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Journal of Chemical and Pharmaceutical Research, 2017, 9(2):244-251

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Spectrophotometric Method for the Determination of Sulfa Drug in Pharmaceuticals Based on Charge Transfer Reaction

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ABSTRACT

New, simple, sensitive, accurate, selective and cost effective method has been developed for the determination sulfa drugs (sulfamethoxazole and sulfamerazine) in bulk and in pharmaceutical dosage forms. The developed method , is a chemical derivatization method involving proton transfer from 2,4,6-trinitrophenol (picric acid) as Lewis acid to the secondary amino group of sulfamethoxazole and sulfamerazine as Lewis base in methanol to form a yellow charge transfer complex exhibiting maximum absorption (λ max) at 420nm and 410nm respectively. The method was linear in the concentration ranges (2-28µg/ml)and(1-30 µg/ml) with correlation coefficient (R2= 0.9992) and (R2=0.9994), to sulfamethoxazole and sulfamerazine sulfa drugs respectively .Intra-day, inter-day precision (as RSD) and accuracy were determined. The results obtained were statistically validated .The method is used to determination sulfa drugs in pharmaceutical preparations.

Keyword: Charge transfer; Sulfa drugs; Sulfamethoxazole; Sulfamerazine; Spectrophotometric determination

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INTRODUCTION

Sulfamethoxazol is N1-(5-methyl-3-isoxazolyl)sulfanilamide;the molecular formula is $C_{10}H_{11}N_30_3S$. It is white, odorless, tasteless compound with a molecular weight of 253.28 and figure 1 show the chemical structural formula of Sulfamethoxazol compound. sulfamerazine is 4-Amino-N-(4-methyl-2-pyrimidinyl)benzenesulfonamide; the molecular formula is $C_{11}H_{12}N_4O_2S$. It is white, odorless, tasteless compound with a molecular weight of 264.3 .and figure 2 shows the chemical structural formula of sulfamerazine compound. Sulfa drugs had attracted specile attention from their therapeutic importance as they were used against a wide spectrum of bacterial ailments [1]. sulfamethoxazole and sulfamerazineare considered as sulfa drugs derived from the parent compound , sulfanilamide,which consider important class of drugs with several types of pharmacological agents possessing antibacterial [2] antyhyroid [3] diuretic [4] In addition, numerous sulfonamide derivatives have been reported as carbonic an hydras inhibitors [5], anti-inflammatory and anticancer agents [6].

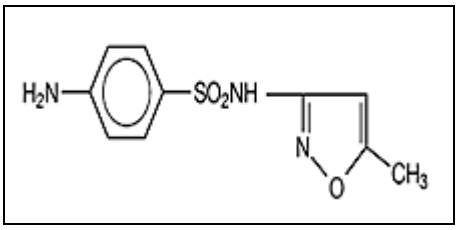

Figure 1: Chemical structure of sulfamerazine compound

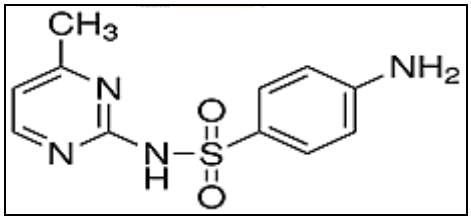

Figure 2: Structure of and sulfamerazine compound

Picric acid (2,4,6-trinitro-1-phenol; PA)figure(3) [7] is a yellow crystalline, bitter [8], toxic [9], explosive solid [10] has been used as a derivatizing reagent in the development of Spectrophotometric studies for the determination many types of pharmaceuticals [11]. It is known that picric acid acts not only as an acceptor to form various π stacking complexes with other aromatic molecules but also as an acidic ligand to form salts through specific electrostatic or hydrogen bond interactions. Bonding of electron donor/acceptor picric acid molecules strongly depends on the nature of the partners [12].

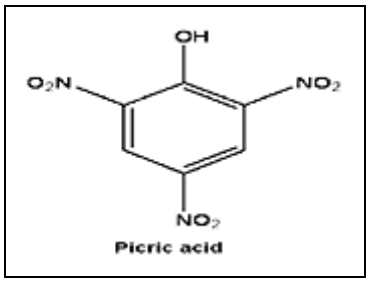

Figure 3: Structure of picric acid (2,4,6-trinitro-1-phenol)

Charge-transfer (CT) complexes were for a long time believed to have very important role in biological systems [13]. Molecular interactions between electron donors and acceptors are generally associated with the formation of intensely colored charge-transfer complexes which absorb radiation in the visible region [14,15]. Charge-transfer complexation is currently achieve the very great importance in biochemical, bioelectrochemical energy transfer process [16,17]. The term charge transfer gives a certain type of complex resulting from interactions of donor and acceptor with the formation of weak bonds [18,19].

Many methods have been developed for determination of sulfa drugs in biological fluids and pharmaceutical preparations. The method includes gas chromatography [20], high performant liquid chromatography [21], electro analytical methods, voltametric determination immune chemical assay, differential scanning calorimetry [22], spectroflurimetry [23], and spectrophotometry [24].

Here in, the charge transfer interaction between π- acceptors picric acid (2,4,6-trinitro-1-phenol; PA) with sulfa drugs like sulfamethoxazole and sulfamerazine as an donor were investigated .The present work describes the simple, and rapid spctrophotometric methods for the quantitative determination of some sulfonamides namely, sulfamethoxazole and sulfamerazine pure form and in Pharmaceutical preparations.

EXPERIMENTAL SECTION

Apparatus

The electronic absorption spectra of methanolic solutions of the donor, acceptors and resulting CT complexes were recorded over a wavelength range of 260-500nm using A Jena Model 1100, UV-Visible spectrophotometer (Germany). The instrument was equipped with a quartz cell with a 1.0cm path length. E. Meter electrical balance is used for weighting the sample.

Chemicals and reagents

The compounds sulfamethoxazole (SMT) and sulfamerazine (SMR) with a purity of 99% were purchased from Aldrich. Picric acid (PA) (Microscopic materials) was obtained from BDH, England. Pharmaceutical formulations were obtained from the local markets, Metheprim tablets (400 mg SDI/ tablet) SDI IRAQ, Bactrim suspension (200 mg SMX/ 5ml), F. Hoffmann-La Roche Ltd., Basel- Switzerland and Septrin suspension (200 mg GSK/120ml), El Salam City , Cairo , A.R.E. Methanol (spectroscopic grade) was obtained from Merck . Distilled water was used wherever required.

Standard solution and reagent

Sulfa drugs stock solutions (0.1% w/v) were Prepared by dissolving 0.1g of sulfamethoxazole and sulfamerazine

pure drugs in 100 ml of methanol and used for the assay in method. Stock solutions were protected from light and kept refrigerated at 4C°. The stock solution was further diluted with same solvent to obtain working solution of concentration 60µg/ml. Picric acid (0.1% w/v) reagent was Prepared by dissolving 0.1 g of picric acid in 100 ml of methanol and used for the assay.

Procedure for pure drug

Aliquots of the working standard solution of Sulfa drug were transferred in a series of 10 ml volumetric flask to give final concentrations of 2-28 µg/ml and 1-30 µg/ml for of sulfamethoxazole and sulfamerazine pure drugs respectively. 1.0 ml of picric acid (0.1%w/v) was added to each flask and volume was made up to the mark with methanol. The solutions were shacked well and allowed to stand for 10 and15minutes for sulfamethoxazole and sulfamerazine pure drugs respectively to complete the reaction. The absorbance was measured at 420nm and 405nm for of sulfamethoxazol-PA and sulfamerazine- PA, complexes respectively against blank reagent prepared similarly. The drug concentration was calculated from the corresponding regression equation of the calibration graph.

Procedure for commercial tablets

Twenty tablets of labeled of drugs were weighed accurately. An average weight of each tablet was determined. An accurately weighed quantity of powder equivalent to100 mg of sulfa drugs was transferred into 100ml volumetric flask and sonicated for 10 min with 40 ml of methanol then made up to the mark with same solvent. The solution were mixed well then filtered rejecting the first portion of filtrate. The prepared solution concentration is 1000 µg/ml. A 6 ml of 1000 µg/ml solution was further diluted to100 ml with methanol to give final concentration of 60µg/ml. Aliquots of the tablets solution were treated as under the general recommended procedures for the reaction with picric acid. Then the concentration of the drug was calculated using calibration curve.

Procedure for commercial suspension

An aliquot 10 ml of the suspension was diluted with 10 ml of methanol by shaking for 10 min., then mixed well and completed to 50 ml in a measuring flask by using methanol then made up to the mark with same solvent. The contents were mixed well then filtered rejecting the first portion of filtrate. The prepared solution concentration is further diluted to100 ml with methanol to give final concentration of 60µg/ml. Solutions of lower concentrations were prepared by appropriate dilution with methanol, then proceeded as detailed under Procedure for pure drug.

RESULTS AND DISCUSSION

Absorption spectra

The reaction of sulfamethoxazole and sulfamerazine pure drugs as n-electron donor and the picric acid as P-acceptors, result in the formation of C-T complexes. The absorption spectra of sulfameethoxazol- PA and sulfamerazine - PA C-T complex resulted in the formation of intense yellow products .These spectra revealed the presence of the absorption bands that correspond to the charge transfer interactions. These bands are observed at 420 nm and 410 nm of sulfamethaxzol - PA and sulfamerazin - PA C-T complex respectively. (Figures 4 and 5) show the electronic absorption spectra of the formed sulfamethoxazole - PA and sulfamerazine - PA C-T complexes respectively. These bands of absorption well known to be characteristic of the formation of new charge-transfer complexes [25,26]. These spectra revealed new absorption bands that are attributed to the charge transfer interactions. The complexes are labile where the broad shape of the curve was observed to indicate that the inflection at this ratio may be attributed to the weak binding forces in the charge transfer complexes due to the bulky size of the two molecules [27,28].

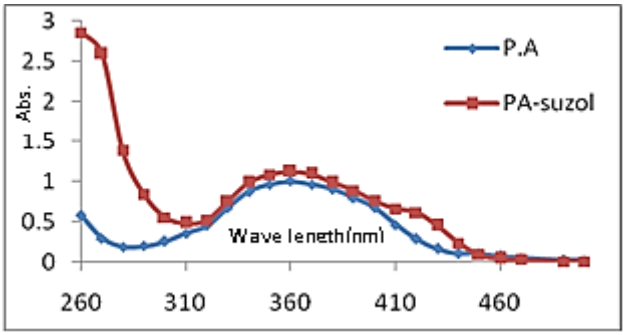

Figure 4: Absorption spectra of sulfamethoxazol –PA complex and PA

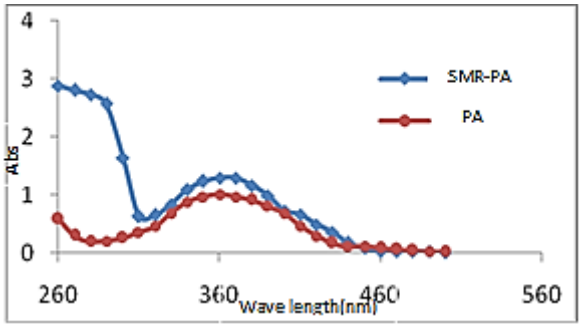

Figure 5: Absorption spectra of sulfamerazine –PA complex and PA

Sulfa drugs show maximum absorption at 420nm and 410nm for sulfamethoxazol and sulfamerazine respectively using methanol as a solvent (Figures 4 and5). The reaction is between electron acceptor (picric acid) and electron donor (Sulfa drugs), at the same time the proton of the hydroxyl group of picric acid will transfer to the secondary amine of Sulfa drugs. The interactions between electron donors and acceptors are generally relate to the formation of intensely colored charge-transfer complexes which absorb radiation in the visible region.(29).The absorption spectra of the yellow colored products were recorded at 420nm and 410 nm for sulfamethoxazol-PA and sulfamerazine-PA respectively against the corresponding blank solution.

Optimization of variables

Many experimental variables which were found to affect the stability and color intensity of the resulting complexes were optimized to produce maximum adherence to Beer's law and sensitivity.

Effect of reagent concentration: The optimum concentration of the reagent(PC) required to achieve maximum sensitivity of the developed color species in the method was ascertained by adding different volumes of the reagent picric acid to a fixed concentration of sulfamethoxazol(18 µg/ml) and sulfamerazine(20 µg/ml). The results showed that 1.0 mL each of 0.1% PA and solution was optimum for the production of maximum and reproducible color intensity (figure 6).

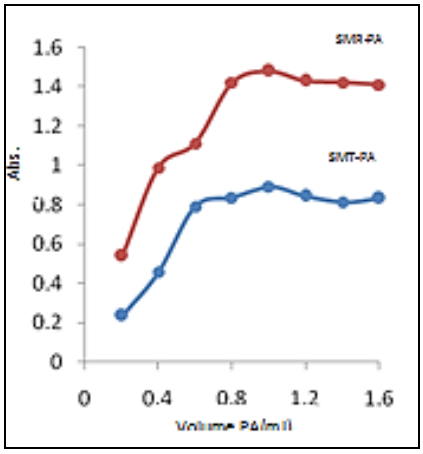

Figure 6: Effect of reagent concentration on the formation of sulfamrthoxazole-PA complex, sulfamerazin-PA complex

Effect of reaction time: The optimum reaction times were determined by measuring the absorbance of the charge transfer complex upon the addition of reagent solution (PA) to sulfa drug solution at room temperature. The reaction of sulfa drug with PA was instantaneous while complete color development was attained after (10 min) with sulfamethoxazol-PA and (5 min)with sulfamerazine-PA .The absorbance of the resulting charge transfer complexes remained stable more than (26h)for sulfamethoxazol-PA and (24h) sulfamerazine-PA complexes.

Stoichiometric relationship: Job's method of continuous variation [30] was employed to determinate the molar ratio between sulfa drugs and picric acid. The concentration of each drug and reagent was adjusted to be (1x 10-3 M). A series of solutions was prepared in which the total volume of the drug and the reagent was kept at 0.1-1.0 ml in 10ml calibrated flasks. The solutions were further manipulated as described under the general recommended procedures described above. The absorbance of each solution (against blank treated similarly) was plotted against the drugs mole fraction [drug]/[drug+ reagent]. The molar ratio was 1:1 as shown in (Figures 7 and 8).

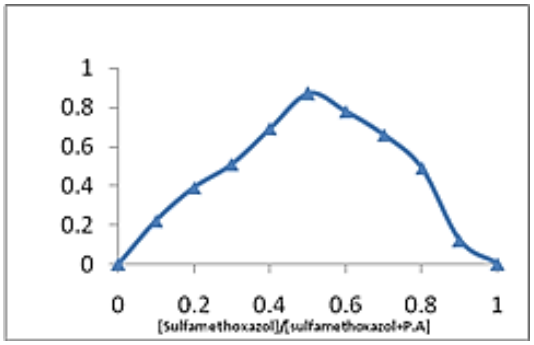

Figure 7: Continuous variation plots of sulfamethaxzol with reagent

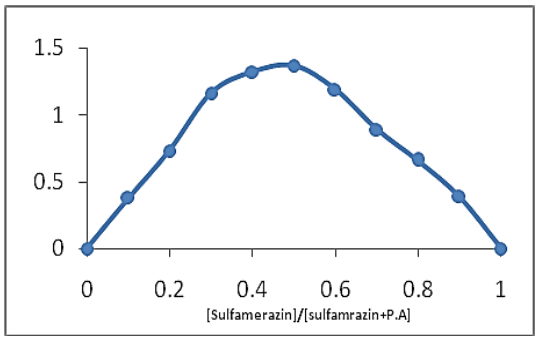

Figure 8: Continuous variation plots of sulfamerazine with reagent

Validation of the proposed method

Linearity:

The correlation coefficient (R2) of the formed product was (0.9992 and 0.9994) for sulfamethoxazole and sulfamerazine respectively; indicating good linearity (Figures 9 and 10) the limit of detection (LOD) and limit of quantification (LOQ) for the proposed method were calculated using the following equations:

$$LOD = 3.3\ a / S$$

Where L is the standard deviation of intercept. S is the slope of calibration curve. The results are summarized in table 1.

$$LOQ = 10\ a / S$$

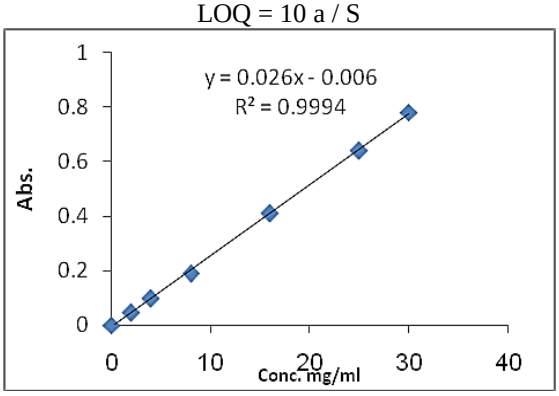

Figure 9: Calibration graph of sulfamerazine

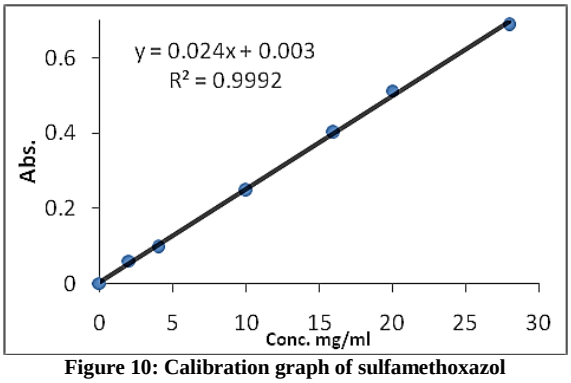

Figure 10: Calibration graph of sulfamethoxazol

Table 1: Quantitative parameters of the proposed method

Parameter	Value	
Complexes name	**Sulfamethoxazole-PA complex**	**Sulfamerazine-PA complex**
Time of reaction(min)	10	5
Stability of color (hour)	26	24
Berr,s law limits (µg/ml)	Feb-28	Jan-30
Regression equation(y = b + ac)*	Y=0.024x+0.003	Y=0.026x+0.003
Correlation coefficient(R^2)	0.9992	0.9994
Slope , a	0.024	0.026
Intercept ,b	0.003	0.006
Standard deviation of slope	0.00032	0.00069
Standard deviation of intercept	0.0016	0.0021
LOD(mg/ml)	0.22	0.27
LOQ(mg/ml)	0.67	0.81

Y = aX+ b, where X is the concentration of Sulfa drug µg/ml

Accuracy

They were checked at three concentration levels, five replicate measurements were recorded at each concentration level. Accuracy was recorded as percent recovery, and by standard addition method. The results are summarized on tables 2 and 3 show a good accuracy.

Table 2: Accuracy of the proposed method for the determination of sulfamethoxazole

Concentration taken (µg/ml)	Concentration found(µg/ml)	Accuracy
4	4.02	100.58%
12	11.89	99.08%
24	23.95	99.79%
		Mean ± SD=99.82±0.152 RSD=0.153

Table 3: Accuracy of the proposed method for the determination of sulfamerazine

Concentration taken (µg/ml)	Concentration found(µg/ml)	Accuracy
4	4.13	103.25%
12	11.95	100.58%
24	23.97	99.88%
		Mean ± SD=101.31±0.182 RSD=0.186

Precision

To determine the precision of the proposed methods, pure sulfa drug sulfamethoxazole and sulfamerazine solution at three different concentration levels 4 , 12 ,and 24 µg/ml (with in the working range) were prepared and analyzed in five replicates during the same day (intra-day precision) and on five consecutive days (inter-day precision) .The results are listed in Tables 4 and 5. Percentage of relative standard deviation for intra-day and for inter-day it indicating usefulness and repeatability of the proposed methods in the routine analysis, the results are listed in tables 4 and 5.

Table 4: Precision of the proposed method for the determination of sulfamethoxazole

Precision			
Intra-day		Inter-day	
Recovery±SD %	**RSD***	**Recovery±RSD %**	**RSD**
98.87±1.625	1.644	97.89±1.631	1.666
99.56±1.253	1.259	98.58±1.233	1.251
99.97±1.098	1.098	100.65±1.091	1.084

Table 5: Precision of the proposed method for the determination of sulfamerazine

Precision			
Intra-day		Inter-day	
Recovery±SD %	**RSD***	**Recovery±RSD %**	**RSD***
99.03±0.896	0.904	98.56±0.891	0.904
99.66±1.538	1.543	99.06±1.672	1.688
100.67±0.826	0.821	99.54±1.591	1.598

Application

Application to analysis of tablets containing sulfa drugs. The proposed method indicating successfully applied for the determination of sulfa drugs in three brands of tablets and suspension the results are compiled in Table 6.The results obtained were statistically compared with those obtained by the reference method [31] by applying the Student's t-test for accuracy and F-test for precision at 95% confidence level. As it can be seen from table 6

the calculated t and F- values at 95% confidence, for four degrees of freedom. This indicates that there are no significant differences between the proposed methods and the reference method with respect to accuracy and precision.

Table 6: Application of the proposed method to the determination of sulfa drugs in dosage form

Preparation	Taken µg.ml-1	Found* µg.ml-1	Recovery%	Official method**
metheprim tablets	10	10.1	101	100.09±0.52
	20	20.04	100.2	
	30	30.07	100.23	
Mean±s.d			100.47±0.36	
t-test			0.97	
f-test			0.65	
Sptazole suspension	10	10.06	100.6	99.69±0.65
	20	20.11	100.55	
	30	29.96	99.86	
Mean±s.d			100.34±0.45	
t-test			1.26	
f-test			0.72	
Septrin suspension	10	10.09	100.9	100.11±0.67
	20	20.12	100.6	
	30	30.33	100.11	
Mean±s.d			100.87±0.55	
t-test			1.18	
f-test			0.59	

*Each value is the average of five separated determination; **Each value is the average of three separated determination; *** The values of t and f values are 1.943 and 6.94 respectively at p=0.05

CONCLUSIONS

Simple, sensitive spectrophotometric methods based on charge transfer complex formation reactions for the determination of sulfa drugs was developed and validated. The suggested methods used a single step reaction and single solvent. No great differences among the proposed method arose from analysis of the experimental results. The recovery data and statistical parameters reveal good precision and accuracy of the method. This method can be used as general methods for the determination of sulfa drugs in bulk powder, dosage forms and suspension, have many advantages over the separation techniques such as reduced cost, and high accuracy.

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