Hacettepe University Journal of the Faculty of Pharmacy
Volume 29 / Number 2 / July 2009 / pp. 95-104

Spectrophotometric Determination and Stability Studies of Sulfamethoxazole and Trimethoprim in Oral Suspension by Classical Least Square Calibration Method

Received : 04.01.2010
Revised : 22.03.2010
Accepted : 30.03.2010

Şahver Ege Hişmioğulları*º, Ender Yarsan**

Introduction

Sulfamethoxazole is N^1-(5-methylisoxasole-3-il) sulfanilamide. Its closed formula $C_{10}H_{11}N_3O_3S$ and molecular weight 253.3 g/mol (Figure 1). White and yellowish white colored, crystallized powder. It does not dissolve in chloroform and ether. Its solubility in water very low, it dissolves in ethanol 1 : 50 and in acetone 1 : 30. On the other hand, it dissolves in alkaline hydroxide solutions. It is a drug which using in both systemic and urinary infections. Generally, it is combined with trimethoprim in commercial drugs [1, 2].

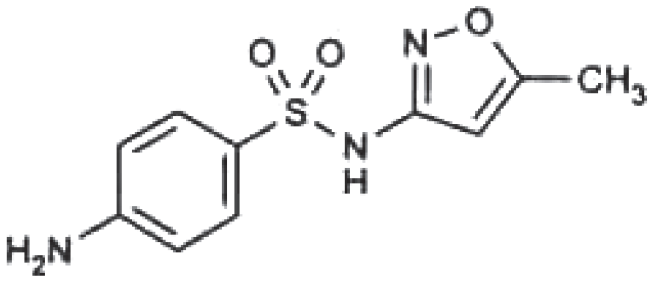

Figure 1

The chemical structure of sulfamethoxazole (3)

*º Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology Antakya, Hatay, Turkey
** Ankara University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Ankara, Turkey
*º Corresponding author: Tel +903262455845, E-mail:sahverege@yahoo.com

Trimethoprim is 5-(3, 4, 5-trimethoxybenzyl) pyrimidin-2,4-diyl-diamine. Its closed formula $C_{14}H_{18}N_4O_3$ and molecular weight 290.3 g/mol (Figure 2). White and yellowish white colored crystal or crystallized powder. It dissolves in water 1 : 2500, in ethanol 1 : 300, in chloroform 1:55 and in methanol 1:80 and it does not dissolve in ether. Trimethoprim structurally resembles in pytheridine of dihydrofolic acid and is strong inhibitor of *dihydrofolat reductase* which converted dihydrofolat into tetrahydrafolat. This situation blocks purins and finally DNA, RNA and protein synthesis. Therefore, sulfonamide and trimethoprim combination is real example of synergism and lead to the sequential inhibition of folic acid synthesis. Bacterial *dihydrofolat reductase* is susceptible to trimethoprim 20-60.000 times than mammalian enzyme. Trimethoprim has bacteriostatic effect with broad-range of *Gram positive* and *Gram negative* bacteria and generally is ineffective to anaerobes [1, 4].

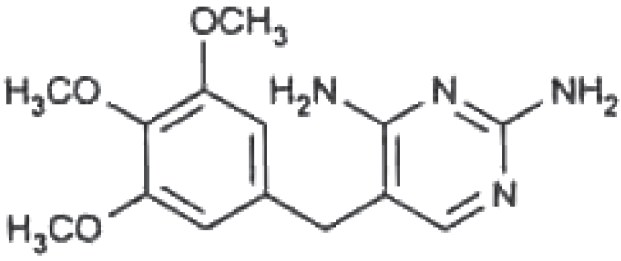

Figure 2.

The chemical structure of trimethoprim (3)

Several analytical methods including spectrophotometry [5-9], HPLC [10-12] and capillary zone electrophoresis [13] were reported for the analysis of the samples consisting of sulfamethoxazole and trimethoprim.

In the analytical studies, several methods such as derivative spectrophotometry, HPLC, PLS and PCR have been used for the quantitative analysis of multicomponent mixtures. As it is known, some disadvantages (the graphical procedure of spectra, the chromatographic condition optimization for separation, the complex calculation and abstract theory for PLS and PCR) for the above methods in the applications have been reported. For these reasons, simple numerical calibration methods instead of the complex analysis methods have been preferred for the quantitative analysis.

In this study, a simple spectrophotometric classical least square calibration was proposed and successfully applied to simultaneous analysis and stability test of the commercial veterinary formulation containing sulfamethoxazole and trimethoprim. A good coincidence was observed for the experimental results obtained by application of the proposed classical least square calibration.

Experimental section

Instruments

A Shimadzu UV-160 double beam UV-Vis spectrophotometer possessing a fixed slit width (2 nm) connected to a computer loaded with Shimadzu UVPC software and a LEXMARK E-320 printer were used to record the absorption spectra. Data analysis was performed by using the Microsoft EXCEL software.

Commercial Veterinary Formulation

A commercial veterinary product (ZOCATRİN oral suspension, Sanofi Dif İlaç A.Ş., İstanbul, Turkey) was assayed. Its declared content was as follows: 400 mg sulfamethoxazole, 80 mg trimethoprim per mL were obtained as a donation from Sanofi Dif İlaç A.Ş., İstanbul, Turkey.

Standard Solutions

Stock solution of 25 mg/50 ml sulfamethoxazole and trimethoprim were prepared in methanol-water (50:50, v/v). A standard series in the concentration range of 2-11 µg/mL sulfamethoxazole and 3-18 µg/mL trimethoprim in the same solvent were obtained from the above stock solutions. The synthetic mixtures solutions as a validation set in the above working concentration ranges.

Sample solutions preparation

In the commercial sample analysis, 1 mL of the commercial oral suspension containing sulfamethoxazole and trimethoprim was transferred into 250 mL calibrated flask and the volume was completed with methanol-water (50:50, v/v). The content of the flask was mechanically shaken for 10 min and then, clear solution was obtained. In the following step, by adding 5 mL of the buffer solution (ammonium chloride/ammonium hydroxide, pH=10) to 25 mL flask and 312.5 µL of the above sample solution was dissolved in 25 mL flask in methanol-water (50:50, v/v).

The final solution was diluted to the working concentration range for the method application.

In the preparation of the sample for the stability tests, at the beginning of study, the formulations with the same batch numbers were opened up (twice from each formulation) and their analysis were done, the control data were obtained. Following that the other formulations in their original containers were stored in different conditions (room temperature, refrigerator, etuve) for next experimental periods. Their analysis was done every 3 month during 12 months. The temperature and humidity of storage conditions were 3.9-4.1 ^{0}C and 30-32 % for refrigerator, +37 ^{0}C and 17-19 % for etuve and 20-29 ^{0}C and 30-33 % for room temperature, depends on season.

Results and Discussion

Figure 3 indicates the absorption spectra of sulfamethoxazole and trimethoprim in the concentration range between 2-11 µg/mL sulfamethoxazole and 3-18 µg/mL trimethoprim in methanol-water (50:50, v/v).

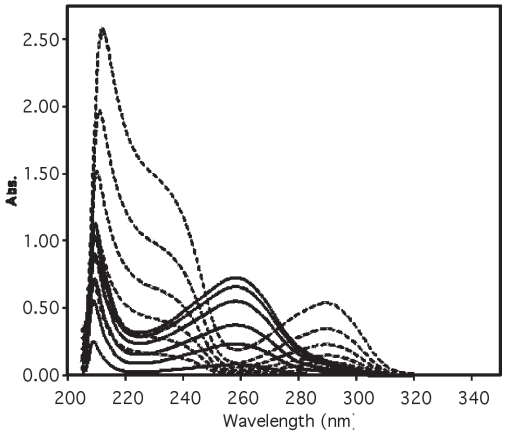

Figure 3
Absorption spectra of calibration solutions of sulfamethoxazole (2-11 µg/mL) (¾) and trimethoprim (----) (3-18 µg/mL).

As it can be seen from Figure 3, the simultaneous determination and test of veterinary drugs in the same mixture is not possible by classical spectral determination due to the overlapping spectra of sulfamethoxa-

zole and trimethoprim. In order to overcome this problem, a simple spectrophotometric classical least square method was proposed for simultaneous analysis and test of the subjected matter compounds.

Application of the Spectrophometric Classical Least Square Method

In this method, the standard series of each compound in the concentration range 2-11 µg/mL for sulfamethoxazole and 3-18 µg/mL for trimethoprim in methanol-water (50:50, v/v) were prepared. Their absorption spectra were recorded between 200-350 nm. Similar procedure was applied to the sample solutions. The absorption spectra of standard series were measured at three-wavelength set (237, 257 and 288 nm). At the above wavelength set, the absorptivity value for each compound was calculated by using the following equation:

$$\varepsilon = A / C \quad \text{(pathlength, } l\text{=1 cm)} \qquad (1)$$

where ε is the absorptivity, A represents the absorbance and C is the concentration of the compound in solution in, expressed in µg/mL. The mean absorptivity values calculated at three-wavelength set were presented in Table I.

TABLE I

The absorptivities calculated from the spectrophotometric measurements at three-wavelength set for sulfamethoxazole and trimethoprim

	λ (nm)	µg/mL	4	8	12	16	20	22	Mean absorptivities
Sulfamethoxazole	237	Abs.	0.0747	0.1395	0.2137	0.3201	0.3775	0.4001	
		Absorptivity	0.0187	0.0174	0.0178	0.0200	0.0189	0.0182	0.0185
	257	Abs.	0.1269	0.2311	0.3724	0.5504	0.6572	0.7256	
		Absorptivity	0.0317	0.0289	0.0310	0.0344	0.0329	0.0330	0.0320
	288	Abs.	0.0199	0.0382	0.0593	0.0823	0.1059	0.1132	
		Absorptivity	0.0050	0.0048	0.0049	0.0051	0.0053	0.0051	0.0050
Trimethoprim	λ (nm)	µg/mL	3	4	6	9	13	18	
	237	Abs.	0.1968	0.2647	0.3819	0.5824	0.8631	1.2003	
		Absorptivity	0.0656	0.0662	0.0637	0.0647	0.0664	0.0667	0.0655
	257	Abs.	0.0210	0.0269	0.0388	0.0601	0.0903	0.1193	
		Absorptivity	0.0070	0.0067	0.0065	0.0067	0.0069	0.0066	0.0067
	288	Abs.	0.0777	0.1039	0.1530	0.2300	0.3311	0.4612	
		Absorptivity	0.0259	0.0260	0.0255	0.0256	0.0255	0.0256	0.0257

According to the calculated absorptivities at the three-wavelength set, the spectrophotometric classical least square calibration was constructed by using the following equation:

$$\lambda_{237}\ A_{mix} = 0.0185\ C_{sulfamethoxazole} + 0.0655\ C_{Trimethoprim}$$
$$\lambda_{257}\ A_{mix} = 0.0320\ C_{sulfamethoxazole} + 0.0067\ C_{Trimethoprim} \quad (2)$$
$$\lambda_{288}\ A_{mix} = 0.0050\ C_{sulfamethoxazole} + 0.0257\ C_{Trimethoprim}$$

Method Validation

In the validation study, 12 synthetic mixtures containing sulfamethoxazole and trimethoprim in the working concentration range (in methanol-water 50:50, v/v) were prepared by using the stock solutions of sulfamethoxazole and trimethoprim (Table II). The absorption spectra of these synthetic mixtures were recorded in the spectral region 200-350 nm. The determination of sulfamethoxazole and trimethoprim in mixtures were performed by using the equation (2). The mean recovery and relative standard deviation calculated and presented in Table II.

TABLE II

Recovery results of sulfamethoxazole and trimethoprim in the synthetic mixtures by spectrophotometric classical least square calibration

Added (mg/mL)		Found (mg/mL)		Recovery (%)	
SMT	TMP	SMT	TMP	SMT	TMP
20	3	19.83	3.05	99.2	101.7
20	4	22.34	4.20	101.7	105.0
20	6	22.33	6.12	101.2	102.0
20	9	22.22	9.14	101.1	101.6
20	13	22.71	13.41	103.6	103.2
20	18	22.45	18.60	102.3	103.3
4	4	4.06	3.98	101.7	99.5
8	4	8.51	3.88	106.5	97.0
12	4	11.49	3.97	95.8	99.3
16	4	15.53	4.06	97.1	101.5
20	4	20.11	4.25	100.6	106.3
22	4	22.13	4.25	100.6	106.3
			Mean	100.9	102.2
			SD	2.78	2.81
			RSD	2.75	2.75

SD = Standard deviation, RSD = % Relative standard deviation

SMT =sulfamethoxazole, TMP =Trimethoprim

In the method validation procedure, good precision and accuracy were observed for the application of the spectrophometric classical least square calibration to the analysis of synthetic mixtures.

Analysis and stability test of commercial veterinary formulation

The study is based on the application of the proposed spectrophotometric classical least square method to the simultaneous quantitative analysis and stability test of the commercial veterinary suspensions of sulfamethoxazole and trimethoprim without a chemical separation step because of the overlapping absorption spectra of the active compounds.

Firstly, the proposed classical least square calibration based on the absorptivities at three-wavelenght set was applied to quantitative analysis of sulfamethoxazole and trimethoprim suspension as explained in the *"sample solutions preparation"* section. The experimental results were presented in Table III.

TABLE III

Experimental results of sulfamethoxazole and trimethoprim by spectrophotometric classical least square method

	mg/mL	
Sample no.	SMT	TMP
1	421.50	78.56
2	402.43	81.05
3	402.83	81.14
4	403.73	80.29
5	394.09	79.34
6	406.02	81.75
7	400.97	80.80
8	406.17	81.19
9	406.17	81.19
10	400.99	80.35
Mean	404.49	80.57
SD	6.97	0.97
RSD	1.72	1.20

SD = Standard deviation, RSD = % Relative standard deviation

SMT =Sulfamethoxazole, TMP =Trimethoprim

Secondly, the veterinary formulations were analysed each 3 months during 12 months. These formulations kept up in their original containers until analyzing procedure and stored in different conditions (room temperature, refrigerator, etuve). Standard and sample solutions preparations were done as explained above. Their absorption spectra were recorded between 200-350 nm. Similar procedure was applied to the sample solutions. The absorption various of standard series were measured at the 3-wavelength set (237, 257 and 288 nm). At the above wavelength set the absorptivity various for each compound were calculated by using the formula $\varepsilon = A$ (absorbance) / C (concentration, µg/mL).

The shelf life of veterinary formulation is declared as 24 months. In USP, there are criteria for oral suspension of sulfamethoxazole and trimethoprim. According to USP, the claimed label quantities of sulfamethoxazole and trimethoprim in veterinary formulation should not be less than 90 % and should not be exceed 110 %. At the end of study, the degradation in the active compounds of veterinary formulation during 12 months was compatible with USP [13].

Conclusion

In spite of overlapping spectra of sulfamethoxazole and trimethoprim in the same spectral range 200-350 nm, the spectrophometric classical least square method was successfully applied for simultaneous analysis and test of the above active compounds in samples. This method is very easy to use and easy to apply and besides very cheap to quality control and routine analysis of two active veterinary compounds in commercial samples.

Summary

Quantitative determination and stability test of sulfamethoxazole and trimethoprim in oral suspension were carried out by spectrophometric classical least square calibration method. Stability test of the related veterinary drugs was performed by using the following conditions: Room temperature, refrigerator and etuve (37 ^{0}C) for each 3 month during 12 months. For the spectrophotometric analysis, the standard series of sulfamethoxazole and trimethoprim was prepared in the concentration range of 2.0-11.0 µg/mL and 3.0-18.0 µg/mL respectively. The absorbances of standard series were measured at three-wavelength set (237, 257 and 288 nm). A classical least square calibration was obtained by using the absorptivities at three-wavelength set. The validity of the spectral method was done by analyzing the synthetic binary mixtures. Af-

terwards the method was successfully applied to simultaneous analysis and stability test of the commercial veterinary formulation containing sulfamethoxazole and trimethoprim.

Keywords: Spectral classical least square, Simultaneous determination, Stability test, Sulfamethoxazole, Trimethoprim, Chemometry

Özet

Oral Süspansiyondaki Sülfametoksazol ve Trimetoprimin Klasik Küçük Kare Kalibrasyon Metodu ile Spektrofotometrik Tayinleri ve Stabilite Çalışmaları

Oral süspansiyondaki sülfametoksazol ve trimetoprimin kantitatif tayini ve stabilite testi, spektrofotometrik klasik küçük kare kalibrasyon metodu ile çalışıldı. Sözkonusu veteriner ilaçlarının stabilite testleri, şu koşullarda gerçekleştirildi : Bir yıl süresince, oda ısısı, buzdolabı ve etüvde (37 ^{0}C) bekletilen ilaçların, her üç ayda bir, analizleri yapıldı. Spektrofotometrik analiz için sülfametoksazol ve trimetoprimin, sırasıyla, 2.0-11.0 µg/mL ve 3.0-18.0 µg/mL konsantrasyon aralığındaki standart serileri hazırlandı. Standart serilerin absorbansları, üç ayrı dalga boyunda ölçüldü (237, 257 ve 288 nm). Bir klasik küçük kare kalibrasyonu, üç ayrı dalga boyunda absorbtiviteler ölçülerek elde edildi. Spektral metodun validasyonu, sentetik ikili karışımlar analiz edilerek yapıldı. Ardından da metot, sülfametoksazol ve trimetoprim içeren ticari veteriner formülasyonun eş zamanlı (simultane) analizi ve stabilite testine başarıyla uygulandı.

Anahtar kelimeler: Spektral klasik küçük kare, Simultane tayin, Stabilite testi, Sülfametoksazol, Trimetoprim, Kemometri

REFERENCES

1. Barragry, T.B. : "Chapter 12 : "Sulfonamides" in Veterinary Drug Therapy, Lea & Febiger, Philadelphia, Baltimore, Hong Kong, London, Munich, Sydney, Tokyo (1994), sayfa 297-301.
2. Budavari, S., O'Neil, M.J., Smith, A., Heckelman, P.E., Kinneary, JF. : The Merck Index. An Encyclopedia of Chemicals, Drugs and Biologicals. 12th ed., Merck Research Laboratories, Division of Merck & Co., Inc. Whitehouse Station, NJ (1996).
3. United States Pharmacopoiea USP 23, NF 18. United States Pharmacopoeial Convention Inc., Rockville (1995).
4. Bishop, Y.M. : The Veterinary Formulary, Handbook of Veterinary Medicines used in Veterinary Practice, 3rd ed., Bishop, Y.M. (Ed.), Royal Pharmaceutical Society of Great Britain and British Veterinary Association, London (1996), page 113.

5. Dinç, E., Baleanu, D., Kadıoğlu, Y., Kadıoğlu, E., Demirkaya, F. : New approach for simultaneous spectral analysis of a complex mixture using the fractional wavelet transform, Communications in Nonlinear Science and Numerical Simulation, 15 (4) : 812-818 (2010).
6. Markopoulou, C.K., Malliou, E.T., Koundourellis, J.E. : Chemometric and derivative methods as flexible spectrophotometric approaches for dissolution and assaying tests in multicomponent tablets, Farmaco, 59 (8) : 627-636 (2004).
7. Granero, G., Garnero, C., Longhi, M. : Second derivative spectrophotometric determination of trimethoprime and sulfamethoxazole in the presence of hydroxypropyl-Ð-cyclodextrine (HP-Ð-CD), J Pharm Biomed Anal, 29 (1-2) : 51-59 (2002).
8. Lopez-Martinez, L., Lopez-de-Alba, P.L., Manuel de-Leon-Rodriguez, L., Yepez Murrieta, M.L. : Simultaneous determination of binary mixtures of trimethoprim and sulfametoxasole or sulphamethoxypyridazine by the bivariate calibration spectrophotometric method, J Pharm Biomed Anal 30 (1) : 77-85 (2002).
9. Ribone, M.E., Pagani, A.P., Olivieri, A.C. : Determination of the minor component bromhexine in cotrimoxazole-containing tablets by absorbtion spectrophotometry and partial least-square (PLS-1) multivariate calibration, J Pharm Biomed Anal, 23 (2-3) : 591-595 (2000).
10. Dinç, E., Bilgili, A., Hanedan, B. : Simultaneous determination determination of trimethoprim and sulphamethoxasole in veterinary formulations by chromatographic multivariate methods, Pharmazie 62 (3) : 179-184 (2007).
11. Akay C., Özkan S.A. : Simultaneous LC determination of trimethoprim and sulfamethoxazole in pharmaceutical formulations, J Pharm Biomed Anal, 30 (4) : 1207-1213 (2002).
12. Berzas Nevado, J.J., Castaneda Penalvo, G., Guzman Bernardo, F.J. : Simultaneous determination of sulfamethoxypyridazyne, sulfamethoxazole, sulfadimetoxine and their associated compounds by liquid chromatography, Anal Chim Acta, 442 (2) : 241-248 (2001).
13. Berzas Nevado, J.J., Castaneda Penalvo, G., Guzman Bernardo, F.J. : Determination of sulfamethoxasole, sulfadiazine, and associated compounds in pharmaceutical preparations by capillary zone electrophoresis, J Chromatogr A, 918 (1) : 205-210 (2001).