Asian Journal of Chemistry Vol. 22, No. 8 (2010), 6569-6579

Combination of Ratio Derivative Spectrophotometry with Simultaneous Standard Additions Method for Determination of Sulfamethoxazole and Trimethoprim

R. HAJIAN*, R. HAGHIGHI† and N. SHAMS†
Young Researchers Club, Department of Chemistry, College of Science, Islamic Azad University Branch of Gachsaran, Gachsaran, 75818-63876, Iran
Fax: (98)(742)3334750; Tel: (98)(742)3334751; E-mail: hajian@iaug.ac.ir; hajian@ch.iut.ac.ir

Ratio derivative spectrophotometrie method has been developed for the simultaneous determination of sulfamethoxazole (SMX) and trimethoprim (TMP) at micromolar levels in Britton Robinson buffer (pH 9) medium. In this method, the overlapping spectra of sulfamethoxazole and trimethoprim were well resolved by making use of the first derivative of the ratios of their direct absorption spectra. The derivative ratio absorbances of sulfamethoxazole and trimethoprim were measured at 256 and 293 nm, respectively for their quantification. The method is simple, fast and does not require separation of sulfamethoxazole and trimethoprim. Another salient feature of the method is that simultaneous standard additions of both analytes permitted to resolve matrix effect and quantification at a unique standard addition plot. Sulfamethoxazole and trimethoprim were determined in the concentration range of 5-150 μmol L^{-1} (SMX/TMP ratio varying from about 1 to 5) in the same aliquot with a good precision and accuracy. The recommended procedure was successfully applied for analysis of sulfamethoxazole and trimethoprim in combination dosage forms.

Key Words: Sulfamethoxazole, Trimethoprim, Spectrophotometry, Ratio derivative spectra.

INTRODUCTION

Sulfamethoxazole (SMX) or 5-methyl-3-sulfanylamidoisoxazole is a chemotherapeutics agent widely used as antibacterial drug. Its individual determination has been carried out by spectrophotometry[1,2] and by fluorimetry[3,4]. HPLC has also been proposed for determination of SMX and its main metabolites in human plasma and urine[5]. The pharmaceuticals containing sulphonamides consist only of one drug or one sulphonamide associated with another drug, which increases the power of the sulphonamide. So, SMX is usually combined with trimethoprim (TMP) in a fixed proportion 5:1, respectively, this association being called cotrimoxazol.

Trimethoprim (TMP) or 2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine is also a bacteriostatic drug. Its determination in pharmaceutical preparations has been usually carried out by spectrophotometric methods[6,7] and sometimes by electroanalytical

†Department of Chemistry, College of Science, Islamic Azad University Branch of Gachsaran, Gachsaran, 75818-63876, Iran.

methods[8,9]. Liquid chromatography[10-12] and gas chromatography have also been proposed for its individual determination or together with its major metabolites of oxidation in different matrices[13].

The simultaneous determination of both analytes has been usually carried out by spectrophotometric methods with multicomponent analysis based on the use of second derivative and diode-array detection[14], PLS[15] and CLS[16]. These methods are complicated and need the expensive instruments. No official procedure is given in well known pharmacopeias for simultaneous determination of sulfamethoxazole and trimethoprim. There is only one report on the application of derivative spectrophotometry for determination of sulfamethoxazole and trimethoprim[14]. The main disadvantage of this technique is its dependence on instrumental parameters like speed of scan and the slit width[17]. The instrumental conditions of recording parent zero-order spectrum have strong influence on the shape and intensity of its derivative generations. The acquired spectrum is more or less distorted by instrumental noises and as the consequence the derivative spectrum is distorted too. The derivatization can amplify the noise signals in the resulted curves. The appropriate selection of mathematical parameters of derivatization allows obtaining intense and shapely derivative spectra of analyte while the spectra of others (matrix) undergo quenching. So the application of this technique required careful selection the mathematical parameters as well as working parameters of spectrophotometer. The close dependence of derivatization results on the instrumental conditions of acquisition of spectra lowers the reproducibility of the elaborated methods.

Since UV-visible spectrophotometry is a rapid, sensitive and inexpensive analytical tool, it is appropriate for dosage control of pharmaceutical preparations. Despite the mentioned advantages, spectroscopy techniques (such as other analytical techniques) suffer from multiplicative (matrix effect) and additive (direct interference) errors. The problem of multiplicative errors can be simply solved by using method of standard addition (MOSA). The applicability of MOSA is limited to the cases where no direct interference (additive error) is present. Additive errors are observed when two or more species in sample have spectral overlapping. In such cases determination of one analyte in the presence of interferent(s) by classical methods (*e.g.*, standard addition or external standard calibration) is not possible.

Various multivariate methods for handling nonselective signals in spectrophotometric analysis have been proposed. Principal component and partial least squares solutions require no explicit data about the individual interferences to be known in order to model and to eliminate them[18]. However, they require a set of calibration samples with known concentrations of the analyte to be determined and the concentration of each interferent in different samples to be varied to allow the calibration algorithm to model its effect. In the most applications of multivariate chemometric methods the matrix effects are not considered.

Following the work of Salinas *et al.*[18], ratio derivative spectrophotometry based on the use of first-derivative of the ratios spectra was tried and found feasible in resolving the spectra of sulfamethoxazole and trimethoprim. Therefore in this work

the application of ratio derivative spectrophotometry combined with simultaneous standard addition of both analytes have evaluated for resolving of matrix effect and additive errors simultaneously.

EXPERIMENTAL

Sulfamethoxazole and trimethoprim were kindly donated by (Inventaa Chemical Limited, India) and used without further purification. All the solvents used in spectrophotometric analysis were of analytical reagent grade.

For preparation of 1×10^{-3} mol L^{-1} sulfamethoxazole, 0.0126 mg sulfamethoxazole (99 %) was weighted and dissolved in distilled water to 100 mL. A 1×10^{-3} mol L^{-1} trimethoprim solution was prepared daily by dissolving 0.0145 g trimethoprim (99 %) in water and diluted to 100 mL.

UV-Vis absorption spectra are measured on an Agilent UV-vis spectrophotometer, Perkin-Elmer (Lambda 25), with the use of 1 cm quartz cells. A Pentium IV (2.53 MHz) computer controlled all of the setting and data processing. A pH-meter (Metrohm, Model 691) with a double junction glass electrode was used to check the pH of the solutions.

Recommended procedure: Suitable volumes of sulfamethoxazole and trimethoprim stock solutions (up to 100 μmol L^{-1}) were mixed in a 25 mL calibrated flask and diluted to volume with 0.1 mol L^{-1} Britton Robinson buffer (pH 9). According to the theory of the ratio-spectra derivative method, the absorption spectrum of the mixture was divided, wavelength by wavelength, by a standard spectrum of trimethoprim (TMP, 15 μmol L^{-1}) for determining of SMX and by a standard spectrum of SMX (2 μmol L^{-1}) for determining TMP. Then, the 1st derivative of the above ratio-spectra was recorded ($\Delta\lambda = 5$) and the values of the derivatives were measured at suitably selected wavelengths. In particular, the concentration of SMX was proportional to the value of the 1st-derivative of the ratio-spectra at 256 nm (one maxima). The concentration of TMP was proportional to the value of the 1st derivative of the ratio spectra at 293 nm (one maxima), respectively. The concentration of SMX and TMP in the mixture was computed from the simultaneous standard additions of both analytes for resolving of matrix effect.

Principle of ratio derivative spectrophotometry: Consider a mixture of two compounds A and B. The absorption spectrum of the mixture is given by the equation

$$A_{M,\lambda_1} = E_{A,\lambda_1}C_A + E_{B,\lambda_1}C_B \quad (1)$$

where A_{M,λ_1} is the absorbance of mixture at wavelength λ_1, E_{A,λ_1} and E_{B,λ_1} are the molar absorbivity of A and B at λ_1, C_A and C_B are the concentrations of A and B in the mixture.

If eqn. 1 is divided by the absorbance at λ_1 of a standard solution of A whose concentration is A^0_A *i.e.*, $A_{A,\lambda_1}A^0_A$ then eqn. 1 becomes:

$$\frac{A_{M,\lambda_1}}{E_{A,\lambda_1}C^0_A} = \frac{C_A}{C^0_A} + \frac{E_{B,\lambda_1}C_B}{E_{A,\lambda_1}C^0_A} \quad (2)$$

which can be simplified to:

$$\frac{A_{M,\lambda_1}}{E_{A,\lambda_1}} = C_A + \frac{E_{B,\lambda_1}}{E_{A,\lambda_1}} C_B \quad (3)$$

Differentiating eqn. 3 with respect to λ, gives

$$\frac{d}{d\lambda}\left(\frac{A_{M,\lambda_1}}{E_{A,\lambda_1}}\right) = C_B \frac{d}{d_\lambda}\left(\frac{E_{B,\lambda_1}}{E_{A,\lambda_1}}\right) \quad (4)$$

Eqn. 4 indicates that the "derivative ratio spectrum" of the mixture is dependent only on the values of C_B and is independent of the value of C_A in the mixture.

RESULTS AND DISCUSSION

The absorption spectra of sulfamethoxazole and trimethoprim under certain experimental conditions are shown in Fig. 1. As it is shown, the maximum wavelengths of two compounds are very close to each other and their spectra are highly overlapped. Therefore, direct determination of two pharmaceutical compounds in the presence of each other is impossible by spectrophotometry. Therefore, the combination of ratio derivative spectrophotometry and standard addition methods was used for resolving of additive interference and matrix effect simultaneously.

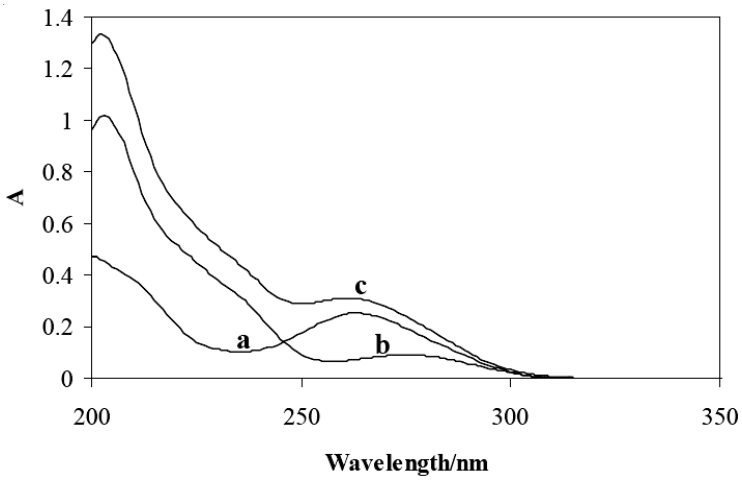

Fig. 1. Absorption spectra of (a) sulfamethoxazole, (b) trimethoprim and (c) a mixture of sulfamethoxazole and trimethoprim (10 μmol L^{-1} for each) in Britton Robinson buffer at pH 7

Effect of operational parameters: In order to optimize the procedure for the simultaneous determination of sulfamethoxazole and trimethoprim, we studied the effect of parameters including pH, wavelength selection and divisor concentrations on the sensitivity and selectivity of the method. As it has been shown in Fig. 2(A). There weren't significant changes in the absorption spectra of sulfamethoxazole

and trimethoprim at the pH range of 2-10. Therefore pH of 9 was selected for obtaining higher selectivity [Fig. 2(B)] and omitting some ion interferences in alkali media.

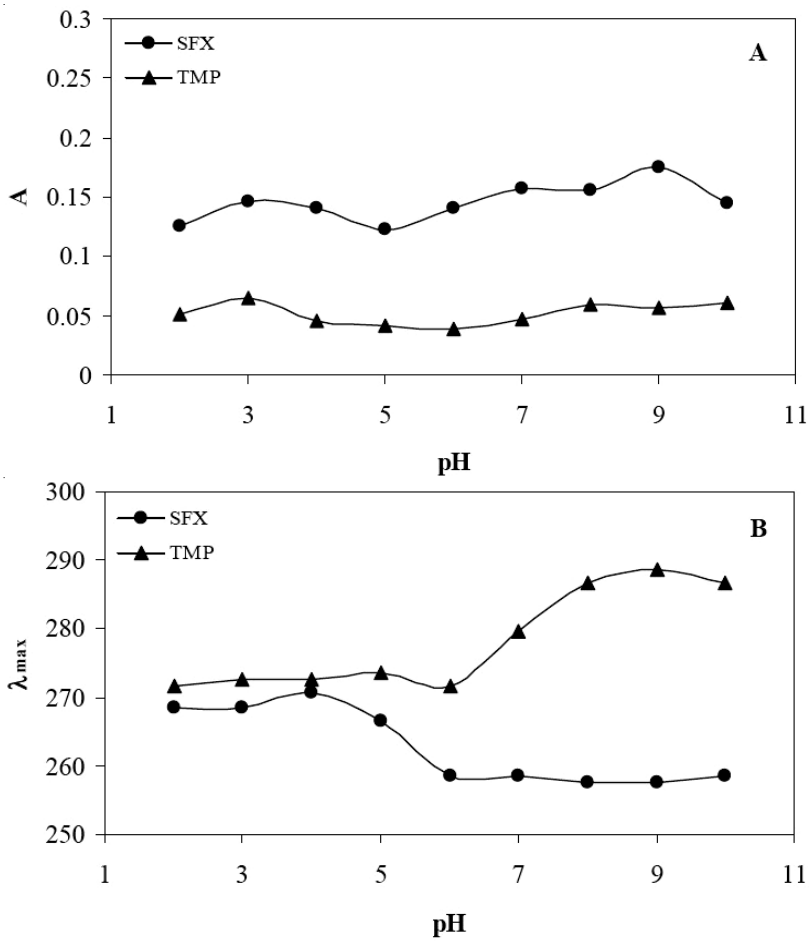

Fig. 2. Effect of pH on the maximum absorption spectra (A) and maximum wavelength (B) of sulfamethoxazole and trimethoprim. Conditions: Britton Robinson buffer (0.01 M), sulfamethoxazole 10 μmol L^{-1}, trimethoprim 10 μmol L^{-1}

Wavelength selection: In a preliminary investigation, different concentrations of SMX and TMP as divisors were examined. An accurate choice of both standard divisors and working wavelengths is fundamental for several reasons[19-21]. In the wavelength range where the absorbance of the standard spectrum used as divisor approaches zero, the noise of the ratio-spectra is greatly enhanced. Consequently, a certain overlap of spectra in the working wavelengths region is actually desirable. Then, by increasing or decreasing the concentration of divisor, the resulting derivative values (hence, the slope of calibration graphs) are proportionately decreased or

increased, with potential variation of both sensitivity and linearity range. From several tests, we found the best results in terms of signal-to-noise ratio and sensitivity by using as divisors standard spectra of both 1-150 μmol L^{-1} SMX and TMP, with minimal differences. Outside the above concentration ranges, the noise was greatly increased; hence it required too high a level of smoothing, with consequent distortion and variation of the shape of curves and location of peaks. For all subsequent measurements, standard divisors of 2 μmol L^{-1} of SMX and 15 μmol L^{-1} of TMP were selected.

Fig. 3 showed two series of ratio-spectra of SMX [from 10-100 μmol L^{-1}, Fig. 3 (A)] and TMP [from 10-100 μmol L^{-1}, Fig. 3 (B)]. Fig. 4 showed the corresponding 1st derivative of the ratio-spectra of Fig. 3. For calibration graphs, we selected the wavelengths which exhibited the best linear response to the analyte concentration, *i.e.*, in the 1st-derivative mode 256 and 293 nm to determine SMX and TMP, respectively.

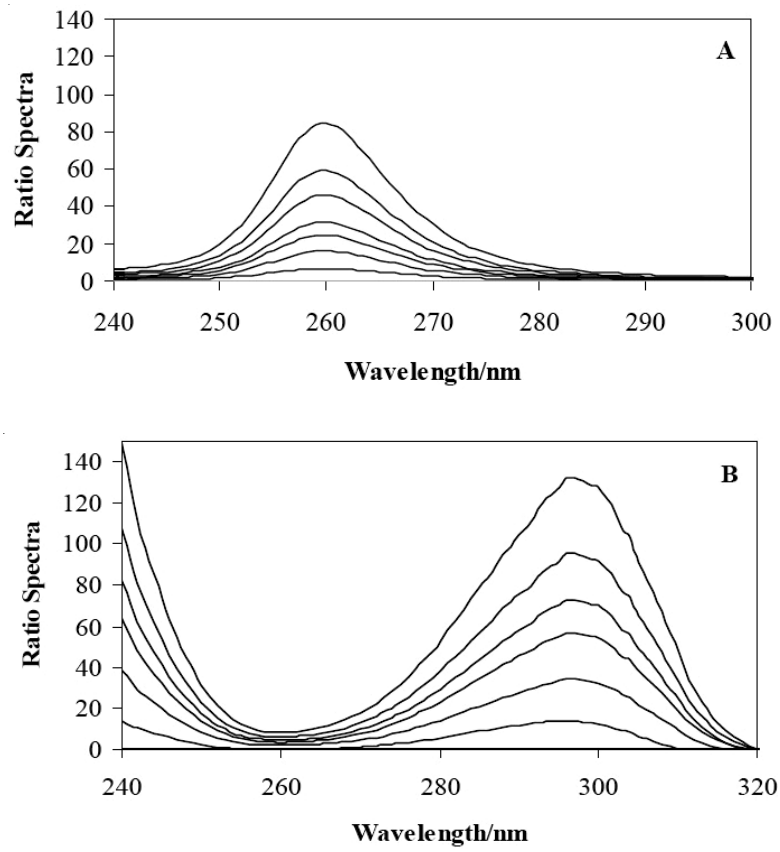

Fig. 3. Ratio-spectra for different concentrations of sulfamethoxazole (10, 20, 30, 40, 50, 70 and 100 μmol L^{-1}. (A) 1-7, divisor TMP, 15 μmol L^{-1}) and of trimethoprim (10, 20, 30, 40, 50, 70 and 100 μmol L^{-1}; (B) 1-7, divisor SMX, 2 μmol L^{-1})

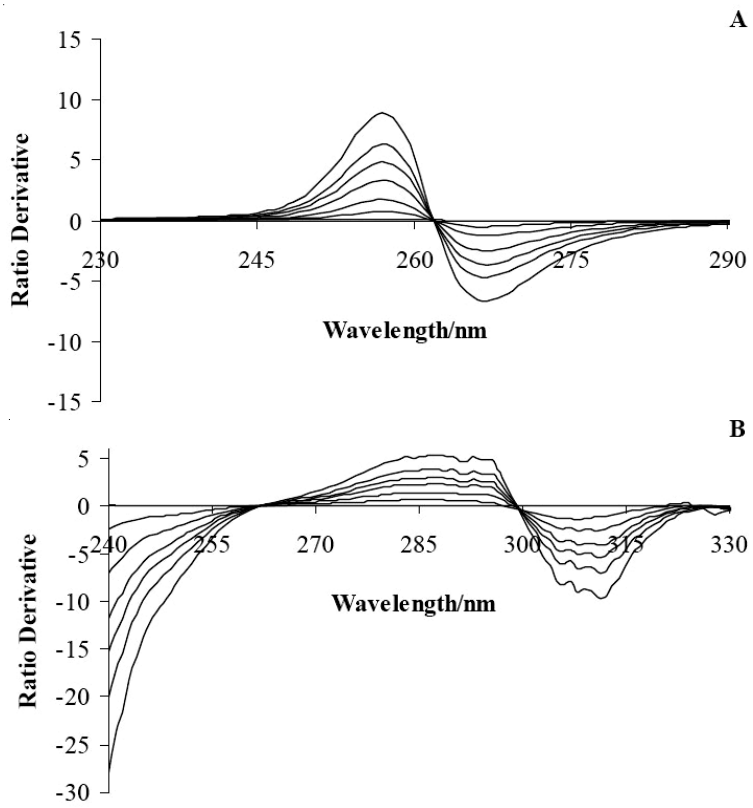

Fig. 4. First-derivative spectra of the ratio-spectra of sulfamethoxazole (10, 20, 30, 40, 50, 70 and 100 μmol L^{-1}. (A) in 15 μmol L^{-1} TMP as divisor and trimethoprim (10, 20, 30 and 40 μmol L^{-1}; (B) shown in 2 μmol L^{-1} SMX as divisor

The calibration graphs for each drug in both derivative modes were achieved by plotting the values of the 1st derivative of the ratio-spectra SMX/TMP and TMP/SMX, with variable concentrations of SMX and TMP, at the above working wavelengths against the concentrations of SMX and TMP in the standards.

Divisor concentration: According to the theory[19], the slope of the calibration graphs proportionally increases if the concentration of the divisors decreases. This is shown in Fig. 5. These results confirm the reliability of the ratio-spectra method in the present instance. According to the above results, divisor concentration of 2 was chosen for divisor SMX because of the higher sensitivity. But because of the low selectivity at 10 and 15 μmol L^{-1} TMX as divisor (Fig. 6), we chose 15 μmol L^{-1} TMX with the high selectivity *versus* SMX concentration up to 90 μmol L^{-1}.

Accuracy: Accuracy of the proposed method was tested by determinations of various synthetic mixtures of SMX and TMP using the simultaneous standard additions of both analyes. The results shown in Table-1 indicate that first derivative ratio spectra method is very effective for the simultaneous determination of SMX and TMP in presence of each other.

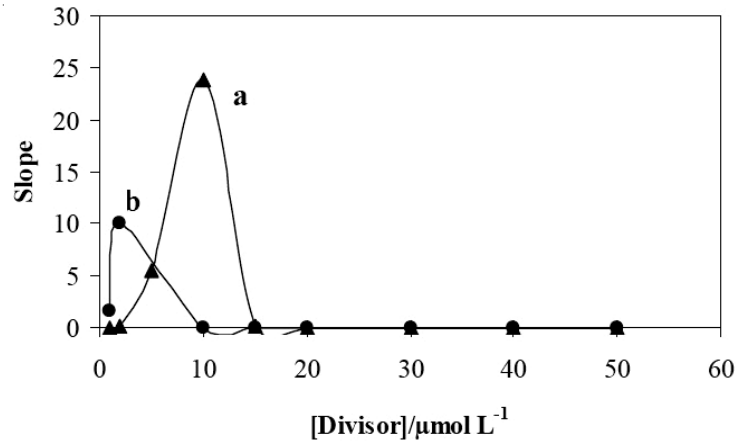

Fig. 5. Graphs of the slopes of the calibration curves of sulfamethoxazole and trimethoprim for different divisor concentrations *versus* divisor concentration. Curve (a) sulfamethoxazole, divisor TMP, 1st (256 nm) derivative spectrophotometry and curve (b) trimethoprim, divisor SMX, 1st (293 nm) derivative spectrophotometry

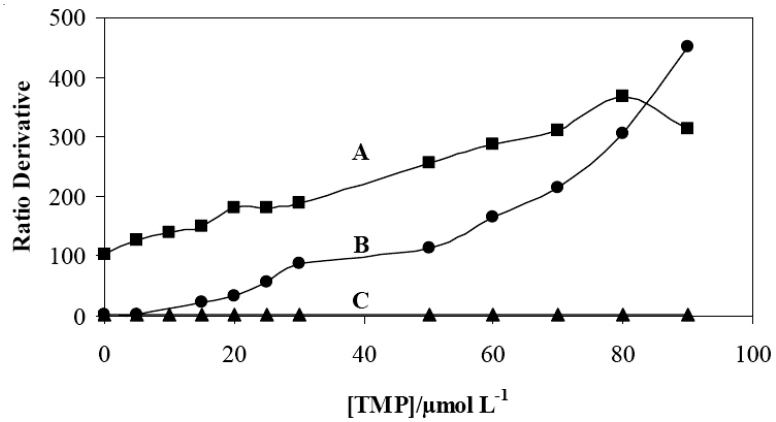

Fig. 6. First ratio derivative spectra at 256 nm for mixes of trimethoprim (0-90 µmol L^{-1}) and sulfamethoxazole (10 µmol L^{-1}) in different concentrations of TMP divisors: (A) 5 µmol L^{-1} (B) 10 µmol L^{-1} and (C) 15 µmol L^{-1}

Precision: To check the reproducibility of the method, three replicate experiments for the analysis of sulfamethoxazole and trimethoprim mixtures were designed (Table-2). As it shown relative standard deviations of calculated concentrations are satisfactory.

Interferents: An attractive feature of an analytical procedure is its relative freedom from interferences. The selectivity of the proposed procedure for the assay of sulfamethoxazole and trimethoprim was identified by studying the effect of excipients that often accompany with SMX and TMP in pharmaceutical formulations. Therefore, samples containing 10 µmol L^{-1} SMX and/or TMP in the absence and

TABLE-1
DETERMINATION OF SULFAMETHOXAZOLE AND TRIMETHOPRIM IN SOME SYNTHETIC MIXTURES

Synthetic samples	C_{added} (μmol L^{-1})		C_{found} (μmol L^{-1})		Recovery (%)	
	SMX	TMP	SMX	TMP	SMX	TMP
Synthetic 1	20.0	20.0	20.20	20.47	101.0	102.3
Synthetic 2	40.0	50.0	41.20	49.79	103.0	99.6
Synthetic 3	10.0	50.0	9.10	49.96	91.0	99.9
Synthetic 4	50.0	10.0	47.62	10.34	91.0	95.2
Synthetic 5	10.0	40.0	10.01	37.05	101.6	100.1
Synthetic 6	40.0	10.0	37.50	10.89	93.7	108.9
Synthetic 7	5.0	5.0	5.30	4.95	106.0	99.0

TABLE-2
REPLICATE MEASUREMENTS RESULTS OF SULFAMETHOXAZOLE AND TRIMETHOPRIM IN SOME SYNTHETIC MIXTURES

C_{added} (μmol L^{-1})		C_{found} (μmol L^{-1})		Average		Standard deviation	
SMX	TMP	SMX	TMP	SMX	TMP	SMX	TMP
10.0	40.0	11.10	42.65				
10.0	40.0	11.80	42.39	11.60	42.53	0.51	0.13
10.0	40.0	12.10	42.54				
20.0	20.0	20.3	19.92				
20.0	20.0	21.5	21.93	21.20	20.54	0.83	1.21
20.0	20.0	21.9	19.77				
40.0	10.0	42.5	10.02				
40.0	10.0	42.6	9.60	42.50	10.26	0.10	0.81
40.0	10.0	42.4	11.17				

presence of excipients were analyzed by means of the proposed procedure. For other constituents, tolerance limit was defined as the concentrations which give an error of ≤ 5 % in the determination of each sulfamethoxazole and trimethoprim. The effects of all examined compounds at several molar ratios over SMX and TMP on the measured analytical concentrations are given in Table-3. The results show no significant interference from excipients. This is due to the fact that in alkali media, pH (> 7), many cationic ions precipitate in aqueous media.

TABLE-3
MAXIMUM TOLERABLE CONCENTRATION OF INTERFERING SPECIES WITH SULFAMETHOXAZOLE (10 μmol L^{-1}) AND TRIMETHOPRIM (10 μmol L^{-1})

Species	Tolerance concentration (mmol L^{-1})
NO_3^-, I^-, K^+, Mg^{2+}, Ca^{2+}, Na^+, CH3COO$^-$, Cu^{2+}, Co^{2+}, Ag^+, Br^-, Cd^{2+}, Fe^{2+}, Zn^{2+}, Bi^{3+}, Cr^{3+},Ca^{2+}, Al^{3+}, Fe^{3+}, PO_4^{3-}, SO_4^{2-}, Mn^{2+}, CH_3OH, C_2H_5OH	10.0
Vitamin B_1, vitamin C, folic acid, glucose, sodium benzoate	0.1

Analyses of SMX and TMP in pharmaceutical formulations: Commercial pharmaceutical formulations (tablets) containing the mixture of SMX-TMP (Chemi Darou and Tehran Darou Pharmaceutical companies, Tehran, Iran), were employed to test the applicability of the proposed method. For tablets, a total of 2 g were ground to a very fine powder and homogenized. Sample solutions were prepared by weighing 0.1322 g of the powder, dissolving it in 0.1 mol L^{-1} sodium hydroxide by using an ultrasonic bath. For resolving of matrix effect, simultaneous standard additions of both SMX and TMP were designed. The absorbance spectrum of each solution was then recorded and transferred to the ratio first derivative spectrum to predict the concentrations of the TMP and SMX by standard addition plot. In the other trials, different amounts of TMP/SMX were spiked into the original dissolved drug and the procedure for the analysis of TMP and SMX were repeated. Each analysis was repeated three times. The results are listed in Table-4. In this table, the resulting values of TMP and SMX as the mean of three replicates and the mean recoveries are represented. The confidence intervals for TMP and SMX are varied in a small range indicating good reproducibility of the proposed analytical method. Indeed, the recoveries (relative to the declared amounts of drugs in tablets and the spiked amounts) varied between 90.0-104.0 and 97.0-110.4 % for TMP and SMX, respectively. This confirms the high accuracy of the proposed method for simultaneous analyses of TMP and SMX in a commercial formulation.

TABLE-4
DETERMINATION OF SULFAMETHOXAZOLE AND TRIMETHOPRIM IN SOME PHARMACEUTICAL FORMULATIONS

Sample	Added		Found		Recovery (%)	
	SMX (mg)	TMP (mg)	SMX	TMP	SMX	TMP
Cotrimoxazol*	–	–	388.09	77.14	97.02	96.43
	224 mg	224	635.34	308.18	110.38	103.14
Cotrimoxazol**	–	–	409.67	83.19	102.42	103.98
	220	224	640.82	277.25	103.19	90.13
Mean	–	–	–	–	103.25	98.42
RSD (%)	–	–	–	–	5.31	6.58

*Label 80 mg trimethoprim and 400 mg sulfamethoxazole manufactured by Chimi Darou Co. (Tehran, Iran). **Label 80 mg trimethoprim and 400 mg sulfamethoxazole manufactured by Tehran Darou Co. (Tehran, Iran).

Conclusion

The 1st order ratio-spectra derivative method enable the quantitation of mixtures of SMX and TMP with good accuracy and precision, either in laboratory samples or in pharmaceutical products. The procedure is fast and specific and works without solving equations or separation steps. As a further advantage of the ratio-spectra method proposed over the zero-crossing derivative method, is the possibility of performing measurements in correspondence of peaks, hence a potentially greater

sensitivity and accuracy. Disadvantages of the zero-crossing method are the risk of small drifts of the cross over points and the fact that the working wavelengths do not coincide with the peaks. This may be particularly dangerous when the slope of the spectra is very high, with consequent loss of precision and accuracy.

ACKNOWLEDGEMENTS

The authors gratefully acknowledged the support of this work by Islamic Azad University Branch of Gachsaran. Also the assistance of Chemi Darou & Tehran Darou Pharmaceutical Companies for offering pure drugs is gratefully acknowledged.

REFERENCES

1. F.A. Mohamed, A.I. Mohamed and S.R. El-Shabouri, *J. Pharm. Biomed. Anal*., **6**, 175 (1988).
2. S. Husain, A.S.R. Krishna Murty, P.R. Prasad and R. Sekar, *Indian Drugs*, **32**, 336 (1995).
3. A. Munoz de la Pena, F. Salinas, I. Duran-Meras and M.D. Moreno, *Anal. Lett*., **27**, 1893 (1994).
4. C. Cruces-Blanco, A. Segura Carretero, S. Fernandez Peinado, M. Roman Ceba and A. Fernandez Gutierrez, *Fresenius J. Anal. Chem*., **365**, 444 (1999).
5. T.B. Vree, A.J.A.M. Van der Ven, C.P.W.G.M. Verwey-Van Wissen, E.W.J. Van Ewijk-Beneken Kolmer, A.E.M. Swolfs, P.M. Van Galen and H. Amatdjais-Groenen, *J. Chromatogr., B: Biomed. Appl*., **658**, 327 (1994).
6. A.L. El-Ansary, Y.M. Issa and W. Selim, *Anal. Lett*., **32**, 955 (1999).
7. S.Z. Qureshi, M.I.H. Helaleh, N. Rahman and R.M.A. Jamhour, *Fresenius J. Anal. Chem*., **357**, 1005 (1997).
8. M. Aboudan, *J. Chem. Tech. Biotech*., **61**, 31 (1994).
9. M.A. Ahmed and M.M. Elbeshlawy, *Anal. Lett*., **28**, 2123 (1995).
10. G.A.E. Van't Klooster, H.J. Kolker, F.M.A. Woutersen- Van Nijnanten, J. Noordhoek and A.S.J.P.A. Van Miert, *J. Chromatogr*., **579**, 355 (1992).
11. P. Nachilobe, J.O. Boison, R.M. Cassidy and A.C.E. Fesser, *J. Chromatogr., Biomed. Appl.*, **616**, 243 (1993).
12. G. Chen, Y.H. Tu, L.V. Allen and D.P. Wang, *Int. J. Pharm*., **46**, 89 (1988).
13. U. Ernemann, D. Schirmer, A. Woeber, P. Langmann, K. Warzecha, R. Joeres, H. Klinker and E. Richter, *J. Chromatogr*., **528**, 243 (1990).
14. C. Altesor, P. Corbi, I. Dol and M. Knochen, *Analyst*, **118**, 1549 (1993).
15. M.E. Ribone, A.P. Pagani and A.C. Olivieri, *Anal. Lett*., **32**, 1389 (1999).
16. M.E.M. Hassouna, *Anal. Lett*., **30**, 2341 (1997).
17. S. Kus, Z. Marczenko and N. Obarski, *Chem. Anal., (Warsaw)*, **41**, 899 (1996).
18. B.G.M. Vadeginste, D.L. Massart, L.M.C. Budens, S. De Jong, P.J. Lewi and J. Smeyers-Verbeke, Handbook of Chemometrics and Qualimetrics, Elsevier, Amsterdam (1998).
19. F. Salinas, J.J.N. Berzas and A.E. Mansilla, *Talanta*, **37**, 347 (1990).
20. J.J.B. Nevado, C. Guiberteau and F. Salinas, *Talanta*, **39**, 547 (1992).
21. B. Morelli, *Talanta*, **41**, 673 (1994).

(*Received*: 25 January 2010; *Accepted*: 22 May 2010) AJC-8731