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Research Article

SIMULTANEOUS DETERMINATION OF TRIMETHOPRIM AND SULFAMETHOXAZOLE IN IMMEDIATE-RELEASE ORAL DOSAGE FORMS BY FIRST-ORDER DERIVATIVE SPECTROSCOPY: APPLICATION TO DISSOLUTION STUDIES

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ABSTRACT

Objective: To develop and validate a new and easy zero-crossing derivative method for the simultaneous determination of trimethoprim and sulfamethoxazole in immediate-release oral dosage forms and to demonstrate its application for the analysis of the drugs during dissolution studies.

Method: Measurement was achieved using the first-derivative signals at 247.8 nm for trimethoprim and at 257.9 nm for sulfamethoxazole. The method was validated according to ICH guidelines. The proposed method was applied in the quantification of both drugs in samples taken during the study of dissolution profiles (USP Apparatus 2, 75 rpm and 900 ml of 0.1 N hydrochloric acid) of Bactrim® and Bactrim® F products (80/400 mg and 160/800 mg, respectively). Dissolution samples were also analyzed by the HPLC pharmacopeial method. Dissolution data of trimethoprim and sulfamethoxazole, percent of drug dissolved at 60 min (*Q*) and dissolution efficiency, obtained by UV and HPLC methods were compared by Student *t*-test.

Results: The method was linear (R^2> 0.98, p< 0.05) in the range of 10 – 50 µg/ml and 250 – 350 µg/ml for trimethoprim and sulfamethoxazole, respectively. The within-day and between-day precision and accuracy were within the acceptable criteria (RSD< 1.9% and 100 ± 3%). No significant differences were found between the results obtained by the first-derivative spectrophotometric method and the HPLC analysis (p> 0.05).

Conclusion: The proposed method can be used for the simultaneous determination of trimethoprim and sulfamethoxazole in dissolution studies. The method is rapid, simple, accurate, and precise without the need of high cost investment.

Keywords: Trimethoprim, Sulfamethoxazole, Derivative spectroscopy, Zero-crossing method, Dissolution profiles.

INTRODUCTION

Trimethoprim [5-(3,4,5-trimethoxybenzyl)pyrimidine-2,4-diamine] and sulfamethoxazole [N^1-(5-methylisoxazol-3-yl)sulfanilamide] are a combined antimicrobial agents that act synergistically, Fig. 1. Trimethoprim/sulfamethoxazole combination is valuable in the prophylaxis and therapy of a number of infectious processes in bladder, ear, intestine and lung [1]. In Mexico, as in other parts of the world, immediate-release oral dosage forms of trimethoprim/sulfamethoxazole are widely marketed as generic products. So, in the development of a new formulation or as a quality control test between lots, dissolution studies of these products are essential. Dissolution test for trimethoprim/sulfamethoxazole tablets is described in the United States Pharmacopeia (USP) [2]. The method indicates the use of USP Apparatus 2 (paddles) at 75 rpm and 900 ml of 0.1 N hydrochloric acid solution at 37.0 ± 0.5ºC as dissolution medium. Under these conditions not less than 70% (*Q*) of the labeled amount of trimethoprim/sulfamethoxazole is dissolved in 60 min. Simultaneous determination of these drugs are currently performed by high-performance liquid chromatography (HPLC) however, and as an alternative method, derivative spectrophotometric analysis of trimethoprim and sulfamethoxazole has been previously published. Derivative spectrophotometry is a useful technique for the suppression of additive interferences due to compounds with overlapping spectra.

To quantify trimethoprim and sulfamethoxazole in dissolution medium some analytical methods using methanolic [3], alkaline [4,5], or a combination of methanolic/alkaline solutions [6] has been reported. Methanolic/alkaline medium is not a suitable reflection of the natural environment following the oral ingestion of the tablets. Pharmacopoeial dissolution test is carried out in acidic medium (0.1 N hydrochloric acid solution) and this environment will prevail for some minutes after *in vivo* tablets intake. On the other hand, external addition of components for the manufacture of trimethoprim/ sulfamethoxazole tablets as hydroxypropyl-β-cyclodextrin; in order to improve the performance and therefore, determining both drugs by UV detection, was also reported [7]. The use of special equipment as fiber-optic UV/VIS spectrophotometer [8], dissolution vessel connected to an FIA manifold [9], multicommutation flow-assembly [10], and photodiode-array spectrophotometer [11] was reported by others authors. The purpose of this study was to develop and validate a simple, economic and rapid zero-crossing first-derivative (^{1}D) spectrophotometric method for the simultaneous determination of the dissolution profiles of trimethoprim and sulfamethoxazole in immediate-release oral dosage forms. The results were compared to those obtained with the HPLC pharmacopeial method recommended for the determination of the drugs in the dissolution medium.

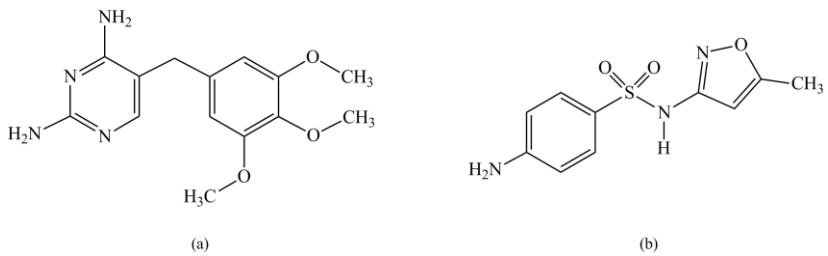

Fig. 1: Chemical structures of trimethoprim (a) and sulfamethoxazole (b).

MATERIALS AND METHODS

Materials

Two trimethoprim/sulfamethoxazole immediate-release oral dosage forms: 80 mg of trimethoprim and 400 mg of sulfamethoxazole as well as 160 mg of trimethoprim and 800 mg of sulfamethoxazole were used in this study. Both products are the Mexican reference products Bactrim® and Bactrim® F (Productos Roche, SA de CV, Mexico), respectively. Acetic acid, hydrochloric acid, and methanolanalytical grade were purchased from J. T. Baker-Mexico as well as acetonitrile HPLC grade. Trimethoprim and sulfamethoxazole standards were purchased from Sigma-Aldrich Co. (St. Louis MO, USA). All samples were filtered through 0.45 µm nitrocellulose Millipore® filters.

Content uniformity and assay

Content uniformity and assay tests were performed with both products, according the procedures described in the United States Pharmacopeia [2].

Standard solutions

Stock solutions of trimethoprim (0.2 mg/ml) and sulfamethoxazole (1 mg/ml) in 0.1 N hydrochloric acid were separately prepared by dissolving 10 mg of trimethoprim in 50 ml of 0.1 N hydrochloric acid and 10 mg of sulfamethoxazole in 10 ml of 0.1 N hydrochloric acid (previously, each drug was dissolved in 1 ml of methanol). Standard solutions were prepared by serial dilutions of the stock solutions to contain the required concentrations for the calibration curves. Trimethoprim and sulfamethoxazole calibration curves in 0.1 N hydrochloric acid were prepared in the concentrations of 10 – 50 µg/ml and 250 – 350 µg/ml, respectively.

Instruments

For dissolution studies, an USP Apparatus 2 (Sotax AT-7 Smart, Switzerland) with a piston pump (Sotax CY7-50, Switzerland) was used. Dissolution tests were carried out according the procedures described in the United States Pharmacopeia [2]. Trimethoprim/sulfamethoxazole intact tablets were added on 900 ml of 0.1 N hydrochloric acid at 37.0 ±0.5ºC as dissolution medium (n = 6). Rotational speed of 75 rpm was tested. After addition of tablets, 10 ml of filtered dissolution sample was withdrawn at 15, 20, 30, 45, and 60 min and replaced with an equal volume of fresh medium to maintain a constant total volume. For spectrophotometric measurement, a double beam UV/VIS spectrophotometer (Perkin Elmer Lambda 35, Waltham MA, USA) with 0.1 cm quartz cells was utilized. The operating conditions for UV analysis were ^{1}D mode with scan speed 240 nm/min, slit width 2.0 nm and sampling interval 1.0 nm. Chromatographic analysis was performed onto an HPLC system (Agilent Model 1100, Santa Clara CA, USA) with variable wavelength UV detector. The compounds were separated with a mobile phase consisting of a mixture of buffer pH 5.9: acetonitrile (85:15, v/v). The mobile phase was filtered, sonicated before used, and delivered at a flow rate of 1.5 ml/min. Injection volume was 20 µl.A Shodex C18 column (4.6 mm × 150 mm) was used. Standard solutions of trimethoprim and sulfamethoxazole were prepared with mobile phase at concentrations of 32 and 160 µg/ml, respectively.

Analytical method validation

The proposed analytical method was validated according to the International Conference on Harmonization (ICH) guidelines [12]. The system linearity, accuracy, precision, and stability were analyzed.

Linearity

To verify the validation of Beer's law, three series of calibration curves in 0.1 N hydrochloric acid were plotted using the ^{1}D spectra in the range of the calibration curve of each drug. Data obtained were fitted by linear regression analysis and the coefficients of regression and regression analysis of variance (ANOVA) were calculated. The response *versus* drug concentration proportionality was demonstrated for each drug by calculating the percentage relative standard deviation (RSD): [((standard deviation)/mean) × 100] of the response factor across the entire range of the calibration curve.

Accuracy and precision

In order to verify the accuracy and precision of the proposed derivative analysis, the added standard method was used, thus matrix effects can easily be removed. This method can be used for resolving binary mixtures in complex samples with unknown matrices as commercial oral dosage forms have. Twenty tablets were accurately weighed and powdered in a mortar; then, quantities of powder of trimethoprim/sulfamethoxazole tablets plus a quantity of trimethoprim or sulfamethoxazole standard (5 mg) to finally give the equivalent of 30, 80 and 120% of the dose of each drug, were separately dissolving in 900 ml of 0.1 N hydrochloric acid at 37.0 ±0.5ºC. The USP Apparatus 2 at 75 rpm was used. At 60 min, the amounts of trimethoprim and sulfamethoxazole dissolved in each sample was calculated with reference to a calibration curve prepared on the day of the experiment. Each determination was performed in triplicate. The percentage relative error (RE): [((found–added)/added)× 100] was taken as a measure of the accuracy and the RSD as a measure of precision. Experiments were done in three consecutive days.

Stability

Stability of analytical solutions was evaluated analyzing two solutions of trimethoprim (15 and 45 µg/ml) and two solutions of sulfamethoxazole (260 and 340 µg/ml) prepared in 0.1 N hydrochloric acid. These solutions were analyzed by the proposed ^{1}D spectrophotometric method at 0, 24 and 48 h after stored at room temperature (25ºC). At 24 and 48 h, the percentage absolute difference AD: [((initial–final)/initial)× 100] recovered of each drug was determined.

Data analysis

Trimethoprim and sulfamethoxazole dissolution data of each product were used to calculate model-independent parameters: % dissolved at 60 min (Q) and dissolution efficiency (DE) [13]. Student's t-test was used for data comparison between obtained results with the proposed ^{1}D spectrophotometric method and HPLC analysis. Differences were considered significant if $p< 0.05$.

RESULTS AND DISCUSSION

Content uniformity and assay

All products met the content uniformity and assay tests specified in the United States Pharmacopeia. The percentages of trimethoprim and sulfamethoxazole on the content uniformity test ranged from 85 to 115% and the assay test was between 90 to 110%, Table 1.

Table 1: Content uniformity and assay results

Drug	Dose (mg)	Content uniformity (min – max)	Assay (%)
T	80	99.1 (97.4 – 102.7)	98.3
S	400	99.0 (97.8 – 100.8)	99.0
T	160	91.30 (89.52– 94.77)	97.6
S	800	98.88 (96.35– 101.83)	98.5

Trimethoprim (T) and sulfamethoxazole (S). Mean, *n* = 10.

Absorption spectra

The zero-order spectra of trimethoprim (50 µg/ml) and sulfamethoxazole (350 µg/ml) standard solutions in 0.1 N hydrochloric acid were separately and combined measured at 240 – 320 nm using 0.1 N hydrochloric acid as blank, Fig. 2a. The zero-order spectra demonstrated a marked overlapping. As a result, simultaneous direct spectroscopy determination of trimethoprim and sulfamethoxazole in commercial tablets was not possible. Then, the ^{1}D spectra of these solutions was obtained, Fig. 2b. As seen in Fig. 2b, the ^{1}D spectra of trimethoprim and sulfamethoxazole revealed three zero-crossing points for their simultaneous determination; these points were found in the wavelengths.

The ^{1}D spectra of trimethoprim (10– 50 µg/ml) and sulfamethoxazole (250 – 350 µg/ml) standard solutions were determined, Fig. 3a and Fig. 3b, respectively. The suitable zero-crossing points were selected based on the best linear response to the trimethoprim concentration in the presence of sulfamethoxazole or the sulfamethoxazole concentration in the presence of trimethoprim. Only the ^{1}D response at 247.8 and 257.9 nm were proportional to the trimethoprim and sulfamethoxazole concentrations, respectively.

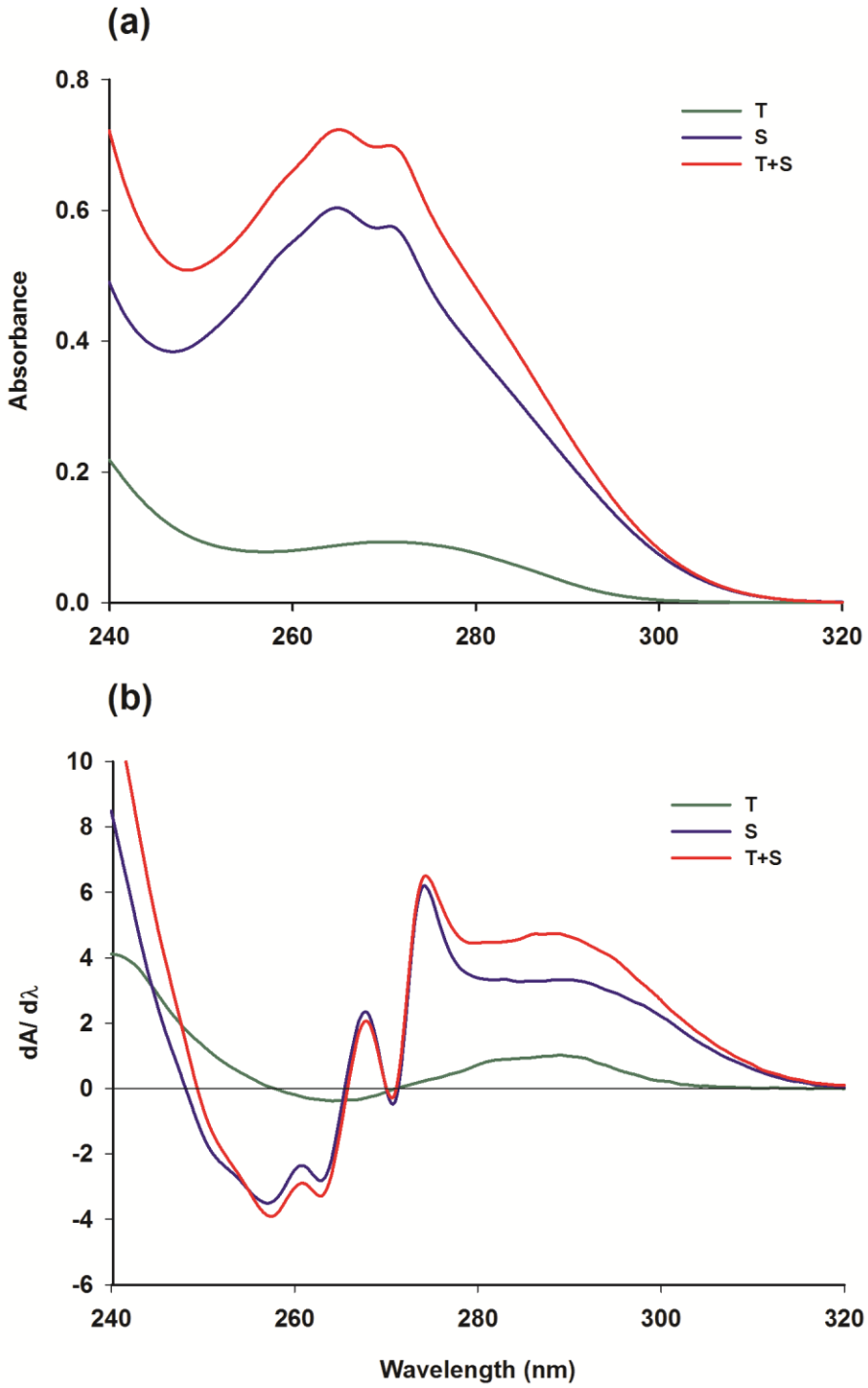

Fig. 2: Zero-order spectra of (a) 50 µg/ml of trimethoprim (T), 350 µg/ml of sulfamethoxazole (S) and their mixture (T+S) at the same concentrations and (b) first-derivative spectra of the same solutions. Drugs were dissolved in 0.1 N hydrochloric acid.

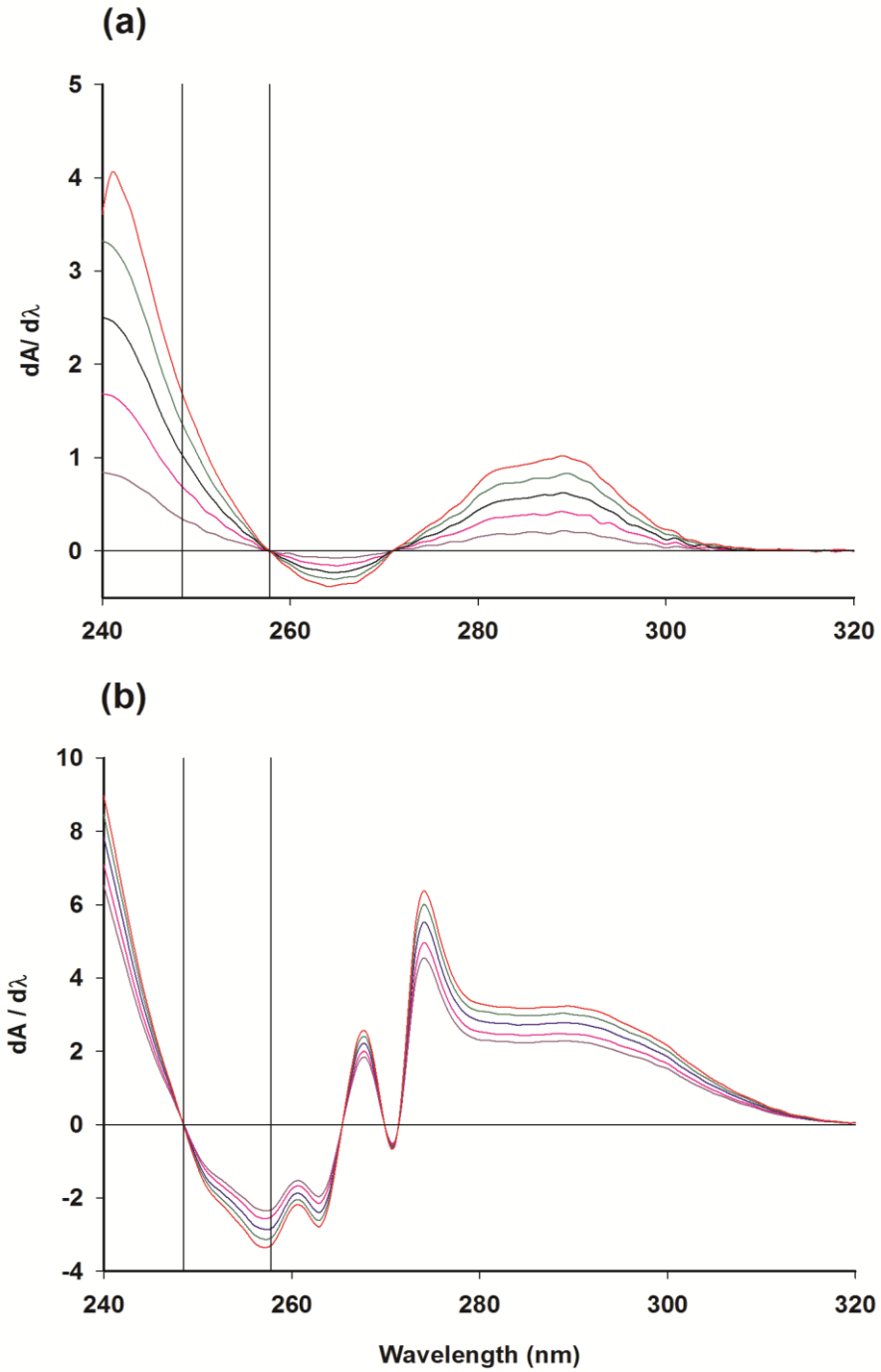

Fig. 3: First-derivative spectra of 10 – 50 µg/ml of trimethoprim (a) and 250 – 350 µg/ml of sulfamethoxazole(b) solutions. Vertical lines indicate 247.8 and 257.9 nm, respectively. Drugs were dissolved in 0.1 N hydrochloric acid.

Method validation

Linearity

The mean regression equation from three standard calibration curves was: $y = 0.0344\,x + 0.034$ for trimethoprim and $y = -0.0102x + 0.1646$ for sulfamethoxazole. Both linear regressions were significant (R^2= 0.98; p<0.05).The RSD values of response factor were 3.3 and 1.7% for trimethoprim and sulfamethoxazole ranges, respectively.

Accuracy and precision

In order to prove the accuracy and precision of the ^{1}D spectrophotometric method, analysis of varying percentage of dose of each drug was done for three days (n = 3/day). The within-day and between-day precision and accuracy were calculated, Table 2. The RSD obtained was in the range of 0.35 – 1.88% and the RE was lower than 1.17% for both drugs in all selected dose percentages which indicate good accuracy and precision of the method. According to ICH guidelines [12], 80% of dose should be the minimum percentage of dose included in the validation scheme, but we choose the 30% of dose as the lower level that 80/160 mg product can be quantified without problem.

Stability

The stability of the ^{1}D spectrophotometric method was assessed analyzing two solutions of trimethoprim and two solutions of sulfamethoxazole at different times. Absolute difference at 24 and 48 h are shown in Table 3. As seen in Table 3, sulfamethoxazole solutions were less stable at the second day of have been prepared.

Table 2: Accuracy and precision data for simultaneous determination of trimethoprim (T) and sulfamethoxazole (S) by first-order derivative method Mean±SD.

		Within-day (*n* = 3)			Between-day (*n* = 9)		
Drug/dose (mg)	Added (mg)	Found (mg)	RSD (%)	RE (%)	Found (mg)	RSD (%)	RE (%)
T/80	24	23.91±0.26	1.09	−0.37	23.94±0.19	0.79	−0.25
	64	64.19±1.19	1.86	0.29	63.43±0.97	1.53	−0.90
	96	95.93±1.21	1.27	−0.07	95.60±1.77	1.85	−0.42
T/160	48	48.11±0.63	1.31	0.23	48.11±0.89	1.85	0.23
	128	129.50±0.67	0.52	1.17	129.20±0.79	0.61	0.94
	192	191.93±1.15	0.60	−0.04	192.18±1.62	0.84	0.09
S/400	120	120.77±0.69	0.57	0.64	120.74±0.73	0.60	0.62
	320	319.71±3.60	1.12	−0.09	319.73±2.48	0.78	−0.09
	480	481.46±5.82	1.21	0.31	483.17±2.90	0.60	−0.66
S/800	240	240.46±4.53	1.88	0.19	240.98±2.50	1.04	0.41
	640	639.52±3.15	0.49	−0.07	641.82±3.67	0.57	0.29
	960	962.22±3.33	0.35	0.23	964.79±4.37	0.45	0.50

RSD: Relative standard deviation; RE: Relative error

Table 3: Absolute difference (%) respect zero time to evaluate stability at 25ºC of trimethoprim (T) and sulfamethoxazole (S) in 0.1 N hydrochloric acid solution. Mean, *n* = 8.

Drug	Conc. (µg/ml)	24 h	48 h
T	15	1.71	2.14
	45	0.31	0.78
S	260	0.38	−5.75
	340	−0.69	−7.46

Data obtained indicate good linearity, accuracy, precision and robustness of the proposed zero-crossing derivative method for simultaneous determination of trimethoprim and sulfamethoxazole in immediate-release oral dosage forms. According to complementary ICH guideline [14], limit of detection and limit of quantitation are characteristics not normally evaluated in dissolutions assays. For both drugs, lack of linearity, accuracy, and precision was determined working out of the proposed range calibration curves.

Dissolution profiles

Trimethoprim and sulfamethoxazole dissolution profiles obtained withthe ^{1}D spectrophotometricmethod and the HPLC analysis (80/400 mg and 160/800 mg products) are shown in Fig. 4a and 4b, respectively.

As can be seen in Figs. 4a and 4b, dissolution profiles of trimethoprim and sulfamethoxazole obtained with both methods (UV and HPLC) were similar. For the determination of dissolution data equivalence, % dissolved at 60 min (*Q*) and DE mean values ± standard error medium (SEM) for products under study are shown in Table 4.

The ^{1}D spectrophotometric method could be applied with great success for the simultaneous determination of trimethoprim and sulfamethoxazole in the immediate-release reference products containing its binary mixture without interference of each other and the matrix effect.

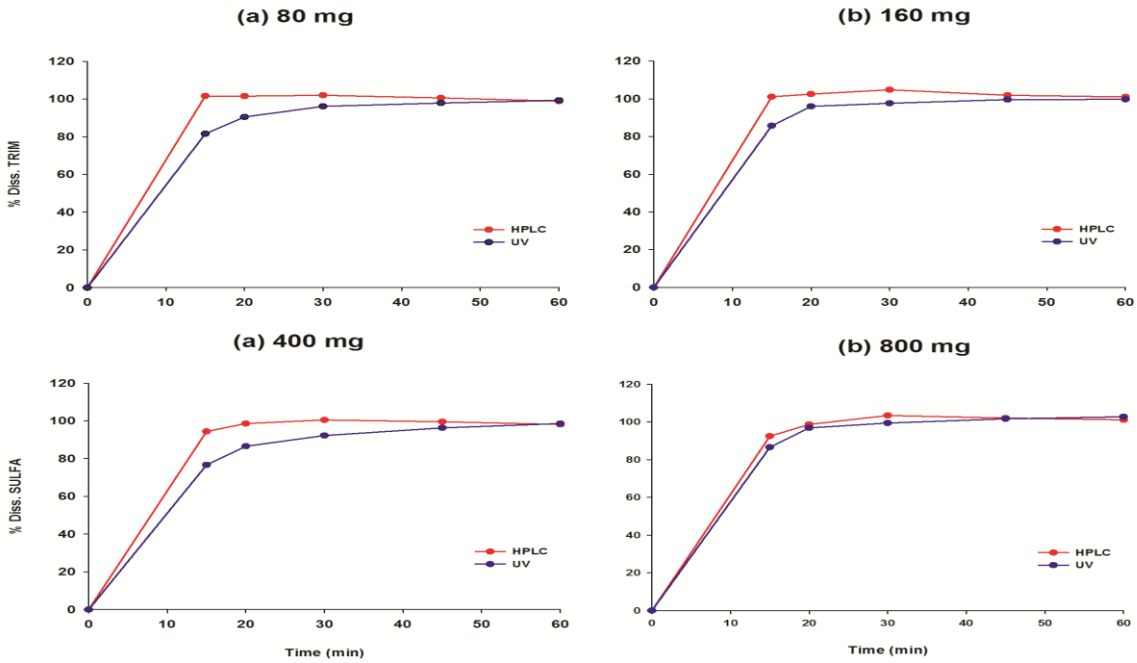

Fig. 4: Dissolution profiles of trimethoprim (TRIM) and sulfamethoxazole (SULFA) from immediate-release commercial products obtained with the first- derivative spectrophotometric method (UV) and chromatographic analysis (HPLC). (a) 80/400 mg and (b) 160/800 mg products. Mean, *n* = 6.

Table 4: Dissolution parameters % dissolved at 60 min (*Q*) and dissolution efficiency (DE). Trimethoprim (T) and sulfamethoxazole (S). Mean±SEM, *n* = 6.

Drug	Method	Dose (mg)	*Q* (%)	DE (%)
T	HPLC	80	98.92±1.77	88.40±0.51
	UV	80	99.28±0.45	81.81±0.21
	HPLC	160	100.99±1.38	89.59±0.22
	UV	160	99.74±0.23	83.99±0.12
S	HPLC	400	98.19±1.69	86.20±0.41
	UV	400	98.63±0.26	79.25±0.18
	HPLC	800	101.15±1.34	87.46±0.15
	UV	800	102.77±0.39	85.52±0.14

No significant differences were found in data obtained by the ^{1}D spectrophotometric method and the HPLC analysis (p >0.05).

When data obtained with our proposed method are compared with HPLC data using model-independent methods, no significant differences were found. The most striking feature of the proposed ^{1}D spectrophotometric method is its simplicity and rapidity, no requiring time-consuming sample preparation such as filtration, degassing or using toxic solvents that are need for HPLC procedure.

CONCLUSION

It was concluded that the ^{1}D spectroscopic method could be used for simultaneous determination of trimethoprim and sulfamethoxazole in immediate-release oral dosage forms. This method could be used for the analysis of active pharmaceutical ingredients in dissolution studies and for quality control purposes. The method is rapid, simple, and economic without the need of high cost investment.

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