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o-PHTHALALDEHYDE BASED SPECTROPHOTOMETRIC DETERMINATION OF SULFONAMIDES

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ABSTRACT

o-Phthalaldehyde (OPA) reacts with primary amino group in a compound in presence of a thiol (RSH) and in an alkaline medium to give a soluble product called isoindole. This is a fluorescent compound, yet it has been exploited for spectrophotometric study by measuring the absorbance at 340 nm which is the wavelength for excitation of the molecule. The spectrophotometric method has successfully been employed in the quantitative estimation of sulfonamides such as sulfadiazine, sulfanilamide and sulfamethoxazole, all containing a primary amino group. The detection ranges from 0.01 to 0.24 mg/ml of the active ingredient in different samples containing sulfonamides. The respective standard curves were linear in the given range and the relative standard deviation of the mean response factor ranged from 1.95-2.08%. 2-methyl-2-propane thiol (2MPT) has been used in the derivatization reaction for the formation of OPA – adduct and borate buffer of pH 10.0 has been used to maintain the medium alkaline.

INTRODUCTION

Sulfonamides are a class of drugs commonly used for their bacteriostatic activity especially in the treatment of urinary-tract infections (Martindale, 1999). Various methods have been developed and used for the separation and quantitative estimation of sulfonamides in food matrices, biological samples, veterinary products and in unfinished, and different final dosage forms. These methods include thin layer chromatography (Bieganowska and Petruczynik, 1996; Szumilo and Flieger, 1996), high performance liquid chromatography (Lin *et al*., 1995; Caballero *et al*., 2001; Pon *et al*, 2001). Capillary zone electrophoresis (Lin *et al*., 1997), Supercritical fluids method (Combs, 1999), antomated dialysis (McGrane *et al*., 1999), liquid chromatography with post column fluorescence derivatization (Vinas *et al*, 1996) and micellar electrokinetic capillary chromatographic method (Nevado *et al*., 1999). All these reported methods suggest that quantification of sulfonamide in any matrix require elaborate and sophisticated instruments which may or may not be available in every laboratory. It is therefore deemed necessary to develop a simple method capable of producing reliable and accurate results. The present study thus deals with the development of a novel method by which sulfonamides such as sulfadiazine (SDZ), sulfanilamide (SNA) and sulfamethoxazole (SMZ) have been estimated spectrophotometrically on the basis of their derivatization with o-phthaladehyde (OPA). OPA is a fluorigenic reagent which possesses the capability of reacting with the organic molecule containing primary amino ($-NH_2$) group in presence of a thiol and in an alkaline medium to give a highly fluorescent product called isoindole (Roth, 1971; Taylor and Tappel, 1973). The chemistry of the reaction has been studied in detail (Simons and Johnson, 1976, 1977a, 1977b) and a variety of compounds have been determined, using different techniques, on the premise of this derivatization procedure (Chow and Karmen, 1980; Mendez and Marco, 1980; Momose and Momose, 1981; Ochiai *et al*., 1983;

Simpson *et al*., 1983; Buteau *et al*., 1984; Frister *et al*., 1987, 1990; Qureshi, 1987; Medina *et al*., 1990a, 1990b, 1990c; Wenck *et al*., 1991; Merino *et al*., 1992). Since sulfonamides also contain the primary amino group on their skeleton they have been derivatized with OPA. The wavelength of excitation (340 nm) of the OPA-adduct has been used as wavelength of absorbance and quantitative determination of the sulfonamide drugs has been made on the basis of this fact that there exists a linearity between absorbance and concentration.

EXPERIMENTAL

Instrumentation

A Shimadzu UV-Visible spectrophotometer (Model UV-240) was used.

Reagents

All chemicals used were of Analar grade.

o-Phthalaldehyde solution

0.134 g accurately weighed amount of OPA was dissolved in 5.8 ml ethanol and to this was then added distilled water upto the mark in 100 ml measuring flask. 10 ml of this solution was further diluted with distilled water to 100 ml to obtain a 10^{-3} M solution.

Thiol solution (0.4% v/v)

0.4 ml thiol was taken in a 100 ml volumetric flask and to this was then added distilled water upto the mark and shaken well to obtain a 0.4% v/v thiol solution. The entire preparation was carried out in a fuming cupboard to avoid rotten smell.

Borate buffer (pH 10.0)

Accurately weighed quantities of borax (4.8 g) and sodium hydroxide (0.8 g) were dissolved in water in a 1000 ml volumetric flask. The flask was filled upto 900 ml with distilled water and pH was measured. Any adjustment of pH was carried out by using either sodium hydroxide or hydrochloric acid and the volume was finally made upto 1000 ml with distilled water.

Sulfadiazine solution

Two tablets of sulfadiazine containing 500 mg sulfadizine each were crushed to powder. To this was then added 3 ml analytical grade HCl to extract soluble material. Filtered to get clear solution and diluted to 1000 ml with distilled water in a measuring flask. Each ml of the solution contains 1 mg sulfadiazine.

Sulfanilamide solution

1.2 g sulfanilamide was dissolved in 3 ml analytical grade HCl and the filtrate was diluted to 500 ml with distilled water to obtain a solution containing 2.4 mg / ml of sulfanilamide.

Sulfamethaxazole solution

A 1.2 g Septran tablet containing 800 mg sulfamethaxazole was crushed and extracted by 3 ml concentrated HCl. The filtrate was then diluted to 500 ml with distilled water to obtain 1.6 mg/ml solution of sulfamethaxazole.

General Procedure

A measured volume 0.1 to 1.0 ml of the different sulfonamide drugs was taken in different volumetric flasks of 10 ml. To these was then added OPA (10^{-3}M) solution to match the mole ratio

of 1:1 followed by the addition of thiol (0.1 to 1.0 ml) and borate buffer of pH 10.0 (1 ml) to each of the flask. Shaken well and the volume was made upto the mark with distilled water. The final concentration of the samples varied from 0.01 to 0.24 mg/ml for different samples. The absorbance was measured at 340 nm.

RESULTS AND DISCUSSION

Sulfonamides and its derivatives have bacteriostatic (growth inhibiting) action at low concentrations but are bacteriocidal at higher levels. These have been reported to be against streptococcal and staphylococcal infections. Structurally, the presence of $-NH_2$ group at the p-position to the substituent group on the amido nitrogen is a pre-requirement for the drugs to be active or the substituted $-NH_2$ group is required to be converted back to the primary amino group in the body.

The primary amino group $-NH_2$ of the sulfonamides has been derivatized with OPA in presence of a suitable thiol (RSH) in an alkaline medium (pH 10.0) to produce an isoindole. This shows to absorb radiation at 340 nm. This wavelength has been used for measuring the absorbance of the derivatives which are soluble in water. Table-1 presents the experimental data of OPA-SDZ indole absorbance against varying concentrations of sulfadiazine (SDZ). The mole ratio of SDZ to OPA remained 1:1. The absorbance readings varied from 0.01 to 0.101 for a concentration of sulfadiazine ranging from 0.01 to 0.1 mg/ml after final dilution. The plot of absorbance versus concentration gave a linear relationship (Fig. 1). 2-methyl-2-propane thiol (2 MPT) was successfully used as an agent responsible for the formation of the derivative. Its quantity required for OPA-amino compound derivatization was determined in which the quantity of OPA to sulfonamide was kept in 1:1 mole ratio. Fig. 2 showed that a minimum quantity of 1 ml of the thiol (2MPT) in a concentration of 0.4% v/v was sufficient to give maximum absorbance which remained unchanged even on further increasing the amount of the thiol. Thus this mole ratio was utilized for the derivatization reaction in all the experiments.

Having set the experimental conditions, sulfanilamide (SNA) which is a parent sulfa drug was then derivatized and determined in the concentration range of 0.024 to 0.240 mg/ml. The values of absorbance in this case varied from 0.005 to 0.049 for the given concentration range (Table-1) and a linear relationship was observed between the absorbance and concentration as shown in Fig. 3. The OPA based derivatization reaction was again followed for the determination of a drug called “Septran” which contained sulfamethoxazole (SMZ) as the active ingredient. The absorbance measured for the concentration varying from 0.016 to 0.16 mg/ml varied from 0.003 to 0.040 as shown in Table-1 and Fig. 4. In this case Beer-Lambert’s law relationship was obeyed for a concentration upto 0.144 mg/ml and after which there was deviation from the linearity. This may be due to intermolecular association or the solute-solvent interaction.

In order to check the validity of the method the data obtained as such were subjected to regression analysis for all three sulfonamide drugs. The values of slope and intercept for each compound, within the specified range were statistically calculated through their respective absorbance vs concentration plots. The results of this are presented in Table-2. Furthermore the relative standard deviation of the mean response factors for the three sulfonamide drugs were also determined and found to be 1.95, 2.03 and 2.08 for SDA, SNA and SMZ respectively. The results of these statistical calculations seem to be quite satisfactory and suggest that the method is precise and reliable for the purpose of quantifying sulfonamides within the given range.

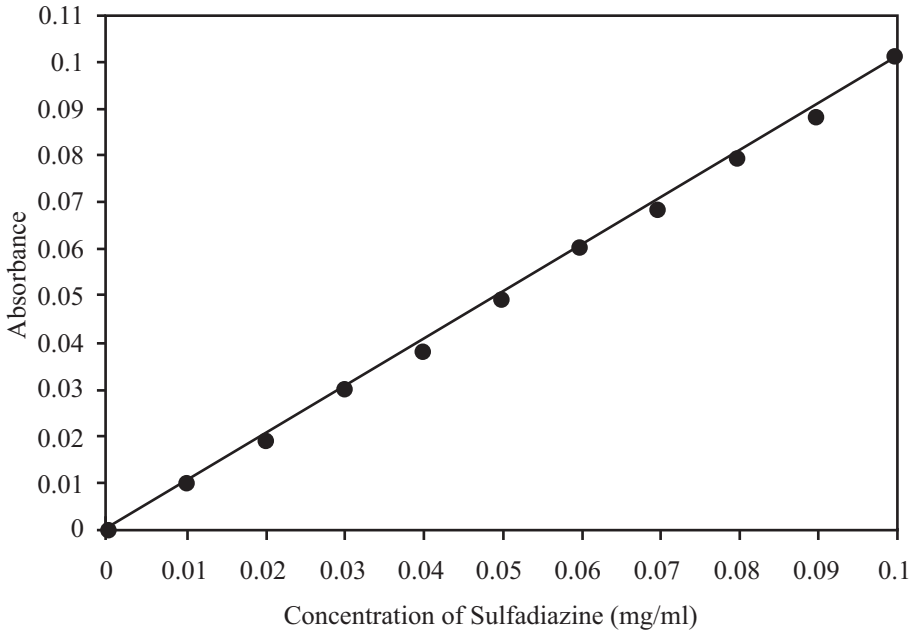

Fig. 1: Absorbance of OPA-SDZ derivative at varying concentrations of sulfadiazine.

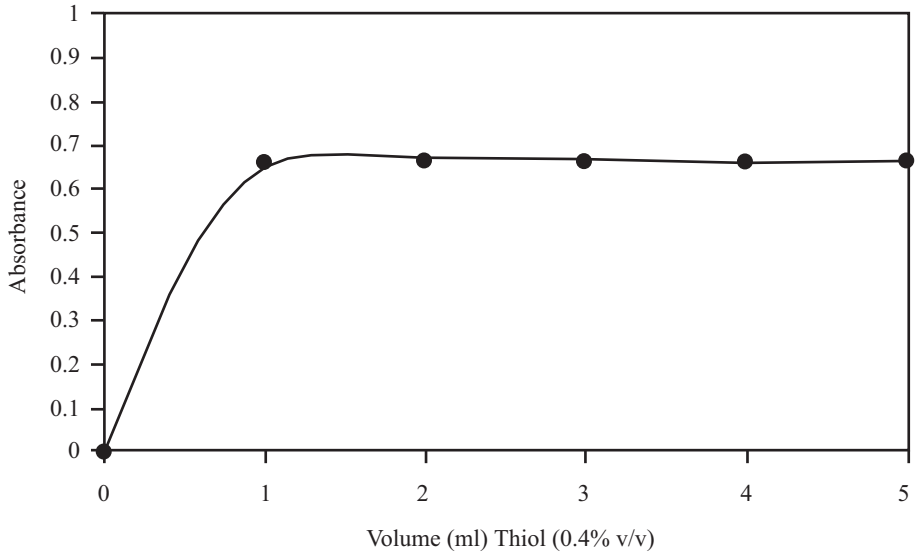

Fig. 2: Absorbance of varying volumes of Thiol solution (0.4% v/v).

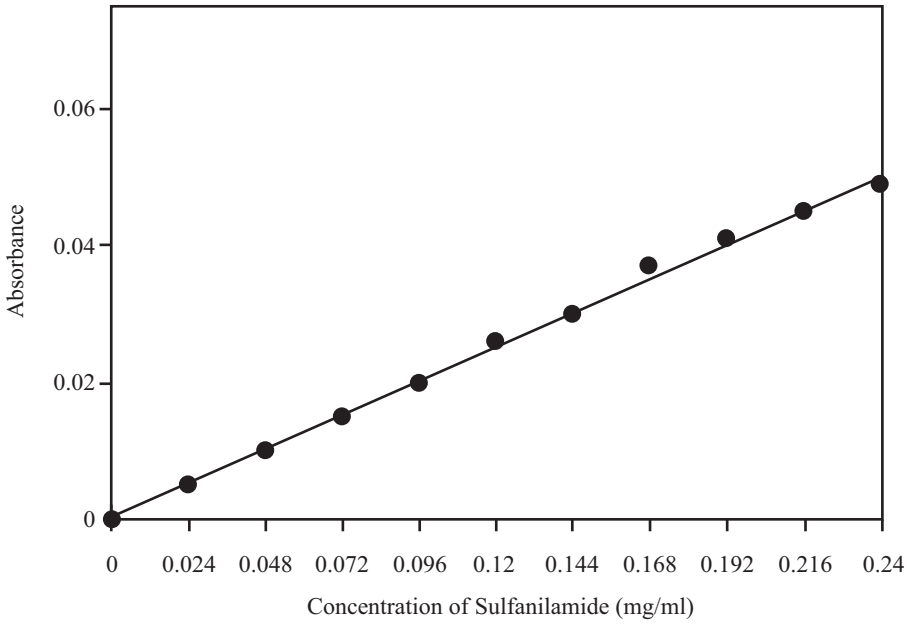

Fig. 3: Absorbance of OPA-SNA derivative at varying concentrations of sulfanilamide.

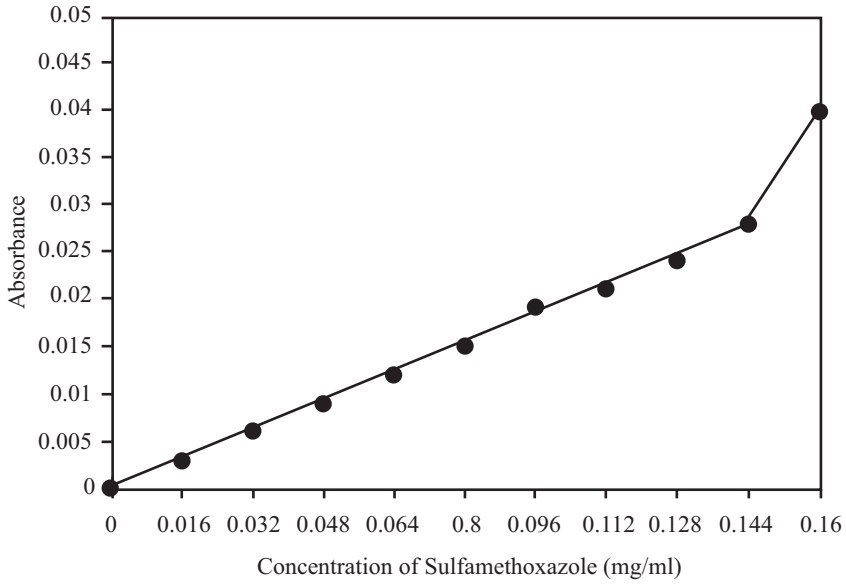

Fig. 4: Absorbance of OPA-SMZ derivative at varying concentrations of sulfamethoxazole.

Table-1
Absorbance of OPA-adduct at varying concentration of SDA, SNA and SMZ

S. No.	Sulfonamide drug sample (ml)*			Absorbance at 340 nm**		
	SDZ 1mg/ml	SNA 2.4 mg/ml	SMZ 1.6 mg/ml	SDA	SNA	SMZ
1	0.1			0.010	0.005	0.003
2	0.2			0.019	0.010	0.006
3	0.3			0.030	0.015	0.009
4	0.4			0.038	0.020	0.012
5	0.5			0.049	0.026	0.015
6	0.6			0.060	0.030	0.019
7	0.7			0.068	0.037	0.021
8	0.8			0.079	0.041	0.024
9	0.9			0.088	0.045	0.028
10	1.0			0.101	0.049	-

*Each sample was taken individually to measure its respective absorbance.
**Each value represent mean of five readings.

Table-2
Regression analysis* of the Calibration Data

Sulfonamide drug	Range (mg/ml)	Slope (± S.D)	Intercept (± S.D)	Correlation Coefficient
Sulfadiazine (SDA)	0.01 – 0.10	0.9988 (0.0121)	-0.0007 (0.0007)	0.9988
Sulfanilamide (SNA)	0.024 – 0.240	0.2086 (0.0040)	0.0003 (0.0006)	0.9971
Sulfamethoxazole (SMZ)	0.016 – 0.144	0.1927 (0.0033)	-0.0002 (0.0003)	0.9980

*Values represent mean of five determinations.

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