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Determination of theophylline and ephedrine HCL in tablets by ratio-spectra derivative spectrophotometry and LC

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Abstract

Two methods are described for the determination of theophylline (THP) and ephedrine hydrochloride (EPH) in combined pharmaceutical tablet forms. The first method depends on the use of the first derivative of the ratio-spectra obtained by dividing the absorption spectrum of binary mixtures by a standard spectrum of one of the compounds. The first derivative amplitudes at 231.8 and 250.3 nm were selected for the assay of THP and EPH, respectively. Calibration graphs were established for 20–180 μg ml^{-1} for THP and 10–50 μg ml^{-1} for EPH. The second method is based on high-performance liquid chromatography on a reversed-phase column using a mobile phase of methanol–water (40 + 60,v/v) (pH 3) with detection at 217 nm. Linearity was obtained in the concentration range of 5–150 μg ml^{-1} for THP and 15–75 μg ml^{-1} for EPH. The detection limits for THP and EPH were 0.73 and 0.92 μg ml^{-1} by ratio-spectra derivative spectrophotometry and 0.59 and 0.86 μg ml^{-1} by HPLC, respectively. The proposed methods were successfully applied to the determination of these drugs in laboratory-prepared mixtures and in tablets. The relative standard deviations were found to be less than 1.5%, indicating reasonable repeatibility of both methods.

Keywords: Theophylline and ephedrine hydrochloride determination; Ratio-spectra derivative spectrophotometry; High-performance liquid chromatography; Pharmaceutical formulation

1. Introduction

Theophylline (THP), a member of xanthine-based alkaloids, which relaxes smooth muscles and relieves broncospasm, has a stimulant effect on respiration. Ephedrine hydrochloride (EPH), a sympathomimetic agent, is used as an expectoran. The association of these drugs might produce a synergy effect in the therapy. THP has been marketed in combination with EPH in pharmaceutical formulations for being used in the symptomatic treatment of bronchial asthma and other bronchospastic conditions [1,2].

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Several methods have been described for the quantitative determination of THP and EPH in dosage forms containing these drugs either alone or in combination with other active ingredients, including high-performance liquid chromatography [3–10], gas chromatography [11–14], micellar electrokinetic capillary chromatography [9,15,16], high-performance thin layer chromatography [17,18], densitometry [19], potentiometry using ion-selective membran electrode [20–22], and spectropotometry [4,23–29].

Although many methods exist for the assays of both drugs as seen above, only a few procedures deal with the determination THP and EPH in presence of each other. Most of them, which include liquid chromatography [30,31], CPA-matrix UV-spectrophotometry [32], capillary electrophoresis [33], involve the assay of both drugs in multicomponent mixtures with other drugs, such as phenobarbital, amobarbital, papaverine and hydroxyzine. Recently, differential-derivative spectrophotometry has been published, which allows specifically the determination of THP and EPH together in their dosage forms [34]. Pharmacopoeial method involves the HPLC determination of both drugs, including conbination with phenobarbital in tablets [35].

One of the classic analytical problem of spectrophotometric multicomponent analysis is that the analyte of interest is often accompanied by other compounds absorbing in the same spectral region. Under computer-controlled instrumentation, derivative spectrophotometry are playing a very important role in the resolution of band overlapping in quantitative analysis. However, for binary mixtures, the classical zero-crossing method requires often to use a wavelength with low sensitivity in the measurements. Salinas et al. [36] designed a new spectrophotometric method, which is based on the derivation of the ratio-spectra for resolving binary mixtures. The main advantage of the ratio-spectra derivative spectrophotometry is the chance of doing easy measurements in correspondence of peaks so it permits the use of the wavelength of highest value of analytical signals (a maximum or a minimum) [37–39]. Moreover, the presence of a lot of maxima and minima is another advantage by the fact that these wavelengths give an opportunity for the determination of active compounds in the presence of other compounds and excipients which possibly interfere the assay.

The present work is a continuation of the authors' research [40–44] on the possibility of the application of ratio-spectra derivative spectrophotometry in the analysis of multi-component systems, allowing an increase in the selectivity of spectrophotometric determination. This ratio-spectra derivative method has been applied to the determination of THP and EPH in both pure forms and pharmaceutical tablets. As a comparison method, a reversed-phase high performance liquid chromatography (RP-HPLC) has also been developed since chromatographic techniques have been mainly used for the analysis of multicomponent mixtures in pharmaceutical preparations reported in pharmacopoeias.

2. Experimental

2.1. Materials

THP and EPH were kindly donated by Carlo Erba Pharm. Ind. (Turkey) and were used as received. Methanol (Merck) was of HPLC grade; water was doubly distilled. All other chemicals were of analytical-reagent grade.

The two analytes in solution did not show any appreciable change in assay values for both techniques for at least 24 h.

2.2. Apparatus

Spectrophotometric analysis was carried out on a Shimadzu 1601 double beam UV–vis spectrophotometer with a fixed slit width (2 nm) connected to an IBM-PC computer loaded with Shimadzu UVPC software, and equipped with a Lexmark 1020 model printer.

A liquid chromatographic system consisting of a Waters Isocratic LC pump 510, with an automatic sample injection system (Waters 717 plus Autosampler), equipped with a Waters 996 photodiode dedector was used.

2.3. Procedures for ratio-spectra derivative spectrophotometry

2.3.1. Calibration

Stock solutions of 1 mg ml^{-1} of the drugs were prepared in methanol. Standard solutions of THP and EPH containing concentration ranges of 20–180 μg ml^{-1} and 10–50 μg ml^{-1} were prepared in methanol, respectively.

The absorption spectra of the binary mixtures prepared at different concentrations of THP were recorded and stored IBM PC computer. According to the theory of the ratio-spectra derivative method [36], the stored spectra of the mixtures were divided, wavelength by wavelength, by a standard spectrum of EPH solution (25 μg ml^{-1} in methanol). Then, the first derivatives of the above ratio-spectra calculated with $\Delta\lambda = 8$ nm, were recorded. In the binary mixtures, we can determine the amount of THP by measuring the first derivative signals at suitably selected wavelengths in the range of 220–260 nm and plotting against the corresponding concentration to obtain the calibration graph.

The similar procedure was followed for the different concentrations of EPH when THP was 100 μg ml^{-1}. In the same way as described above, the calibration curve was obtained by plotting versus the drug concentration, the signal in the first derivative of ratio spectrum between 220 and 280 nm.

2.3.2. Analysis of tablets

An accurately weighed portion of the powder (mixed contents of 20 tablets) corresponding to one tablet, was dissolved in 50 ml methanol and extracted by shaking mechanically for 10 min. The resulting suspension was filtered by washing through Whatman filter paper No.42 into a 100 ml calibrated flask and then diluted to volume with methanol (solution A). Further dilutions were made using the same solvent.

2.4. Procedure for HPLC

2.4.1. Chromatographic conditions

Chromatographic separation was performed on a Bondopak C_{18} reverse phase column packed with 10 μm dimethyl octadecylsilyl bonded amorphous silica (300 × 3.9 mm). The mobile phase consisted of a mixture of methanol + water (40 + 60, v/v) with the pH of the water adjusted to three with 10% v/v *o*-phosphoric acid. All analysis were done under isocratic conditions at a flow rate of 1.2 ml min^{-1} and the effluent was monitored by UV measurements at 217 nm. All solutions were filtered through 0.45 μm milipore filter to use and degassed in an ultrasonic bath. 20 μl of each solutions was injected and chromatograms were recorded.

2.4.2. Calibration

Stock solutions were prepared by dissolving THP and EPH in methanol to obtain a concentration of 5 and 1 mg ml^{-1}, respectively. The standard solutions of THP and EPH containing a fixed concentration (10 μg ml^{-1}) of sulfamethoxazole (internal standard) were prepared in mobile phase by varying the concentrations in the range of 5–150 and 15–75 μg ml^{-1}, respectively. Triplicate 20 μl injections were made for each solution and the peak area ratio of each drug to the internal standard was plotted against the corresponding concentration to obtain calibration graph.

2.4.3. Analysis of tablets

Further dilutions of solution A were made with mobile phase, adding an appropriate amount of internal standard.

3. Results and discussion

3.1. Ratio-spectra derivative spectrophotometric method

The absorption (zero-order) UV spectra of THP, EPH and their mixture in the 220–300 nm wavelength region are shown in Fig. 1. The extensive overlap of the spectral bands of the two drugs prevents the use of the conventional UV spectrophotometry for assaying binary mixtures.

Fig. 2 shows the ratio-spectra of different amounts of THP (spectra divided by the standard spectrum of a 25 μg ml^{-1} solution of EPH) and

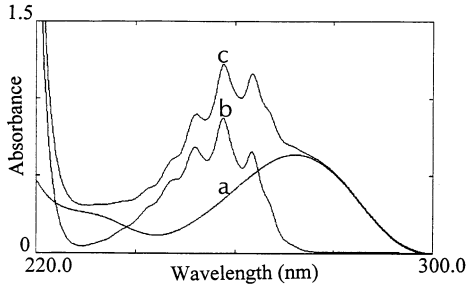

Fig. 1. Zero-order spectra of (a) 100.0 μg ml^{-1} THP (b) 40.0 μg ml^{-1} EPH and (c) their mixture in methanol.

their first derivatives. The first derivative amplitudes at 231.8 nm corresponding to a minimum are proportional to the TPH concentration.

For determining the other component, EPH, an analogous procedure was followed. Fig. 3 shows the ratio-spectra of different standard solutions of EPH and their first derivatives, using the spectrum of a 100 μg ml^{-1} solution of THP as the divisor. The concentration of EPH is proportional to the amplitude of the maximum at 250.3 nm

Statistical analysis of the regression equations is reported in Table 1.

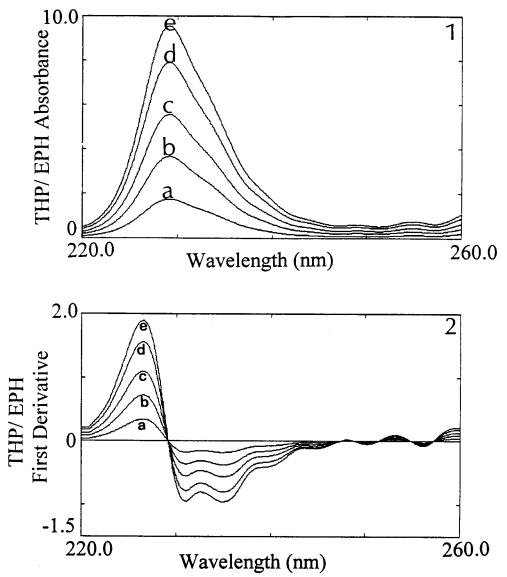

Fig. 2. Ratio spectra (1) and first derivatives (2) of THP of (a) 20.0 μg ml^{-1} (b) 60.0 μg ml^{-1} (c) 100 μg ml^{-1} (d) 140.0 μg ml^{-1} (e) 180.0 μg ml^{-1}, using a 25.0 μg ml^{-1} EPH as divisor.

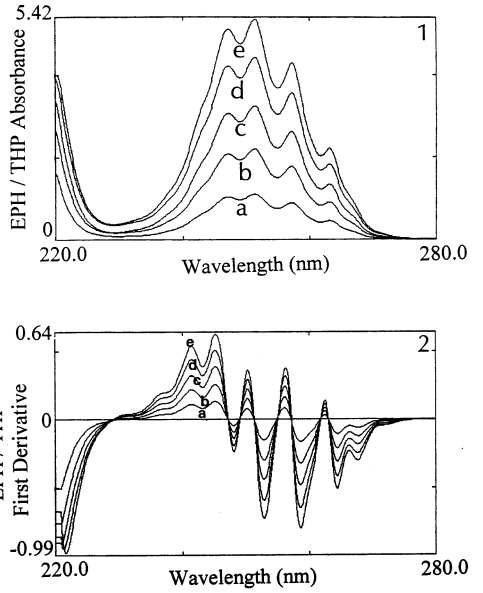

Fig. 3. Ratio spectra (1) and first derivatives (2) of EPH of (a) 10.0 μg ml^{-1} (b) 20.0 μg ml^{-1} (c) 30.0 μg ml^{-1} (d) 40.0 μg ml^{-1} (e) 50.0 μg ml^{-1}, using a 100.0 μg ml^{-1} THP as divisor.

The ratio-spectra derivative method permits the use of the different concentrations as the divisor to obtain the different calibration graphs. In a preliminary investigation, for selecting the standard solution as divisor at an appropriate concentration, concentrations of EPH and TPH in the range 10–50 and 20–180 μg ml^{-1} were tested, respectively. A concentration of 25 μg ml^{-1} of EPH and 100 μg ml^{-1} of TPH as divisor gave best results in terms of signal-to-noise ratio and highest correlation coefficient values, being an indication of the quality of fitting of the data to the straight line.

A value of $\Delta\lambda = 8$ nm, i.e. the width of the boundaries over which the derivative is calculated, was found optimal in connection with both slit width and wavelength interval.

3.2. Chromatographic method

The reversed-phase HPLC method was developed to provide a specific procedure suitable for the rapid quality control analysis of binary mixtures containing TPH and EPH, and as referee

Table 1
Statistical analysis for the calibration graphs of THP and EPH in mixtures by use of the first derivative ratio spectra and HPLC methods

Method	Analyte	Wavelength (nm)	Linearity range (μg ml^{-1})	Regression equation[a]	Correlation coefficient	RSD of slope	RSD of intercept	Detection limit (μg ml^{-1})	Quantitation limit (μg ml^{-1})
Ratio spectra derivative	THP	231.8	20–180	$Y = 1.89C + 0.936$	0.999	0.45	0.12	0.73	2.43
	EPH	250.3	10–50	$Y = 0.79C + 0.998$	0.999	0.97	0.75	0.92	3.07
	THP	217.0	5–150	$Y = 0.026C + 0.048$	0.997	0.78	0.55	0.59	1.97
HPLC	EPH	217.0	15–75	$Y = 0.012C - 0.1343$	0.997	0.83	0.12	0.86	2.92

[a] Where C is the concentration of the analyte (μg ml^{-1}).

Table 2
Within- and between-day precision of THP and EPH standards by using HPLC method

Theoretical concentration (μg ml^{-1})	THP				EPH			
	Within-day measured concentration (μg ml^{-1})[a]		Between-day measured concentration (μg ml^{-1})[b]		Within-day measured concentration (μg ml^{-1})[a]		Between-day measured concentration (μgml^{-1})[b]	
	Mean	R.S.D.%	Mean	R.S.D.%	Mean	R.S.D.%	Mean	R.S.D.%
10	9.98	0.087	9.96	0.20	–	–	–	–
50	49.94	0.47	49.86	0.18	–	–	–	–
15	–	–	–	–	14.96	0.18	14.90	0.36
25	–	–	–	–	25.05	0.28	25.03	0.28

[a] Mean values represent five different standard solutions for each concentration.
[b] Between-day reproducibility was determined from five different runs over a 2 weeks period.

method for the ratio-spectra derivative assay. Chromatographic investigations revealed that a mixture of THP and EPH could be resolved from the coformulated excipients using C_{18} stationary phase. In order to use the similar sample that in the spectrophotometric analysis, the mobile phase was chosen as methanol–water mixture. After several trials in various proportions and different pH values, a satisfactory separation was obtained with a mobile phase consisting of methanol-water (40 + 60, v/v) (pH 3). The optimum wavelength for detection was 217 nm, at which much better detector response for both drugs was obtained. This chromatographic system allowed complete base line separation with a resolution factor of 1.9 between adjacent peaks. Selectivity factor for this system was found 1.76. Tailing factors were obtained as 1.69 for EPH and 1.50 for THP. Under the described chromatographic conditions, the analyte peaks were well defined, resolved and almost free from tailing. At a flow rate of 1.2 ml min^{-1}, the retention times for EPH, THP and sulfamethoxazole (internal standard) were 2.76, 4.04 and 4.96 min, respectively. Fig. 4 shows a typical chromatogram of extracts from pharmaceutical tablet form. As shown in the figure, no interfering peaks were found in the chromatogram due to tablet excipients. The analytical data for the calibration graphs are listed in Table 1.

Table 2 represents the results obtained for intra- and inter- day variability studies of THP and EPH samples. These results show the accuracy and reproducibility of the assay. Thus, it was concluded that there was no significant difference for the assay, which was tested within day and between days.

3.3. Analysis of tablets

In order to assess the validity and applicability of the proposed methods (ratio-spectra derivative and HPLC), recovery studies were performed by analyzing laboratory-made mixtures with different proportions of the two drugs. The results obtained (Table 3) were statistically compared using Student's t-test. As shown from the Table, the calculated t values were less than theoretical value, indicating no significant difference between the two methods. Such results encourage the use of the methods described for the assay of THP and EPH in commercial tablets. As far as we know, no official method is described in pharmacopeias related to the pharmaceutical dosage forms of TPH–EPH mixture, except their ternary mixture in combination with phenobarbital [35]. For this reason, the differential-derivative spectrophotometric method based on pH changes [34], which was recently performed in our laboratory, was chosen as an reference method. In comparison with the literature spectrophotometric method, higher sensitivity in the measurements and no need to work only at zero-crossing points were advantages for the proposed ratio-spectra derivative method.

Table 3 compares the results between proposed and literature methods. For tablet formulation

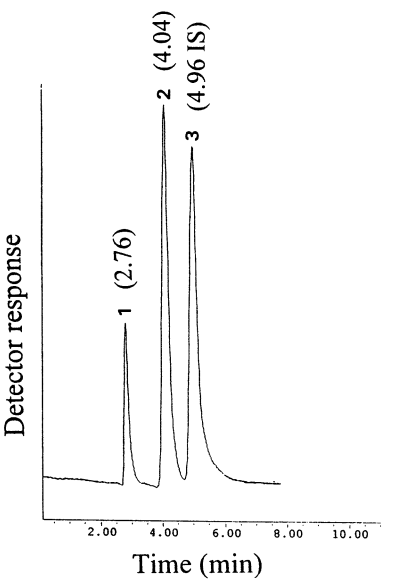

Fig. 4. Chromatogram of a diluted solution of tablet formulation containing THP (1) and EPH (2) with internal standard (IS) (3).

Table 3
Assay results of THP and EPH in laboratory-made mixtures and commercial tablets using ratio spectra derivative and HPLC methods

Preparation	Recovery (mean ± S.D.)[a] (%)					
	THP			EPH		
	Literature method [34]	Ratio spectra derivative	HPLC	Literature method [34]	Ratio spectra derivative	HPLC
Laboratory-made mixtures		99.88 ± 0.52 $t = 1.12$ (2.31)[c]	99.60 ± 0.42		99.68 ± 1.21 $t = 1.21$ (2.31)[c]	100.0 ± 0.6
Commercial[b] tablets	99.28 ± 1.14 ($t_{theo.}$: 2.31)[c] ($F_{theo.}$: 2.60)[c]	99.47 ± 0.65 $t = 1.07$ $F = 0.29$	99.84 ± 0.25 $t = 0.33$ $F = 0.15$	99.0 ± 0.51 ($t_{theo.}$: 2.31)[c] ($F_{theo.}$: 2.60)[c]	99.12 ± 0.91 $t = 0.26$ $F = 0.31$	99.84 ± 1.15 $t = 1.49$ $F = 0.01$

[a] Mean and standard deviation for five determinations; percentage recovery from the label claim amount.
[b] Each tablets was labeled to contain 125 and 25 mg of theophylline and ephedrine hydrochloride, respectively.
[c] Theoretical values for t and F at $P = 0.05$ level.

examined the assay results were in good agreement with the declared content. The precision of compared spectrophotometric method is more for EPH, whereas its precision is less for THP than proposed ratio-spectra derivative and HPLC methods.

According to the Student's t- and variance ratio F-test, the calculated (experimental) t and F values were less than the tabulated (theoretical) values in either test at the 95% confidence level. This indicates that there is no significant difference between the performance of the literature and proposed methods as regards to mean values and standard deviations (Table 3). Moreover, high percentage recovery data shows that the methods are free from the interferences of the excipients used in the formulation.

4. Conclusions

In this study, HPLC method was especially used as a versatile reference method and could be also satisfactory for biological fluids since its high separation power. The applicability of this method to serum is under investigated. However, this chromatographic technique is in need of longer analysis time and requires expensive equipment and materials such as column and significant amount of HPLC grade organic solvent.

As a result, the ratio-spectra derivative method is proposed for rapid, simple, cheap and sensitive technique for the reliable analysis of THP and EPH in simple multi component formulations and in the presence of any interferences due to the matrix formulation, and is easily applied for routine use.

Since the aim of this study was not to set up stability indicating power of the methods, there are no data presented with such intention.

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