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QUALITY CONTROL OF POTENTIATED SULFONAMIDE COMMERCIAL VETERINARY FORMULATIONS BY UV-SPECTROPHOTOMETRY

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ABSTRACT

Combinations of a sulfamethoxazole with trimethoprim in a fixed ratio in commercial preparations, very often are used for chemotherapeutic practice in veterinary medicine. As well as in human medicine, veterinary formulations with this combination of active drugs ingredients, which are commonly termed "potentiated sulfonamide", must be of good quality, safe and effective.

For a reliable quality control of the active compound's content it is essential to apply selective, accurate and precise analytical procedures. Liquid chromatographic and spectrophotometric methods that include specific sample preparation are the most frequently utilized. In this investigation, a simple ultraviolet-visible (UV-Vis) spectrophometric method has been developed for determination of sulfamethoxazole/trimethoprim content.

The method has excellent linearity in the concentration range 1–30 μg/mL. Correlation coefficient ranges from 0.9997 to 0.9999, going from classical to derivatives UV spectroscopy. Statistical analysis confirmed that the method has satisfactory accuracy and precision according to the Horwitz criterion, where RSD < 4.00 % for commercial veterinary medicinal product Hemosul-P (oral powder) and RSD < 3.40 % for Hemosul-S (inj. sol.). Applying the suggested procedure, it was possible to perform selective analysis for SMX in the pharmaceutical preparations without removing the excipients. Since there is no need for sample preparation and sophisticated apparatus, the described method presents rather inexpensive procedure for quantitative determination of SMX in these veterinary medicinal products (VMPs). The obtained results are in a good agreement with the declared contents.

The proposed UV method is suitable to be utilized for quality control of these VMPs in terms of assuring proper and effective drug administration. Finally, this results in presence of safe levels of drug residues in the food of animal origin.

Key words: potentiated sulfonamide, sulfamethoxazole/trimethoprim, UV spectrophotometry, veterinary medicinal products

INTRODUCTION

In the practice of veterinary medicine several separate combinations of a sulfonamide with trimethoprim in a fixed ratio (5 : 1) are used clinically. These combinations are commonly termed "potentiated sulfonamide antimicrobial agents". Examples of such potentiated sulfonamide preparations include sulfamethoxazole/trimethoprim (co-trimoxazole), sulfadiazine/trimethoprim (co-trimazine) and sulfadoxine/trimethoprim (co-trimoxine). Potentiated sulfonamides have th e desirable property of reducing, by several folds, the minimum inhibitory concentration (MIC) of both the sulfonamide and the trimethoprim against a wide range of pathogenic organisms. Lowered MICs needed to control infections result in small doses of drugs used in each animal and thereby a reduction in the total dose of drug administered to the animal [1].

Veterinary drugs Hemosul-S (inj. sol.) and Hemosul-P (oral powder) are sulfamethoxazole/trimethoprim products, in parenteral and oral dosage forms, approved for use in veterinary practice of domestic animals in the Republic of Macedonia.

In veterinary medicine, as well as in human medicine, drugs must be of good quality, safe and effective. For a reliable quality control of the active compound's content it is essential to apply selective, accurate and precise analytical procedures. Potentiated sulfonamide combinations, like sulfamethoxazole/trimethoprim, are analyzed by a variety of chromatographic and conventional methods.

From an analytical point of view, methods for drugs analysis in commercial preparations are considerably less complex than methods for analysis of drugs and their metabolites in biological samples as blood, plasma, hair or urine. However, the unequivocal determination of a drug in veterinary formulations is as important as determination in complex matrices, because the veterinary product quality is directly related to safe levels of residues in food of animal origin.

Several UV/VIS spectrophotometric methods have been widely developed to quantify active drugs ingredients. As most active components possess chromophore groups, they can be determined directly in the ultraviolet region without the need for a derivatization reaction.

Multicomponent derivative spectroscopic method at 280 and 294 nm was employed for determination of the two substances in mixture [2]. 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 has been published [3]. By the suggested procedure, it was possible to analyze SMX and TMP in pharmaceutical preparations without separating from each other or from the excipients. Two methods, namely first derivative and classical last squares methods were selected and applied for comparative purposes to analyze UV-spectra of the methanol solutions of the SMX and TMP in synthetic binary mixtures and in number of antibacterial pharmaceutical preparations produced by Egyptian companies [4].

The formulation of Sulfamethoxazole and Trimethoprim in a combination mixture is very good pharmacologically since it enhances the efficacy of the individual drugs. However in these combination difficulties in analysis on ordinary UV spectrophotometry are introduced because the two components give overlapping spectral bands on zero-order. The method is rapid, simple and can be applied successfully to assay a mixture of the two drugs in pharmaceutical preparations [5].

A micellar electrokinetic capillary chromatography was performed for the determination of sulfamethoxazole and trimethoprim. Recoveries were optimal and acceptable after extraction with ethanol/deionized water for both investigated compounds from laboratory mixtures of standards. The method was applied to determine sulfamethoxazole and trimethoprim in tablets, powder and solution for infusion [6].

A rapid, reliable and sensitive UV-spectrophotometric method has been developed for the determination of the Sulphamethoxazole. The developed method was employed in *in-vitro* protein binding studies, the drug shows 50-60 % binding and it was good agreement with reported pharmacokinetic data and drug release kinetics [7].

A rapid, simple and sensitive spectrophotometric method for the determination of some sulfa drugs is described. The method is based on the formation of orange yellow colored azo product by the diazotization of sulfonamides: dapsone (DAP), sulfathiazole (SFT), sulfadiazine (SFD), sulfacetamide (SFA), sulfamethoxazole (SFMx), sulfamerazine (SFMr), sulfaguanidine (SFG) and sulfadimidine (SFDd) followed by a coupling reaction with 3-aminophenol in aqueous medium. The method is successfully employed for the determination of sulfonamides in various pharmaceutical preparations and common excipients used as additives in pharmaceuticals do not interfere in the proposed method [8].

The common availability of the instrumentation, the simplicity of procedures, economy, speed, precision and accuracy of the technique still make spectrophotometric methods attractive. So, in this present investigation, an attempt has been made to develop accurate, precise, environmental friendly and economically UV spectrophotometry method for the estimation of sulfamethoxazole in different dosage form.

MATERIALS AND METHODS

Standard stock solutions

Sulfamethoxazole (SMX) was purchased from Sigma-Aldrich. Standard stock solutions (1 mg mL^{-1}) were prepared by weighing 50 mg of SMX and by dissolving in 50 mL 96 % ethanol (Riedel-de Haën). Applying suitable dilutions, spiking solution for veterinary formulation samples with concentration of 100 µg mL^{-1} and calibration standards for the UV method in the concentration range of 1–30 µg mL^{-1} (1, 2, 5, 8, 10, 15, 20 and 30 µg mL^{-1}) were prepared using 10 % ethanol and acetate buffer (pH 4.8).

Commercial veterinary formulation

A commercial veterinary product Hemosul-P (oral powder) and Hemosul-S (inj. solution) from producer "Veterinarski zavod Subotica" was assayed. It's declared content was as follows: 100 mg sulfamethoxazole and 20 mg trimethoprim in 1 g powder of Hemosul-P; 200 mg sulfamethoxazole and 40 mg trimethoprim in 1 mL solution of Hemosul-S.

Sample solutions preparation

For the commercial sample analysis, an adequate mass portion (equal to concentration of 100 µg mL^{-1} SMX) of Hemosul-P (or volume of Hemosul-S) was transferred into 100 mL flask, 2 ml buffer solution (pH=4.8) and 10 % ethanol were added and the sample was sonificated in ultrasonic bath for 30 minutes. The flask was filled to the mark with 10 % ethanol. In the following step, an aliquot of the filtrate was transferred into 10 mL flask, then 2 ml buffer solution (pH=4.8) were added and the volume was filled up with 10 % ethanol, mechanically shaken and filtrated through white Whatman filter paper.

Spectrophotometric measurements and Data analysis

The UV spectra of the standard and sample solutions, and appropriate blanks were recorded on a Varian Carry 50 UV/Visible Spectrophotometer, at room temperature in 1 cm quartz cell, in the wavelength range from 190 to 350 nm, with resolution 0.5 nm and scan rate of 300 nm/min. Varian Carry software was used for the calculated derivative spectra and Microsoft Office Excel for determination of statistical parameters.

RESULTS AND DISCUSSION

The spectra of the standard solutions of SMX (Figure 1) are characterized by two absorption band, one at lower wavelengths, with a maximum of absorbance that exhibits bathochromic shift by increasing concentration of SMX (from 206 nm to 212 nm). Therefore this absorption band is not suitable for quantitative determination. On the other hand, the spectral analysis clearly indicates that the absorbance band at the higher wavelength (268 nm) is more suitable for quantitation.

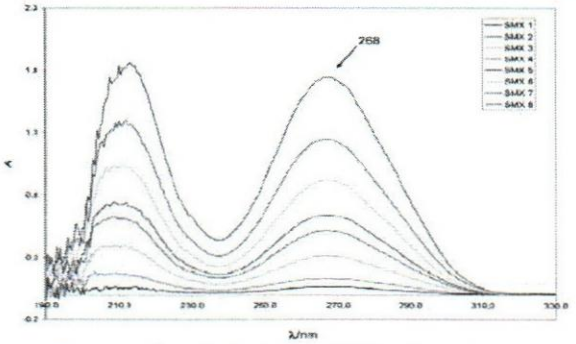

Figure 1. UV spectra of standard solutions of sulfamethoxazole (1–30 μg mL^{-1})

With aim to eliminate the possible interferences of the excipients, and improvement of the method precision, the first-order derivative spectra are obtained (Figure 2).

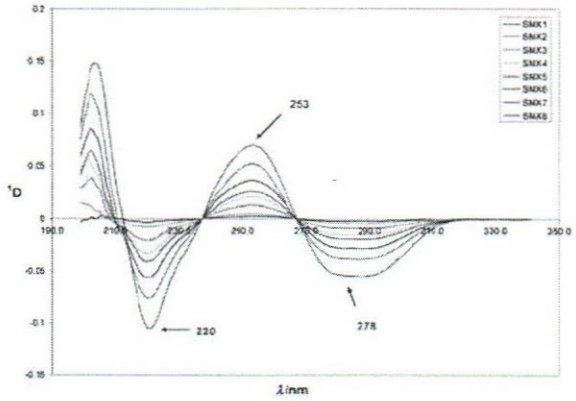

Figure 2. First-order derivative spectra of standard solution of SMX (1–30 μg mL^{-1})

The spectral analysis from first-order derivative spectra indicate that the obtained derivative signals at 220 nm, 253 nm and 288 nm are sufficiently well-defined for quantitative determination of the SMX. Under the optimum conditions described above, the linear correlation was obtained between the concentrations of SMX in the tested range and their corresponding absorbances at 268 nm, as well as and for the derivative signals of three selected wavelengths (Table 2). The equations of the calibration curves, correlation coefficients (R), number of data (n) and standard errors of estimate ($SE_{y,x}$) are listed in Table 1.

Table. 1. Equation of calibration curves for zero-order and first-order spectroscopy at different wavelengths

Signal*	y=ax+b #	$SE_{y,x}$	R	n	γ(SMX)/ μg mL^{-1}
A_{268}	y = 0.0586x + 0.0281	0.0295	0.9997	8	1 - 30
$^1D_{220}$	y = 0.0035x + 0.0033	0.0031	0.9985	8	1 - 30
$^1D_{255}$	y = 0.0024x + 0.0010	0.0020	0.9990	8	1 - 30
$^1D_{278}$	y = 0.0017x + 0.0007	0.0007	0.9999	8	1 - 30

**A – absorbance from UV spectra, ^1D – derivative signals from first derivative spectra*

 # a – slope, b – intercept, y – A or ^1D, x – mass concentration = γ(SMX)

From the data in the Table it may be clearly seen that the correlation coefficient for the calibration equation calculated from the $^1D_{278}$ signals is the highest. The standard error of the estimate is the lowest, indicating that the accuracy of prediction is highest.

Accuracy testing

To test the method suitability in terms of accuracy and precision, recovery experiments were conducted by adding known quantities of standard solution of SMX (5, 10 and 15 $\mu g mL^{-1}$) to the different sample formulations of SMX, and then the mixtures were analyzed by the proposed method. Figure 3 presents the spectra of solutions of Hemosul-P spiked with a standard solution of SMX. The results from the recovery experiment are shown in Table 2. The obtained satisfactory values for the recoveries (95–105 %), except for those obtained from derivative signal $^1D_{220}$, indicate that the method is accurate.

The same procedure was performed for Hemosul-S and the corresponding results are given in Table 3. In this case, the values for recovery are quite variable, probably due to insufficient homogeneity of Hemosul-S as a matrix. It may be concluded that the obtained values from the signals 1D220 and 1D253 are unacceptable.

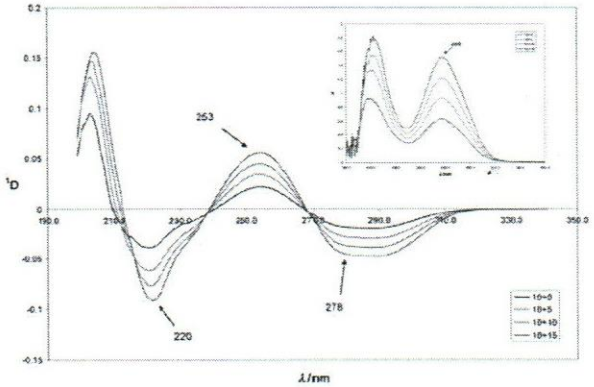

Figure 3. First-order (and zero-order in small picture) derivative spectra of spiking solution (sample of Hemosul-P containing 10 $\mu g mL^{-1}$ SMX + 0, 5, 10 and 15 $\mu g mL^{-1}$ of standard solution SMX)

Method Repeatability

The precision for the proposed methods were investigated by intra-day determination of six replicates [9] at working concentrations of 15 $\mu g mL^{-1}$ SMX. The intra-day precisions are expressed as relative standard deviation and the data presented in Table 2 for Hemosul-P and Table 3 for Hemosul-S, respectively.

The obtained values for RSDs are compared with the maximal theoretical values (Horwitz criterion) calculated according to the declared content of SMX in both veterinary drugs.

Table 2. Analytical Validation Parameters for Hemosul-P

	A_{268}	$^1D_{220}$	$^1D_{233}$	$^1D_{278}$
Accuracy				
Recovery/%	100.82-101.45	93.53-110.60	98.80-103.59	96.14-96.23
Repeatability				
m(SMX) / mg	107.27	108.69	89.18	107.02
n	6	6	6	6
SD / mg	2.09	4.48	2.15	1.78
RSD / %	1.94	4.12	2.41	1.66
Horwitz criterion RSD ≤ 4.00 %				

The results for repeatability are quite expected, from the perspective of the previous test accuracy. For Hemosul-P, the results for the content of sulfamethoxazole obtained from the derivative signal $^1D_{220}$ are unsatisfactory according to the Horwitz criteria (4.00 % in case of Hamosul-P). The best results with lowest RSD were obtained by absorbance at 268 nm and the derivative signal at 278 nm.

In Hemosul-C, problems with insufficient homogeneity of the samples came to full expression. As a result, any RSD value, except that the $^1D_{278}$, failed Horvitz criterion, which in this case is lower (3.40 %) because of higher content of SMX in Hemosul-S, compared with the same in Hemosul-P.

Table 3. Analytical Validation Parameters for Hemosul-S

	A_{268}	$^1D_{220}$	$^1D_{253}$	$^1D_{278}$
Accuracy				
Recovery/%	96.62-103.48	66.77-103.59	84.83-88.08	87.51-93.77
Repeatability				
m(SMX) / mg	171.82	247.67	176.04	200.27
n	6	6	6	6
SD / mg	7.30	28.40	7.81	4.88
RSD / %	4.25	11.47	4.44	2.44
Horwitz criterion RSD ≤ 3.40 %				

Generally, it can be concluded that the best results are obtained from derivative signal $^1D_{278}$. This was expected and logical and is strongly related to previous conclusions for the calibration curves on different wavelengths (Table 1). Accordingly, it is confirmed that the possible effects of matrix or excipients are eliminated by application of first-order derivative spectroscopy *vs.* the classical (zero-order) spectroscopy.

CONCLUSION

The proposed UV method is suitable for quantitative analysis of commercial veterinary formulation Hemosul-P and Hemosul-S. Statistical analysis showed the method is accurate and precise. There was no interference from excipients in the tablets. Since there is no need for sample preparation or sophisticated apparatus, the proposed methods may be used successfully applied for quantitative determination of sulfamethoxazole, for performing the official quality control. The results obtained for the analyzed compound are in a very good agreement with the declared contents.

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КОНТРОЛА НА КВАЛИТЕТ НА ПОТЕНЦИРАНИ СУЛФОНАМИДНИ КОМЕРЦИЈАЛНИ ВЕТЕРИНАРНИ ФОРМУЛАЦИИ СО УВ-СПЕКТРОФОТОМЕТРИЈА

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АПСТРАКТ

Комбинацијата на сулфометоксазол и триметоприм во фиксен однос во различни комерцијални препарати, многу често се користи во хемотерапевската пракса во ветеринарната медицина. Како во хуманата, така и во ветеринарната медицина овие формулации на активни компоненти најчесто се нарекуваат "потенцирани сулфонамиди" кои мораат да бидат со добар квалитет, безбедни и ефикасни.

За сигурна контрола на активната компонента, од суштинско значење е применување на селективни, точни и прецизни аналитички постапки. Најчесто користени методи, кои вклучуваат специфична подготовка на примерокот, се течна хроматографија и спектрофотометрија. Во ова истражување развиен е едноставен УВ-ВИС спектрофотометриски метод за определување на содржината на сулфометоксазол во присуство на триметоприм.

Овој метод има одлична линеарност во концентрацискиот опсег од 1-30μg/mL. Коефициентот на корелација се движи од 0.9997 до 0.9999, одејќи од класична кон деривативна УВ спектроскопија. Со статистичката анализа утврдено е дека овој метод има задоволителна точност и прецизност во согласност со критериум на Horwitz, каде RSD<4,00% за комерцијални ветеринарни медицински производи Hemosul-P (прашок за орална примена) и RSD<3,40% за Hemosul-S (инјекција). Со примена на предложената постапка, можно е да се изврши селективна анализа на сулфометоксазол во фармацевските препарати, без отстранување на екципиенси. Бидејќи не постои потреба за подготовка на пробата и за софистицирани апарати, опишаниот метод претставува прилично евтина постапка за квантитативно определување на сулфометоксазолот во овие ветеринарни медицински производи. Добиените резултати се во согласност со декларираната содржина.

Предложениот УВ метод е погоден за користење на контрола на квалитетот на овие ветеринарни медицински производи во смисла на обезбедување на соодветна и ефикасна администрација на лекарства. На крајот, ова резултира со присуство на безбедно ниво на резидуи од лековите во храната од животинско потекло.

Клучни зборови: потенцирани сулфонамиди, сулфометоксазол/триметоприм, УВ спектрофотометрија, ветеринарни медицински производи