Bratton-Marshal reagent to get colored azo dye which can be utilized for the quantitative estimation of Amisulpride by visible spectrophotometry. The chromogen exhibit absorption maxima at 530 nm and obeyed Beer's law in concentration range of 1-5 µg/mL with a correlation coefficient of 0.9999. Method M_2 is depending on the oxidative coupling of AMS with MBTH in the presence of Fe (III) to form to bluish-green colored chromogen and the absorbance was estimated at 580 nm against the reagent blank. Beer's law obeyed in the concentration range of 2-10 µg/mL with a correlation coefficient of 0.9995. In method M_3 the drug with Folin's Ciocalteu reagent to form blue colored chromogen which exhibit absorption maxima at 700 nm and obeyed Beer's law in concentration range of 10-50 µg/mL with a correlation coefficient of 0.9999. The three methods have been validated statistically with respect to linearity, precision, and accuracy according to ICH guidelines.

INTRODUCTION

Amisulpride (Coukell *et al*, 1996) belongs to second generation antipsychotic, a substituted benzamide derivative which is widely utilized drug to treat schizophrenia as well as mania episodes in patients with bipolar disorder (Costa e Silva, 1998). Hence there is wide scope for the development of new analytical methods for the assays of Amisulpride. Developments of some new instrumental methods are in need for the quantitative estimation of Amisulpride in bulk drug and pharmaceutical dosage forms with high sensitivity, accuracy, precision and economical too. The prominent analytical important functional groups of the drug are Primary aromatic amino group, ketone functional group, 3° amino group etc. have not been exploited completely. Hence there is a scope for developing more spectrophotometric methods for Amisulpride. Thus, the three new analytical methods are planned to develop with high sensitivity, accuracy and precision. The commercial formulations of Amisulpride in tablet form with 50 mg, 100 mg and 200 mg manufactured on the brand name of Sulpitac by sun pharma and Solian by Sanofi Aventis pharma were obtained locally and utilized in the analysis of the drug. Amisulpride (AMS) is not official drug in IP, BP and USP. Extensive literature studies on the developed analytical methods on AMS revealed that very few analytical methods have been forthcoming to determine this drug in pharmaceutical tablet dosage form. Literature survey reveals that for quantification of amisulpride in biological samples by LC-MS/MS ((Pehourcq *et al*, 2003; Gschwend *et al*, 2006; Nirogi *et al*, 2008; Zou *et al*, 2009; Balasekhara Reddy *et al*, 2011; Couchman *et al*, 2011) HPLC (Nishihara *et al*, 1983; Bohbot *et al*, 1987; Mokrim *et al*, 1993; Malavasi *et al*, 1996; Ascalone *et al*, 1996; Sachse *et al*, 2003; Skibinski *et al*, 2007; Das *et al*, 2009; Kudris *et al*, 2011; Devadasu *et al*, 2011) pharmaceuticals by spectrophotometric, electrophoretic (Syeda Humaira *et al*, 2008; Sharma *et al*, 2010; Ravisankar *et al*, 2012) and voltametric (Süzen, 2003) were reported.

Of all of the studies very few reports had been brought to light that the AMS in the tablet form was estimated by using visible

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spectrophotometry. Therefore keeping in view the comparative need 3 visible spectrophotometric methods were evolved depending on the main functional groups existed in the drug. Even though the above mention reported methods were estimated with spectrophotometric methods utilizing several reagents but the present author producing the thesis utilized other than the reagents such as BM reagent, MBTH, FC reagent while estimating the AMS with spectrophotometric methods which attain better results for quantification of Amisulpride in terms of sensitivity, precession, time saving and highly reliable methods. The chief aim and theme of this analysis study on AMS is to evolve simple, fast, efficient, accurate, precise and highly reliable spectrophotometric methods for the estimation of AMS in bulk as well as pharmaceutical dosage forms subject to official guide lines. The structural formula of the drug is shown in Figure 1.

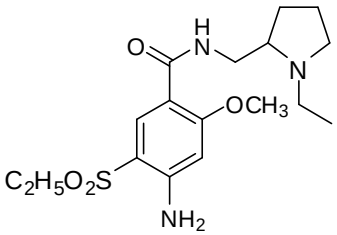


Figure 1 Chemical structure of Amisulpride.

Experimental

MATERIALS AND METHODS

All the chemicals and reagents utilized in the present study were of Anal R grade. The pure drug of Amisulpride was supplied by Sun pharmaceutical Industries Ltd., Mumbai, India as a sample gift. The commercial formulation such as sulpitec obtained from local market. Reagents like MBTH from Fluka company, Sodium corbonate and ferric chloride relating to Merck company, Folin-Ciocalteu reagent, ammonium sulphamate, sodium nitrite and HCL of Loba company, were procured locally.

Instruments used

A Systronics Double beam Ultra violet visible spectrophotometer 2201 with 1 cm correctly suited quartz cells was utilized for all spectral and absorbance measurements. A Systronics digital pH meter was employed for all measurements of pH.

Preparation of Reagents and standards

Analytical grade chemicals and reagents were utilized and solutions were made with double distilled water.

Method M_1

N-(1-napthyl) ethylene diamine dihydrochloride (NED, B.M reagent)

NED solution was prepared with 100 mg of N-(1-napthyl) ethylene diamine dihydrochloride correctly weighed, duly allowed to dissolve in 100 mL of distilled water and got 0.1 % of NED solution (Merck, 0.1 % w/v, 3.86 x 10^{-3} M).

Ammonium sulfamate solution (AS)

AS solution was prepared with 500 mg of ammonium sulfamate precisely weighed duly dissolved in 100 mL of distilled water and got 0.1 % w/v of AS solution (Loba, 0.5 % w/v, 4.38 x 10^{-2} M).

Sodium nitrite solution

$NaNO_2$ solution was prepared with 100 mg of sodium nitrite accurately weighed and duly dissolved in 100 mL of distilled water and obtained 0.1 % w/v of $NaNO_2$ solution (Loba, 0.1 % w/v, 1.45 x 10^{-2} M).

Hydrochloric acid solution

HCl solution was made with 44.5 mL of concentrated hydrochloric acid and clearly diluted with 100 mL of distilled water and got 5 N of HCl solution (Loba, 5N).

Method M_2

3- Methyl-2-Benzothiazolinone hydrazone solution

MBTH solution was prepared with 200 mg of 3-methyl-2-benzothiazolinone hydrazone aptly weighed and got dissolved in 100 mL of distilled water and obtained 0.2 % of MBTH solution (Fluka, 0.2 % w/v, 1.12 x 10^{-2} M).

Ferric chloride solution

$FeCl_3$ solution was prepared with 100 mg of ferric chloride accurately weighed and allowed to dissolve in 100 mL of distilled water and got 0.1 % w/v of $FeCl_3$ solution (Merck, 0.1 %, 6.17 x 10^{-3} M).

Method M_3

Folin-Ciocalteu reagent

FC reagent solution was prepared and perfectly diluted three times of Folin-Ciocalteu reagent (2 N) with distilled water. (Loba, 0.67 N).

Sodium carbonate solution

Na_2CO_3 solution was prepared by dissolving 2.0 g of sodium carbonate in 100 mL of distilled water. (Merck 2 % w/v, 1.89 x 10^{-1}M).

Preparation of standard drug solutions

Methods M_1, M_2, M_3

Exactly 100 mg pure drug of Amisulpride was put into a 100 mL volumetric flask which consists of 20 mL of 0.1N hydrochloric acid and sonicated for 20 minutes. The volume was filled to the mark with 0.1N hydrochloric acid solution and

got the stock solution 1 mg/mL and it was again diluted with same solvent and got working standard solution.

Recommended procedures

After systematic and complete study of various parameters concerned as described under results, discussion in this present chapter the following procedures were recommended for the estimation of AMS in bulk and pharmaceutical formulations.

For bulk samples

Method M_1

Aliquots of standard AMS drug (0.1 - 0.5 mL, 100 µg/mL) solution in 0.1N hydrochloric acid solution were poured into a series of 10 ml volumetric flasks. Duly adding 1mL of 5 N hydrochloric acid and 1 mL of sodium nitrite (0.2 % w/v) solution and mixed perfectly for 5 minutes.

Then 1mL of ammonium sulphamate (0.1 % w/v) was added to this solution duly shaking to get it neutralized with the excess of nitrous acid. Eventually 1mL of N-(1-Naphthyl) ethylenediamine dihydrochloride has to be added and mixed thoroughly duly keeping separately as it is for about 20 minutes to get the reaction. The final volume was filled up to the mark with 0.1 N hydrochloric acid and the absorbance of resultant purple-red chromogen was evaluated at 530 nm against the reagent blank. The quantity of AMS obtained was calculated from its calibration plot.

Method M_2

The aliquots of standard AMS drug (0.2 - 1.0 mL, 100 µg/mL) solution in 0.1N hydrochloric acid were put into a series of 10 mL volumetric flasks duly adding 1.5 mL of $FeCl_3$ (0.033 M) and 2 mL of 3-Methyl-2-benzothiazolinone hydrazone hydrochloride. After mixing thoroughly, the said solution was kept for 20 minutes at room temperature to get it reacted.

The solution was filled up to the mark with the 0.1N hydrochloric acid. Due to reaction the solution changed to bluish-green colored chromogen and the absorbance was estimated at 580 nm against the reagent blank. Basing on calibration plot the amount of AMS was calculated.

Method M_3

Aliquots of standard AMS drug (0.1 - 0.5 mL, 1000 µg/mL) solution in 0.1N hydrochloric acid were transferred into a series of 10 mL volumetric flasks.

To this added 1ml of Folin-Ciocalteu reagent and 2 mL of sodium corbonate (2 % w/v) solution, shacked well and the reaction mixture was allowed to stand for 15 minutes and the resultant solution was made to the volume with 0.1 N hydrochloric acid. The absorbance of blue colored chromogen thus formed was measured at 625 nm against the reagent blank. The amount of AMS was calculated from its calibration plot.

For pharmaceutical formulations

For methods M_1, M_2 and M_3

Twenty tablets of AMS were weighed crushed to a fine powder out of which a quantity of tablet powder weighing exactly and equivalent to 50 mg of AMS was transferred into a100 mL volumetric flask containing 50 mL of 0.1N HCl and sonicated for 15 minutes and the extract of the drug filtered through a cotton wool and the filtered solution was filled up with 0.1N HCl and aptly diluted with the same solvent and utilized for M_1, M_2 and M_3 methods. The same procedure was adopted for the rest of the methods but different solvents were used for extraction of AMS and with regard to M_{15} method by using methanol. Working sample solutions were prepared duly adopting the procedure intended for bulk samples.

RESULTS

Spectral Characteristics

To determine the optimum wavelength relating to maximum absorption (λ_{max}) of the colored species formed in all the five methods, specified amounts of AMS were taken and the colors were developed separately duly adopting the above stated procedures for each method. The absorption spectra scanned in the spectrophotometer in the wavelength range of 400 - 800 nm against respective reagent blank. Reagent blank absorption spectrum was noted down for each method against appropriate solvent (0.1N hydrochloric acid for methods M_1, M_2 and M_3 and the results obtained are shown in the Figures from 2 to 4. The absorption curves of colored species formed in every method showed characteristic absorption maxima (Table 4) but the blank in every method exhibited low or negligible absorption in this region.

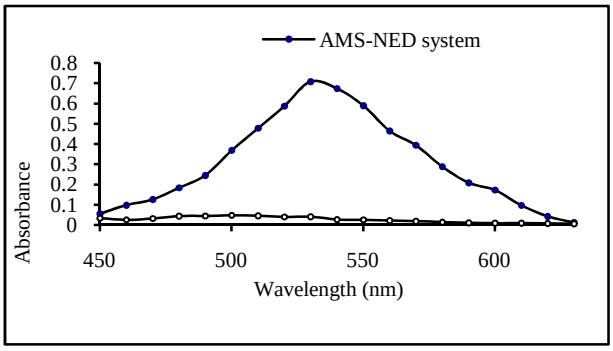


Figure 2 Absorption spectra of AMS with NED system and its reagent blank

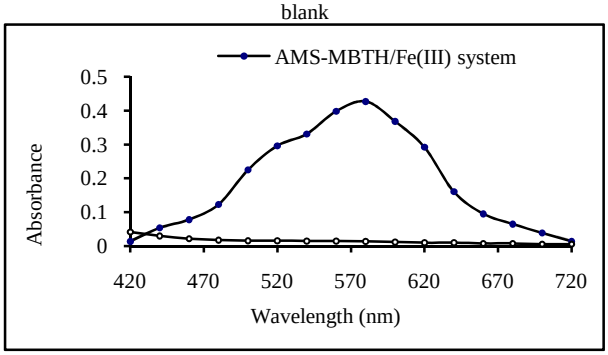


Figure 3 Absorption spectra of AMS with MBTH system and its reagent blank

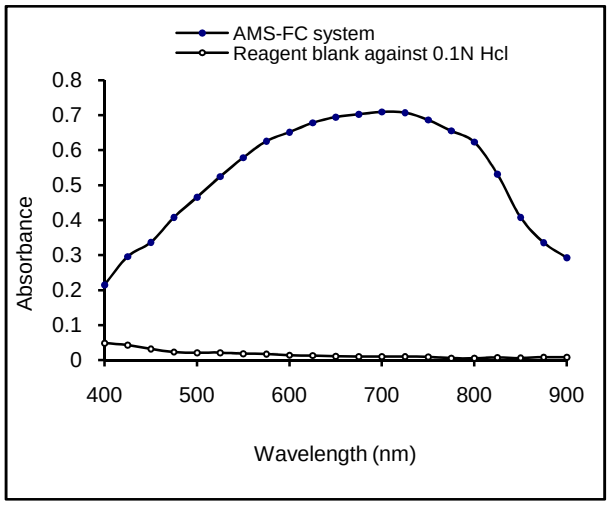


Figure 4 Absorption spectra of AMS with FC system and its reagent blank

Optimum conditions fixation in procedures

The optimum conditions inducted in the procedures of each method were confirmed by executing systematic and perfect investigations which are shown in Tables from 1 to 3.

Fixation of parameters

While developing M_1 to M_3 methods, a systematic study of the effects of numerous related parameters in the methods concerned were undertaken by changing one parameter at a time duly controlling all other parameters as they were to achieve optimum color development, least blank color,

reproducibility and the reasonable period of stability of finishing colored species formed and for this purpose the following methods were taken up.

Method M_1

In this method the coupling reaction of the diazonium salt of AMS with N-(1-napthyl) ethylene diamine dihydrochloride were involved and the necessary optimum conditions for formation of colored species were established.

By going through the effects of concentration and volume of 5N HCl, concentration of $NaNO_2$, NED and ammonium sulphamate effect of order of addition of reagents and solvent for eventual dilution and the ultimate results achieved are tabulated in Table 1.

Table 1 Optimum conditions established for method M_1.

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	530– 540	530	----
Volume of 5 N HCl	0.8 - 1.2 mL	1.0 mL	Less than 0.8 mL of acid, the diazotization reaction was found to be incomplete.
Concentration of sodium nitrite solution	1.40 x 10^{-2} to 1.50 x 10^{-2} M	1.45 x 10^{-2} M	With greater than 1.45 x 10^{-2} M sodium nitrite solution there was no added advantage.
Volume of sodium nitrite Solution (1.45 x 10^{-2} M)	0.8 - 1.2 mL	1.0 mL	With more than 1.5 mL of sodium nitrite solution, blank interference was observed.
Temperature	0 - 35 °C	Lab. Temperature	Most of the diazotization reactions will takes place at low temperatures (< 15 °C). For the present experiment room temperature was found to be enough. Beyond 35 °C the reaction was found to be progressively decreased.
Volume of ammonium sulfamate solution (4.38 x 10^{-2} M)	0.8 - 1.2 mL	1.0 mL	A minimum of 0.8 mL of ammonium sulfamate is necessary to destroy excess nitrous acid. More than 1.2 mL of the solution has no benefit.
Time between the additions	2 - 6 minutes	5 minutes	A minimum period of two minutes time was necessary for completion of reactions (Diazotization, neutralization of excess nitrous acid and coupling with NED)
Stability of the colored species	5 - 6 hours	15 minutes	Absorbance of the colored product decreased slowly after the stability period.
Solvent for the final dilution	---	Water	Final dilution with other water miscible solvents (methanol, acetone, acetonitrile) did not enhance the intensity of coloured species. Water is sufficient for final dilution.

Table 2 Optimum conditions established for method M_2.

Parameter	Optimum range	Conditions in the procedure	Remarks
λ_{max}	570 - 590	580	----
Nature of oxidant	Fe (III)	Fe (III)	When the other oxidants such as Ce (IV), Cr (VI), CAT, IO_4^-, $S_2O_8^{2-}$, Fe $(CN)_6^{3-}$ used instead of Fe (III), the absorbance of coloured species reduced.
Volume of MBTH, 1.12 x 10^{-2} M and keeping time	0.5 -1.5 mL	1.0 mL	For more than 1.5 mL, the absorbance of test solution remains the same against the reagent blank.
Volume of Iron (III) chloride	1.5 - 2.5 mL	2 mL	If Iron (III) chloride volume exceeds 2.5 mL, the blank absorption increases.
Keeping time and temperature	10 - 25 minutes at room temperature	15 minutes at room temperature	The other organic solvents were tested for the procedure includes ethanol and acetonitrile. Methanol was chosen for keeping the formed hydrazone product in solution form.
Order of addition of reagents	--	Drug, MBTH and Fe (III)	The change in the order of addition of reagents resulted in decreasing the absorbance.
Solvent for final dilution	--	Distilled water	There is no variation in the intensity of colored species formed even if water miscible organic solvents such as ethanol, propane-2-ol, 1, 4 dioxan or acetonitrile is used instead of water for final dilution.
Stability of colored species after final dilution	Immediate - 60 minutes	20 minutes	After 60 minutes the absorbance of colored species was found to be slowly decreasing.

Method M_2

Depending on the reaction of AMS with MBTH in the presence of Fe (III), this method was developed. The maximum conditions in this method were fixed, depending on the study of various relevant parameters, which is the nature of oxidant, concentration of oxidant, concentration of MBTH, solvents utilized and stability period of colored species. The necessary optimum conditions were keenly examined and results achieved are presented in Table 2.

Method M_3

The involvement of this method is the redox reaction between AMS and Folin-Ciocalteu reagent. The effects of nemerous concerned parameters that is to say nature of alkali, reaction time, volume of FC reagent, volume of alkali, temperature as well as stability of colored species formed were carefully observed. The optimum conditions were noted and results achieved are mentioned in Table 3.

Optical characteristics

In order to test whether the coloured species obtained in the proposed methods adhered to beer's law, the absorbance at appropriate wavelength of a set of solutions containing varying amounts of AMS and specified amounts of reagents as given in indicated procedures for each method were recorded against the corresponding reagent blanks.

Table 4 Optical characteristics, regression data, Precision and accuracy of the proposed methods for AMS

Parameter	M_1	M_2	M_3
λ_{max} (nm)	530	580	700
Beer's law limits (µg / mL)	1 - 5	2 - 10	10 - 50
Molar absorptivity (L. mole^{-1} cm^{-1})	3.731x 10^5	2.752 x 10^4	1.112 x 10^4
Detection limits (µg / mL)	0.028938	0.433523	0.17633
Sandell's sensitivity (µg /cm ^{2}/0.001 absorbance unit)	0.009901	0.01342	0.03322
Optimum photometric range (µg / mL)	1.0 - 8.0	1.5 - 15	5 - 60
Regression equation (Y = a+ bc):			
Slope (b)	0.1022	0.036479	0.0098
Standard deviation of slope (S_b)	1.39 ×10^{-5}	3.96×10^{-3}	3.96 x 10^{-3}
Intercept (a)	0.000333	0.00029	0.0014
Standard deviation of intercept (S_a)	2.298 ×10^{-3}	4.792 x 10^{-3}	4.792 x 10^{-2}
Standard error of estimation (S_e)	4.94 x 10^{-3}	3.33 x 10^{-3}	4.77 x 10^{-3}
Correlation coefficient (R^2)	0.99996	0.9995	0.9999
% Relative standard deviation*	0.5976	0.2743	0.9247
% Range of Error (Confidence limits)*			
0.05 level	0.627	0.2879	0.699
0.01 level	0.983	1.4515	1.5210
% Error in bulk samples*	0.33	-0.51	0.25

Method validation

Sensitivity of the method

To check and ascertain the validity of Beer's law for these six developed methods, the absorption at suitable wavelengths of a set of solutions with varied quantities of AMS and determined amounts of reagents as explained in the procedures undertaken for each method were indicated against the relevant reagent blanks.

The Beer's law plots pertaining to these systems are exhibited graphically in Figures from 5 to 7. In respect of Beer's law limits, molar absorptivity, sandell's sensitivity and optimum photometric range for AMS for each developed method with afore said reagents are computed duly showing the results in Table 4. Least square regression analysis was under taken to obtain the values of the slope, intercept and correlation coefficient values and the results are mentioned in Table 4.

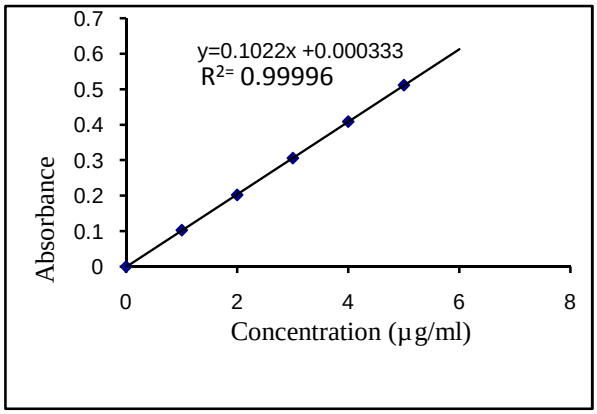


Figure 5 Beer's law plot of AMS with NED system

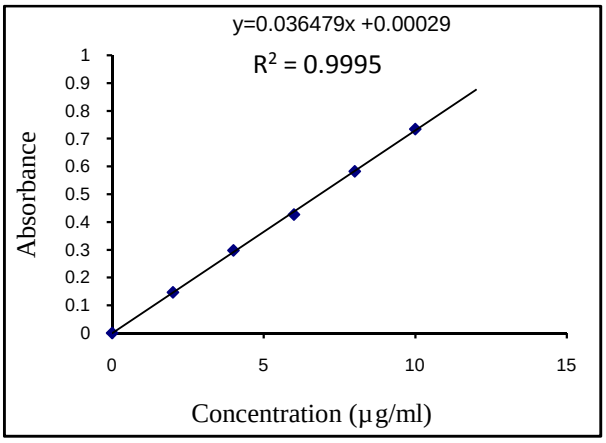


Figure 6 Beer's law plot of AMS with MBTH system

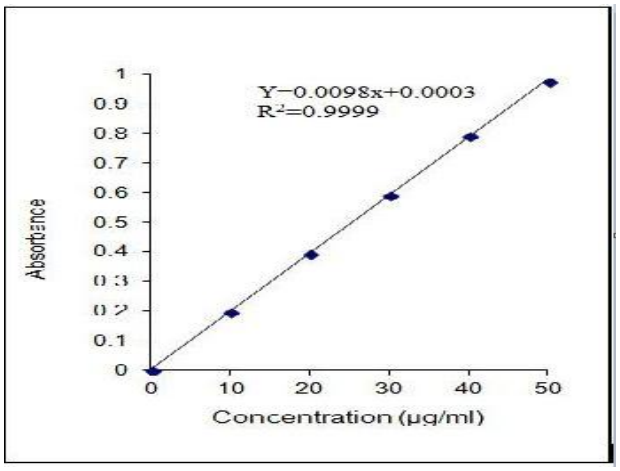


Figure 7 Beer's law plot of AMS with FC system

Precision

The precision of each one out of 6 proposed visible spectrophotometric methods were decided individually from the absorbance values achieved by actual determination of 6 replicates of a fixed quantity of AMS in eventual solution.

Accuracy/ Recovery studies

The accuracy of the method was decided by taking aliquots of known quantities of AMS bulk samples within the beers law limits and estimated them with the newly proposed methods by the author and reported methods as well. The results of percent error so obtained are shown in Table 5.

Interference studies

Separate examinations were held to assess the effect of modified range of excipients and other additives normally existed in the AMS formulations while determining under suitable conditions. The excipients and additives usually utilized such as microcrystalline cellulose, lactose monohydrate, povidone, sodium starch glycolate, colloidal silicon dioxide and magnesium stearate by the proposed method even if they exist in big amounts than they commonly exist in the manufacture of AMS tablet formulations did not interfere while determining AMS.

Method M $_1$

Method M_1 is based on the diazotization of primary aromatic amine of AMS with nitrous acid followed by coupling with Bratton-Marshal reagent (NED) to get colored azo dye which is shown in scheme 1.

Method M_2

This method is depending on the oxidative coupling of AMS with MBTH in the presence of Fe (III). Under the reaction conditions, MBTH owing to oxidation with $FeCl_3$ loses two electrons and one proton forming an electrophilic intermediate which has been assumed as the active coupling species. One mole of this intermediate reacts with AMS by an electrophilic attack on most electrophilic site of AMS to form colored species as exhibited in scheme 2.

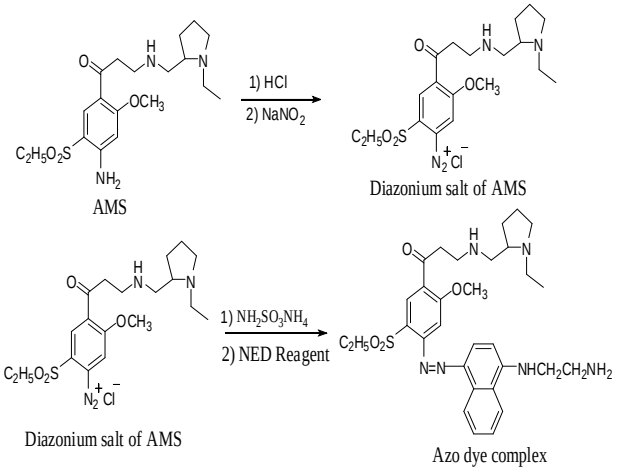
Table 5 Assay and recovery of AMS in pharmaceutical formulations

Method	Pharmaceutical formulation	Labeled Amount (mg)	Proposed Method			Found by reference method ï S.D	% recovery by proposed methods** ï S.D
			Amount found* (mg) ï S.D	t (value)	F (Value)		
M_{12}	Tablet –I	50	50.02 ± 0.018	0.588	1.215	50.3 ± 0.012	100.05 ± 0.21
	Tablet –II	100	99.8 ± 0.012	0.826	1.457	100.4 ± 0.008	99.89 ± 0.18
	Tablet –III	150	149.9 ± 0.011	1.023	2.043	150.1 ± 0.005	101.15 ± 0.31
M_{13}	Tablet –I	50	49.4 ± 0.013	0.543	1.452	49.7 ± 0.015	100.12 ± 0.09
	Tablet –II	100	100.2 ± 0.015	1.327	1.978	100.8 ± 0.011	99.89 ± 0.96
	Tablet –III	150	150.9 ± 0.021	0.562	1.758	149.05 ± 0.016	100.11 ± 0.14
M_{14}	Tablet –I	500	49.8 ± 0.012	0.719	2.531	49.9 ± 0.011	99.26 ±0.55
	Tablet –II	100	100.8 ± 0.009	0.541	1.233	100.4 ± 0.084	100.04 ± 0.12
	Tablet –III	150	150.6 ± 0.017	1.023	1.651	148.99 ± 0.022	99.95 ± 0.11

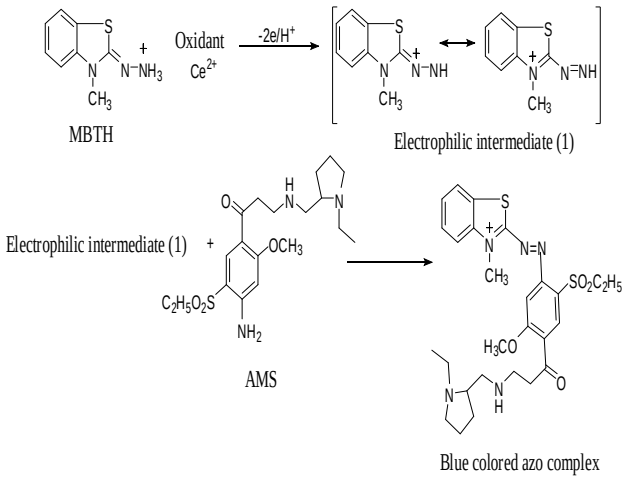
* Average ± standard deviation of six determinations, the t and F- values refer to comparison of the proposed method with reference method.
Theoretical values at 95 % confidence limits t = 2.571 and F = 5.05. ** Average of six determinations.

Chemistry of the colored species

AMS contains separate functional groups of different reactivity like primary aromatic amine, secondary amine, tertiary amine and corbonyl functional group. In total six methods have been developed depending on the reactivity of the said functional groups. The chemistry involved in the above proposed methods showed and explained several colored chromogens in the following schemes 1 and 2 as shown bellow.



Scheme 1 AMS reaction with NED reagent



Scheme 2 AMS reaction with MBTH

Method M_3

The color formed by FC reagent with AMS combination is explained as follows based on the analogy with the reports of earlier workers. The following chemical species are involved in the preparation of mixed acids in the FC reagent.

$3H_2O.\ P_2O_5.\ 13\ WO_3.\ 5MoO_3.\ 10\ H_2O$ and
$3H_2O.\ P_2O_5.\ 14\ WO_3.\ 4MoO_3.\ 10\ H_2O$

Probably AMS effects reduction of 1, 2 or 3 oxygen atoms from the tungstate and/or molybdate, and produced one or more of several possible reduced species, which have characteristic intensity of blue color.

DISCUSSION

In the present work, three visible spectrophotometric methods were developed and validated for the assay determination of AMS. In order to ascertain the optimum wavelength of the maximum absorption of the color species formed in each of the three methods specified amounts of AMS were taken and the colors were developed separately the above said procedures individually. The absorption spectra were scanned on a UV visible spectrophotometer in the wave length region of 400 - 800 nm against a corresponding reagent blank. Reagent blank absorption spectrum was noted down for each method against appropriate solvent (0.1N hydrochloric acid for methods M_1, M_2 and M_2) and the results obtained are shown in the Figures from 2 to 4. The absorption curves of colored species formed in every method showed characteristic absorption maxima (Table 4) but the blank in every method exhibited low or negligible absorption in this region. The absorption maxima of NED, MBTH, Folin-Ciocalteu reagent, were observed to be 530 nm, 580 nm, 700 nm, respectively.

The beer's law plots of these systems were recorded and are graphically exhibited in Figures from 5 to 7. Beer's law limits, molar absorptivity, sandell's sensitivity and optimum photometric range for AMS in each developed method with the chosen reagents are calculated and explained in Table 4. Least square regression analysis was carried out for getting slope, intercept and correlation coefficient values are shown in Table 4. The response of each drug was found to be linear in the specified concentration ranges from 1 - 5 µg/mL, 2 - 10 µg/mL, 10 - 50 µg/mL for NED, Fe(III)/ MBTH, FC, respectively. The correlation coefficient found to be more than 0.9995. The percent relative standard deviation and percent range of errors at 0.05 and 0.01 confidence limits were calculated in respect of proposed methods which are shown in Table 4. These values indicate that all proposed methods were precise.

By analyzing pharmaceutical formulation for the active ingredient, initially recovery studies were taken up under the proposed methods. Three different quantities of pure drug were added to the previously analyzed formulations and the total quantity of the drug was once more estimated by M_1 to M_3 proposed methods. The ultimate results so obtained are recorded and shown in Table 5.

It was also found that the excipients and additives such as microcrystalline cellulose, magnesium stearare lactose monohydrate, povidone, sodium starch glycolate, and colloidal silicon dioxide existed in the preparation of AMS tablet formulation did not interfere while determining the AMS by the proposed methods even if they are existed in huge quantities than normally present. To find out the suitability of the proposed methods for the assay of pharmaceutical formulations (Tablets) containing AMS were analysed by each proposed method and reference method. The results obtained from each of the proposed and reference methods were compared statistically by the t - and F - tests and were found that these proposed methods do not differ significantly in precision and accuracy from reference method. The results are tabulated in Table 5.

CONCLUSION

Three visible spectrophotometric methods have been developed for the estimation of AMS in bulk and pharmaceutical tablet dosage forms. The author developed these three visible spectrophotometric methods as explained above based on the reactivity of different functional groups such as primary aromatic amine, tertiary amino group and carbonyl functional in AMS. Specific reagent was used for each method and the ε_{max} values of each method are different. The sensitivity order of various proposed methods is $M_1 > M_2 > M_3$. The correlation coefficients (r^2) were found to be greater than 0.9995 which indicates that the developed methods were linear. Statistical analysis of the results showed that all the proposed procedures have good precision and accuracy. Results of analysis of pharmaceutical formulations reveal that the proposed methods are suitable for their analysis with virtually no interference of the common additives presented in pharmaceutical formulations. These methods can be adopted for routine quality control of AMS in bulk and pharmaceutical preparations.

References

Ascalone V, Ripamonti M, Malavasi B. 1996. Stereospecific determination of amisulpride, a new benzamide derivative, in human plasma and urine by automated solid-phase extraction and liquid chromatography on a chiral column. application to pharmacokinetics. J Chromatogr B Biomed Appl. 676, 95-105.

Balasekhara Reddy CH, and Babu Rao. 2011. Development and validation of Amisulpride in human plasma by HPLC coupled with tendem mass spectrometry and its application to a pharmacokinetic study, Sci.Pharm.79, 583-599.

Bohbot M, Doare L, Diquet B. 1987. Determination of a new benzamide, amisulpride, in human plasma by reversed-phase ion-pair high-performance liquid chromatography. J Chromatogr. 416, 414-419.

Costa e Silva JA. 1998. The public health impact of anxiety disorders: a WHO perspective.Acta Psychiatr Scand Suppl. 393, 2-5.

Couchman L, Morgan PE, Flanagan RJ. 2011. Basic drug analysis by strong cation-exchange liquid chromatography-tandem mass spectrometry simultaneous analysis of amisulpride and metamfetamine and amphetamine in serum/plasma. Biomed Chromatogr25, 867–872.

Coukell AJ, Spencer CM, Benfield P. 1996. Amisulpride: A Review of its Pharmacodynamic and Pharmacokinetic Properties and Therapeutic Efficacy in the Management of Schizophrenia. CNS Drugs. 6, 237-243.

Das A, Bhaumik U, Chakrabarty US, Sarkar AK, Ghosh A, Bose A, Chatterjee B, Pal TK. 2009. Comparative bioavailability study of amisulpride tablets in healthy Indian volunteers. Arzneimittelforschung. 59, 166-170.

Devadasu Ch and Ravisankar P. 2011. Validated RP-HPLC method for the microgram determination of Amisulpride in pure and pharmaceutical formulations. 2, 202-211.

Gschwend MH, Arnold P, Ring J, Martin W. 2006. Selective and sensitive determination of amisulpride in human plasma by liquid chromatography-tandem mass spectrometry with positive electrospray ionisation and multiple reaction monitoring. J Chromatogr B Analyt Technol Biomed Life Sci. 831, 132-139.

International Conference on Harmonization. Validation of Analytical Procedures: Text and Methodology Q2 (R1), 2005.

Kudris IV, Skakun NN, Orlova IN, Libina VV, Kulikov AU. 2011. Analysis of Amisulpride in Human Plasma by SPE and LC with Fluorescence Detection. Chromatographia.73, 67-74.

Malavasi B, Locatelli M, Ripamonti M, Ascalone V. 1996. Determination of amisulpride, a new benzamide derivative, in human plasma and urine by liquid-liquid extraction or solid-phase extraction in combination with highperformance liquid chromatography and fluorescence detection. application to pharmacokinetics. J Chromatogr B Biomed Appl. 676, 107–115.

Mokrim R, Brunet C, Cazin M, Gressier B, Luyckx M, Dine T, Robert H, Cazin JC. 1993. Amisulpride evaluation bv radioreceptor assav comparison with HPLC procedure after single administration in the rabbit. Methods Find Exp Clin Pharmacol.15, 41–47.

Nirogi R, Bhyrapuneni G, Kandikere V, Mudigonda K, Ajjala D, Suraneni R, Mukkanti K. 2008. Liquid chromatography tandem mass spectrometry method for the quantification of amisulpride with LLOQ of 100 pg/mL using 100 microL of plasma. Biomed Chromatogr. 22, 1424-1433.

Nishihara K, Kohda Y, Tamura Z. 1983. Determination of sultopride in serum and saliva by high-performance liquid chromatography. Chem Pharm Bull (Tokyo) 31, 4144-4146.

Pehourcq F, Ouariki S, Begaud B. 2003. Rapid high-performance liquid chromatographic measurement of amisulpride in human plasma: application to manage acute intoxication. J Chromatogr B Analyt Technol Biomed Life Sci. 789, 101-105.

Ravisankar P, Devadasu Ch, Sowjanya S, Srinivasa Babu P and Devala Rao G. 2012. Analysis of Amisulpride in pharmaceutical dosage forms by novel spectrophotometric methods. Int. J. Chem. Sci., 10, 203-212.

Sachse J, Hartter S, Weigmann H, Hiemke C. 2003. Automated determination of amisulpride by liquid chromatography with column switching and spectrophotometric detection. J Chromatogr B Analyt Technol Biomed Life Sci. 784, 405-410.

Sharma S, and Neog M. 2010. Development and Validation of Spectrophotometric Methods for Estimating Amisulpride in Pharmaceutical Preparations. Anal. Sci. 26, 485-489.

Skibinski R, Komsta L, Hopkala H, Suchodolska I. 2007. Comparative validation of amisulpride determination in pharmaceuticals by several chromatographic, electrophoretic and spectrophotometric methods. Anal Chim Acta. 590, 195–202.

Süzen S, Demírcígíl BT, Buyukbingol E, Özkan SA. 2003. Electroanalytical evaluation and determination of 5-(3′-indolyl)-2-thiohydantoin derivatives by voltametric studies: possible relevance to in vitro metabolism. New J Chem. 27,1007-1011.

Syeda Humaira, Day A.K, and Raju S.A. 2008. Development and validation of Spectrophotometric methods of Amisulpride in pharmaceutical dosage forms. International Journal of chemical sciences, 6, 437-440.

Zou S, Wen Y, Lin Q. 2009. Determination of amisulpride in plasma by LC/MS/MS.Zhongguo Xiandai Yingyong Yaoxue. 26, 483-486.

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