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Second derivative spectrophotometric determination of trimethoprime and sulfamethoxazole in the presence of hydroxypropyl-β-cyclodextrin (HP-β-CD)

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Abstract

An easy and rapid second-derivative spectrophotometric method for the simultaneous analysis of trimethoprime (TMP) and sulfamethoxazole (SM) is described. These drugs have been used as antibacterial against a wide spectrum of organisms and combinations of these drugs are commonly used for the treatment of a variety of infections. The most advantageous approach of this method is the use of HP-β-CD, which allows to improve the performance of the second-derivative ultraviolet spectrophotometry. For both compounds, a shift of the absorption bands and variations of their intensity were observed. The calibration graphs were linear in the concentration range of TMP (1.92–19.2 μg ml^{-1}) and SM (1.60–16.5 μg ml^{-1}), the correlation coefficient for the calibration graphs was better than 0.9994 and the precision was satisfactory (CV% < 4.96) in HP-β-CD solutions. The proposed method was succesfully applied to the assay of commercial tablets. The results were compared to those obtained by second-derivative ultraviolet spectrophotometry in the absence of HP-β-CD. Thereby, the details of the statistical treatment of the analytical data are also presented.

Keywords: Antibacterial drugs; Hydroxypropyl-β-cyclodextrin; Second-derivative ultraviolet spectrophotometry; Inclusion complexes; Validation

1. Introduction

Trimethoprime (TMP) and sulfamethoxazole (SM) are well known as antibacterial drugs inhibiting consecutive steps in the biosynthesis of nucleic acids and proteins essential to many bacteria [1]. This synergy between TMP and the sulfonamide drug occurs against a wide spectrum of organisms and combinations of these drugs, commonly used for the treatment of a variety of infections (urinary tract, respiratory tract and enteric infections) [2]. SM and TMP are the active ingredients in several oral suspension and solid dosage forms. The analytical methodology for this type of drugs has been based mainly on liquid chromatography [3–6] and UV–visible spectroscopy [7,8] including derivative procedures [9].

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Nevertheless, these methods are time consuming and/or relatively difficult. Also, Altesor et al. [10] presented a derivative method which has need of an equation system and a photodiode-array spectrophotometer for the simultaneous determination of the two drugs. In our experience, however, the determinations are greatly simplified by the use of a cyclodextrin (CD), which allows to enhance specificity and sensitivity, and a conventional wavelength scanning.

CDs are water-soluble cyclic oligosaccharides composed of six (α-), seven (β-) and eight (γ-) units of D-(+)-glucopyranose arranged in a truncated cone-shape structure. The hydrophobic cavity of CDs can host a large variety of organic and inorganic compounds [11]. Entire molecules or parts of them can penetrate in the semi polar cavity forming an inclusion complex. The chemical reactivity and the spectroscopic properties of the guest molecules are modified as a result of the inclusion [12]. From an analytical point of view, the formation of inclusion complexes allows, in certain cases, to improve the performance of the methods for the determination of different analytes including pharmaceuticals [13]. Several chromatographic and spectrofluorimetric techniques benefit from cyclodextrin inclusion of the analytes, and from the associated enhanced selectivity and sensitivity [14–16]; however, no analytical spectrophotometric studies using CDs have been found in the literature consulted. In the present work, a simple, rapid and sensitive method, based on UV-derivative spectrophotometry using hydroxypropyl-β-cyclodextrin (HP-β-CD) solutions, is proposed for the simultaneous quantification of TMP and SM. The objective of this work is to demonstrate the capability of the second-derivative method in the presence of HP-β-CD to solve and overcome the problem of overlapping spectral bands, allowing the simultaneous determination of these drugs in laboratory mixtures without the need of prior separation. The developed method was extended to determine the content of both drugs in commercial tablets. Also, the results obtained from this method were compared to those obtained in the absence of HP-β-CD.

2. Experimental

2.1. Apparatus

UV–visible spectroscopy determinations were performed using a Shimadzu UV 260. The measurements were carried out at room temperature. Second-order derivative spectra were obtained using the following conditions: wavelength range, 220–330 nm; $\Delta\lambda = 4$; slit width, 2 nm.

Data analysis was performed with Sigma Plot (Scientific Graph System) Jandel Scientific, version 3.0.

2.2. Reagents and samples

All the experiments were performed with analytical grade chemicals and solvents. HP-β-CD (MW = 1325; degree of molar substitution, 7.0) was a present from Ferromet S.A. (agent of Roquette in Argentina). Cotrimoxazol Forte (Richet, Argentina) and Dosulfin Fuerte (Labinca S.A., Argentina) tablets were labeled to contain 160 mg of TMP and 800 mg of SM. The excipients present in tablets are: magnesium trisilicate, aluminum hydroxide, lactose, polyvinylpyrrolidone, magnesium stearate and talc.

2.3. Standard solutions and calibration graphs

Stock solutions of both TMP (96.0 μg ml^{-1}) and SM (91.5 μg ml^{-1}) were prepared in ethanol. The working standard solutions were prepared by dilution of appropriate volume aliquots of the stock solutions with 10% w/v HP-β-CD solution or distillated water to reach concentration ranges of 1.92–19.2 μg ml^{-1} for TMP, 1.83–18.3 μg ml^{-1} and 1.6–16.5 μg ml^{-1} for SM.

Similarly, two series of each mixture solution were also prepared from the stock solutions. The first series contained a constant concentration of SM (14.4 μg ml^{-1}) and a varying concentration of TMP (1.92–19.2 μg ml^{-1}). The second series contained a constant concentration of TMP (2.97 μg ml^{-1}) and a varying concentration of SM (0.83–18.3 and 1.6–16.5 μg ml^{-1}).

2.4. UV measurements

The second-order derivative spectra (^{2}D) of the HP-β-CD or the working water standard containing the varying amount of each drug and those containing mixture of both drugs were scanned in the range of 220–330 nm against HP-β-CD solution or against water as blanks. The values of the ^{2}D amplitudes at 254 nm (zero-crossing of SM) were measured for the determination of TMP in the presence of SM, and the ^{2}D amplitudes values at 264 nm (zero-crossing for TMP) were used for the determination of SM in presence of TMP.

The ordinate values ^{2}D of the regression lines were calculated from the amplitude measurements (cm) and standardized as follows:

$$^2\mathrm{D} = \frac{\text{Recorder divisions (h cm)} \times \text{scale expansion}}{\text{7.65 cm full scale}}.$$

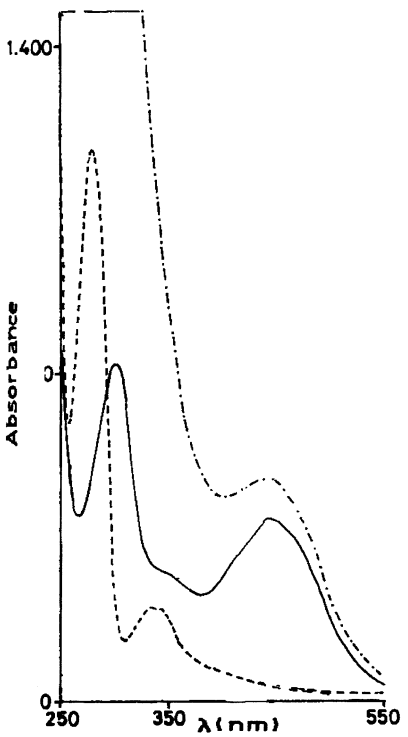

Fig. 1. Absorption (zero-order) UV spectra of TMP (···), SM (—) and their mixture (-·-·-·-) in water.

2.5. HPLC measurements

The HPLC determinations were carried out as directed the USP XXIV [17].

2.6. Sample preparation for solid dosage forms

Ten tablets of Cotrimoxazol Forte (Richet®, Argentina) and 10 tablets of Dosulfin® Fuerte (Labinca S.A., Argentina) labeled to contain 160 mg of TMP and 800 mg of SM as active ingredients were weighed and finely powdered. Portions of the powder equivalent to about 100 mg of SM were accurately weighed and transferred into 25 ml volumetric flasks using ethanol. The flasks were completed to volume with ethanol and sonicated for 10 min. The suspensions were filtered through 0.45 μm membrane filter. Appropriate volume aliquots of filtrate were diluted in 10% w/v HP-β-CD solution and in distilled water to suit the calibration graphs for the derivative measurements.

3. Results and discussion

A thorough investigation was conducted in order to choose the optimum HP-β-CD quantity for the spectrophotometric determination of TMP and SM. The best results for their simultaneous determination in tablet formulations were obtained in 10% HP-β-CD solutions.

Fig. 1 shows the absorption (zero-order) spectra of TMP (A) (19.5 μg ml^{-1}) with maxima at 207 and 282 nm, SM (B) (19.5 μg ml^{-1}) with a maximum at 265 nm and a mixture of TMP and SM (C) (both 19.5 μg ml^{-1}). Because of the large overlapping with the spectral bands of the two compounds, conventional UV spectrophotometry cannot be used for the simultaneous determination of TMP and SM in mixtures. Fig. 2 shows the effect of the addition of HP-β-CD on the absorption spectra of TMP and SM. The spectra of these compounds are altered in the presence of HP-β-CD. A shift of bands and a variation of their intensity were observed for both compounds. Nevertheless, these changes did not permit the simultaneous determination of these drugs. The

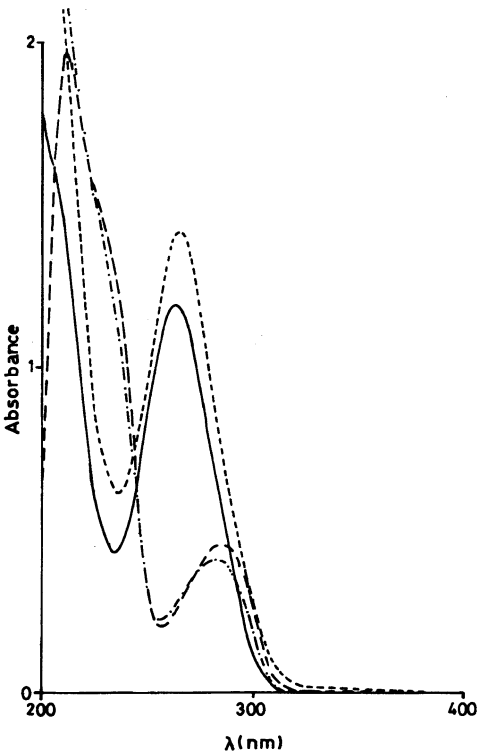

Fig. 2. Absorption (zero-order) UV spectra of TMP in water (-·-·-·-) and in 10% w/v HP-β-CD solution (-----); and SM in water (—) and in 10% w/v HP-β-CD solution (···).

second-order derivative spectrophotometric method (^{2}D) was considered to be ideal for solving the overlapping over its first-derivative spectra and for being used for the simultaneous determination of TMP and SM. ^{2}D UV spectra of TMP and SM exhibited sharp bands with great amplitudes that may offer more selective identification and determination of the two compounds. The ^{2}D spectra permit the determination of SM at 264 nm (zero crossing of TMP) by using the values of the ^{2}D amplitudes. The spectra were recorded in the range of 220–330 nm. The addition of HP-β-CD produced a shift of the 264 nm band with an increasing intensity, giving higher analytical sensitivity and selectivity. In the same way, TMP was determined at 254 nm (zero crossing of SM) through ^{2}D amplitude measurements over the range 220–330 nm (Fig. 3). However, it was observed pronounced changes in the molar absorptivity (ε) of TMP in the presence of SM, resulting in Beer's law deviation of the measurements, and leading to inaccurate results. Fortunately, this problem was solved satisfactorily by using HP-β-CD.

The main instrumental parameter that affects the shape of the derivative spectra and the signal-to-noise ratio is the wavelength increment over which derivatives are obtained ($\Delta\lambda$). This parameter needs to be optimized to give a well resolved large peak, i.e. to give good selectivity and higher sensitivity in determination. Increasing $\Delta\lambda$, the signal-to-noise ratio improves, thus the fluctuation in a derivative spectrum decreases. However, if the value of $\Delta\lambda$ is too large, the spectral intensity signal of second derivative deteriorates. Various values of $\Delta\lambda$ were tested and $\Delta\lambda = 4$ nm was chosen as the most optimus in order to give an adequate signal-to-noise ratio.

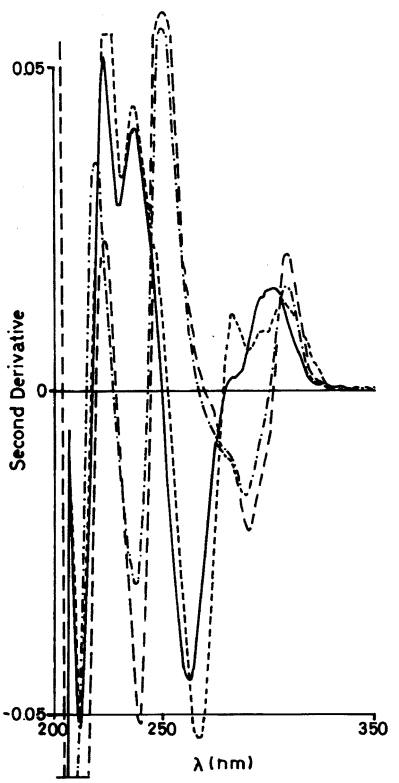

Fig. 3. Second-derivative spectra of TMP in water (-·-·-·-) and in 10% w/v HP-β-CD solution (-----); and SM in water (—) and in 10% w/v HP-β-CD solution (···).

Table 1
Analytical data of the calibration graphs for the determination of TMP and SM in standard solutions by second-derivative spectrophotometry

Compound	Solvent	Derivative mode (nm)	Linearity range (μg ml^{-1})	Regression equation (H (cm) = $bC+a$)[a]	r[b]	S_a[c]	S_b[d]
SM	Water	$^2D_{264}$	(1.6–16.5)	$H = 2.24\times10^{-3}\,C - 1.11\times10^{-3}$	0.9966	0.67×10^{-3}	0.07×10^{-3}
SM	10% w/v HP-β-CD	$^2D_{264}$	(1.83–18.3)	$H = 2.91\times10^{-3}\,C - 0.09\times10^{-3}$	0.9997	0.45×10^{-3}	0.02×10^{-3}
SM (TM 2.97 μg ml^{-1})	Water	$^2D_{264}$	(1.6–16.5)	$H = 2.17\times10^{-3}\,C + 1.61\times10^{-3}$	0.9929	0.89×10^{-3}	0.09×10^{-3}
SM (TMP 2.97 μg ml^{-1})	10% w/v HP-β-CD	$^2D_{264}$	(1.83–18.3)	$H = 2.73\times10^{-3}\,C - 0.39\times10^{-3}$	0.9994	0.65×10^{-3}	0.05×10^{-3}
TMP	Water	$^2D_{254}$	(1.92–19.2)	$H = 2.94\times10^{-3}\,C - 4.16\times10^{-3}$	0.9975	0.93×10^{-3}	0.08×10^{-3}
TMP	10% w/v HP-β-CD	$^2D_{254}$	(1.92–19.2)	$H = 3.38\times10^{-3}\,C - 3.07\times10^{-3}$	0.9997	0.70×10^{-3}	0.06×10^{-3}
TMP (SM 14.4 μg ml^{-1})	10% w/v HP-β-CD	$^2D_{254}$	(1.92–19.2)	$H = 3.43\times10^{-3}\,C - 2.41\times10^{-3}$	0.9993	0.72×10^{-3}	0.06×10^{-3}

[a] Derivative value (^2D) versus concentration (C) of each drug in μg ml^{-1}; standard specimen: $n = 10$.
[b] Correlation coefficient.
[c] S_a, standard deviation of intercept of regression line.
[d] S_b, standard deviation of slope of regression line.

Table 2
Detection and quantification limits for the determination of TMP and SM by second-derivative spectrophotometry

Compound	Solvent	C_L (μg ml^{-1})[a]	C_Q (μg ml^{-1})[b]
SM	Water	0.90	2.97
SM	10% w/v HP-β-CD	0.46	1.52
SM (TMP 2.97 μg ml^{-1})	Water	1.23	4.06
SM (TMP 2.97 μg ml^{-1})	10% w/v HP-β-CD	0.71	2.34
TMP	Water	0.95	3.14
TMP	10% w/v HP-β-CD	0.62	2.05
TMP (SM 14.4 μg ml^{-1})	10% w/v HP-β-CD	0.63	2.08

[a] $C_L = 3S_a/b$.
[b] $C_Q = 10S_a/b$.

Under the described experimental conditions, the calibration graphs, prepared by plotting ^{2}D values against TMP or SM concentrations in HP-β-CD solution or in water, respectively, gave significant linearity with negligible intercepts, confirming the mutual independence of the two components derivative signals. At the same time, the similarity observed between regression equations of pure drug and mixture solutions suggested no interferences in the estimation of one drug in the presence of the other. In Table 1, the statistical parameters were given, the regression equations calculated from the calibration graphs, along with the standard deviations of the slope (S_b) and the intercept (S_a) on the ordinate. The linearity of calibration graphs and conformity of the ^{2}D measurements to Beer's law were proved by the high values of the correlation coefficients (r) of the regression equations mainly in HP-β-CD solutions. The detection limits (C_L) and the quantification limits (C_Q) were calculated from the calibration data and are shown in Table 2. These results showed that both, the detection and the quantification limits in HP-β-CD solutions, were higher than in water. Therefore, the use of HP-β-CD enhanced the sensibility of the method. In order to assess the precision, solutions of TMP, SM and their synthetic mixtures at three different levels of concentrations were analyzed five times in a single run (within-day precision), and five times in separate runs over a period of 2 weeks (between-day precision). The mean values obtained with the coefficients of variation (CV%) are shown in Table 3. These data indicate that the proposed derivative spectrophotometric method is highly precise during one analytical run and between different runs. The accuracy and selectivity of the proposed method were verified by means of recovery assays for TMP and SM in the pure form and for the synthetic admixtures of both drugs and the excipients present in the tablets. Five successive determinations of each solution were carried out and the percentage of recovery in each case was calculated. The results obtained from the recovery of both drugs (Table 4) showed that accuracy and selectivity were better in the presence of HP-β-CD.

The proposed method was evaluated in the assay of commercial tablets. Five replicate determinations were made. Excellent results (Table 5) were obtained for the recovery of both drugs in HP-β-CD solutions and were in good agreement with the label claims.

To validate the derivative spectrophotometric method, the results were compared with those obtained by an HPLC method described in USP XXIV [17]. Results of the two commercial tablets are given in Table 5. The results found according to the proposed method in the presence of HP-β-CD, differ from those found by the official USP method by less than 1.6%. This difference is small enough to be considered negligible for most routine purposes.

The stability of aqueous solutions with and without HP-β-CD of TMP and SM was studied by recording their absorption spectra. The data obtained showed that the drugs remained stable for at least 20 days when the solutions are stored at 25 °C.

4. Conclusions

The most considerable advantage of this derivative spectrophotometric method using HP-β-CD solutions in comparison to previously reported derivative method, for the determination of TMP and SM, is the lack of need for particular requirements (mainly a complex calculation procedure and a photodiode-array spectrophotometer). Another advantages of this method are the simple and rapid sample preparation and assay procedure. The wide range of linearity with considerable accuracy, precision and selectivity allows the method to be used to assay TMP and SM in tablets. The statistical analysis of the results indicated that the presence of one of the components does not interfere with the determination of the other. Because of the lack of an available and simple derivative method with no special requirements, this can be of remarkable importance for the quality control tests on finished products. For example, the method was successfully applied in our laboratory in the determination of the studied compounds in commercial tablets.

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Table 3
Data from precision assays for TMP and SM

Compound	Solvent	Within-day assay ($n = 5$)		Between-day assay ($n = 5$)	
		Mean ± SD (μg ml^{-1})	CV%	Mean ± SD (μg ml^{-1})	CV%
SM	Water	2.11 ± 0.21	9.82	1.93 ± 0.19	9.87
		9.34 ± 0.30	3.25	8.28 ± 0.79	9.55
		18.10 ± 0.12	0.68	17.09 ± 0.95	5.57
SM	10% w/v HP-β-CD	1.61 ± 0.07	4.32	1.71 ± 0.11	6.19
		7.73 ± 0.31	3.98	7.90 ± 0.74	9.42
		15.97 ± 0.62	3.86	16.62 ± 1.30	7.80
SM (TMP 2.97 μg ml^{-1})	Water	2.32 ± 0.17	7.44	2.32 ± 0.17	7.44
		8.74 ± 0.92	10.50	8.76 ± 0.94	10.70
		17.37 ± 1.56	8.95	17.32 ± 1.55	8.92
SM (TMP 2.97 μg ml^{-1})	10% w/v HP-β-CD	1.67 ± 0.08	4.96	1.76 ± 0.12	7.03
		8.48 ± 0.32	3.82	8.85 ± 0.58	6.50
		16.39 ± 0.50	3.04	17.15 ± 0.79	4.59
TMP	Water	2.44 ± 0.09	3.83	2.44 ± 0.09	3.83
		9.52 ± 0.28	2.97	9.52 ± 0.28	2.97
		18.81 ± 0.27	1.42	18.81 ± 0.27	1.42
TMP	10% w/v HP-β-CD	2.56 ± 0.10	4.05	2.64 ± 0.24	8.97
		8.47 ± 0.18	2.17	9.21 ± 0.65	7.05
		19.09 ± 0.62	3.26	18.70 ± 0.61	3.28
TMP (SM 14.4 μg ml^{-1})	10% w/v HP-β-CD	3.14 ± 0.14	4.50	2.96 ± 0.10	3.53
		9.95 ± 0.15	1.50	10.41 ± 0.47	4.51
		19.12 ± 0.30	1.56	19.24 ± 0.17	0.89

Table 4
Recoveries of TMP and SM in pure forms and in synthetic admixtures

Compound	Solvent	Nominal value ($\mu g\ ml^{-1}$)	Found value mean ($\mu g\ ml^{-1}$) ± SD ($n = 3$)	CV%	% Recovery
SM	Water	1.6	2.25 ± 0.25	11.24	140.5
		8.2	9.15 ± 0.22	2.44	111.6
		16.5	18.08 ± 0.10	0.57	109.6
SM	10% HP-β-CD	1.83	1.82 ± 0.15	8.02	99.5
	w/v	9.15	8.90 ± 0.51	5.74	97.2
		18.3	18.23 ± 0.07	0.41	99.6
SM (TMP 2.97 $\mu g\ ml^{-1}$)	Water	1.6	2.66 ± 0.09	3.64	160.3
		8.2	9.16 ± 0.06	1.65	110.3
		16.5	18.50 ± 0.09	0.49	112.1
SM (TMP 2.97 $\mu g\ ml^{-1}$)	10% HP-β-CD	1.83	1.82 ± 0.03	1.66	99.6
	w/v	9.15	9.03 ± 0.09	0.99	98.7
		16.47	17.01 ± 0.15	0.81	103.3
TMP	Water	1.92	2.49 ± 0.07	2.65	129.6
		9.60	9.60 ± 0.23	2.41	99.9
		19.20	19.00 ± 0.17	0.89	98.9
TMP	10% HP-β-CD	1.92	1.94 ± 0.12	4.60	100.9
	w/v	9.6	9.96 ± 0.12	1.19	103.7
		19.2	19.84 ± 1.03	5.17	103.3
TMP (SM 14.4 $\mu g\ ml^{-1}$)	10% HP-β-CD	1.92	1.95 ± 0.21	7.25	101.8
	w/v	9.6	9.69 ± 0.51	4.90	100.9
		19.2	19.31 ± 0.27	1.41	100.6

Table 5
Results of analysis of commercial tablets containing TMP and SM

Sample	SM (mg per tablet)[a]	% Recovery	SM (mg per tablet)[b]	% Recovery	TMP (mg per tablet)[b]	% Recovery
Second derivative method						
Dosulfin® Fuerte	962.5 ± 10.1	120.3	805.9 ± 16.7	100.7	160.1 ± 7.1	100.1
Cotrimoxazol Forte	936.5 ± 22.3	117.1	803.9 ± 22.3	100.5	164.6 ± 9.9	102.9
HPLC method						
Dosulfin® Fuerte	798.7 ± 9.3	99.8			160.3 ± 7.5	100.2
Cotrimoxazol Forte	800.7 ± 8.8	100.1			162.0 ± 8.9	101.3

[a] In water.
[b] In HP-β-CD solution.

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