Simultaneous Quantification of Trimethoprim and Sulphadiazine in Pharmaceutical Preparations by Derivative Ultraviolet Spectroscopy

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Received on July 28, 2004

Abstract

The application of derivative UV-spectrophotometry for simultaneous determination of sulphadiazine (SDZ) and trimethoprim (TMP) in their binary mixtures is presented. The determination of SDZ and TMP was made using the first-order derivative (Δλ = 0.5 nm) at 259 and 261.5 nm, respectively. The values obtained linearly related to the concentration of SDZ and TMP and have relative standard deviations of 1.07 and 0.53 %, respectively. The quantification was carried out in 0.001 mol l⁻¹ methanolic potassium hydroxide. The calibration graphs are linear up to 60 μg ml⁻¹ of TMP and up to 25 μg ml⁻¹ of SDZ in the presence of each others (r > 0.997). The limit of detection was about 1 μg ml⁻¹ of SDZ and about 0.5 μg ml⁻¹ of TMP. The method is cheap, time saving and successfully useful to determine analytes in commercial TMP/SDZ pharmaceuticals. The results obtained were compared with the results of HPLC. It was found that the results obtained from the use of derivative spectrophotometry compare favorably with those obtained with HPLC.

Keywords: trimethoprim, sulphadiazine, derivative spectrophotometry, HPLC, simultaneous determination.

Introduction

Sulphadiazine (SDZ) - trimethoprim (TMP) mixture is widely used in the treatment and prevention of various infectious diseases in poultry [1]. SDZ is mostly mixed with TMP in a ratio of 5:1; usually each 100 g of this premix contains 400 mg SDZ and 80 mg TMP [2].

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Sulfadiazine and trimethoprim have been determined simultaneously by liquid chromatography with ultraviolet detection [3-6] and Liquid chromatography with mass spectrometric detection [5-6].

It has been shown that derivative spectrophotometry is a useful analytical technique for the enhancement of the resolution of overlapping peaks, and the elimination of background or reduction of matrix effect. [7-9]. Several applications of derivative spectrophotometry have been described for determination of pharmaceutical products in binary and ternary mixtures [10-17].

Sulfadiazine and trimethoprim have overlapping absorbance spectra, therefore it is difficult to use peaks for reliable direct absorbance measurements. The aim of this work is to demonstrate the ease with which the derivative method circumvents this problem of overlapping spectral bands, so allowing the simultaneous determination of these drugs without prior separation.

In this study, a SDZ/TMP mixture is analyzed simultaneously by UV-derivative spectrophotometry. The mixture was also analyzed by high performance liquid chromatography (HPLC) and the results obtained by the two methods were comparable.

Experimental

A. Spectroscopy

Instrumentation: The instrument used was a Unicam UV/VIS, model UV2, double-beam spectrophotometer. It was operated under the following conditions: wavelength range of scan: 200-325 nm; mode: first-derivative; scan rate: 120 nm/min; and wavelength interval: 0.5 nm. The spectra of test and standard solutions were recorded in 1 cm quartz cells. This instrument has also a build-in software capability that permits derivative (D1-D4) calculations. The instrument is equipped with a built-in hard disk and a floppy disk drive for bulk data storage. The scan spectra can be stored and digitized by the use of software program supplied with the spectrophotometer.

Reagents: Analytical grade reagent chemicals and distilled water were used to prepare all solutions. Pure sulfadiazine and pure trimethoprim were supplied by AVICO (Arab Veterinary Industrial Corporation), Amman, Jordan and VAPCO (Veterinary and Agricultural Products Corporation), Amman, Jordan.

Samples: All drugs analyzed; Vapcotrim (VAPCO, each gm contains 400 mg SDZ and 80 mg TMP), Coliprim (AVICO, each ml contains 200 mg SDZ and 40 mg TMP), and Norodine (NORBROOK, each ml contains 200 mg SDZ and 40 mg TMP) were purchased from the market.

Sample Preparation and Procedure:
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Stock solutions of SDZ and TMP (100 µg ml\(^{-1}\) of each) were prepared in methanolic potassium hydroxide such that a 100 µg of each drug was weighed into a 100 ml volumetric flask, and 50 ml of 0.001 mol l\(^{-1}\) methanolic potassium hydroxide added. The flasks were shaken and filled to the mark with the same solution. Working solutions in the range of 2-25 µg ml\(^{-1}\) SDZ and 4-60 µg ml\(^{-1}\) TMP were prepared by dilution with the same solvent to construct the calibration graphs. Several mixtures of SDZ and TMP standards in the respective ranges were also prepared in the same solvent. Stock solutions of the drug samples were also prepared in methanolic potassium hydroxide, and then the solutions were filtered through a 0.45 µm filter to get rid of insoluble additives. Several concentrations of each drug were prepared by withdrawing calculated volumes of the drug solutions and diluting them by the same solution. The zero and first derivative spectra were recorded for drug solutions and binary mixtures from 220 to 320 nm against the methanolic potassium hydroxide solution. The absolute values of the first derivative were measured at 259 nm and 261.5 nm for the determination of SDZ and TMP, respectively.

B. Liquid Chromatography

Instrumentation: The high performance liquid chromatograph used was TSP- P1000 unit equipped with UV-VIS lamp TSP-UV1000; Altec-210A manual injector with 20 µl sample loop; Chromo-Quest computing integrator software.

Reagents: Acetonitrile (CH\(_3\)CN), Tetrahydrofuran (THF), Doubly distilled water (DDW), all are HPLC grades, Formic acid (85%), Triethylamine (TEA), analytical grade. SDZ, TMP both provided by AVICO and VAPCO, Amman, Jordan

Chromatographic conditions: flow rate: 1.0 ml/min, wavelength: 254 nm, Pressure: 2.00 Kpsi, column oven temperature 35 °C, Chromatographic column: Hyprsil C-18 silica based column; particle size: 5 µm; stainless steel (250 mm long x 4.6 mm i.d.), run time 10 min.

Mobile phase: CH\(_3\)CN : THF : H\(_2\)O : TEA : Formic acid (4 : 3 : 92.2 : 0.3 : 0.5).

Preparation of standard solution:

SDZ standard stock solution: Accurately weigh 0.0395 - 0.0405 g SDZ (USP Convention) into 100 ml volumetric flask; dissolve and dilute to volume using water.

TMP standard stock solution: Accurately weigh 0.0795-0.0805 g TMP (USP Convention) into 100 ml volumetric flask; dissolve and dilute to volume using mobile phase.

Mixed working standard stock solution: Take 10.0 ml from SDZ standard stock solution above and 10.0 ml from TMP standard stock solution dilute to 100 ml using mobile phase to get final concentration of 40 and 80 µg/ml SDZ and TMP.

Preparation of sample for injection:
TMP sample solution: Take 2 ml finished product and dilute to a total of 100 ml using distilled water, sonicate until complete dissolution; from this solution take 10 ml and dilute to a total of 100 ml using mobile phase, (final concentration is 80 μg/ml TMP).

SDZ sample solution: Take 2 ml finished product and dilute to a total of 100 ml using water, sonicate until complete dissolution; from this solution take 1 ml and dilute to a total of 100 ml using mobile phase, (final concentration is 40 μg/ml SDZ).

The whole analysis was monitored using two working standard preparation; first standard was injected 6 times while the other standard injected twice; the standard deviation for the six injections were less than 1% that proves that the instrument was stable over the analysis time, while the difference between the two standards was less than 2% that indicates that there was no difference in the two standards prepared; i.e., no significant personal errors such as errors that might occur in weighing. Peak areas were used for quantification based on the external standard method. Reported numbers are average of four replicates and each replicate was injected twice.

Results and Discussion

The absorption spectra of SDZ, TMP as well as a mixture of both (40 μg ml SDZ: 20 μg ml TMP) in the 250-310 nm wavelength range are shown in Fig. 1. As can be seen from Fig. 1, the spectrum of the mixture is the sum of the two spectra. Both spectra are partially overlapped, and the presence of one prevents the accurate determination of the other.

To separate the spectra, a derivatization procedure was applied for simultaneous determination of the two drugs in the mixture. Fig. 2. shows the first derivative absorption spectra of SDZ and TMP and their mixture. Sulphadiazine exhibits characteristic signals at 259 nm and 289.5 nm, where TMP display zero D1 values. Accordingly, the concentration of SDZ may be determined at λ = 289.5 or at 251.5 nm in the presence of TMP without interferences. Meanwhile, TMP may be estimated through D1 measurement at 261.5 nm or at 251.5 nm at which SDZ gives zero D1 values. In this study, the wavelengths 259 and 261.5 nm were chosen to determine SDZ and TMP respectively.

Fig. 3 shows a series of first derivative spectra obtained for mixtures containing 10 μg ml⁻¹ TMP and variable SDZ concentrations over the range 2-25 μg ml⁻¹. As can be seen, the height at 259 nm (h1) is proportional to the amount of SDZ present in the mixture and there is an isobestic point at 261.5 nm corresponding to the zero crossing of the pure TMP solution. Similarly, Fig. 4. shows series of first derivative spectra obtained for mixtures containing 20 μg ml⁻¹ SDZ and variable TMP concentrations over the range 4-60 μg ml⁻¹. As can be see the height at 261.5 nm (h2) is proportional to the amount of TMP present in the mixture and there is an isobestic point at 259 nm corresponding to the zero crossing of the pure SDZ solution. The measured D1 values for TMP and SDZ at the selected wavelengths are linearly correlated to the concentrations over the range of 2-25 μg ml⁻¹ and 4-60 μg ml⁻¹ for SDZ and TMP, respectively.

Figs. 5 and 6 show the effect of acidity on the absorption spectra of SDZ and TMP, respectively. Fig. 5 shows the spectra of SDZ in methanol containing various
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concentrations of hydrochloric acid and potassium hydroxide (0.001, 0.01, 0.1 and 1 mol l\(^{-1}\)). Fig. 6 shows the spectra of TMP in methanol containing various concentrations of hydrochloric acid and potassium hydroxide (0.001, 0.01, 0.1 and 1 mol l\(^{-1}\)). From these two figures, we notice the significant effect of the acidity on the characteristics of the absorption spectra for SDZ and TMP. For example, Fig. 5 shows that there is a significant shift in the absorption spectrum of SDZ and a significant decrease in the amplitude upon dissolving the SDZ drug in 0.01 mol l\(^{-1}\) HCl instead of dissolving it in 0.001 mol l\(^{-1}\) HCl. Fig. 6 also shows a significant decrease in the amplitude in the spectrum of TMP in 0.1 mol l\(^{-1}\) HCl instead of dissolving it in 0.001 mol l\(^{-1}\) HCl. However, the difference in the spectra of each drug is insignificant when methanolic potassium hydroxide solutions are used, especially for TMP. Because of the dependence of the spectra of the two drugs on the acidity, and because of the small changes in the spectra in the methanolic potassium hydroxide solutions, the same batch of methanolic potassium hydroxide was used for the preparation of all solutions involved, in order to ensure maximum reliability of the results.

Four calibration graphs were constructed from first derivative signals for standards containing between 4 to 60 µg ml\(^{-1}\) of TMP in the presence of 2, 10, 15 and 20 µg ml\(^{-1}\) of SDZ. Similarly, four calibration graphs were prepared for standards containing between 2-25 µg ml\(^{-1}\) of SDZ in the presence of 2, 10, 15 and 20 µg ml\(^{-1}\) of TMP.

The slope, intercept, correlation coefficient and standard deviations obtained are summarized in table 1. The linearity of the calibration graphs and the adherence of the system to Beer's law are validated by the high value correlation coefficient of the regression equation. From table 1, we can see that the slopes of the calibration graphs for TMP are independent of the SDZ concentrations. Similarly, the slopes of the calibration graphs for SDZ are independent of the TMP concentrations.

The limit of detection (LOD) was defined as the lowest concentration of SDZ and TMP that could be recognized by the detector with a signal-to-noise (S/N) ratio of ≥3. LOD in this method was about 1 µg ml\(^{-1}\) for SDZ and about 0.5 µg ml\(^{-1}\) of TMP.

The applicability of the proposed method to routine analysis was investigated by analyzing some of the commercially available pharmaceutical preparations. The closeness of the results to the label claim supports the accuracy of the method.

Conclusion

In conclusion, it can be stated that the selectivity, accuracy and precision of the first order UV-derivative assay was satisfactory for the simultaneous determination of SDZ and TMP. The proposed method is cheap, time saving and does not need any complicated apparatus.

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The same samples were also analyzed by a standard HPLC method used to analyze such mixture. The results obtained by the proposed method were in excellent agreement with those obtained by HPLC method (Table 2).

Acknowledgments

The financial support from Yarmouk University is gratefully acknowledged. The author would like to thank Rafat Kitaneh from Avico Company, Amman-Jordan, for providing the standards used in this study and for helping in the HPLC analysis.

تقدير السلفاديازين والتراميتوبريمي في آن واحد في مستحضرات صيدلانية

باستخدام اختراق الطيف فوق البنفسجي

أحمد العمري

ملخص

في هذا البحث استخدمت المشكلتة الأولى لطيف الأشعة فوق البنفسجية لتقدير السلفاديازين والتراميتوبريمي كل بوجود الآخر وقد تم ذلك على الطول الموجي 259 نانومتر للسلفاديازين و261.5 للتراميتوبريمي في القيم التي تم الحصول عليها خطية مع التركز لكل من السلفاديازين والتراميتوبريمي وكان معدل الانحراف 1.07% للسلفاديازين و0.53% للتراميتوبريمي. وقد تم تقدير هذه المركبات في محلول هيدروكسيد البوتاسيوم في الميثانول 0.001 ج. كانت المنحنيات المرجعية المستخدمة لتقدير هذه المركبات خطية حتى تركيز 25 ميكروجرام/مل للسلفاديازين و60 ميكروجرام/مل للتراميتوبريمي. كان أقل تركيز يمكن قياسة لهذه الطريقة (Limit of detection) 0.5 ميكرو جرام/مل للسلفاديازين و1.0 ميكرو جرام/مل للتراميتوبريمي. تبين أن الطريقة المستخدمة في هذا البحث طريقة ناجحة لتقدير هذه المركبات في المستحضرات الدوائية التجارية وتهدف لتحليل متابعة تلك التي تم الحصول عليها باستخدام جهاز الكروماتوغرافيا السائلة ذات الداء العالي (HPLC).
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References:


Table (1): Statistical analysis for calibration graphs used in the determination of SDZ (2-25 μg ml⁻¹) and TMP (4-60 μg ml⁻¹) in mixtures by first-derivative spectrophotometry.

<table>
<thead>
<tr>
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<td></td>
<td></td>
<td>µg ml⁻¹</td>
<td>x 10⁻³</td>
<td>x 10⁻³</td>
<td>Coeff.</td>
<td>x 10⁻³</td>
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<td>6.765</td>
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Table (2): Simultaneous determination of TMP and SDZ in pharmaceutical products.

<table>
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<th>Proposed Method</th>
<th>% Found</th>
<th>HPLC Method</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>SDZ</td>
<td>TMP</td>
<td>SDZ</td>
<td>TMP</td>
</tr>
<tr>
<td>Vapocotrim</td>
<td>400 µg g⁻¹</td>
<td>80 µg g⁻¹</td>
<td>99.1</td>
<td>98.6</td>
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<tr>
<td>Narodine</td>
<td>200 µg ml⁻¹</td>
<td>40 µg ml⁻¹</td>
<td>100.5</td>
<td>98.8</td>
</tr>
<tr>
<td>Coliprim</td>
<td>200 µg ml⁻¹</td>
<td>40 µg ml⁻¹</td>
<td>98.4</td>
<td>99.6</td>
</tr>
<tr>
<td>Mean ± standard deviation</td>
<td>99.5 ± 1.01</td>
<td>69.0 ± 0.53</td>
<td>101.8 ± 3.77</td>
<td>100.5 ± 0.89</td>
</tr>
</tbody>
</table>

Means values of three determinations

Fig.1. Absorption spectra of TMP, trimethoprim; SDZ, sulphadiazine; and Mix, a mixture of TMP and SDZ.
Fig. 2. First-derivative spectra of TMP, trimethoprim; SDZ, sulphadiazine; and Mix, a mixture of TMP and SDZ.

Fig. 3. The first derivative spectra of TMP, trimethoprim; SDZ, sulphadiazine; [TMP] = 10 µg ml⁻¹, with 1. 2; 2. 4; 3. 6; 4. 10; 5. 15; 6. 20; and 7. 25 µg ml⁻¹ of SDZ.
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Fig. 4. The first derivative spectra of TMP, trimethoprim; SDZ, sulphadiazine; [SDZ] = 20 μg ml⁻¹, with 1. 4; 2. 10; 3. 20; 4. 30; 5. 40; 6. 50; and 7. 60 μg ml⁻¹ of TMP.

Fig. 5. Effect of acidity on absorption spectra of SDZ, sulphadiazine solutions (10 μg ml⁻¹ in methanol). A. 0.001 mol l⁻¹ KOH; B. 0.01 mol l⁻¹ KOH; C. 0.1 mol l⁻¹ KOH; D. 0.001 mol l⁻¹ HCl; E. 0.01 mol l⁻¹ HCl; F. 0.1 mol l⁻¹ HCl.
Fig. 6. Effect of acidity on absorption spectra of TMP, trimethoprim solutions (15 μg ml⁻¹ in methanol). A. 0.001 mol l⁻¹ KOH; B. 0.01 mol l⁻¹ KOH; C. 0.1 mol l⁻¹ KOH; D. 0.001 mol l⁻¹ HCl; E. 0.01 mol l⁻¹ HCl; F. 0.1 mol l⁻¹ HCl.