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Original Article

Determination of Sulfamethoxazole and Trimethoprim in Pharmaceuticals by Visible and UV Spectrophotometry

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Abstract

A method for the simultaneous determination of sulfamethoxazole (SMX) and trimethoprim (TMP), based on a direct determination of SMX after diazotization and coupling with 2-naphthol by visible spectrophotometry and an indirect determination of TMP in the UV region by difference was developed. By the suggested procedure, it was possible to analyze SMX and TMP in pharmaceutical preparations without separating from each other or from the excipients. Primarily a portion of the sample was diluted to have an absorbance of 0.4-0.7 at 271 nm, which corresponds to both SMX and TMP. By adding sodium nitrite, HCl and 2-napthol to 1 ml of the diluted solution, only SMX is diazotized and coupled with 2-naphthol forming a colored product with λ max at 482 nm where the quantity of SMX could be obtained directly from a calibration curve. By plotting another calibration curve for SMX at 271 nm, the influence of SMX on the total absorbance of a mixture of SMX and TMP at 271 nm could be deduced. The absorbance corresponding to TMP only was obtained by difference, which was transferred to quantity using a calibration curve at 271 nm. This procedure was applied successfully for the analysis of SMX and TMP in pharmaceutical preparations without prior separation and with acceptable errors.

Keywords: UV-visible Spectrophotometry; sulfamethoxazole; trimethoprim; diazotization; 2-naphthol.

Introduction

The sulfonamide drugs were the first effective chemotherapeutic agents to be employed systematically for the prevention and cure of bacterial infections in human beings. The considerable medical and public health importance of their discovery and their subsequent widespread use were quickly reflected in the sharp decline in morbidity and mortality figures for the treatable infectious diseases. The advent of penicillin and subsequently of other antibiotics has diminished the usefulness of the sulfonamides, and they presently occupy a relatively small place in the therapeutic armamentarium of the physician. However, the introduction in the mid-1970s of the combination of trimethoprim (TMP) and sulfamethoxazole (SMX) has resulted in increased use of sulfonamide for the treatment of specific microbial infections (1). The introduction of TMP in combination with SMX constitutes an important advance in the development of chemically effective antimicrobial agents and represents the practical application of a theoretical considerations, that is, if two drugs act on sequential steps in the

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pathway of an obligate enzymatic reaction in bacteria, the result of their combination will be synergistic. The anti microbial activity of the combination of TMP and SMX results from its action on two steps of the enzymatic pathway for the synthesis of tetrahydrofolic acid (2). In many countries the combination is known as Co-trimoxazole.

The UV absorption spectra of SMX and TMP show overlapping with each other, creating a serious problem for their analysis in the pharmaceutical products. Therefore, various techniques for the simultaneous determination of SMX and TMP in pharmaceuticals have been developed (3-9). However, while most of these methods are simpler than HPLC techniques, they still require a lot of data manipulation, which makes it difficult for their application as standard routine methods. For example, the method suggested by Lopez-Martinez et al. (3) was based on the bivariate calibration spectrophotometric method. In this method a Milton Roy (Rochester, NY, USA) Spectronic 3000 diode array spectrophotometer coupled to a Milton Roy 486 PC and User Data software for spectral data acquisition, storage and manipulation were used. All data treatment operations were carried out using a Hewlett Packard Vectra micro computer equipped with the GRAMS/32^{TM} software package. In addition to the above sophisticated facilities, all calculations for the bivariate method were performed using a GWBASIC program. Thus, it is highly desirable to develop even simpler methodologies with minimal sample and data manipulation. The standard USP (10) procedure utilizes the expensive HPLC technique with UV detection for the simultaneous determination of the SMX and TMP. The simultaneous determination of SMX and TMP by visible and difference spectrophotometry is not yet developed.

Previously, aromatic amines containing drugs such as the sulfonamides were determined by a diazotization reaction (11). It is based on the conversion of the free aryl amine into a diazonium salt at 0-5°C by a reaction with nitrous acid; the salt rapidly forms an azo-dye with a chromogenic reagent, such as 1-naphthol, 2-naphthol and N-(1-naphthyl)–ethane- 1,2 diammonium dichloride (the Bratton-Marshall reagent).

In the present procedure, a simple and sensitive spectrophotomertic method was developed for the simultaneous determination of TMP and SMX in pharmaceuticals. This procedure was based on diazotizing SMX, then coupled with 2-naphthol to give a yellow compound, without being interfered by TMP. The absorbance of the same sample which contains both SMX and TMP was measured at 271nm. The absorbance of SMX alone at 271nm was obtained via the quantity of SMX measured directly at 482 nm. The absorbance of TMP alone was obtained by difference, which was transformed to TMP quantity using a calibration curve at 271 nm.

Experimental

Apparatus

Spectrophotometric measurements were performed using a Shimadzu UV-160 A spectrophotometer. For a comparison, Waters associates high-performance liquid chromatography was also used for the determinations. The procedure described in United States Pharmacopia 28 (10) was followed closely.

Reagents

Sulfamethoxazole and trimethoprim (Sigma Co.) stock solutions (1 mg ml^{-1}) were prepared by dissolving 0.1 g of SMX or TMP in 100 ml of aqueous alcohol (1:1). Working solutions were prepared by suitable dilution. A sodium nitrite (Merck) stock solution (6.9 mg ml^{-1}) was prepared by dissolving 0.690 g $NaNO_2$ in 100 ml distilled water. A 2-naphthol (Merck) solution (7.5 mg ml^{-1}) was prepared by dissolving 0.75 g of 2-naphthol in 100 ml ethanol. Sulfamic acid powder (Merck) was used to destroy the excess of nitrous acid.

Calibration curves of TMP and SMX at 271 nm

To 10 ml volumetric flasks, TMP (110-350 µg) or SMX (80-200 µg) was sampled. After adding HCl (5N, 0.1 ml), the volumes were adjusted by aqueous ethanol (1:1). The absorbance was measured at 271 nm and the calibration curves were drawn (Fig. 1 and 2).

Calibration curve of SMX after diazotization and coupling with 2-naphthol

To 10 ml volumetric flasks in ice water, SMX (20-130 µg), and sodium nitrite solution (0.1 M, 0.2 ml) were transferred, followed by HCl (5N, 0.1 ml) and mixed thoroughly. After about 2 minutes, sulfamic acid (100 mg) was added and mixed well, followed by 2-naphthol solution (0.5 ml) and mixed. The flasks were kept at 60 °C for 50 minutes, followed by dilution with aqueous ethanol (1:1). The absorbance of the solutions was measured at 482 nm and a calibration curve was drawn (Fig. 3).

Results and Discussion

By reacting SMX with nitrous acid at 0-5 °C, the aromatic amine of SMX is diazotized. The diazotized SMX derivative is reactive toward aromatic compounds such as 2-naphthol forming a colored product with λmax at 482. Under controlled conditions, the intensity of the reaction product color is proportional to the quantity of SMX used. Quantities of 40-130 µg SMX which correspond to absorbance of 0.21-0.66 obeys Beer's-Lambert law and has a molar absorbance of 1.34×10^4. Maximum temperature and minimum reaction time for maximum formation of the colored product were 60° C and 50 minutes respectively. Many pharmaceutical additives and TMP do not interfere with the analysis of SMX. Using sulfamic acid to destroy the excess of nitrous acid was necessary, otherwise it reacts with 2-naphthol to give a colored product. After transforming SMX quantity to absorbance at 271 nm, the absorbance of TMP alone in admixture could be calculated by subtraction. The TMP absorbance was transformed to quantity using a suitable calibration curve for TMP at 271 nm.

Effect of temperature on the coupling reaction

The coupling reaction of the diazotized SMX with 2-naphthol proceeds slowly. Therefore the temperature effect on the progress of the coupling reaction was examined. After diazotizing SMX (100 µg), it was left with 2-naphthol at different temperatures (30-80° C). The volume was made to 10 ml and the absorbance was measured at 482 nm. It was observed that the reaction

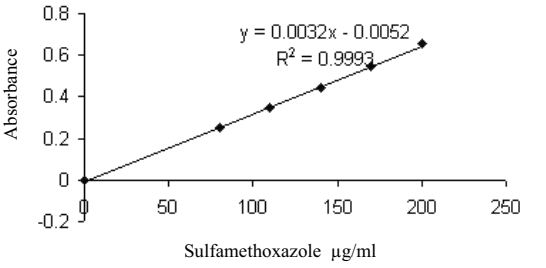

Figure 1. Calibration curve for SMX at 271 nm.

continued to increase till 60° C after which it decreased. Therefore all the coupling reactions were performed at 60 °C.

Effect of time on the coupling reaction

The time necessary for the maximum reaction product formation was studied as follows. 8 samples of diazotized SMX (100 µg) were left with 2-naphthol at 60 °C at different intervals of time (10, 20, 30, 40….. 80 min). After following the general procedure, the absorbance was measured at 482 nm. It was observed that maximum absorbance occurred after 50 min. Therefore all the coupling reactions were performed at 60°C for 50 min.

Effect of TMP on SMX analysis

TMP contains primary amine groups attached to a pyrimidine ring. An interference by TMP was expected, but experimentally was not observed which could be attributed to the presence of the nitrogen atoms in the pyrimidine ring of trimethoprim. To verify this observation, three different solutions of SMX and TMP were prepared separately as follows:

1. TMP (100 µg) 2. SMX (100 µg) 3. TMP + SMX (100 µg + 100 µg)

The above solutions were diazotized and coupled with 2-naphthol and the absorbance was measured at 482 nm. The absorbance of the first

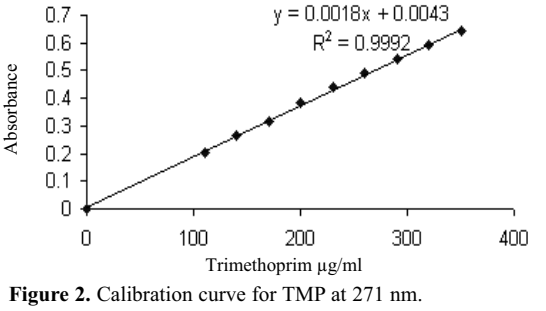

Figure 2. Calibration curve for TMP at 271 nm.

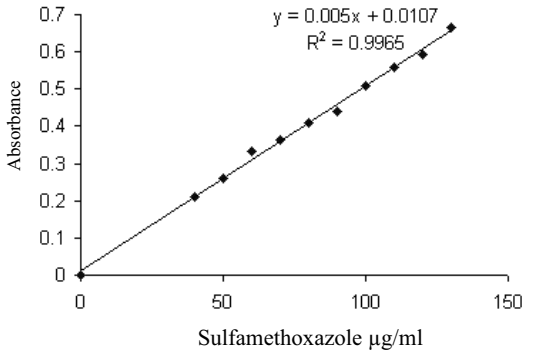

Figure 3. Calibration curve for SMX after diazotization and coupling with 2-naphthol at 482 nm.

solution was negligible and that of the 2nd and 3rd were almost similar, indicating that TMP did not interfere with the analysis of SMX by this procedure.

Effect of common excipients

To evaluate the effect of common excipients on the analysis of SMX and TMP in pharmaceutical preparations, the analysis of SMX (50 mg) and TMP (20 mg) in triplicate was performed in the presence of the compound under study. The presence of the following substances in the indicated amounts was tolerated by SMX (50 mg) and TMP (20 mg). EDTA-2Na (20 mg), glycerine (0.5 ml), cellulose (3 mg), methyl paraben (25 mg), sodium saccharin (15 mg), semithicone (30 mg), sorbitol (15 mg), and sucrose (35 mg).

Recovery of SMX and TMP from pharmaceuticals

For the recovery purposes of SMX and TMP from the known pharmaceutical preparations of SMX, a known amount of SMX and TMP were added to a portion of the pharmaceuticals and processed as the general procedure. The studied products include children and adult Co-trimoxazole tablets and syrup. In this respect, 5 tablets were powdered, mixed with water and spiked with pure SMX and TMP and transferred to 500 ml volumetric flask by ethanol and made to volume. For the syrup, 5 ml is mixed with water, spiked with pure SMX and TMP and transferred to 100 ml volumetric flask and made to volume by ethanol. The diluted solutions were filtered. Aliquot of the solution was processed according to the general procedure. The results are shown in Tab 1.

Application to real sample

The proposed method was applied to the determination of SMX and TMP in pharmaceuticals by using the procedure described in the experimental section. 10 adult or children co-trimoxazole tablets were weighed and powdered. 100 mg of the powder is transferred to a 100 ml volumetric flask and made to volume by ethanol. Suitable dilution of the solution was made to contain 4-13 µg/ml of SMX and diazotized and coupled according to the general procedure, the absorbance was measured at 482 nm, and the quantity of SMX was obtained directly from a calibration curve (Fig 3). For syrup, 5 ml is transferred to a 100 ml volumetric flask and made to volume by ethanol and processed as above. To determine TMP, the same volume sample as that diazotized was sampled into 10 ml volumetric flask, made to volume by aqueous ethanol (1:1) and the absorbance was measured at 271 nm (Total absorbance = TA). The SMX quantity obtained by diazotization is transformed to absorbance (SA) at 271 nm via a calibration curve (Fig 1). The absorbance of TMP alone (TPA) at 271 nm will be:

Table 1. Recovery of TMP and SMX from pharmaceuticals by the proposed procedure (n=5)

Pharmaceutical Products	Labeled mg/ tablet		Added mg		Expected mg		Found mg		Recovery % ± s.d.	
	SMX	TMP	SMX	TMP	SMX	TMP	SMX	TMP	SMX	TMP
Co-trimoxazole Adult tablets	400	80	200	40	600	120	595	122	97.5±1.8	105.0±2.7
Co-trimoxazole Children tablets	100	20	50	10	150	30	148	30.4	96.0±2.1	104.0±2.2
Co-trimoxazole Oral suspension		40/5ml		20/5ml	300	60	297	61.1	97.0±1.3	105.5±2.4

Table 2. Analysis of SMX by diazotization and coupling and TMP by difference method in synthetic and commercial pharmaceuticals (n=5) in comparison with USP method[10].

Pharmaceutical products	Labeled quantity mg/tablet		Measured quantity % ± s.d.			
			Proposed method		USP method	
	SMX	TMP	SMX	TMP	SMX	TMP
Synthetic tablets	30	20	100.0 ± 1.91	97.45 ± 1.23	98.2 ± 1.7	98. 5 ± 1.42
Co-trimoxazole adult tablets	400	80	99.45 ± 1.72	104.16 ± 2.36	99.2 ± 1.6	99.3 ± 1.4
Co-trimoxazole children tablets	100	20	95.29 ± 2.43	106.8 ± 2.65	98.1 ± 2.3	101.3 ± 1.8
Co-trimoxazole oral suspension	200/5 ml	40/5 ml	98.6 ± 2.1	104.15 ± 2.73	98.8 ± 1.7	102.6 ± 2.2

$$TPA_{271nm} = TA_{271nm} - SA_{271nm}$$

TPA was transformed to TMP quantity using a calibration curve (Fig 2). The results were summarized in Table 2. As shown in Table 2, the TMP quantities obtained by this procedure were higher than the labeled quantities, which could be attributed to the excipients, but did not exceed the USP limit.

Selectivity and Specificity of the suggested procedure

As far as the active ingredient TMP and the examined excipients did not interfere with the determination of SMX after diazotization and coupling with 2-naphthol, this method could be considered selective, but not specific because other sulfonamides which usually contain aromatic amines will interfere appreciably with this method.

Precision and Accuracy

Two series of SMX and TMP mixtures each contains 10 samples with 40 and 130 µg of SMX and 20 and 65 µg of TMP were subjected to the general procedure and the absorbance was recorded using a blank. The absorbance was transformed to quantity using a calibration curve. The precision was 99.2 -99.8 %, the accuracy 98.8-99.3% and the confidence limits were 38.96 ± 0.04 and 129.2 ± 0.7 for 40 and 130 µg of SMX respectively. The precision, accuracy and confidence limits for TMP were 99.3-99.6 %, 99.5-99.7 %, 20.2 ± 0.21 and 65.4 ± 0.62 for 20 and 65 µg of TMP respectively. The lower limit of quantification (LOQ) was found 8 µg/ml and 4 µg/ml for TMP and SMX respectively.

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