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A novel spectrofluorimetric method for the determination of amisulpride in bulk and pharmaceutical formulation

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ABSTRACT

A sensitive, simple, selective, reproducible and cost-effective spectrofluorimetric method for the determination of Amisulpride in bulk as well as commercial formulations. For the first time this method is based on reaction of Amisulpride with 1, 2 - Naphthoquinone -4- sulphonate (NQS) in sodium hydroxide to become a product of orange color. The spectrofluorimetric method involved in reduction of this product with potassium borohydrate, derivatized reaction product is obtained which is highly fluorescent product and shows maximum fluorescence intensity at λem = 370 nm after excitation at λex = 275 nm. The different experimental parameters affecting progress of the derivatization reaction were carefully studied and optimized. Under optimum experimental conditions the method has an excellent linear relationship with best correlation coefficient of 0.9999. The fluorescence intensity concentration plot was linear over the range of 0.2-1.2 µg/mL. The mean accuracy was found to be 99.50 % to 99.99 %. The intraday and interday precision was found to be 0.105-0.173 % and 0.116-0.133 % respectively. The limit of detection was found to be 0.0116 µg/mL and the limit of quantification was 0.0350 µg/mL. The interference effects on common excipients on the quantification of drug were investigated and no interference effects were observed. The results obtained were in good agreement with those obtained using a reported spectrophotometric method. Therefore this newly proposed spectrofluorimetric method is most convenient analytical technique and has been successfully feasible for determination of Amisulpride in bulk and pharmaceutical formulations in quality control as well as clinical laboratories.

Key words: Amisulpride, spectrofluorimetric method, 1, 2 - Naphthoquinone - 4- sulphonate.

INTRODUCTION

Systematic IUPAC name of Amisulpride (AMS) is 4- amino-N-[(1-ethylpyrrolidin-2-yl)methyl]-5-ethylsulfonyl-2-methoxybenzamide[1]. AMS used for easing the symptoms of severe or sudden (acute) and ongoing or long term (chronic) schizophrenic disorders in which positive symptoms such as delusions, hallucinations, thought disorders and/or negative symptoms such as blunted affect, emotional and social withdrawal are prominent, including patients characterized by predominant negative symptoms. Amisulpride is not approved by the FDA for use in the U.S.A, but it is used in Europe, Israel, India, Australia and Newzelnd to treat schizophrenia and psychosis. Amisulpride binds

selectively with a high affinity to human dopaminergic D_2/D_3 receptor subtypes whereas it is devoid of affinity for D_1, D_4 and D_5 receptor subtypes. Unlike classical and atypical neuroleptics, Amisulpride has no affinity for serotonin, α-adrenergic, histamine H_1 and cholinergic receptors. In addition, Amisulpride does not bind to sigma sites.

Several techniques were reported for determination of AMS in a variety of matrices. These methods include, non-aqueous titration[2], spectrophotometric methods [3-7], HPLC [8], LC with fluorescence detection[9], LC-MS/MS [10-17], GC/MS [18] for the estimation of AMS were reported.

The above mentioned few techniques are sensitive and need expensive instruments except spectrophotometric method and are occasionally tedious and require much time. Infact the proposed spectrofluorimetric new method need simple solvent and no necessity of complicated sample preparation. The analysis of novel developed method was validated in terms of ICH Q2 (R1)[19] guidelines.

An intensive literature survey on AMS analysis reveled that NQS has not yet so far used as reagent for the determination of AMS tablets with spectrofluorimetric method. NQS has been utilizing to determine many compounds but the reaction between the NQS and AMS in tablet form has not yet been explored. The chief aim of this study is to develop a simple, rapid, accurate, sensitive as well as low cost alternative technique for the determination of AMS in bulk and pharmaceutical formulation. The author clearly noticed that for the quality control of the drugs of commercial formulations duly utilizing simpler matrix among which spectrofluorimetric method is usually adopted for routine analysis in quality control laboratories but literature survey reveals that quantification of AMS tablets are found to be very few but none has attempted and investigated with NQS as reagent with spectrofluorimetric method hitherto. Therefore the current study was mainly focused to investigate NQS as derivatizing reagent in the development of sensitive spectrofluorimetric method to determine AMS in tablet form as this was not investigated previously as explained above. The chemical structure of Amisulpride is shown in figure 1.

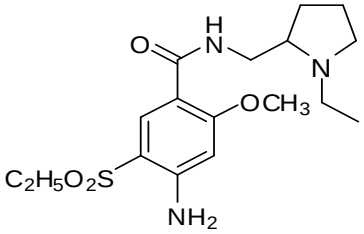


Figure 1. Chemical structure of Amisulpride

MATERIALS AND METHODS

Chemicals and reagents

All the solvents and reagents are analytical grade utilized from the beginning to the end of the estimation process. AMS tablets, pure drug and all the reagents as detailed in the statement (Table 1).

Table 1. Materials used for the present study

S.No.	Materials	From which company procured
1.	Amisulpride pure sample and tablet formulations.	Pure sample procured form Hetero labs Ltd., Hyderabad and tablet formulation Amide from Mesmer Pharmaceuticals, Chennai, and Amisyt from East West inda. Haridwar.
2.	1, 2 - Naphthoquinone - 4 - sulphonate (NQS)	Merck Specialties Pvt. Ltd., Mumbai, India.
3.	Potassium borohydrate (KBH_4)	Merck Specialties Pvt. Ltd., Mumbai, India.
4.	Methanol	Merck Specialties Pvt. Ltd., Mumbai, India.
5.	Water	Merck Millipore, Germany.

Instruments used

Elico Spectrofluorimeter SL-174 was utilized for spectral and fluorescence measurements. ELICO LI-617 digital pH meter was utilized for adjusting pH which was detailed in the table 2.

Table 2. Instruments used for the present study

S.No.	Instruments	Name of company and model
1.	Spectrofluorimeter	ELICO SL-174 Spectrofluorimeter.
2.	pH meter	ELICO LI-617 pH meter, ELICO India Ltd.
3.	Weighing balance	ESSAE VIBRA AJ (0.001g), ESSAE-Teraoka Ltd.
4.	Ultra sonic sonicator	Johnson plasto sonic private limited, Pune.

Selection of wave length

To detect the wavelength the working standard solution of AMS was scanned ranging from 200-600 nm in spectrofluorimeter and got 274 nm as excitation wavelength and 370 nm as the emission wavelength and the results are graphically shown in figures 2 and 3.

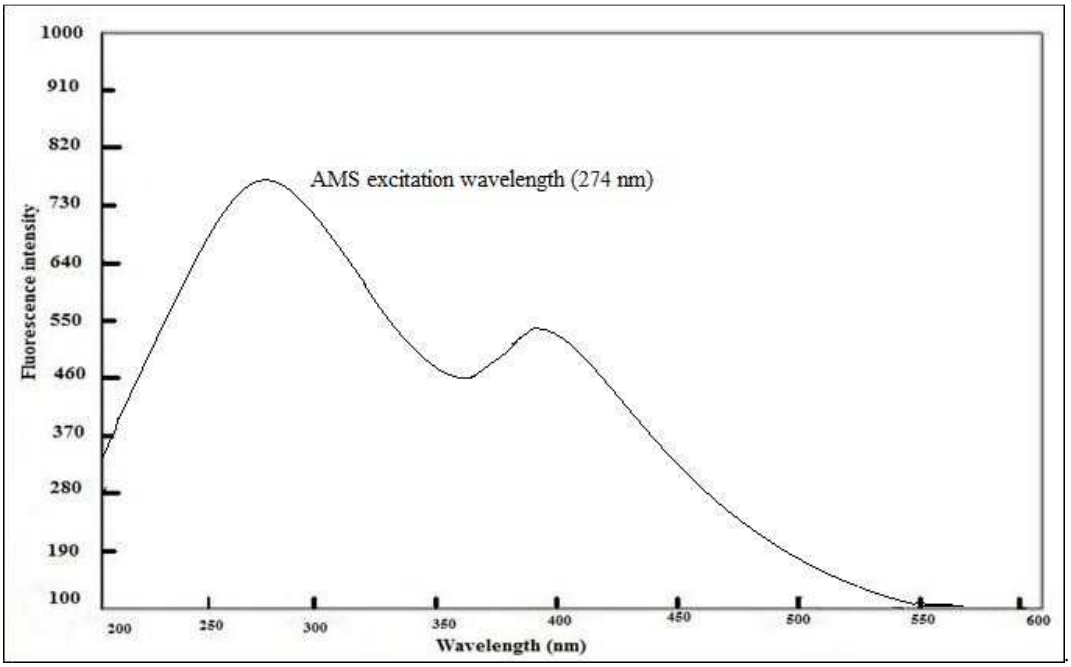


Figure 2. Excitation spectrum of AMS with NQS system

Preparation of reagents and standards

1, 2- Napthoquinone-4-sulphonate as derivatization reagent

150 mg of 1, 2- Napthoquinone-4-sulphonate was precisely weighed and poured in to 25 mL volumetric flask duly allowing dissolving in five milliliters of distilled water and completed up to volume with distilled water and obtained 0.6 v/v solutions which was protected from exposure to light while utilizing.

Preparation of stock solution

100 mg of AMS was accurately weighed and transferred into 100 mL of clean dry volumetric flask. The drug was dissolved in 30 mL distilled water duly sonicated for 5 minutes and solvent so obtained was completed to volume for getting stock solution of 1mg/ml i.e., 1000 µg/ml. This was considered as the standard stock solution and the said solution was used as working standard solution.

Preparation of working standard solutions

For spectrofluorimetry, a quantity of 1 mL of the stock solution was pipette out transferred into the 100 mL volumetric flask and filled up to the mark with milli-Q type 1 ultra pure water to achieve eventual concentration of 10 µg/mL.

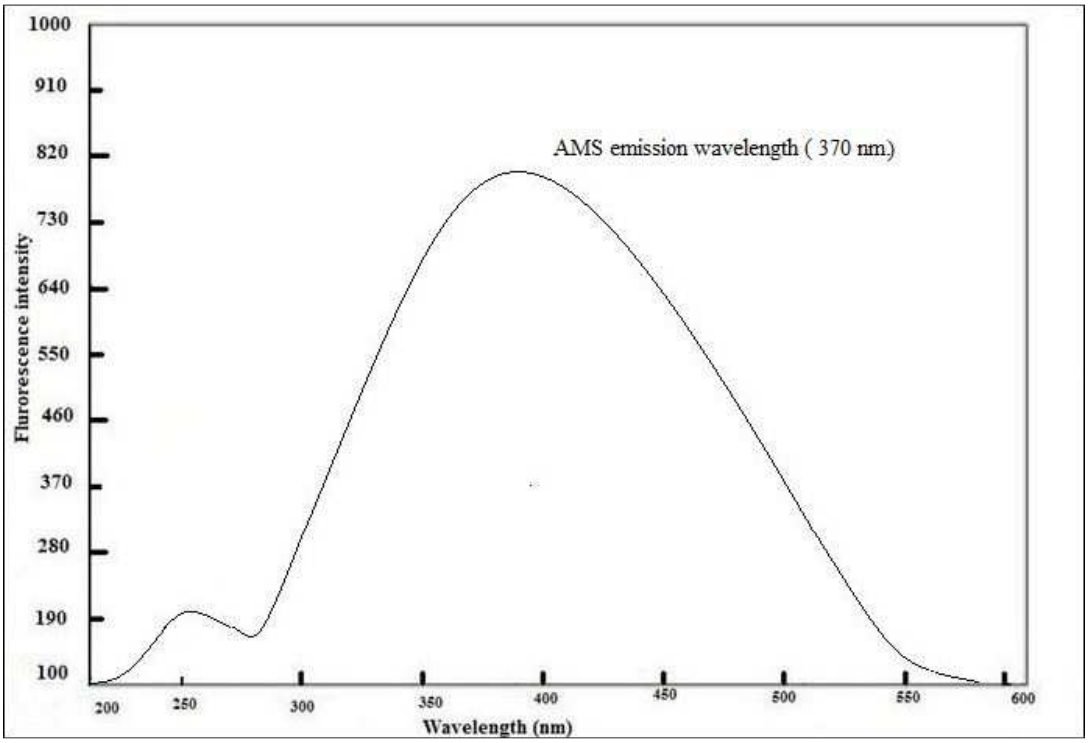


Figure 3. Emission spectrum of AMS with NQS system

Preparation of sample solution

Twenty AMS tablets were correctly weighed and crushed into fine powder, out of it precisely weighted quantity of the tablet powder equivalent to 100 mg of AMS was transferred into 100 mL of volumetric flask consisting of 30 mL distilled water and sonicated for 20 minutes and allowed it entirely dissolved duly filtered and the said filtrate was filled up to the volume with ultrapure water and obtained appropriate concentration to perform analysis in spectrofluorimetric method.

General recommended procedure

Aliquots (0.02 - 0.12 mL; 10 µg/mL) of AMS solution consisting of 0.2 to 1.2 µg/mL and from a standard solution of 10 µg/mL were poured into a series of 10 ml of calibrated flasks and then 1ml quantity of 0.01 M Sodium hydroxide and 1 mL of prepared NQS reagent solution with 0.6 % w/v were added. The entire content in the volumetric flask was allowed to heat in water bath for 40 minutes at 70 ± 5^{o}C subsequently kept it in ice water for 5 minutes to become cooled. The entire content in the volumetric flask was poured into a separating funnel and then duly extracted with 2 portions of 5 mL quantity of chloroform. The said combination of chloroformic extracts was allowed to evaporate beneath stream of air. The resultant remaining portion was reconstituted in 2 mL of methanol and then quantitatively poured into 10 mL volumetric flask, again 1mL of KBH_4 solution with concentration of 0.03 % in CH_3OH was combined and the resultant action occurred was kept at room temperature of 30 ± 5 ^{o}C for 5 minutes. The solution so obtained was diluted to volume with 0.025 M ethanolic hydrochloric and the resultant intensity of the emission and excitation wavelength were recorded at 370 nm and 274 nm respectively against reagent blank duly treating in the same way. The quantum of AMS existing in the sample solution was calculated from the calibration curve.

RESULTS AND DISCUSSION

Method Development and Validation

Reduction action on AMS-NQS product is absolutely essential to develop the spectrofluorimetric method. At the reduced NQS reagent acts as fluorescent processing in the maximum identical excitation as well as emission of the product of AMS - NQS. Therefore before carrying out the reduction process a selective extraction step for the AMS - NQS product out of remaining reagent of NQS is absolutely needed. Depending on the reported efficiency KBH_4 was selected as reducing reagent for derivatives of NQS. The reaction was held by changing the concentrations

ranging 0.001- 0.01% w/v and conducted investigation to find out the effect of KBH_4 using as a reducing agent. The utmost fluorescence intensity was achieved at the concentration of 0.004 % in the final solution, but the concentrations beyond 0.004 % yield no effect on the fluorescence intensity of reaction product of AMS with NQS (Figure 4). A close examination of the effect of pH on the relative fluorescence intensity of the reduced reaction product of AMS - NQS results reveled that optimum fluorescence intensity was achieved at pH 2.1 (Figure 5) When reaction mixture was diluted with 0.025 M ethanolic Hydrochloric acid solution.

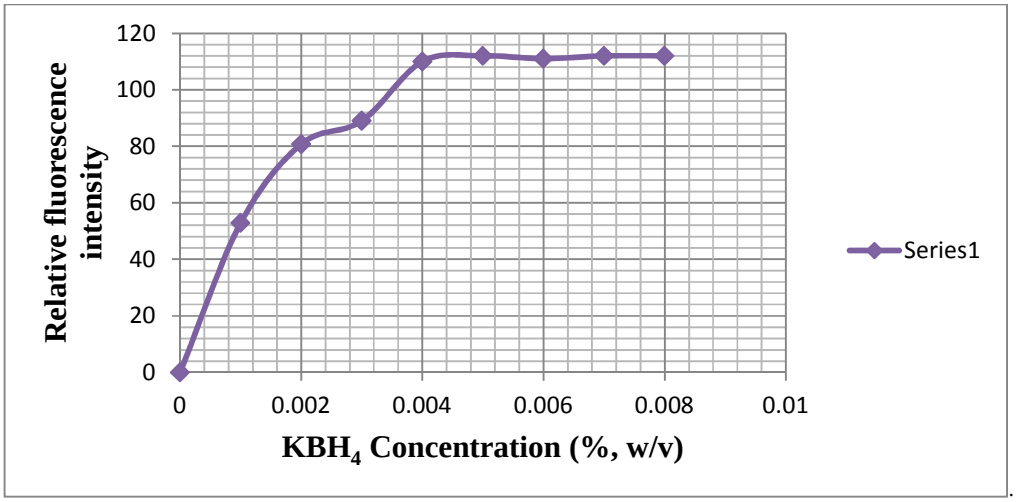


Figure 4. Effect of KBH_4 concentration on the fluorescence intensity

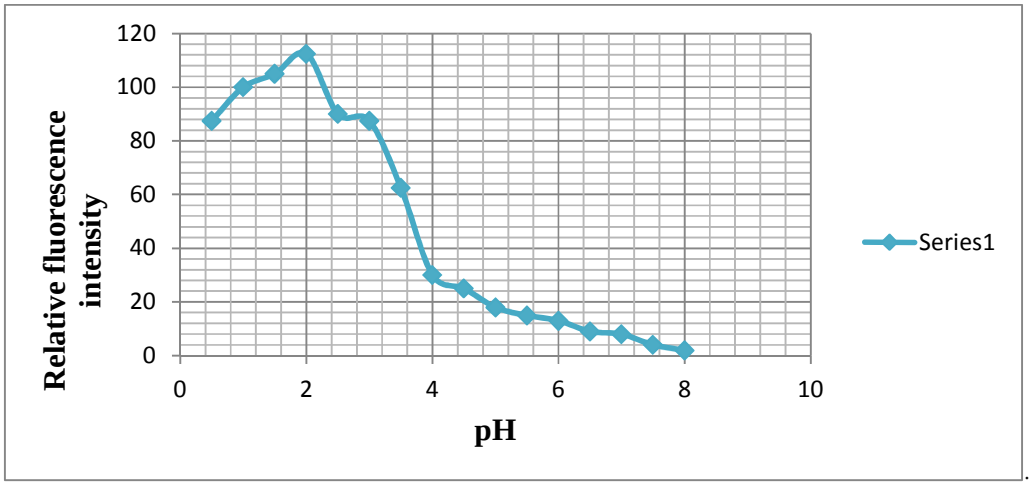


Figure 5. Effect of pH on the RFI

Linearity

From the above prepared stock solution, appropriate dilutions were prepared to get final concentration of 0.2, 0.4, 0.8, 1.0, 1.2 µg/mL and fluorescence intensity was measured at 370 nm over the concentration range of 0.2 - 1.2 µg/mL and the calibration curve was plotted by plotting concentration on X-axis and resultant fluorescence intensity on Y-axis. Their corresponding regression equation was also computed. The calibration data of standard AMS is shown in Table 3. The linear regression data of the proposed method for AMS is presented in Table 4. Figure 6 represents the linearity graph of AMS at different concentrations.

Table 3. Calibration data of standard Amisulpride

Concentration (µg/mL)	Fluorescence intensity
0	0
0.2	132
0.4	256
0.6	387
0.8	512
1	646
1.2	765

Table 4. Linear regression data of the proposed method of AMS

Parameter	Result
λ_{ex} (nm) λ_{em} (nm)	274 370
Linearity range (µg/mL)	0.2 -1.2
Regression equation (Y= a + bc)	Y = 643x + 0.6667
Intercept (a)	0.6667
Slope (b)	643
Standard deviation of intercept (S_a)	2.2562
Standard deviation of slope (s_b)	3.1288
Standard error of estimation (S_e)	3.31123
Correlation coefficient (R^2)	0.99999
% Range of Error (Confidence limits)* 0.05 level 0.01 level	 0.06023 0.07915

** Average of six determinations.*

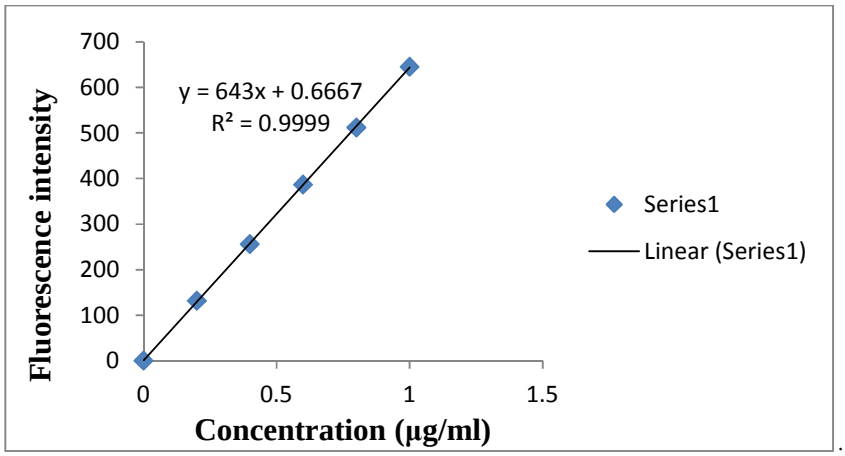


Figure 6. Calibration graph of AMS

Precision

System precision

Six replicate recording of Fluorescence intensity at 370 nm of 0.6 µg/ml concentration standard solution showed % RSD (Relative Standard Deviation) less than 2, which indicates acceptable reproducibility and precision of the system. System precision results are tabulated in Table 5.

Table 5. Results of system precision

S.N0	Concentration	Fluorescence intensity
1	0.6 µg/mL	387
2	0.6 µg/mL	387.1
3	0.6 µg/mL	387
4	0.6 µg/mL	387.18
5	0.6 µg/mL	387.1
6	0.6 µg/mL	387
	Average	387.06
	Standard Deviation	0.075
	% Relative Standard Deviation (RSD)	0.019

Method Precision

Method precision was estimated by performing assay of sample under the tests of (A) repeatability (Intraday precision) and (B) Intermediate precision (Inter day precision) performed during 2 consecutive days by two different analysts, at different working concentrations. Results of Intraday and Interday precision are summarized in table 6. The calculated % RSD were below 2 % indicating excellent precision of the developed procedure at both levels of repeatability and intermediate precision.

Table 6. Intraday and interday precision of AMS

Concentration (µg/mL)	Intraday precision[$]		Interday precision[$]	
	Fluorescence intensity Mean[a] ± SD (n = 6)	% RSD	Fluorescence intensity Mean[a] ± SD (n = 6)	% RSD
0.4 µg/mL	256 ± 0.425	0.166	256 ± 0.342	0.133
0.6 µg/mL	387 ± 0.672	0.173	387 ± 0.458	0.118
0.8 µg/mL	512 ± 0.541	0.105	512 ± 0.597	0.116

[a]Average of six determinations, SD = Standard deviation, % RSD = Relative standard deviation.

Accuracy

Accuracy was determined by performing recovery experiments in which determination of % mean recovery of sample by percentage method at three different levels 80 %, 100 %, 120 %. 80 to 120 % of the sample solutions were prepared as per the procedure given in the methods from the dilutions used for linearity. At each level, three analyses were performed. Percent mean recovery was calculated as shown in Table 7**.** The accepted limits of recovery are 98 %-102 % and all observed data are within the required range and low % RSD are also indicates that the high accuracy of the proposed method.

Table 7. Accuracy data of AMS

Type of recovery in % level	Fluorescence intensity	% Recovery	Mean % Recovery	% RSD
80	204.8	100	99.50	0.25
80	203.4	99.5		
80	202.8	99.02		
100	257	100.39	99.99	0.53
100	256	100		
100	255	99.62		
120	306.8	99.87	99.88	0.32
120	307	99.93		
120	306.8	99.86		

% RSD = Relative Standard Deviation.

Limit of Detection (LOD)

Depending on the standard deviation of the response and the slope, the detection limit may be expressed as: LOD = 3.3*s/S. Where s = the standard deviation of the response. S = the slope of the calibration curve. The result is summarized in table 8. The calculated detection limit indicates that the good sensitivity of the proposed method.

Limit of Quantitation (LOQ)

Depending on the standard deviation of the response and the slope, the quantitation limit (QL) may be expressed as: LOQ = 10* s/S where s = the standard deviation of the response. S = the slope of the calibration curve. The calculated QL for the studied drug is as shown in table 8 which indicates the good sensitivity of the proposed method.

Table 8. LOD and LOQ results of AMS

Limit of Detection (LOD)	0.0116 µg/mL
Limit of Quantitation (LOQ)	0.0350 µg/mL

Robustness and Ruggedness

Robustness was studied by evaluating the influence of small variation of method variables, including concentration of analytical reagents and reaction time on the performance of the proposed methods. In these experiments, one parameter was changed whereas the others were kept unchanged, and the recovery percentage was calculated each time. It was found that tiny variation of method variables did not significantly affect the procedures. This provided an indication for the reliability of the proposed method during its routine application for the analysis of Amisulpride. Ruggedness was also tested by applying the proposed methods to the assay of Amisulpride using the same operational conditions but utilizing two different instruments at two different laboratories and different elapsed time. Results obtained from lab-to-lab and day-to-day variations were found to be reproducible, the full range of recovery values was 98.6–100.4% and the RSD was 0.42 % and 0.62 % for the spectrofluorometric method.

Specificity and interference studies

The specificity of the method was investigated by observation of any interference encountered from the common tablet excipients such as Lactose monohydrate, maize starch, magnesium sterate, methyl cellulose, colloidal anhydrous silica are not found to be interfered with the determination of AMS drug by the proposed method**.** This study indicates that presence of these excipients did not interfere with the developed method as proved the excellent recoveries obtained.(table 9).

Table 9. Analysis of the AMS in presence of some common excipients using proposed spectrofluorimetric method

Excipients	Amount added (µg/mL)	% Recovery ± SD (n=6)
Maize starch	25	100.21±0.54
Lactose	25	99.83±0.55
Mg Sterate	25	98.86±0.62
Methyl cellulose	25	99.88±0.55
Talk	25	99.98±0.44

Determination of Amisulpride in bulk

The concentrations of the drug were calculated from linear regression equations. The % amount found was found to be 99.933 % (table 10).

Table 10. Analysis of Amisulpride in bulk

Concentration (0.6 µg/ml)	Amount found in micrograms*	Amount found in (%)*
Mean±SD	0.5996±0.01	99.933 ±1.62
% RSD	1.66	1.621

***= Average of 6 determinations.*

Application to pharmaceutical formulation:

It is evident from the above mentioned results that the proposed methods yield satisfactory results with AMS in bulk. Thus the said tablets were subjected to the analysis of their contents from the active ingredient by the proposed method. To determine the content of AMS in tablet (label claim 100 mg /tablet) the contents of 20 tablets were weighed and then their mean weight determined and finely powdered. An equivalent weight of the tablet content was transferred into a 100 mL volumetric flask containing 60 mL of milli-Q type 1 ultra pure water, sonicated for 20 minutes and filtered through whatman filter paper. The procedure followed under general recommended procedure which was previously discussed was followed. Concentration of sample solution was found from calibration curve of AMS. The tablet contents, as percentage were 98.87± 0.15 and 99.85 ± 0.18 for Amide and Amysyt respectively. These results were juxtapose with those obtained from the official method by statistical analysis with respect to the accuracy (t-test) and precision (F-test). No significant differences were found between the calculated and theoretical values of t- and-F-tests at 95 % confidence limit proving similar accuracy and precision in the analysis of AMS in its dosage form. The determination of AMS in tablet dosage form by spectrofluorimetry is shown in Table 11.

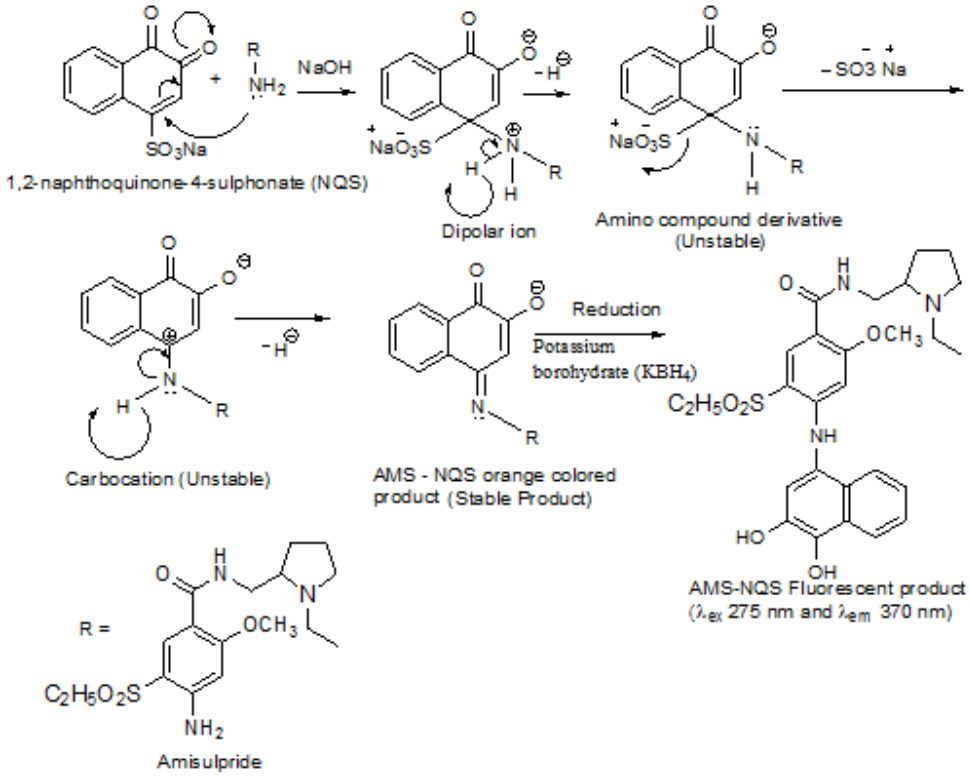
Table 11. Estimation of AMS in tablet formulation by spectrofluorimetry

Tablet formulation	Labeled claim	Amount found (mg/tablet)	% Recovery* ± SD	t-Value[(a)]	F-Value[(a)]
Amide (Mesmer)	100 mg	99.87 mg	99.87 ± 0.15	0.18	1.76
Amysyt (East West)	100 mg	99.85 mg	99.85 ± 0.18	0.17	1.83

** = six times repetitions were done, SD = Standard deviation, (a) Theoretical values for t and F at 95 % confidence limits t=2.571 and F=5.05, respectively.*

Strategy for Reaction Involved

The current study is based on the reaction of AMS with 1, 2 - Naphthoquinone -4- sulphonate in sodium hydroxide, to become dipolar ion and then amino compound derivative which is unstable from, which lose -$S0_3Na$ and forms unstable carbocation can lose one proton forms stable AMS-NQS orange colored product is obtained. When this AMS-NQS orange coloured product is reduced with potassium borohydrate AMS-NQS highly fluorescent product is obtained. This highly fluorescent product shows maximum fluorescence intensity at λem = 370 nm after excitation at λex = 275 nm. The scheme 1 presents the reaction pathway proposed between AMS and NQS.



Scheme 1. Suggested Reaction pathway of AMS with NQS

CONCLUSION

Simple, reliable, sensitive and selective spectrofluorimetric method has been developed for determination of Amisulpride. After examining the fact through relevant literature on the subject the author observed that no analyst hitherto analyze AMS drug in tablet form with NQS as reagent with spectrofluorimetric method. The developed spectrofluorimetric method has the advantage of being simple, highly sensitive and low cost method for determination of the investigated Amisulpride drug in pure form, pharmaceutical formulations, without any interference from common excipients present and with minimum detection limits. The developed method is better than previously reported methods with regard to its selectivity and sensitivity features. The linearity range of the proposed spectrofluorimetric method existed in between 0.2-1.2 µg/mL which is less than previously reported methods on AMS. More over all the reagents connected with this analytical method are less cost, have excellent shelf life and are available in any analytical laboratory. Therefore, the developed method can be considered as suitable for routine analysis of investigated Amisulpride in quality control and clinical laboratories.

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REFERENCES

[1] Current Index of Medical Specialities (CIMS-133, Apr-July [update 2]) UBM Medica India Private Limited, Bangalore, **2016**; 195.
[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, 195-202.
[4] P. Ravi Sankar, Ch. Devadasu, S. Sowjanya, P. Srinivasa Babu, G. Devala Rao, *Int. J. Chem. Sci.*, **2012**, 10, 203-212.
[5] Ch. Devadasu, P. Ravisankar, P. Srinivasa Babu, G. Devala Rao, *The indian Pharmacist*, **2011,** Volume IX, No,7, 70-74.
[6] P. Ravisankar, G. Devala Rao, *International journal of recent scientific research*, **2015,** 6, 4830-4837.
[7] Syeda Humaira, AK. Day, SA. Raju, *Int. J. Chem. Sci.*, **2008**, 6 , 437-440.
[8] P. Ravisankar, G. Devala Rao, *Journal of chemical and pharmaceutical research*, **2015,** 7(6), 815-824.
[9] IV. kudvis, NN. Skakun, IN. Orlova, VV. Libina, AU. Kulikov, *Chromatographia*, **2011**,73,67-74.
[10] R. Nirogi, G. Bhyrapuneni, V. Kandikere, K. Mudigonda, D. Ajjala, R. Suraneni , K. Mukkanti . *Biomed Chromatogr*, **2008**; 22: 1424–1433.
[11] C. Kratzsch , FT. Peters , T. Kraemer, AA. Weber, HH. Maurer, *J Mass Spectrom*, **2003**, 38, 283–295.
[12] MH. Gschwend , P. Arnold, J. Ring , W. Martin, *J Chromatogr B Analyt Technol Biomed Life Sci*., **2006**, 831,132–139.
[13] H. Kirchherr , WN. Kühn-Velten , *J Chromatogr B Analyt Technol Biomed Life Sci*., **2006**, 843,100–113.
[14] L.Couchman , PE. Morgan, RJ. Flanagan, *Biomed Chromatogr*, **2011**, 25, 867–872.
[15] S. Zou , Y. Wen , Q. Lin, *Zhongguo Xiandai Yingyong Yaoxue*, **2009**, 26, 483–486.
[16] C. Frahnert , ML. Rao , K. Grasmader, *J Chromatogr B Analyt Technol Biomed Life Sci*., **2003**,794, 35–47.
[17] M. Ramakotaiah, K. Kanchanamala,C.Bala Sekhar Reddy, C.Babu Rao, K. Chandrasekhar, *Sci.Pharm*.**2011**, 79 (3), 583-599.
[18] I. Paportsis, A. Rizopoulou, P. Nikolaou, C.Pistos, C. Spiliopoulou, S. Athanaselis, *J Chromatogr B Analyt Technol Biomed Life Sci*., **2014, 1, 111-116.**
[19] International Conference on Harmonization. Validation of Analytical Procedures: Text and Methodology Q2 (R1), **2005**.