ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXIII, N 1, 3 SECTIO DDD 2010

Department of Applied Pharmacy, Medical University of Lublin

ŁUKASZ ZIMMER, WIKTOR CZARNECKI

Derivative spectrophotometric method for simultaneous determination of sulfadimidine and trimethoprim

Metoda równoczesnego oznaczania sulfadymidyny i trimetoprimu z zastosowaniem spektrofotometrii pochodnych

Sulfonamides are a group of synthetic organic compounds that have played an important role as effective chemotherapeutics in bacterial and protozoal infections in medicine and veterinary practice [13, 14, 18]. Sulfonamides are bacteriostatic antimicrobials that interfere with the biosynthesis of folic acid in bacterial cells; they compete with para-aminobenzoic acid (PABA) for incorporation into dihydrofolic acid. By replacing the PABA molecule in dihydrofolic acid, they prevent the formation of folic acid required for nucleic acid synthesis and multiplication of the bacterial cell [2]. In clinical practice, sulfonamides have been administered individually or in mixtures include sulfadiazine, sulfadimidine, sulfamethoxazole, sulfanilamide and trimethoprim, which increases the power of sulfonamide [16]. They are used in the treatment of otitis, bronchitis, sinusitis and pneumoystis pneumonia and urinary tract infections in combination with trimethoprim [1, 10]. In veterinary, they cover infectious diseases of the digestive and respiratory tracts, secondary infections, mastitis, metritis and foot rot [4, 18].

Sulfadimidine (SDD) N-*(4,6-dimethylpyrimidin-2-yl)sulphanilamide* is perhaps one of the most widely used sulfonamide. SDD is regularly used in food-producing animals for therapeutic, prophylactic, or growth-promoting purposes. The use of this sulfonamide in dairy cows is of great concern because the sulfonamide residues are turning up in milk, an important component in the diets consumed by almost all young and growing children and most adults every day [7]. It has a spectrum of antimicrobial action similar to other sulfonamides [2].

Trimethoprim (TMP) *5-(3,4,5-trimethoxybenzyl)pyrimidine-2,4-diamine* is one of the most widely used antibacterial additives that acts synergistically in combination with sulfonamides.

It inhibits the conversion of bacterial dihydrofolic acid to tetrahydrofolic acid which is necessary for the synthesis of certain amino acids, purines, thymidine, and ultimately DNA synthesis. It acts in the same metabolic pathway as sulfonamides [5, 6].

Several analytical methods have been reported in the literature for the determination of sulfonamides individually or in combination with other sulfonamides and TMP in pharmaceutical preparations or biological samples. The mixture of sulfonamides and TMP has been studied in a number of reports and the latter provide several analytical methods for the determination, including

voltammetry [12], spectrophotometry [8, 13, 14, 16, 21] derivative spectrophotometry [9, 15] and HPLC [1, 3, 19].

To determine the total content of sulfonamides and TMP, spectrophotometric methods including derivative spectrophotometry have been developed. Ni et al. [17] applied a UV- spectrophotometric method with the aid of chemometrics methods comparing the classical last squares (CLS), principal component regression (PCR) and partial last squares (PLS) models for determination of sulfadiazine, sulfadimidine, sulfamethoxazol, sulfanilamide and trimethoprim.

Two derivative spectrophotometric (ratio derivative spectra and algorithm bivariate calibration) methods and a chemometric (PLS) were proposed by Markopoulou et al. [15] for simultaneous determination of binary mixtures of trimethoprim (TMP) with sulfamethoxazole (SMX) or sulfamethazine (SMZ) or sulfafurazole (SFZ) withouth prior separation. For the application of the bivariate calibration, two optimum wavelengths sets were selected using the method of Kaiser. The wavelengths pair with the highest absolute sensitivity values for the TMP/SMZ (sulfadimidine) binary mixture, was 303.3 and 232.4 nm. Mean recoveries in synthetic binary mixture for derivative bivariate method were 99.9 ± 1.69% for TMP and 99.3 ± 1.26% for SMZ. Bivariate calibration spectrophotometric method was applied to simultaneous determination of trimethoprim (TMP), sulfamethoxazole (SMX) or sulfamethoxypyridazine (SMP) binary mixtures by Lopez-Martinez et al. [14].

Binary mixture of sulfadiazine-trimethoprim with overlapping spectra was determined by Moreno Galvez et al. [16]. The chosen mathematical method for solving this problem was zero crossing in the first derivative spectra. The whole process was carried out by using a Flow Injector Analysis (FIA) and provided with a UV-vis spectrophotometer detector. The zero crossing of the first derivative spectra was observed at 248.5 nm and 242.25 nm for sulfadiazine and 288.0 nm and 258.8 nm for trimethoprim. Suitable wavelengths for analyses were 288.0 and 248.5 for sulfadiazine and trimethoprim, respectively.

Derivative sectrophotometry was introduced in the early 1950s by Hammond and Price and has been applied to many chemical systems, such as pharmaceuticals, foods, cosmetics, and environmental samples, giving rise to accurate and precise analytical results [16]. This technique, based on the use of derivative spectra resulting from derivatisation of zero-order spectra of UV-vis absorption, keeps all laws of classical spectrophotometry. The derivatisation of zero-order spectrum can lead to separation of overlapped signals and it allows quantification of one or a few analytes without initial separation. A major success was achieved by derivative treatment of the absorbance curves-plotting of the first or a higher order mathematical derivative of absorbance against wavelength ($dA/d\lambda$) [11, 20]. The main advantage of the derivative method is the presence of a large number of maxima and minima, which provides an opportunity for the determination of active compounds in the presence of other pharmaceuticals and excipients, which possibly interfere with the analysis. In zero order bivariate calibration method a particular case arises when one or both of the analytes present broad bands with no well-defined maximum [15].

In this work, a derivative spectrophotometric method is reported to accomplish the simultaneous determination of sulfadimidine and trimethoprim in mixtures without the need for prior separation.

EXPERIMENTAL DESIGN. MATERIALS AND METHODS

R e a g e n t s. All chemicals were of analytical-reagent grade. Sulfadimidine Natrium (SDD-Na) and trimethoprim (TMP) were purchased from POCH SA(Gliwice Poland), ethanol was from P.P.H, „STANLAB". The ammonium buffer solution pH 10 was prepared from POCH reagents. Ammonium hydroxide 25% solution (HPLC grade) and ammonium chloride (HPLC grade) were obtained from POCH SA (Gliwice Poland).

Stock solutions containing respectively 1.000g l^{-1} of SDD-Na and TMP in ethanol were prepared and stored at 40C. Working solutions were prepared daily by appropriate dilution. The water was purified by Cobrabid-Aqua CA-ROD 3 ECO system.

A p p a r a t u s. A Thermo Scientific Helios Omega UV-VIS spectrophotometer connected to PC fitted with VISION pro software was used for all measurement and treatment of data.

P r o c e d u r e s. Samples were prepared in 25 ml volumetric flasks by adding 20 mg l^{-1} of SDD-Na and between 2.0 and 20 mg l^{-1} of TMP or 20 mg l-1 of TMP and between 2.0 and 20 mg l^{-1} of SDD-Na, 0.5 ml of ethanol, 5 ml of ammonium buffer solution (pH 10) and volume adjusted with purified water [14]. Spectra of the solutions were recorded between 210 and 360 nm. A 25 ml solution containing 5 ml of buffer and 0.5 ml of ethanol was used as reference. The absorption spectra of the samples thus prepared were recorded and stored in the computer. Two series of solutions containing constant amount of TMP (20 mg l^{-1}) and variable amount of SDD-Na (2, 4, 8, 12, 16, 20 mg l^{-1}) or variable amount of TMP (2, 4, 8, 12, 16, 20 mg l^{-1}) and constant amount of SDD-Na (20 mg l^{-1}) were prepared for the bivariate calibration. The accurate volumes of stock solutions of SDD-Na and TMP were added to 25 ml volumetric flasks followed by 0.5 ml of ethanol, 5 ml of ammonium buffer solution (pH 10) and volume was adjusted with purified water.

B i v a r i a t e c a l i b r a t i o n m e t h o d. The concentration of two components SDD-Na (S) and TMP (T) in a mixture can be determined according to Lambert-Beer's law through a system of four calibration curves by measuring the first derivative spectra for each component in the mixture at two selected wavelengths.

The linear calibration regression function for the first derivative spectrophotometric determination of an analyte S at a selected wavelength (λ1) is given by

$$D_S = a_S C_S + int_S$$

and analyte T (λ_2) is given by

$$D_T = a_T C_T + int_T$$

where a_{S1}, a_{T1} and a_{S2}, a_{T2} are the linear regression calibration curve slope values of the first derivative spectra, C_S and C_T the concentration of analytes S and T, respectively and int_S , int_T are the intercept values of calibration curves.

As the additivity law is kept, the derivative spectrum of binary mixture (S,T) is the sum of derivative spectra of each individual component and at two selected wavelengths (λ1, λ2) we have two equations set:

$$(\lambda_1)\ D_{ST1} = D_{S1} + D_{T1} = a_{S1} C_S + a_{T1} C_T + int_{ST1}$$
$$(\lambda_2)\ D_{ST2} = D_{S2} + D_{T2} = a_{S2} C_S + a_{T2} C_T + int_{ST2}$$

where int_{ST1}, int_{ST2} are the sum of the interceps of the linear calibration of two analytes at two wavelengths ($int_S + int_T = int_{ST}$). The values of CS and CT can be evaluated as follows:

$$C_S = \frac{a_{S2}(D_{ST1} - int_{ST1}) + a_{S1}(int_{ST2} - D_{ST2})}{a_{S2}a_{T1} - a_{S1}a_{T2}}$$

$$C_T = \frac{(D_{ST1} - int_{ST1}) + a_{T1}C_T)}{a_{S1}}$$

RESULTS AND DISCUSSION

Optimum experimental conditions were set according to Lopez-Martinez et al. [14]. Analytical characteristics for individual zero order determination of SDD-Na and TMP were evaluated at the maximum absorption wavelengths. For the first derivative determination of SDD-Na and TMP wavelengths were chosen based on the slope values of the linear regression and determination coefficient from three analytical wavelengths based on maxima and minima. The results are summarized in Table 1 and Table 2.

Table 1. Analytical characteristics and statistical parameters for single component determination of sulfadimidine natrium (SDD-Na) and trimethoprim (TMP)

Component	SDD-Na	TMP
Absorbance		
λ_{max}	259 nm	289 nm
Linearity range (µg/ml)	2-20 µg/ml	2-20 µg/ml
Equation	A=0.0665[SDD]+0.0094	A=0.0252[TMP]-0.003052
Determination coefficient	0.999	0.998
RSD(%) (n = 6)	0.47	0,34
Average recovery R%±SD	99.4±4.1	101.3±5.5
Detection limit (µg/ml)	0.14	0.09
First derivative		
λ_{max}	268 nm	246 nm
Linearity range (µg/ml)	2-20 µg/ml	2-20 µg/ml
Equation	^{1}D=-0.1611[SDD]-0.0103	^{1}D=-0.2749[TMP]+0.0347
Determination coefficient	0.999	0.999
R.S.D(%) (n = 6)	0.41	0.48
Average recovery R%±SD	99.6±1.4	100.2±2.6
Detection limit (µg/ml)	0.07	0.11

Detection limit = 3.3Sy/a; Sy – standard deviation, a – slope of calibration

Table 2. Analytical wavelengths for SDD-Na and TMP determination in single component solutions (aS, aT - slope value of linear regression calibration for SDD-Na and TMP)

Wavelenghts (nm) TMP	a_T	Determination coefficient r^2 (p = 0.05)
246	-0.2748	0.999
268	0.0595	0.999
303	-0.0899	0.999
Wavelenghts (nm) SDD-Na	a_S	
232	0.1725	0.998
254	0.0381	0.999
268	0.1614	0.999

Fig.1 shows absorption spectra and Fig. 2 shows first derivative spectra of TMP and SDD-Na for individual components and their binary mixture. As shown, there is a high spectra overlap – an analytical problem, which makes it difficult to simultaneously determine two or more compounds in the sample without previous chemical separation. For this reason the first derivative bivariate calibration technique was applied for simultaneous determination of SDD-Na and TMP in the mixture. Fig. 3 shows first derivative spectra of sulfadimidine (SDD-Na) 2 - 20 mgl^{-1} and trimethoprim (TMP) 20 mgl^{-1} binary mixtures and Fig.4 shows first derivative spectra of sulfadimidine (SDD-Na) 20 mgl^{-1} and trimethoprim (TMP) 2- 20 mgl^{-1} binary mixtures.

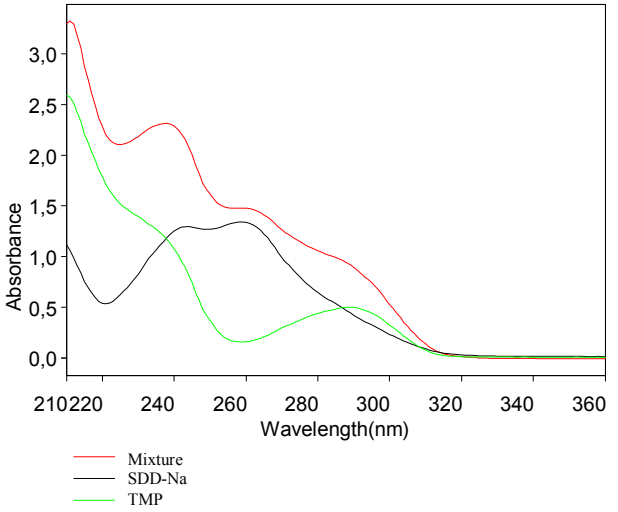

Fig.1. Absorption spectra of sulfadimidine natrium (SDD-Na) 20 mgl^{-1}, trimethoprim (TMP) 20 mgl^{-1} and their mixture

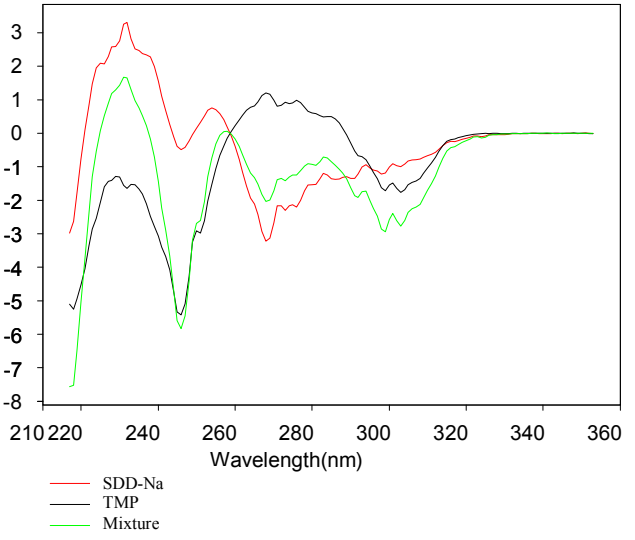

Fig. 2. First derivative spectra of sulfadimidine natrium (SDD-Na) 20 mgl^{-1}, trimethoprim (TMP) 20 mgl^{-1} and their mixture

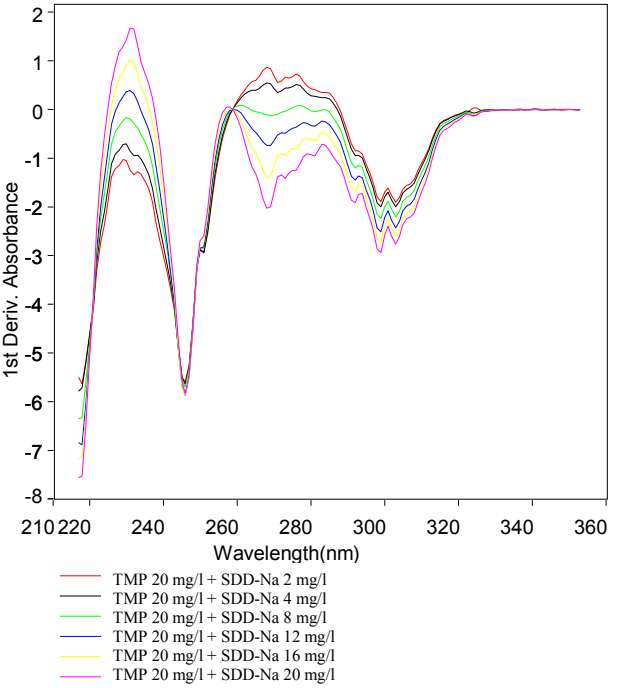

Fig. 3. First derivative spectra of sulfadimidine natrium (SDD-Na) 2 - 20 mgl^{-1} and trimethoprim (TMP) 20 mgl^{-1} binary mixtures

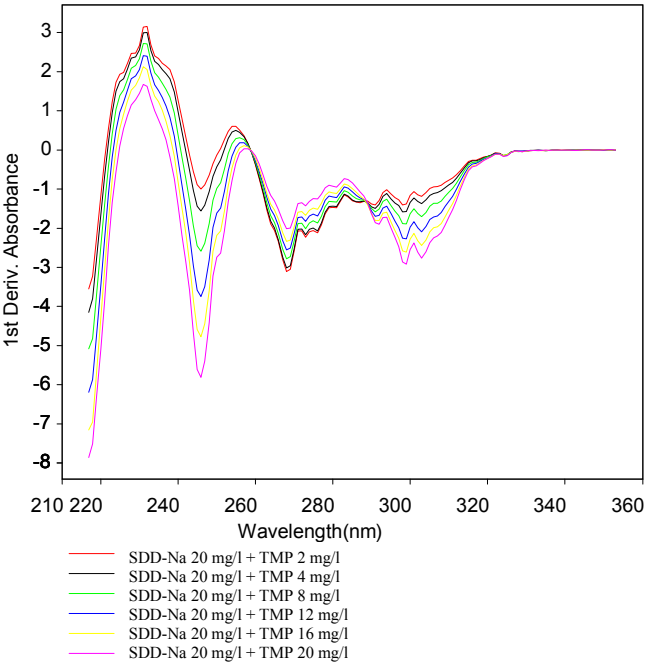

Fig. 4. First derivative spectra of sulfadimidine natrium (SDD-Na) 20 mgl^{-1} and trimethoprim (TMP) 2 - 20 mgl^{-1} binary mixtures

Two optimum wavelengths 249 and 268 nm were proposed for SDD-Na-TMP mixture. These optimum wavelengths were used to determine four linear regression calibration equations – two calibrations for each component at two wavelengths. Linear regression calibration formulas and determination coefficients are presented in Table 3.

Table 3. Linear regression calibration formulae used for first derivative bivariate algorithm (D= aC+ int)

Binary mixture SDD-Na - TMP		
Component	Calibration equations and determination coefficients	
	λ= 249 nm	λ= 268 nm
SDD-Na	^{1}D= -0.001761[SDD-Na]-0.002453 (r^2= 0.960)	^{1}D=-0.161052[SDD-Na]-0.010271 (r^2= 0.999)
TMP	^{1}D=-0.164800[TMP]+0.026986 (r^2= 0.999)	^{1}D=0.059386[TMP]+0.021784 (r^2= 0.999)

Zero crossing first derivative method was also proposed to select the analytical signal. The selected wavelengths were 244 nm for TMP and 289 nm for SDD-Na determination (Fig. 2). The calibration equation and determination coefficient for each component are presented in Table 4.

Table 4. Calibration formulas for TMP and SDD- Na in the binary mixture obtained using zero-crossing method from the derivative spectra

Component	λ (nm)	Calibration equation	Determination coefficient r^2 (p = 0.05)
TMP	244	^{1}D= 0.240099[TMP]+0.015694	0.999
SDD-Na	289	^{1}D= -0.065169[SDD-Na]-0.007260	0.999

The proposed methods were analysed regarding the precision (relative standard deviation), linearity (evaluated by regression equations) and accuracy (% recovery – using the first derivative bivariate and 0 crossing calibration equations). Results are shown in Table 5.

Table 5. Recovery results for SDD-Na and TMP in the binary mixture applying the first derivative bivariate method and zero-crossing method from the derivative spectra

TMP SDD-Na			SDD-Na		
Added (µg/ml)	Bivariate method (% found)	0 crossing method (% found)	Added (µg/ml)	Bivariate method (% found)	0 crossing method (% found)
2.0	102.7	117.5	2.0	99.9	110.5
4.0	100.1	109.1	4.0	98.8	125.9
8.0	96.7	101.5	8.0	100.1	114.7
12.0	99.5	103.1	12.0	100.6	109.8
16.0	98.1	99.9	16.0	99.6	105.7
20.0	97.2	98.1	20.0	98.9	103.8
% Mean recovery	99.1	104.8	% Mean recovery	99.6	111.7
%RSD (n = 6)	2.22	6.9	%RSD (n = 6)	0.71	7.1

The results for bivariate method are satisfactory with %RSD <2.22 and % recovery value 99.1±2.22 for TMP and 99.6±0.71 for SDD-Na. For 0 crossing method %RSD <7.1 and recovery value 104.8±6.9 for TMP and 111.7±7.1 for SDD-Na. Evaluation of the method bias was carried out using statistical t-test (p = 0.05) and there is a statistically significant difference $t_{crit} > t_{stat} = 2.57$ in the proposed methods. The statistical study of the regression line showed that the determination coefficients and interceps do not differ significantly, for all analytes, from the ideal values of 1 and 0, respectively. Comparison of the obtained results shows that the bivariate procedure gave better results than classical derivative spectrophotometry.

CONCLUSIONS

The obtained results show that derivative bivariate algorithm can be applied to simultaneous determination of SDD-Na and TMP with good accuracy and precission in laboratory prepared samples. The first derivative bivariate calibibration method is simple, low cost and good accuracy spectrophotometric method for the simultaneous determination of TMP in combination with SDD-Na.

REFERENCES

1. Akay C., Özkan S.A.: Simultaneous LC determination of trimethoprim and sulphamethoxazole in pharmaceutical formulations. J. Pharm. Biomed. Anal., 30, 1207, 2002.
2. Baroni E. E., Diaz D. C., Picco E. et al.: Effects of age on the pharmacokinetics of single dose sulfamethazine after intravenous administration in cattle. Vet. Res. Commun., 32, 509, 2008.
3. Berzas Nevado J. J., Castañeda Peñalvo G., Guzmán Bernardo F. J.: Simultaneous determination of sulfamethoxypyridazine, sulfamethoxazole, sulfadimethoxine and their associated compounds by liquid chromatography. Anal. Chim. Acta, 442, 241, 2001.
4. Botsoglou N. A., Fletouris D. J.: Drug Residues in Foods: Pharmacology, Food Safety and Analysis. Marcel Dekker, New York 2001.
5. Campero A. R., Escudero B. R., Del Cisne Valle A. et al.: Bioequivalence of Two Commercial Preparations of trimethoprim/sulfamethoxazole: A randomized, single-dose, single-blind, crossover trial. Clin. Therap., 29, 326, 2007.
6. Dodd M. C., Ching-Hua H.: Aqueous chlorination of the antibacterial agent trimethoprim: Reaction kinetics and pathways. Water Research, 41, 647, 2007.
7. Furusawa N.: Simplified determining procedure for routine residue monitoring of sulphamethazine and sulphadimethoxine in milk. J. Chromatogr. A, 898, 185, 2000.
8. Gemperline P. J., JungHwan C., Baker B. et al.: Determination of multicomponent dissolution profiles of pharmaceutical products by in situ fiber-optic UV measurements. Anal. Chim. Acta, 345, 155, 1997.
9. Granero G., Garnero C., Longhi M.: Second derivative spectrophotometric determination of trimethoprime and sulfamethoxazole in the presence of hydroxypropyl-_-cyclodextrin. J. Pharm. Biomed. Anal., 29, 51, 2002.
10. Guneysel O., Onur O., Erdede M., Denizbasi A.: Trimethoprim/Sulfamethoxazole Resistance in Urinary Tract Infections. J. Emerg. Med., 36, 338, 2009.

11. Karpinska J.: Derivative spectrophotometry-recent applications and directions of developments. Talanta 64, 801, 2004.
12. Kotoucek M., Skopalová J., Michálková D.: Electroanalytical study of salazosulfapyridine and biseptol components at the mercury electrode. Anal. Chim. Acta, 353, 61, 1997.
13. López-Martínez L., Guzmán-Mar J. L., López-de-Alba P. L.: Simultaneous determination of uranium(VI) and thorium(IV) ions with carminic acid by bivariate calibration. J. Radioanal. Nucl. Chem., 247, 413, 2001.
14. López-Martínez L., Lopez-de-Alba P. L., de-Leon-Rodriguez L. M., Yepez-Murrietta L.: Simultaneous determination of binary mixtures of trimethoprim and sulfamethoxazole or sulphamethoxypyridazine by the bivariate calibration spectrophotimetric method. J. Pharm. Biomed. Anal., 30, 77, 2002.
15. Markopoulou C., Malliou E, Koundourellis J.: Chemometric and derivative methods as flexible spectrophotometric approaches for dissolution and assaying tests in multicomponent tablets. Il Farmaco, 59, 627, 2004.
16. Moreno Gálvez A., García Mateo J. V., Martínez Calatayud J.: Simultaneous dissolution profiles of two drugs, sulfadiazine-trimethoprim and amitriptyline-perphenazine, in solid oral dosage forms by a FIA manifold provided with a single spectrophotometric detector. J. Pharm. Biomed. Anal., 30, 535, 2002.
17. Ni Y., Qi Z., Kokot S.: Simultaneous ultraviolet–spectrophotometric determination of sulfonamides by multivariate calibration approaches. Chemom. Intell. Lab. Syst., 82, 241, 2006.
18. Papapanagiotou E. P., Fletouris D. J., Psomas I. E.: Sulphamethazine residue depletion study in piglets after oral administration. Anal. Chim. Acta, 529, 325, 2005.
19. Pereira A. V., Cass Q. B.: High-performance liquid chromatography method for the simultaneous determination of sulfamethoxazole and trimethoprim in bovine milk using an on-line clean-up column. J. Chromatogr. B, 826, 139, 2005.
20. Sánchez Rojas F., Bosch Ojeda C.: Recent development in derivative ultraviolet/visible absorption spectrophotometry: 2004–2008: a review. Anal. Chim. Acta, 635, 22, 2009.
21. Silva F. E. B., Ferrão M. F., Parisotto G. et al.: Simultaneous determination of sulphamethoxazole and trimethoprim in powder mixtures by attenuated total reflection-Fourier transform infrared and multivariate calibration. J. Pharm. Biomed. Anal., 49, 800, 2009.

SUMMARY

Two derivative spectrophotometric methods: the first derivative bivariate algorithm and zero-crossing method from the derivative spectra were proposed for simultaneous determination of sulfadimidine natrium (SDD-Na) and trimethoprim (TMP). Calibration model for bivariate method was constructed with the use of four linear regression calibration equations – two calibrations for each component at two wavelengths. The results obtained were compared with those from zero-crossing derivative spectrophotometry which employs one linear regression calibration equation for each component. The results for bivariate method are satisfactory with % recovery value 99.1±2.22 for TMP and 99.6±0.71 for SDD-Na. For 0 crossing method % recovery value 104.8±6.9 for TMP and 111.7±7.1 for SDD-Na. Comparison of the obtained results shows that the bivariate procedure gave better results than classical derivative spectrophotometry. The first derivative bivariate calibibration method is simple, low cost and good accuracy spectrophotometric method for the simultaneous determination of TMP in combination with SDD-Na.

STRESZCZENIE

Dwie metody z zakresu spektrofotometrii pochodnych: metoda podwójnej kalibracji oraz przejścia przez zero zostały zaproponowane do jednoczesnego oznaczenia sulfadimidyny sodowej (SDD-Na) i trimetoprimu (TMP). Do metody podwójnej kalibracji użyto czterech równań kalibracyjnych – po dwa równania dla każdej badanej substancji przy dwóch długościach fal. Wyniki porównano z otrzymanymi metodą przejścia przez zero dla spektrofotometrii pochodnych, która wymaga jednego równania kalibracyjnego dla każdego badanego związku. Uzyskane wyniki są satysfakcjonujące z odzyskiem 99,1%±2,22 dla TMP i 99,6%±0,71 dla SDD-Na. Dla metody przejścia przez zero 104,8%±6,9 dla TMP i 111,7%±7,1 dla SDD-Na. Porównanie wyników otrzymanych dwiema metodami wykazuje, że metoda podwójnej kalibracji daje lepsze rezultaty niż klasyczna metoda spektrofotometrii pochodnych. Metoda podwójnej kalibracji z użyciem pierwszej pochodnej jest prosta i tania o dużej dokładności. Można ją zastosować do oznaczania obok siebie sulfadimidyny i trimetoprimu.