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Research Article

SOLID PHASE EXTRACTIVE SPECTROPHOTOMETRIC DETERMINATION OF SOME SULFA DRUGS

***KANCHAN UPADHYAY[1] , ANUPAMA ASTHANA[1],& NEETU TIWARI[1]**

[1]**Department of Chemistry, Govt. V.Y.T. PG. Autonomous college, Durg(Chhattisgarh), India 491001**,**Email:** kanchanupadhyay83@gmail.com

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ABSTRACT

A simple and sensitive solid phase extractive spectrophotometric method for the determination of three sulfa drugs in bulk, in pharmaceutical dosage forms and in biological fluids was developed. The method is based upon coupling of diazotized sulfa drug with phloroglucinol in an acidic medium. The resulting yellow dye has absorption maximum at 420nm and the resulting dye is stable for several days. The Beer's law range for sulfamethoxazole, sulfacetamide and sulfadiazine in aqueous medium are 0.2-2.0, 0.2-2.0 and 0.1-1.0 µg mL^{-1} respectively and in extractive medium range for above drugs are 0.02-0.2, 0.02-0.2, and 0.01-0.1 µg mL^{-1}. The molar absorptivity for the sulfa drugs is 9.21x 10^5, 7.79 x 10^5 and 1.820 x10^6 L mol^{-1} cm^{-1} respectively. The developed method is free from the interference of common excipients used in pharmaceutical dosage. The method was also used for the determination of sulfa drugs in pharmaceutical dosage as well as in human serum and urine samples.

Keywords: Sulfamethoxazole, sulfacetamide, sulfadiazine, phloroglucinol, spectrophotometry. Solid phase extraction

INTRODUCTION

Sulfonamides, important analogues of p-amino benzoic acid[1] are used in the treatment of urinary track infections, eye infections and as a prophylaxis of rheumatic fever[2]. Antibacterial sulfonamides act as competitive inhibitors of the enzyme dihydropteroate synthetase, DHPS in bacteria. DHPS catalyses the conversion of PABA (p-amino benzoate) to dihydrophorate a key step in folate synthesis, which in necessary for the cell to synthesize nucleic acids and thus exhibits a bacteriostatic effect[3]. Sulfonamides are rapidly absorbed establishing a therapeutic range of 30-150 µg mL^{-1} in plasma and 500-1000 µg mL^{-1} in urine. The major metabolite N-acetylated sulfonamide has no antibacterial activity but retain the toxicity of the parent compound[4]. A regular determination of the plasma concentration of free drug is essential when patients with sever bacterial infections are being treated with large doses of sulfonamides[5]. The most common manifestations of a hypersensitivity reaction of sulfa drugs are "rash" and hives. However there is several life threatening manifestations of hypersensitivity to sulfa drugs including Stevens- Johnson syndromes, toxic epidermal necrosis, agranulocytosis, hemolysis anemia, thrombocytopenia and fulminate hepatic necrosis[6].

Various methods have been developed for determination of sulfa drugs in pharmaceutical preparations and in biological fluids. The method includes GC [7,] HPLC [8, 9, and 10], electro analytical methods [11], voltametric determination[12-14] immune chemical assay[15, 16], differential scanning calorimetry[17], surface enhanced Raman spectrometry[18], spectroflurimety[19], and spectrophotometry [20-27]. Most spectrophotometric methods suffer from low sensitivity high detection limits, tedious experimental conditions and complex procedure for the preparation of samples or standard solutions.

EXPERIMENTAL

Apparatus

A Systronic spectrophotometer (Model 166) was used for absorbance measurement. The pH measurements were made with a Systronic digital pH meter (Model-335).

Reagents

Pharmaceutical grade sulfa drugs (Merck Ltd.) sulfamethoxazole, sulfacetamide and sulfadiazine were used, sodium nitrite and phloroglucinol from Aldrich chemicals. All other reagents and solvents were of analytical grade. Commercial dosage forms were obtained from the local market.

Stock and reagent solution

A 1000 µg mL^{-1} stock solution of drug was prepared by dissolving 100 mg each sulfonamide in 20 ml of 10M H_2SO_4 then diluting with water up to the mark in 100 ml volumetric flask.

Sodium nitrite solution

0.02 %(w/v) sodium nitrite solution was prepared in double distilled water.

Phloroglucinol solution

0.25% (w/v) phloroglucinol solution was prepared in double distilled water.

10M H_2SO_4 and 3% (w/v) sulphamic acid solution was also prepared in double distilled water.

GENERAL PROCEDURE

Preparation of calibration curve

Aliquots of standard sulfonamide solutions sulfamethoxazole (0.02-0.2 µg mL^{-1}), sulfacetamide (0.02-0.2 µg mL^{-1}), and sulfadiazine (0.01-0.1 µg mL^{-1}) were taken into 25 mL graduated flask followed by 0.5 mL sulphuric acid to each. After cooling in an ice bath 1mL of sodium nitrite (0.02%) was added and allowed to stand for 5 min with occasional shaking. Excess nitrite was removed by addition of 1 mL of sulphamic acid. Then 1.5 mL of phloroglucinol was added. A yellow colour appears which enhanced after addition of 1mL 10M sulphuric acid solution. The solution was made up to the mark with distilled water.

The resulting yellow dye was extracted by passing the above solution through a C-18 cartridge that was preconditioned by passing in sequence 3 mL of each of methanol and water. The dye was eluted by passing 2 mL of methanol and the absorbance was measured at 420 nm against a reagent blank, which gave negligible absorbance at this wavelength.

Determination of dosage form

Tablets: The tablet formulations were purchased from local market each containing 500 mg of sulfonamides. Twenty tablets were powdered and mixed thoroughly amount equivalent to 100 mg sulfonamides was then dissolved in 2 mL of 10M sulphuric acid and then diluting with water up to 100 mL and appropriate aliquots of the solution were taken and the above recommended procedure was followed.

Eye drops: The eye drop formulations purchased from local sources containing 10 mg mL^{-1} were taken. A volume of the eye drops equivalent to 100 mg of sulfonamides were diluted with 2 mL of 10M sulphuric acid and made up to 100 mL with double distilled water and the above-mentioned procedure followed.

Suspensions: Suspensions were mixed thoroughly, an amount equivalent to 100mg of sulfonamides was taken dissolved in 10M

sulphuric acid and made up to 100 mL, and appropriate aliquot were taken and above recommended procedure was followed.

Determination in serum and urine

1 mL of blank serum or urine sample was spiked with sulfonamides at a concentration level of 5-50 µg mL^{-1} 2 mL of 20% trichloroacetic acid was added to each sample and vortex for 1 min and centrifuged for 5 min at 1500 rpm. 2 mL of this solution was transferred in to a 10 mL volumetric flask and the above-recommended procedure was followed.

RESULTS AND DISCUSSION

A new colorimetric method was developed for the determination of some sulfonamides with different substituent. The method depends upon diazotization of the sulfonamides followed by coupling with phloroglucinol in acidic medium due to which a yellow azo dye formed immediately. The azo dye formed has an absorption maximum at 420nm.

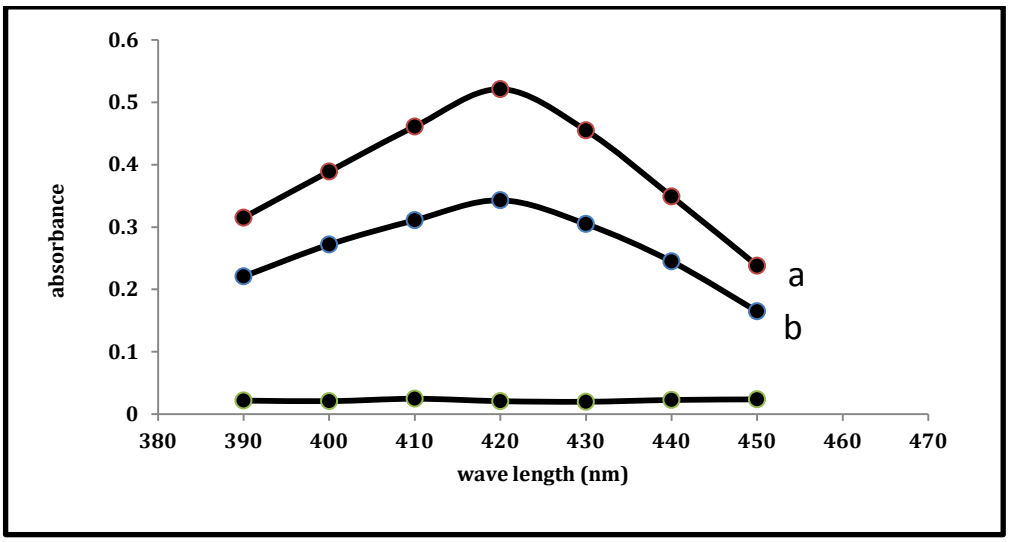

Fig.1 Graph for absorption maxima (λ_{max})

Effect of varying reaction conditions

Effect of reagent concentration

Effect of nitrite concentration was evaluated by adding it in different ratio with respect to sulfonamides. It was found that 0.5-1.5 mL gives maximum absorbance; 1 mL of 0.02% (w/v) sodium nitrite was used for complete diazotization.

Different volume of 0.25% phloroglucinol were added and tested for maximum absorbance. It was found that 1.5 mL of phloroglucinol gives maximum absorbance.

Effect of acid concentration

The effect of acidity on the diazotization reaction was studied by using HCl, H_2SO_4 and H_3PO_4 in the pH range of 0-6. The maximum diazotization was obtained in H_2SO_4 at pH range of 2-4 so 2M H_2SO_4 was used in study.

Effect of time and temperature

Since diazotization for 2 min or more gave the same results so, 5 min was selected for studies. The azo dye was formed after addition of phloroglucinol required 5 min for complete colour development.

Effect of temperature on diazotization and coupling was studied. It was found that diazotization at 0-5 ^oC gives maximum colour intensity. The effect of temperature on coupling rate was studied at different temperature ranges. Coupling rate was slow below 10 ^oC while it was instantaneous at temperature above 10 ^oC hence room temperature was selected for coupling.

Sulphamic acid: 0.5- 5.0 ml of sulphamic acid do not affect the intensity of dye so 1 mL of sulphamic acid was added for removal of excess sodium nitrite.

Stability of colour

Under optimized conditions, the produced dye was stable for several days at room temperature.

Interference

The effect of common excipients such as talc, glucose, dextrose lactose etc commonly used in pharmaceutical preparation of sulfonamide derivatives were investigated under the optimum condition. An amount in 1000 fold excess of that used in the pharmaceutical preparation was added in 0.1 µg mL^{-1} sulfonamide solutions and no effect due to these excipients was found under the proposed experimental conditions.

Method validation

The absorbance vs. time was plotted and a linear correlation was found (Fig 2). Beer's law was obeyed over the concentration range, the molar absorptivity and sandell'ss sensitivity given in Table (1). The limits of detection, limit of quantification, regression equation, and correlation coefficients values are given in Table (1). The limit of detection (LOD) and quantification (LOQ) calculated according to the current ICH guidelines [29] are presented in Table (1).

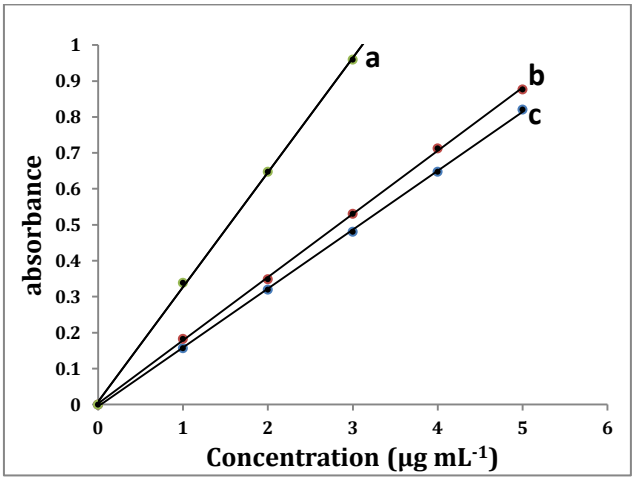

Fig.2 Calibration curve for sulfonamides

Table 1:Analytical parameters for spectrophotometric determination

Parameters in extracted medium	SMX	SFA	SFD
λmax, (nm)	420	420	420
Beer's law limits (μg mL-1)	0.02-0.2	0.02-0.2	0.01-0.1
Molar absorptivity($Lmol^{-1}m^{-1}$)	$0.921x10^6$	$0.779x10^6$	$1.826x10^6$
Sandell's sensitivity (μg cm^{-2})	0.000275	0.00028	0.00014
Limit of detection (μg mL^{-1})	0.0071	0.0068	0.0036
Limit of quantification(μg mL^{-1})	0.02	0.02	0.011
Regression equation (Y=bX*+a)			
Slop(b)	4.13	4.30	8.26
Standard deviation of slop(S_b)	0.089	0.086	0.18
Intercept(a)	0.002	0.001	0.002
Standard deviation of intercept(S_a)	0.0013	0.0024	0.0012
Variance(S_a^2)	$1.69x10^{-6}$	$5.76x10^{-6}$	$1.44x10^{-6}$
Correlation coefficient	0.99	0.99	0.98

The accuracy of an analytical method expresses the closeness between the reference value and the found value [29]. Accuracy was evaluated as percentage relative error between the measured concentrations and taken concentration for sulfonamides. The precision of the method was calculated in terms of intermediate precision (intra-day and inter-day) [30]. Three different concentrations of sulfonamides (within the working limits) were analyzed in seven replicates during the same day and five consecutive days. The SD and RSD values of intra-day and inter-day studies showed that precision was good Table 2-3.

Table2: Intra-day precision and accuracy studies (n=7)

SMX/SFA/SFD	SMX			SFA			SFD		
	Found[a]	Precision[b]	Accuracy[c]	Found[a]	Precision[b]	Accuracy[c]	Found[a]	Precision[b]	Accuracy[c]
0.02	0.021	5.05	5.71	0.022	3.11	10.7	0.020	5.48	1.42
0.05	0.051	2.63	2.28	0.053	3.33	6.3	0.051	2.63	2.28
0.1	0.11	4.32	10.2	0.12	10.8	20.1	0.104	7.54	4.28

[a] Mean value of seven determinations, [b] Relative standard deviation (%), [c] Bias%: (found-taken/taken) x100

Table3: Intra-day precision and accuracy studies (n=5)

SMX/SFA/SFD	SMX			SFA			SFD		
	Found[a]	Precision[b]	Accuracy[c]	Found[a]	Precision[b]	Accuracy[c]	Found[a]	Precision[b]	Accuracy[c]
0.02	0.021	2.10	6.0	0.020	5.53	3.0	0.021	5.26	4.0
0.05	0.051	2.40	2.0	0.051	2.40	2.0	0.052	2.40	2.0
0.1	0.108	4.14	8.0	0.104	5.26	4.0	0.108	4.14	8.0

[a] Mean value of five determinations, [b] Relative standard deviation (%), [c] Bias%: (found-taken/taken) x100

Reproducibility

Proposed method follows Beer's law over the concentration range of 0.01-0.1 μg mL^{-1} (sulfadiazine), 0.02-0.2 μg mL^{-1} (sulfamethoxazole and sulfacetamide). The molar absorptivity for sulfamethoxazole, sulfacetamide and sulfadiazine are $9.21x\ 10^5$, $7.79\ x\ 10^5$ and $1.820\ x10^6$ $Lmol^{-1}cm^{-1}$ respectively. Sandell's sensitivity for these drugs is 0.000275 μg cm^{-2}, 0.000275μg cm^{-2} and 0.000137μg cm^{-2} respectively. The reproducibility of the method was studied by replicate analysis of standard sulfa drug solutions (0.1 μg mL^{-1}). The standard deviations for the proposed method are 0.00339, 0.00737 and 0.00694 and relative standard deviations are 1.06%, 2.11% and 2.05% respectively.

APPLICATIONS

Analysis of pharmaceutical preparations

The applicability of the proposed method for the assay of different pharmaceutical formulations containing sulfamethoxazole, sulfacetamide and sulfadiazine was examined for tablets, eye drops and suspensions. The results were statistically compared with those obtained by the official method based on nitrite titration [28] with sodium nitrite and the reported spectrophotometric method based on reaction of the drug with acetyl acetone formaldehyde reagent. The student's t-test and F-test data show that the proposed method is of comparable accuracy and precession with the other established methods. The results were summarized in Table 4.

Table4: Determination of sulfonamides in pharmaceutical preparations

Sample	Sulfonamides found(mg/tablet)				t-Test (±)	F-Test	Standard error
	Proposed Method[b]	% recovery	Pharmacopial method[b]	% recovery			
A (400mg)	399.8 (±0.26)	99.9	399.4(±0.32)	99.8	1.67	1.53	0.0842
B (500mg)	499.8 (±0.20)	99.9	499.3(±0.26)	99.8	2.630	1.69	0.0670
C (10 mg)	9.8 (±0.22)	98.0	9.8(±0.32)	98.0	0.4457	2.115	0.0793
D (10 mg)	10.06(± 0.17)	100	9.9(± 0.20)	99.0	0.6594	1.38	0.0536

[b]mean of five determinants

Tabulated t-value at the 95% confidence level is 2.78; Tabulated F-value at the 95% confidence level is 6.39.

Assay in serum and urine sample

The amount of sulfa drugs in serum and urine sample was tested by analyzing control serum or urine spiked with sulfa drugs. The concentration of sulfa drugs was calculated from the calibration curve after hydrolysis procedure. The recovery data were compared with other methods and the results were listed in Table 5.

Table5: Determination of sulfonamides in biological samples

Sample	Found		t-Test (±)	F-Test	Standard error
	Reported[b]	Proposed[b]			
Urine sample					
A(100)	99.19(±0.24)	99.42(±0.25)	1.45	1.12	
B(200)	199.02(±0.16)	199.26(±0.23)	1.89	1.96	
C(300)	399.42(±0.37)	399.65(±0.24)	1.16	2.39	
Serum sample					
A(30)	29.5(±0.37)	29.90(±0.22)	1.67	2.80	
B(60)	59.7(±0.20)	58.92(±0.13)	2.06	2.35	
C(120)	119.2(±0.35)	119.5(±0.36)	1.42	1.02	

Table6: Comparison of proposed method with other visible spectrophotometric method

Reagents	λmax, nm	Range of determination ppm	Remarks
p-Benzoquinone	500	10-50	Heating is needed
o-Chloranil	525	10-70	Less sensitive
Dopamine	500	0.1-7.0	Less sensitive
8-hydroxyquinoline	500	0.2-6.0	Less sensitive
Primaquine Phosphate	468-474	0.1-12	Less sensitive
Phloroglucinol (Proposed method)	420	0.01-0.1(SFD) 0.02-0.2 (SMX & SFA)	Simple, sensitive and free from drastic experimental conditions

CONCLUSION

The proposed method is simple, sensitive and free from drastic experimental conditions such as heating. It is also accurate, precise enough to be successfully adopted as an alternative to the existing spectrophotometric method and evaluation of drug in pharmaceutical preparations and in biological samples.

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