SIMPLE AND FACILE SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF AMISULPRIDE IN PHARMACEUTICAL FORMULATIONS

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Abstract

Three simple, sensitive and novel spectrophotometric methods have been established for determination of amisulpride (ASP) in pharmaceutical dosage forms. The proposed methods are based on the diazotization of ASP with sodium nitrite and hydrochloric acid, followed by coupling with ethyl acetoacetate, 1-benzoyl acetone and 8-hydroxyquinoline in alkaline medium for methods A, B and C respectively. The formed azo dyes are measured spectro-photometrically at 438, 446 and 545 nm for methods A, B and C respectively. Different variables affecting the reactions were optimized. Beer's law is obeyed over the concentration ranges of 1.0-24.0, 1.0-24.0 and 1.0-20.0 µg/mL for methods A, B, and C, respectively. The results of the proposed methods are validated statistically. The proposed methods were applied successfully to the determination of ASP in pharmaceutical dosage forms.

Rezumat

Lucrarea prezintă etapele de realizare şi validare a trei metode spectrofotometrice noi pentru determinarea cantitativă a amisulpridului (ASP) din diferite forme farmaceutice. Metodele se bazează pe reacţia amisulpridului cu azotitul de sodiu şi respectiv, cu acid clorhidric urmat de o reacţie de cuplare cu acetoacetatul de etil (pentru prima metodă), cu 1-benzoilacetona (metoda a doua) şi cu 8-hidroxichinolina (cea de-a treia metodă) în mediu alcalin. Compuşii formaţi au fost estimaţi spectrofotometric la 438nm (pentru prima metodă), 446nm (metoda a doua) şi 545nm (metoda a treia). Studiul prezintă deasemenea, rezultatele validării acestor metode.

Keywords: amisulpride, spectrophotometry, diazo coupling reaction, pharmaceutical formulations

Introduction

Amisulpiride (ASP - 4-amino-*N*-[(1-ethylpyrrolidin-2-yl) methyl]-5-ethylsulfonyl-2-methoxy-benzamide) (Figure 1) belongs to the new generation of atypical antipsychotic drugs. Its pharmacological activity is based on the selective binding to D2 and D3 dopaminergic receptors. It has a lower risk of extrapiramidal side effects and it is relatively better tolerated

than conventional antipsychotic drugs. Today ASP is widely used in the treatment of different kinds of schizofrenia. Various analytical methods have been reported for the assay of ASP in biological samples. They include high pressure liquid chromatography with UV [1–3], fluorescence [4, 5] and mass spectrometry detection [6, 7]. Very few analytical methods have reported the determination of ASP in pharmaceutical formulations. The methods include differential pulse and square-wave voltammetry [8], UV spectrophotometry [9], visible methods based on azo dye formation [10] and stability indicating derivative spectrophotometric methods [11]. Some of these methods suffer from narrow range of determination, less sensitive and interference from the tablet matrix, whereas others are time consuming or require expensive equipment. This paper describes three sensitive and simple spectrophotometric methods for the determination of ASP in its pharmaceutical formulations. The methods are based on the aromatic amino group present in ASP which was diazotized with nitrous acid at 5 ^{o}C temperature and the diazonium salt thus formed was coupled with ethyl acetoacetate (method A), 1-benzoylacetone (method B) and 8-hydroxyquinoline (method C) in alkaline medium. The colored chromogens formed were measured at 438, 446 and 545 nm for method A, B and C, respectively. The scientific novelty of the present work is that the reagents used in the proposed methods are easily available and the reactions involved with these reagents are simple, rapid and sensitive in their range of determination. The results obtained by the proposed methods were compared favorably with those of the reference method.

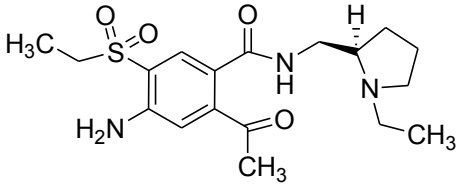

Figure 1
Chemical structure of amisulpride

Materials and Methods

Apparatus

All absorption spectra were recorded using a double beam Ultraviolet-visible-Nearinfrared (UV-Vis-NIR) spectrophotometer (Shimadzu 1601, Japan) equipped with 1 cm matched quartz cells by using a personal computer loaded with the UV-PC 3.9 software package. An

electronic micro balance (Sartorius MC 5, Germany) was used for weighing the substances.

Reagents

All solvents and reagents used were of analytical grade. Double-distilled water was used throughout the experimental study. 0.2 (w/v) % sodium nitrite (S.D. Fine Chem., Mumbai, India), 2% (w/v) sulfamic acid (BDH, Mumbai, India) and 20 % (w/v) sodium hydroxide (S.D. Fine Chem., Mumbai, India) were freshly prepared in double distilled water. 1M hydrochloric acid (S.D. Fine Chem., Mumbai, India) was prepared with double distilled water. 5% (v/v) ethyl acetoacetate (S.D. Fine Chem., Mumbai, India), 0.5 % (w/v)

1- benzoylacetone (Sisco research chemicals, Mumbai, India) and 0.5 % (w/v) 8-hydroxyquinoline (E-Merck, Mumbai, India) were prepared in methanol (Sisco research chemicals Ltd, Mumbai, India).

A standard of ASP was obtained from Hetero Drugs, Hyderabad, India. Different pharmaceutical formulations were purchased from the local market. Standard stock solution of 1000 µg/mL ASP was prepared by dissolving accurately weighed 50 mg of pure drug in 0.1 M hydrochloric acid and diluted to 50 mL in a calibrated flask. The solution was further diluted to 100 µg/mL with 0.1 M hydrochloric acid.

General procedure

Method A

Aliquots of the standard ASP (100 µg/mL) solution ranging from 0.1 – 2.4 mL were transferred into a series of 10 mL volumetric flasks. To each flask, 1 mL of ice-cold 0.2% sodium nitrite and 1 mL of 1 M hydrochloric acid were added. The resultant solution from each flask was well shaken and allowed to stand for 5 min at 0-5 ^{o}C temperature for diazotization to complete. After 5 min, 0.5 mL of 2.0% sulphamic acid was added to each flask. Then volumes of 1.0 mL of 5% ethyl acetoacetate (EAA) and 1.0 mL of 20 % sodium hydroxide solutions were added. The contents were diluted to the mark with distilled water and mixed well. The absorbance of the yellow colored azo dye was measured at 438 nm against the reagent blank.

Method B

Different aliquots of stock reference ASP solution (100 µg/mL) ranging from 0.1 – 2.4 mL were transferred into a series of 10 mL volumetric flasks. To each flask, 1 mL of ice-cold 0.2% sodium nitrite and 1mL of 1M hydrochloric acid were added. The resultant solution from each flask was well shaken and allowed to stand for 5 min at 0-5^{o}C temperature for diazotization to complete. After 5 min, 0.5 mL of 2.0% sulphamic acid was added to each flask. Then volumes of 1.5 mL of 0.5%

1-benzoylacetone (BAC) and 1.0 mL of 20 % sodium hydroxide solutions were added. The contents were diluted to the mark with distilled water and mixed well. The absorbance of yellow colored azo dye was measured at 446 nm against the blank.

Method C

Varying aliquots of stock reference ASP solution (100 µg/mL) in the range from 0.1 – 2.0 mL were transferred into a series of 10 mL volumetric flasks. To each flask, 1 mL of ice-cold 0.2% sodium nitrite and 1 mL of 1 M hydrochloric acid were added. The resultant solution from each flask was well shaken and allowed to stand for 5 min at 0-5 ^{o}C temperature for diazotization to complete. After 5 min, 0.5 mL of 2.0% sulphamic acid was added to each flask. Then volumes of 1.0 mL of 0.5% 8-hydroxyquinoline (8-HQ) and 1.5 mL of 20% sodium hydroxide solutions were added. The contents were diluted to the mark with distilled water and mixed well. The absorbance of the "wine" red colored azo dye was measured at 545nm against the blank.

In three methods, the calibration graph was constructed by plotting the absorbance *versus* concentration of the ASP in µg/mL. The concentration of the unknown was read from the calibration graph or computed from the regression equation.

Application of assays on pharmaceutical formulations

Ten ASP containing tablets were accurately weighed and finely powdered. An amount equivalent to 50 mg of ASP was transferred to a 50 mL volumetric flask and added 25mL of 0.1M hydrochloric acid. This volumetric flask was shaken by a mechanically for 15 minutes. The volume was completed with the same solvent, and the solution was filtered using Whatmann No. 41 filter. The filtrate was diluted with 0.1 M hydrochloric acid to obtain a working concentration of 100 µg/mL of ASP for methods A, B and C. A suitable aliquot was then subjected to analysis.

Results and Discussion

The proposed methods are the diazo coupling reaction of the ASP with EAA, BAC and 8-HQ in alkaline medium. Two steps are involved in the reactions that produce the colored dyes. In the first step, the studied ASP is treated with nitrite solution in acidic medium at 5 ^{o}C temperature, which undergoes diazotisation to obtain the diazonium chloride ion. In the second step, the diazonium cation reacted with the coupling reagents such as EAA, BAC and 8-HQ by electrophilic substitution at active methylene group of EAA and BAC to form the yellow colored azo products in methods A and B in the presence of sodium hydroxide medium. In case of method C an

electrophilic substitution took place in the *para*-position to the hydroxyl group on the benzene ring in 8-HQ to produce a "wine" red colored azo dye in the sodium hydroxide medium. The resulting azo dyes show maximum absorption at 438, 446 and 545 nm for methods A, B and C, respectively (Figure 2). The possible reaction pathways for proposed methods are depicted in figure 3.

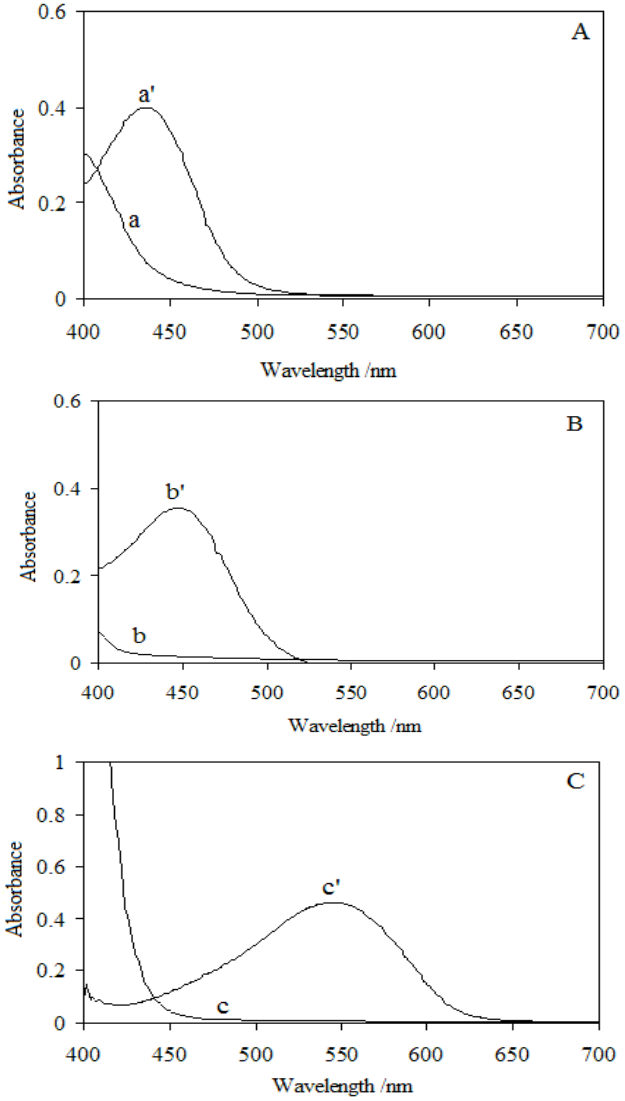

Figure 2

a′, b′, c′ are absorption spectra of reaction products of 10 µg/mL ASP against reagent blanks for methods A, B and C, respectively. a, b, c are absorption spectra of reagent blanks against water for methods A, B and C, respectively.

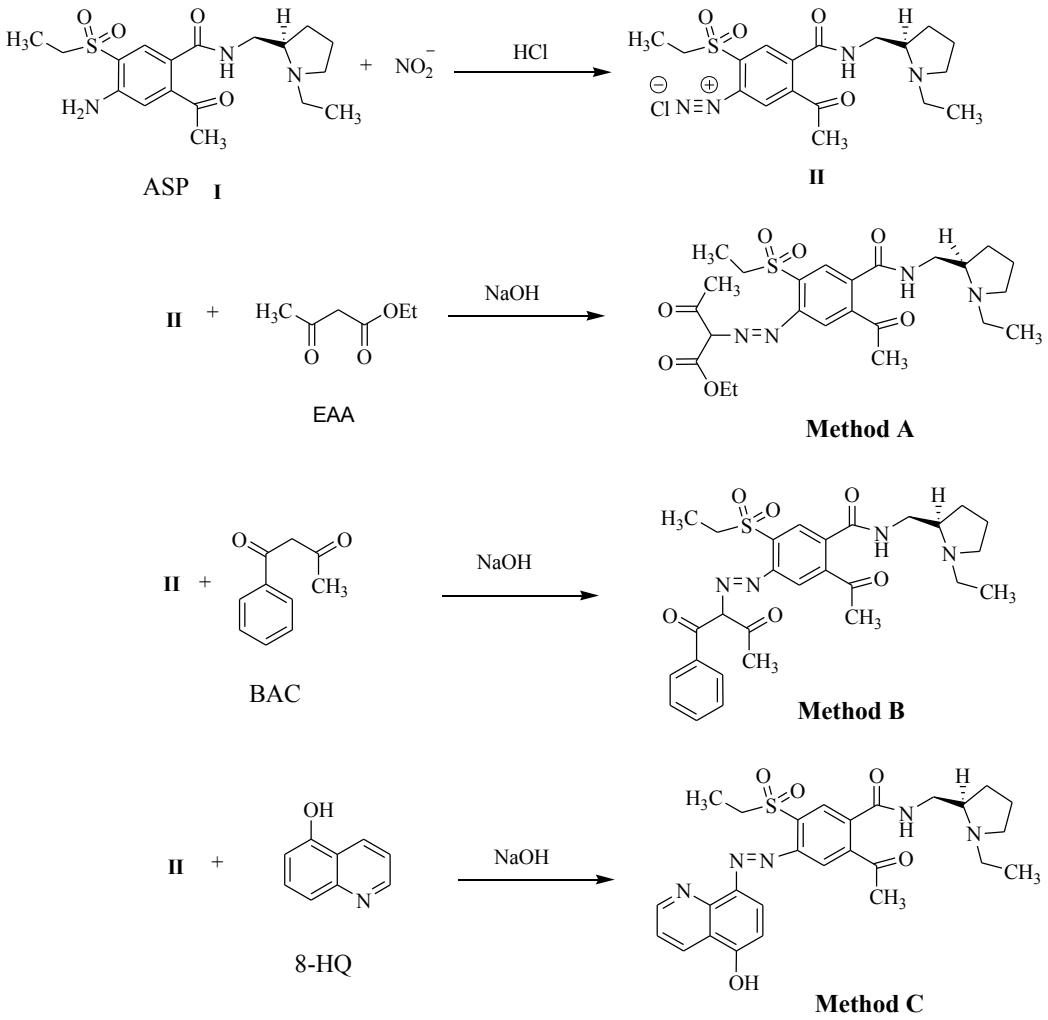

Figure 3
The possible reactions implied by the proposed methods

Optimum reaction conditions

Effect of acid on diazotization

The diazotization reaction occurred in acidic conditions. Hydrochloric acid as reaction medium was found to give more satisfactory results than sulphuric acid. Therefore, the concentration of hydrochloric acid solution was optimized for maximum diazotization. It was found that the absorbance reached its maximum when the amount of hydrochloric acid was 0.8 mL. Evidently, the absorbance was kept to a constant value when the amount of hydrochloric acid was above 0.8 mL (Figure 4). This indicates

that the amount of the formed soluble azo dyes reached its maximum. Thus, 1 mL of 1M hydrochloric acid was used in all methods.

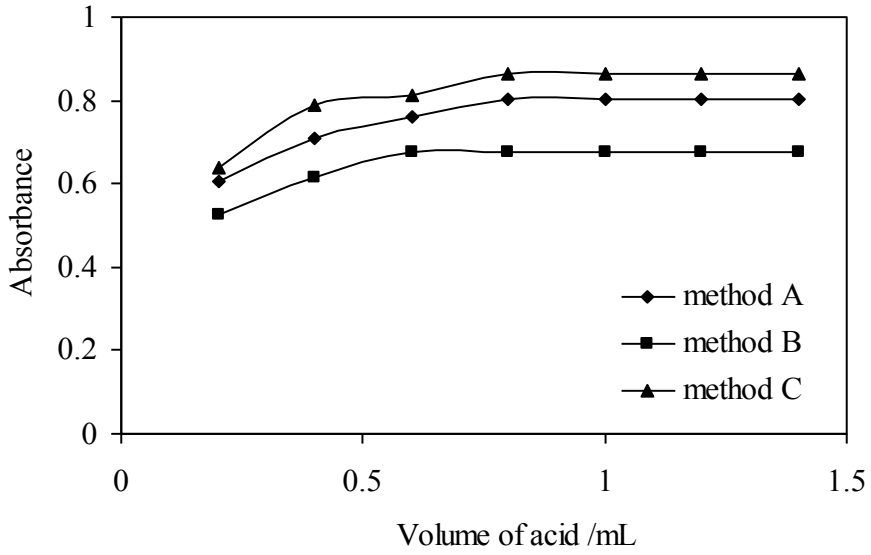

Figure 4
Effect of hydrochloric acid on the 8 µg/ mL ASP in methods A, B, and C.

Effect of Sodium Nitrite Concentration

For the maximum formation of the azo dyes, the influence of sodium nitrite concentration was investigated. The volume of 1mL was selected in order to ensure an excess of reagent in the flask to guarantee the reaction development. The interference of an excess of nitrous acid was removed by adding 0.5 mL of 2 % sulfamic acid before the coupling reaction. The maximum time required for diazotization was found to be 5 min.

Effect of coupling reagents concentration

The effects of the concentration of EAA, BAC and 8-HQ were studied in the proposed methods by measuring the absorbance at specified wavelengths in the standard procedure for solutions containing a fixed concentration of ASP and varying amounts of coupling reagents. These results indicated that the maximum absorbance was obtained when 1.0mL of 0.5% EAA and 8-HQ were added for methods A and C, respectively but in case of method B 1.5mL of 0.5% BAC was found to be optimum for the quantitative determination of the investigated drug. A higher concentration of reagents does not affect the sensitivity of the methods as shown in Figure 5.

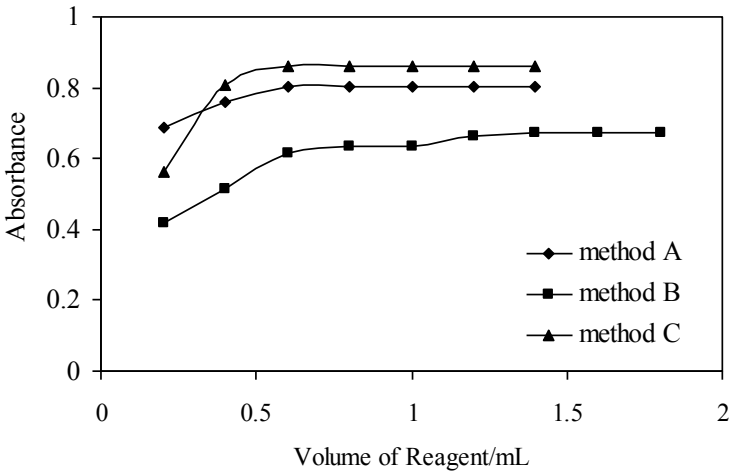

Figure 5
Effect of the concentration of the coupling reagent on 8 µg/mL ASP in methods A, B, and C.

Effect of sodium hydroxide

The stability and formation of azo dyes depends upon the nature of reaction medium. A study was conducted to determine the most effective alkalis and the optimum alkali concentration to be used. Sodium hydroxide was found to be more suitable for the coupling reaction compared to sodium carbonate or aqueous ammonia because the formed dyes were stable and more intense in sodium hydroxide medium. The results of the study reveled that 1mL of 20% sodium hydroxide is optimum for methods A and B. However in the case of method C, 1.5 mL of 20% sodium hydroxide is the optimum volume.

A stability study of the chromogens was carried out by measuring the absorbance values at time intervals of 10 min. It was found that the formed azo dyes were stable for more than 2 hours in method A and 3 hours in methods B and C.

Validation of the proposed methods

Calibration curves, linearity and sensitivity

Under optimum experimental conditions, a linear relation was obtained between absorbance and concentration of ASP in the range 1.0-24.0 µg/mL in method A, 1.0-24.0 µg/mL in method B and 1.0-20.0 µg/mL in method C. The regression analysis of the plot using the method of least squares was performed to evaluate the intercept (a), slope (b), regression

coefficient (r^2) and standard deviations of slope and intercept. In all cases, Beer's law plots were linear with good correlation coefficients as shown Table I. The detection limit or LOD is the lowest amount of analyte in a sample that can be detected not quantitated under the experimental conditions. The LOD and LOQ [12] were determined using the formula: LOD or LOQ= kS.D.a/b, where k is 3.3 for LOD and 10 for LOQ, S.D.a is the standard deviation of the intercept, and b is the slope of regression line.

Table I
Analytical and regression parameters of the proposed methods

Parameter	Method A	Method B	Method C
λ_{max} (nm)	438	446	545
Beers law limit – Linear concentration range (µg/mL)	1.0-24.0	1.0-24.0	1.0-20.0
Molar absorptivity (L/mol. cm)	3.6918×10^4	3.1557×10^4	3.8146×10^4
Sandell`s sensitivity (µg/cm^2)	1.0×10^{-2}	1.17×10^{-2}	0.96×10^{-2}
Slope (b)	0.0999	0.0854	0.1032
Intercept (a)	0.0021	0.0066	0.0069
Correlation coefficient (r^2)	0.9999	0.9996	0.9999
Standard deviation of slope (S_b)	4.3×10^{-4}	6.9×10^{-4}	4.6×10^{-4}
Standard deviation of intercept (S_a)	5.9×10^{-3}	9.3×10^{-3}	5.2×10^{-3}
Detection limit LOD (µg /mL)	0.19	0.36	0.16
Quantification limit LOQ (µg /mL)	0.6	1.1	0.5

Precision and accuracy of the proposed methods

Intra-day precision (repeatability) of the proposed methods was evaluated by carrying out the determination of six replicates of pure drug at three different concentration levels within Beer's limit on the same day. Inter-day precision (intermediate precision) was also evaluated by assaying the pure drug solution in six replicates at the same concentration levels on five consecutive days. Table II summarizes the intra-day and inter-day precision and accuracy of the proposed methods. The results reveal that precision and accuracy of the proposed method were fairly high as indicated

by the low values of %RSD (relative standard error) and %RE (relative error).

Table II

Evaluation of precision and accuracy of the proposed methods

Proposed method	ASP taken (µg/ mL)	Intra-day precision (*n*=6)*			Inter-day precision (*n*=6)*		
		ASP found (µg /mL)	% RSD	% RE	ASP found (µg /mL)	% RSD	% RE
Method A	4	4.02±0.036	0.89	0.50	3.98±0.051	1.25	-0.50
	12	12.05±0.046	0.38	0.42	12.03±0.162	1.33	0.25
	20	20.01±0.044	0.22	0.05	20.02±0.187	0.95	0.10
Method B	4	4.02±0.029	0.77	0.50	3.98±0.055	1.38	-0.50
	12	11.93±0.075	0.64	-0.58	11.95±0.115	1.00	-0.41
	20	19.96±0.047	0.23	-0.20	20.03±0.206	1.05	0.15
Method C	4	3.97±0.061	1.54	-0.75	4.02±0.052	1.30	0.50
	12	12.05±0.088	0.73	0.41	12.01±0.128	1.07	0.08
	20	19.96±0.169	0.85	-0.58	19.98±0.174	0.87	-0.10

*Mean value of six determinations; SD = Standard deviation; RSD = Relative standard deviation ; RE. Relative error

Accuracy and recovery

The validity of the proposed methods was also checked by performing recovery experiments by the standard addition method. In this study, pre analyzed tablet powder was spiked with pure ASP at two different levels and the total amount of drug was determined by the proposed methods. Each determination was repeated three times. The recoveries of the pure drug added to the tablet powder are shown in Table III. The results reveal that the proposed methods are not liable to interference by tablet fillers, excipients and additives usually used in pharmaceutical preparations. The recovery values are indicating that the accuracy of method is not affected by co-formulated substances.

Table III

Results of recovery experiments by standard addition method

Proposed methods	Formulation taken (µg /mL)	Pure drug added (µg /mL)	Sizopride®	Sulpitac®	Amigold®
			(%)Recovery [a] ± SD		
Method A	8	4	100.2±0.71	101.8±1.87	101.15±1.31
	8	8	100.32±0.95	102.07±1.09	101.06±1.12
Method B	8	4	98.05±0.97	100.08±0.65	99.62±0.94
	8	8	99.47±0.58	101.05±0.93	100.65±0.39
Method C	8	4	99.44±0.73	99.6±1.45	100.48±0.42
	8	8	99.21±1.14	100.12±0.5	101.11±1.21

[a]Mean value of three determinations

Application of the method to the analysis of tablets

The proposed methods were successfully applied to the analysis of ASP in various commercially available tablet forms. The results of the

assays are given in table IV and compared favorably with the reference method [9]. As can be seen from table IV, the calculated t-value and F-value at the 95 % confidence level did not exceed the tabulated values of 2.57 and 5.05, respectively, for five degrees of freedom. It is obvious from these results that the proposed methods are applicable to the analysis of drug in its formulations with comparable analytical performance.

Table IV

Results of the assay of ASP in tablet forms by the proposed methods

Pharmaceutical preparations #	Amount per tablet (mg)	% Found* ± SD			
		Reference method	Method A	Method B	Method C
Sizopride®-100[a]	100	99.68±1.09	100.3±1.35 t=0.87 F= 1.50	99.09±0.60 t=0.99 F= 3.36	100.36±1.07 t= 1.08 F= 1.05
Sulpitac®-100[b]	100	101.07±1.60	101.21±1.41 t= 0.16 F= 1.29	100.71±1.83 t=0.37 F= 1.30	100.68±0.92 t=0.52 F= 3.02
Amigold®-100[c]	100	100.5±0.86	100.63±1.07 t= 0.23 F= 1.55	100.20±1.72 t=0.04 F= 4.0	99.97±1.19 t=0.13 F= 1.91

#Marketed by: [a] Crescent Pharma, Baddi; [b]Sun pharma, Mumbai; [c]Unichem, Mumbai.
*Mean value of six determinations. The theoretical values of t (2.57) and F (5.05) at confidence limit at 95% confidence level and five degrees of freedom (p=0.05).

Conclusions

The present paper presents EAA, BAC and HQ as new diazo coupling reagents for the spectrophotometric determination of ASP. The proposed methods are selective as the free aromatic amine group preferentially interacts with nitrous acid to form the diazotized product which is later coupled with EAA, BAC or 8-HQ to form very stable dyes. The developed methods in the present work proved to be an excellent alternative for the ASP determination in pharmaceutical formulations. The proposed method concerning 8-HQ was superior as compared with that of the other coupling reagents and the performance order of the proposed methods is 8-HQ > EAA > BAC according to the higher molar absorpitivity and lower detection limits. The proposed methods provide adequate sensitivity, selectivity and is free from interferences. From the practical point of view, the developed methods need minimum sample treatment, which allowed us to achieve a high analytical productivity. These advantages make the proposed methods very suitable for routine analysis of ASP in quality control laboratories.

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