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NEW COLORIMETRIC METHOD DEVELOPMENT AND VALIDATION OF SULFACETAMIDE IN BULK AND FORMULATION BY DIFFERENT ANALYTICAL REAGENTS

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Abstract

Four simple, sensitive and reproducible spectrophotometric methods (Method A, Method B, Method C and Method D) were developed for the determination of sulfacetamide (SA) and its pharmaceutical formulation. Method A was developed based on diaziatation of the SA by sodium nitrite in acidic medium followed by coupling with B.M reagent having absorption maximum at 530 nm. Method B was developed based on reaction of NQS with primary amine in SA in presence of alkaline medium having maximum absorption at 466nm. Method C was based on reaction of primary amine with MBTH in presence of FeCl3 having maximum absorption at 562nm. Method D was developed based on reduction of phosphomolybdotungstic acid in presence of alkali medium having an absorption maximum at 760 nm. Beer's law was obeyed in the range of 1 to 3 µg/ml for Method A, 5 to 30 µg/ml for Method B, 10 to 50 µg/ml for Method C, and 100 to 300 µg/ml for Method D. These methods were successfully validated and estimated in bulk and pharmaceutical formulations.

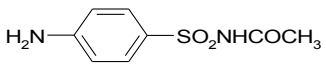
***Keywords*:** B.M reagent, F.C reagent, MBTH reagent, NQS reagent, Sulfacetamide

1. Introduction

Sulfacetamide is a sulfonamide bacteriostatic antibiotic. It is chemically known as N-[4-aminophenyl) sulfonyl]-acetamide [1]. Many bacteria synthesize their own folic acid (FA) of which p-amino benzoic acid (PABA) is a constituent, and is taken up from the medium.Sulfonamides, being are structural analogues of PABA, that competitively inhibit bacterial folate synthase, folic acid is not formed and a number of essential metabolic reactions suffer [2- 4]. They are effective against many gram positive organisms and some gram negative bacilli [5] and are used in the treatment of urinary tract infections and eye infections.SA is a highly soluble compound yielding neutral solution, mildly irritating to the eye in the concentration up to 30%.

Literature survey reveals that the drug is official in Indian Pharmacopoeia[6], United States Pharmacopoeia[7]; British Pharmacopoeia[8]estimated by nitrite titration and can be determined by a variety of analytical techniques such as Column Chromatography[9], liquid Chromatography[10], capillary chromatography[11], micellar liquid chromatography[12], Spectrophotometric determination [13-15] , spectroscopic studies [16,17] , NMR[18] , fluorescent probe study[19]

Fig 1: Structure of Sulfacetamide



2. Experimental

2.1 Chemicals and Materials: All employed chemicals were of analytical grade and highly purified water was used. Sulfacetamide pure sample was obtained as a gift sample from Kanvista formulations, Hyderabad, India.

2.2 Instrumentation: UV/Visible spectrophotometer (LABINDIA UV/3092) with matched quartz cells were used for the present investigation.

2.3 Preparation of reagents:

2.3.1 Sodium nitrite solution (0.1%w/v): 100 mg of sodium nitrite was dissolved in distilled water and made up to 100 ml.

2.3.2 Hydrochloric acid (5N): 425 ml of concentrated HCL was taken and diluted to 1000 ml with distilled water.

2.3.3 Ammonium Sulfamate Solution (0.1%W/V): 500 mg of ammonium sulfamate was dissolved in distilled water and made up to 100 ml with distilled water.

2.3.4 B.M Reagent (N-(1-napthyl) ethylene diamine dihydrochloride solution) (0.1%w/v):

100 mg of B.M reagent was dissolved in 100 ml of distilled water.

2.3.5 Sodium hydroxide solution (0.1N): 400 mg sodium hydroxide was dissolved in distilled water and made up to 100 ml with distilled water.

2.3.6 NQS reagent (β-naphthoquinone-4-sulfonate sodium salt) (0.5%w/v): 500 mg of NQS reagent was dissolved in distilled water and made up to 100 ml with distilled water.

2.3.7 Ferric chloride (1%w/v): 1 gm of ferric chloride was dissolved in distilled water and made up to 100 ml with distilled water.

2.3.8 MBTH reagent (3-Methyl 2 Benzothiazolinone Hydrazone HCL (0.5%w/v) :

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500 mg of MBTH reagent was dissolved in distilled water and made up to 100 ml with distilled water.

2.3.9 F.C Reagent (Folin-Ciocalteu Reagent): F.C reagent is diluted with water in the ratio of 1:2

2.3.10 Sodium carbonate solution (20%w/v): 20 gm of sodium carbonate was dissolved in distilled water and made up to 100 ml.

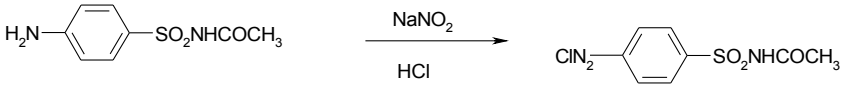
2.3.11 Preparation of Standard Solutions: 100 mg of sulfacetamide was accurately weighed and dissolved in 100 ml of distilled water (stock solution I).

2.3.12 Preparation of sample solution (sulfacetamide eye drops): Prepare 1000 µg/ml solution with distilled water from eye drops (stock A).

2.4 Procedure for Estimation

2.4.1 Method A: Prepared 100µg/ml from the stock solution. To each flask, 1 ml of 5 N concentrated HCl and 1 ml of 0.1 % w/v sodium nitrite were added and shaken for 5 minutes. To this, 1 ml of 0.5 % w/v ammonium sulfamate was added followed by the addition of 1 ml of B.M reagent (scheme.1).The absorbance was measured at 530 nm against the reagent blank (Fig.2).The linearity range from 1 to 3 µg/ml (Fig.2a).The amount was calculated from the calibration graph.

Scheme 1



Sulfacetamide Diazonium Salt

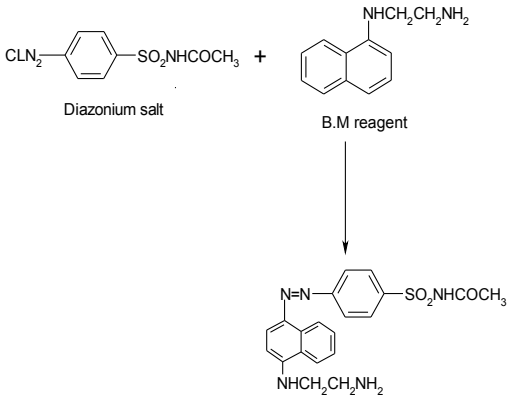


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Fig.2 Absorption spectra of B.M with Sulfacetamide against the reagent blank

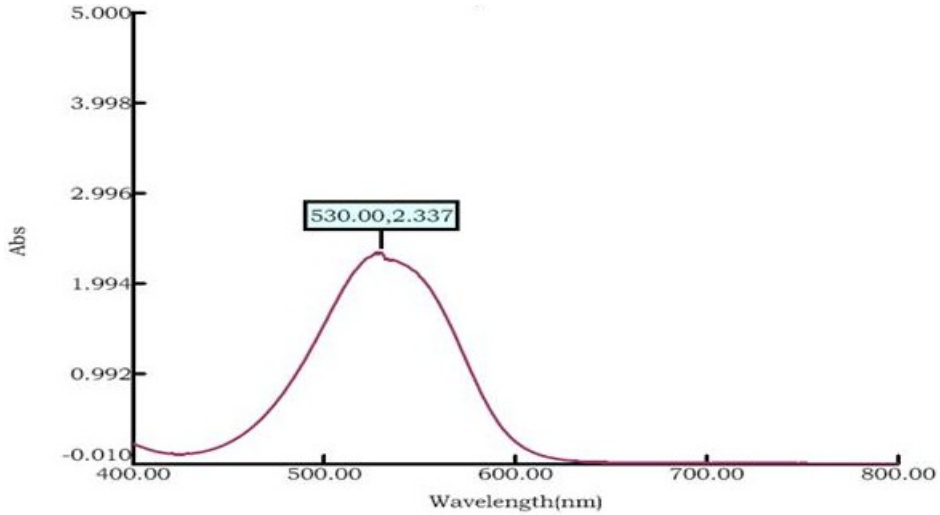
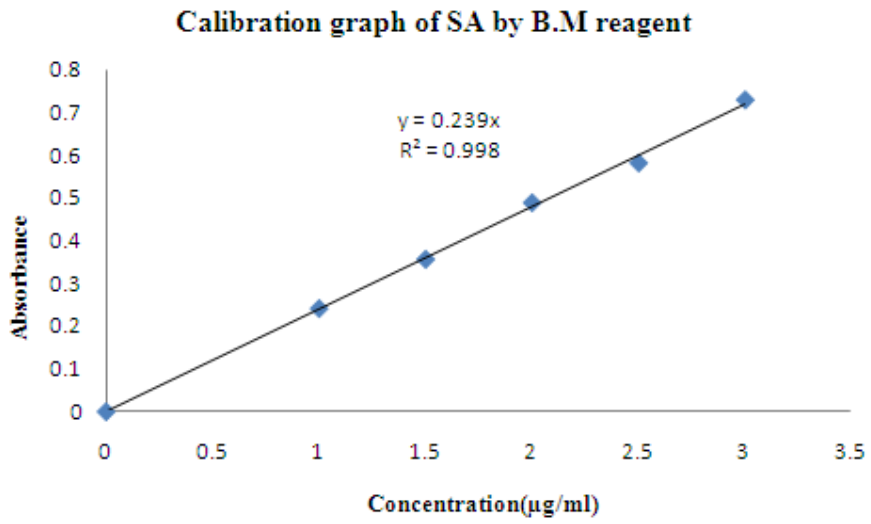


Fig.2a.Calibration graph of SA by B.M reagent



2.4.2. **Method B:** Prepared 100µg/ml from the stock solution. To each flask, 1 ml of 0.5%w/v NQS regent, followed by addition of 1 ml of 0.1 N sodium hydroxide solution made up the volume with distilled water (scheme.2). The absorbance was measured at 466 nm against the reagent blank (Fig.3). The linearity range from 5 to 30 µg/ml (Fig.3a). The amount was calculated from the calibration graph.

Scheme 2:

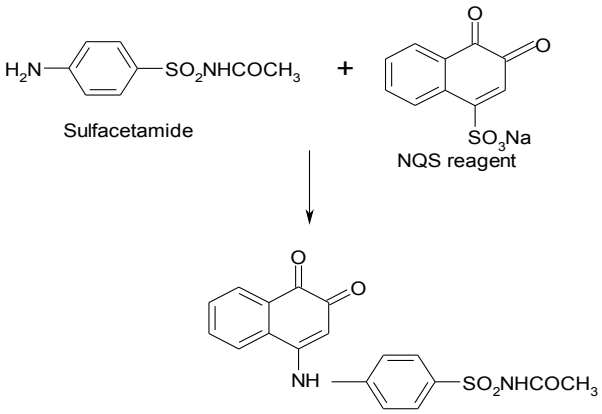


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Fig.3 Absorption spectra of NQS with Sulfacetamide against the reagent blank

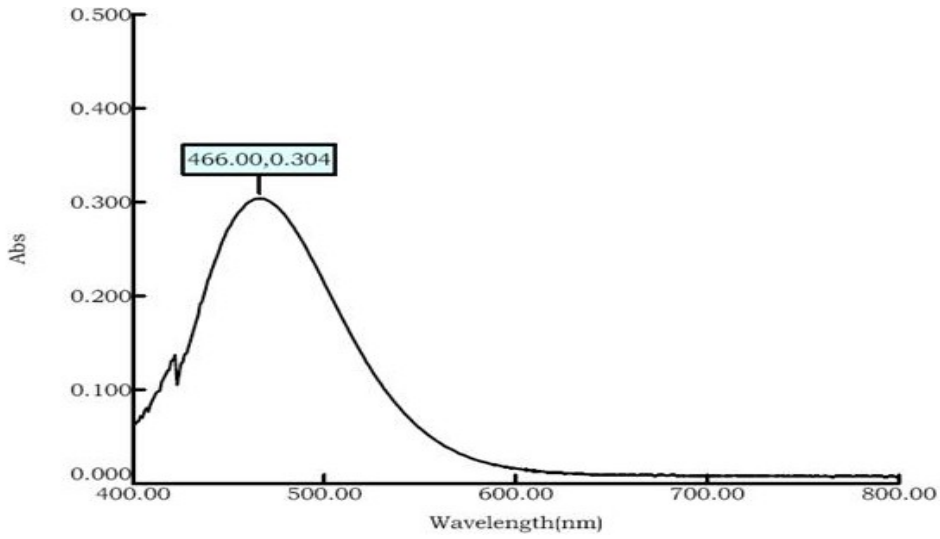
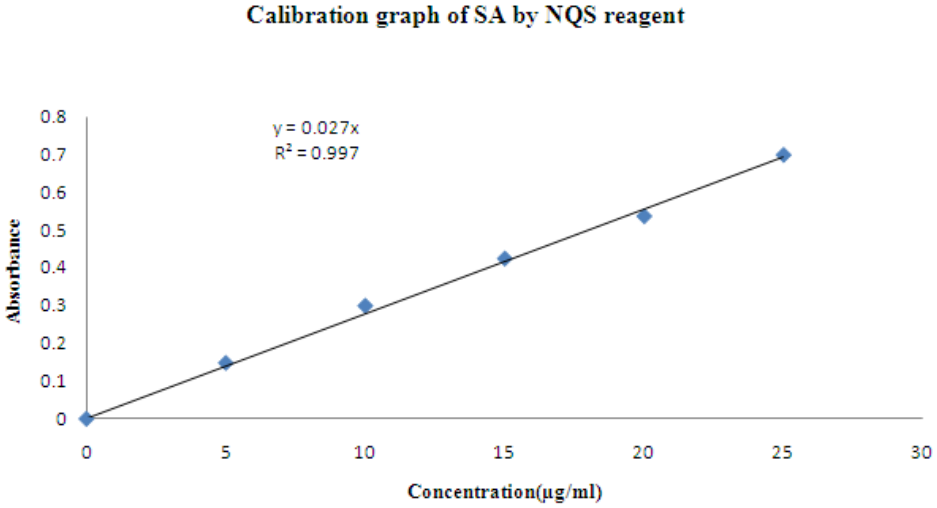
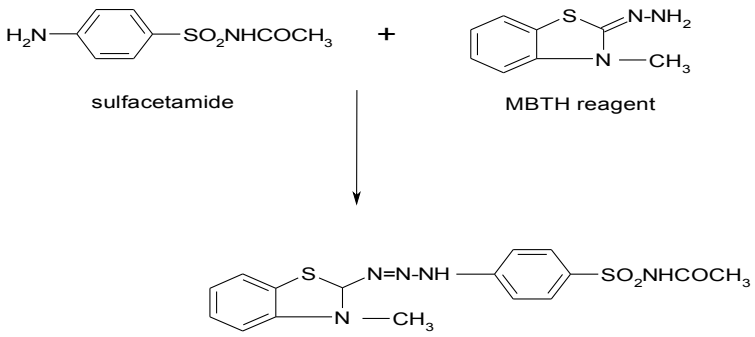


Fig.3a.Calibration graph of SA by NQS reagent



2.4.3 Method C: Prepared 100µg/ml from the stock solution. To each flask, 2 ml of 0.5% MBTH reagent, followed by addition of 2ml of 1% Ferric Chloride solution, kept aside for 20 minutes ,made up with distilled water(scheme.3).The absorbance was measured at 562 nm against the reagent blank (Fig.4). The linearity range from 10 to 50 µg/ml (Fig.4a).The amount was calculated from the calibration graph.

Scheme 3:



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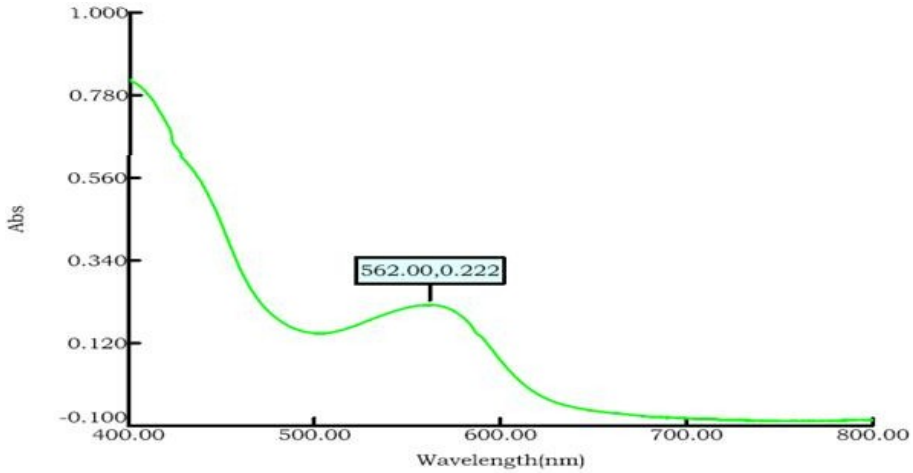

Fig.4a.Calibration graph of SA by MBTH reagent

2.4.4 Method D: Prepared 100µg/ml from the stock solution. To each flask, 1 ml of F.C reagent, followed by addition of 2 ml of 20%w/v sodium carbonate solution made up the volume with distilled water.The absorbance measured was absorbance at 760 nm against the reagent blank (Fig.5). The linearity range from100 to300µg/ml (Fig.5a).The amount was calculated from the calibration graph.

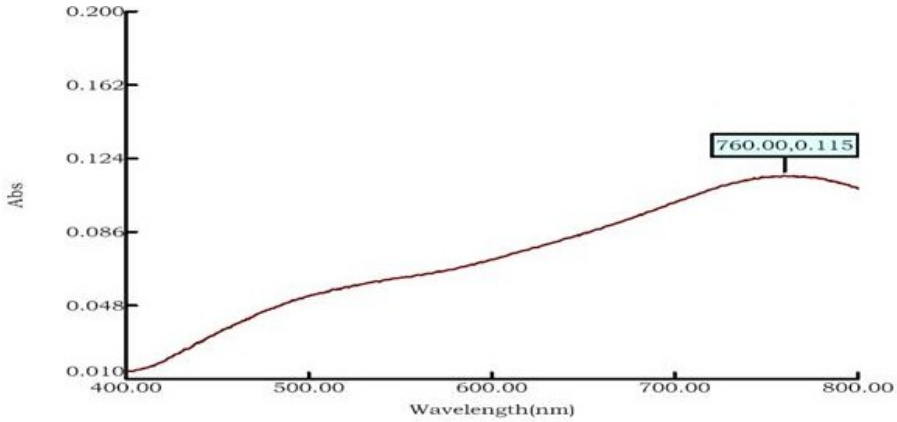

Fig.5. Absorption spectra of F.C with Sulfacetamide against the reagent blank

Calibration graph of SA by F.C reagent

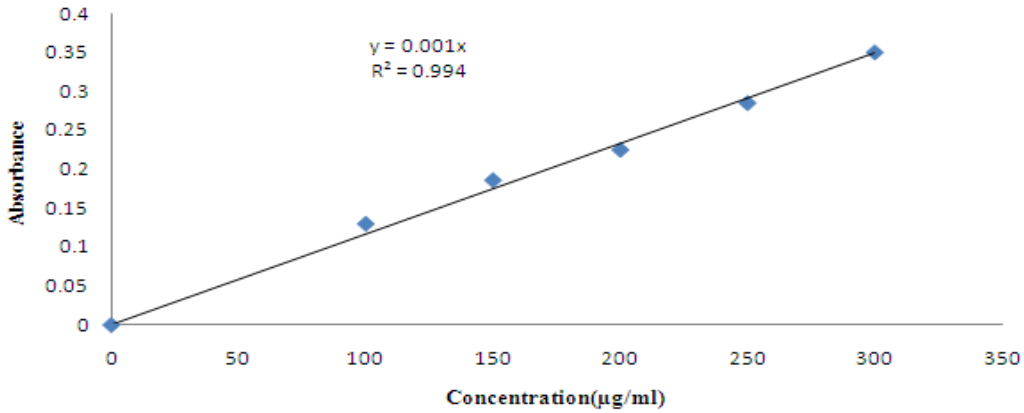

Fig.5a.Calibration graph of SA by F.C reagent

3. Results and Discussion

3.1 Validation parameters: The methods were validated statistically as per ICH guidelines [20] for all the parameters like accuracy, linearity, precision, ruggedness and specificity. Accuracy of the methods were ascertained on the basis of recovery studies, carried out by standard addition method in which pre-analyzed samples were taken and standard drug was added at three different levels (80%,100%,120% of the test concentration). The % recovery studies are given in (Table 1). Precision was studied by analyzing six replicates of sample solutions and concentrations were calculated and given in (Table 2). Ruggedness was established by carrying out experiment at different conditions like intraday, interday and by analyst specificity. By observing validation parameters methods were found to be specific, accurate, precise and economical and can be successfully applied to analyze commercially available formulations containg sulfacetamide. The results obtained are in good agreement with the labeled content.

Table 1. Results of Recovery Studies.

Recovery level (%)	Amount spiked(µg/ml)	Amount recovered(µg/ml)	%Mean recovery(n=3)	%RSD
Method A				
80	1	0.999	99.9	0.320
100	2	2.01	100.50	0.412
120	3	3.02	100.66	0.452
Method B				
80	10	10.03	100.30	0.291
100	15	14.99	99.93	0.301
120	20	19.99	99.95	0.312
Method C				
80	15	15.04	100.26	0.121
100	20	20.01	100.05	0.281
120	25	25.03	100.12	0.321
Method D				
80	100	99.99	99.99	0.134
100	150	149.98	99.98	0.254
120	200	200.02	100.01	0.442

Table 2: Evaluation of Precision

Drug	S.No	Label claim	% Purity	Average (%)	SD	RSD [a]	RSD [b]
Method A Sulfacetamide eye drops IP	1	Albucid® 20%	97.21	98.47	0.78	0.62	0.82
	2		98.02				
	3		98.43				
	4		99.21				
	5		98.63				
	6		99.32				
Method B Sulfacetamide eye drops IP	1	Albucid® 20%	99.32	98.63	0.78	0.65	0.96
	2		98.99				
	3		99.52				
	4		98.50				
	5		97.50				
	6		97.99				
Method C Sulfacetamide eye drops IP	1	Albucid® 20%	99.21	99.65	0.43	0.52	0.98
	2		100.01				
	3		100.12				
	4		99.98				
	5		99.10				
	6		99.50				
Method D Sulfacetamide eye drops IP	1	Albucid® 20%	97.81	98.57	0.47	0.56	1.21
	2		98.29				
	3		98.99				
	4		99.12				
	5		98.50				
	6		98.76				

SD. Standard deviation; RSD.relative standard deviation; a: intraday precision, b: interday precision.

3.2 Spectral characteristics: Method A involves diaziatation of the drug by sodium nitrite in acidic medium followed by coupling with B.M reagent. Method B is based on reaction of NQS with primary amine involves nucleophilic substitution reaction in alkaline medium. Method c is based on reaction with MBTH in presence of Fecl3. Method D is based on reduction of phosphomolybdotungstic acid in presence of alkali medium.

3.3 Quantification: The limits of Beer's law, the molar absorptivity and Sandell's sensitivity values were evaluated which were given in Table3.Graphs of absorbance versus concentration showed zero intercept and are described by the regression equation, Y=bx (where Y is the absorbance of a 1 cm layer, b is the slope and x is the concentration of the drug in µg/ml).

Table 3: Optical characteristics and validation data of Sulfacetamide.

Parameters	Method A	Method B	Method C	Method D
λmax (nm)	530	466	562	760
Beer's law limits (µg/ml)	1-3	5-25	10-50	100-300
Molar absorptivity (l/mol/cm)	$60.75*10^3$	$6.868*10^3$	$2.036*10^3$	$0.254*10^3$
Correlation coefficient (r^2)	0.998	0.997	0.995	0.996
Sandell's sensitivity (ng/cm^2)	$0.4184*10^{-2}$	$3.70*10^{-2}$	$12.48*10^{-2}$	$100.078*10^{-2}$
Regression equation (y)	Y=0.239X	Y=0.027X	Y=0.008X	Y=0.001X
Slope (b)	0.239	0.027	0.008	0.001
%RSD	0.456	0.312	0.0514	0.062
LOD (µg/ml)	0.138	6.11	5.94	0.062
LOQ (µg/ml)	0.4184	18.518	18.00	132.0
Inter day RSD	0.326	0.412	0.102	0.230
Intraday RSD	0.721	0.632	0.203	0.430

Y=mx where x is the concentration of drug in µg/ml; Average of six determinations

4. Conclusion

The proposed methods were found to be simple, economical, selective and sensitive. The statistical parameters clearly indicated the reproducibility and accuracy of the methods.

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