

# Simultaneous spectrophotometric determination of chlorphenoxamine hydrochloride and caffeine in a pharmaceutical preparation using first derivative of the ratio spectra and chemometric methods

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## Abstract

Three new methods are described for the simultaneous determination of chlorphenoxamine hydrochloride (CP) and caffeine (CAF) in their combination. In the first method, ratio spectra derivative spectrophotometry, analytical signals were measured at the wavelengths corresponding to either maxima and minima for both drugs in the first derivative spectra of the ratio spectra obtained by using each other spectra as divisor in their solution in 0.1 M HCl. In the other two methods, chemometric techniques, classical least-squares (CLS) and inverse least-squares (ILS), the concentration data matrix were prepared by using the synthetic mixtures containing these drugs in 0.1 M HCl. The absorbance data matrix corresponding to the concentration data matrix was obtained by the measurements of absorbances in the range 225–285 nm in the intervals with  $\Delta\lambda = 5$  nm at 13 wavelengths in their zero-order spectra, then, calibration or regression was obtained by using the absorbance data matrix and concentration data matrix for the prediction of the unknown concentrations of CP and CAF in their mixture. The numerical values were calculated by using MAPLE V software in chemometric methods. The procedures do not require any separation step. The accuracy and the precision of the methods have been determined and they have been validated by analyzing synthetic mixtures containing title drugs. These three methods were successfully applied to a pharmaceutical formulation, sugar-coated tablet, and the results were compared with each other. © 2002 Elsevier Science B.V. All rights reserved.

**Keywords:** Chlorphenoxamine hydrochloride; Caffeine; Ratio spectra derivative spectrophotometry; Chemometric methods; Pharmaceutical preparation

## 1. Introduction

Combination of chlorphenoxamine hydrochloride (CP) with Caffeine (CAF) is frequently prescribed in medicine as an antihistaminic drug.

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Various methods including spectrophotometry [1–4], TLC densitometry [5] and atomic absorption spectrometry [6] have been used for the determination of CP and CAF in pharmaceutical preparations containing CP + CAF mixture.

Salinas et al. [7], developed a new method for analysis of mixtures with overlapped spectra. Salinas's method is based on the use of the first derivative of the ratio spectra. In this method, the concentrations of active compounds were determined by measuring the amplitudes of the minimum or maximum at points corresponding to the selected wavelengths. Berzas Nevado et al. [8–11] and Dinç and Onur [12–17] applied the same method to determine the active compounds in different mixtures.

Chemometric calibration techniques can be summarized as multiple linear regression (MLR; classical least-squares (CLS) and ILS calibrations), principal component regression (PCR) and partial least regression techniques [18–23]. Chemometric quantitative calibration techniques in spectral analysis is gaining importance in the quality control of drugs in mixtures and pharmaceutical formulations containing two or more drugs with overlapping spectra, due to no need of separation procedure in the drug determinations. In addition, these techniques can be successfully applied to all the analysis methods. Dinç and Onur used these techniques for the simultaneous analysis of a binary and a ternary mixtures [24,25].

In this study, ratio spectra derivative spectrophotometry and two chemometric methods are proposed for the simultaneous determination of CP and CAF in their mixtures and pharmaceutical preparation, sugar-coated tablet.

## 2. Chemometric methods

### 2.1. Classical least-squares (CLS)

CLS involve the application of multiple linear regression (MLR) to the classical expression of the Beer–Lambert law of spectroscopy:

$$A = K \times C$$

This equation is a matrix equation and it can be written as a linear equation system:

$$A_1 = K_{11}C_1 + K_{12}C_2 + \dots\dots\dots K_{1c}C_c$$

$$A_2 = K_{21}C_1 + K_{22}C_2 + \dots\dots\dots K_{2c}C_c$$

$$A_3 = K_{31}C_1 + K_{32}C_2 + \dots\dots\dots K_{3c}C_c$$

... ..

$$A_w = K_{w1}C_1 + K_{w2}C_2 + \dots\dots\dots K_{wc}C_c$$

where,  $A_w$  is the absorbance at the  $w$ th wavelength;  $K_{wc}$  is the calibration coefficient for the  $c$ th component at the  $w$ th wavelength;  $C_c$  is the concentration of the  $c$ th component.

### 2.2. Inverse least-squares (ILS)

ILS also involve the application of MLR to the inverse expression of the Beer–Lambert law of spectroscopy:

$$C = P \times A$$

This equation can be written as a linear equation system:

$$C_1 = P_{11}A_1 + P_{12}A_2 + \dots\dots\dots P_{1w}A_w$$

$$C_2 = P_{21}A_1 + P_{22}A_2 + \dots\dots\dots P_{2w}A_w$$

$$C_3 = P_{31}A_1 + P_{32}A_2 + \dots\dots\dots P_{3w}A_w$$

... ..

$$C_c = P_{c1}A_1 + P_{c2}A_2 + \dots\dots\dots P_{cw}A_w$$

where,  $A_w$  is the absorbance at the  $w$ th wavelength;  $P_{cw}$  is the calibration coefficient for the  $c$ th component at the  $w$ th wavelength;  $C_c$  is the concentration of the  $c$ th component.

## 3. Experimental

### 3.1. Apparatus

Shimadzu 1601 PC double beam spectrophotometer with a fixed slit width (2 nm) connected to a computer loaded with Shimadzu UVPC was used for all the spectrophotometric measurements.

In ratio spectra derivative spectrophotometry, range was selected as 200.0–310.0 nm ( $\Delta\lambda = 4$  nm) for reading the analytical signals. The ordinate maximum and minimum settings were (+ 0.35)–

(−0.40) for CP in 205.0–260.0 nm range and (+5.0)–(−8.0) in 220.0–310.0 nm range for CAF in their mixture.

### 3.2. Materials

CP and CAF were kindly donated by Ibrahim Ethem Pharm. Ind., Turkey and used without further purification.

All the solvents used in spectrophotometric analysis were of analytical reagent grade.

### 3.3. Standard solutions

Solutions of 100 mg/100 ml of CP and 100 mg/100 ml CAF were prepared, respectively, in 0.1 M HCl.

### 3.4. Sample preparation

Twenty sugar-coated tablets were accurately weighed and powdered in a mortar. An amount of the tablet mass equivalent to one tablet content was dissolved in 60 ml of 0.1 M HCl. After 30 min of mechanically shaking the solution was filtered in a 100 ml volumetric flask. The residue was washed three times with 10 ml solvent then the volume was completed to 100 ml with the same solvent. This solution was diluted 1:20 with 0.1 M HCl. All the spectrophotometric methods were applied to the latest diluted solution.

### 3.5. Commercial pharmaceutical preparation

Systral-C® (50 mg CAF and 20 mg CP/sugar-coated tablet) Ibrahim Ethem Pharm. Ind., Turkey (batch no: 6L 7198) was assayed.

## 4. Results and discussion

### 4.1. Ratio spectra first derivative spectrophotometry

The ratio spectra of different CP standards at increasing concentrations in 0.1 M HCl obtained by dividing each with the stored spectrum of the standard solution of CAF by computer aid are

shown in Figs. 1 and 2a and the first derivative of these spectra (<sup>1</sup>DD) traced with the interval of  $\Delta\lambda = 8$  nm are illustrated in Fig. 2b. As seen in Fig. 2b, there exist one maximum (218.3 nm) and one minimum (229.1 nm) and we found that both of them are suitable for the determination of CP in CP + CAF mixture. We selected 229.1 nm for the determination of this compound in the assay of synthetically prepared pharmaceutical preparation, tablet, due to its lower R.S.D. value and more suitable mean recovery among the wavelengths mentioned (Table 1). The ratio and ratio derivative spectra of the solutions of CAF in different concentrations in 0.1 M HCl traced with the interval of  $\Delta\lambda = 8$  nm by using the standard spectrum of CP as divisor by computer aid is demonstrated in Fig. 3a and b, respectively.

In these spectra, two maxima (272.2 and 278.5 nm) and one minimum (288.5 nm) were found suitable for the quantification of CAF in CP + CAF mixture. Measured analytical signals at these wavelengths are proportional to the concentrations of the drugs. We selected 288.5 nm for the determination of this compound in the assay of pharmaceutical preparation, sugar-coated tablet, due to its lower R.S.D. value and suitable mean recovery among the wavelengths mentioned (Table 1).

Calibration graphs were established from analytical signals measured at 218.3 and 229.1 nm for standards containing 8–48 µg/ml of CP and at 272.2, 278.5 and 288.5 nm for standards containing 8–48 µg/ml CAF corresponding to maxima and minima in the absence of each other.

In the method, the mean recoveries and relative standard deviations calculated for synthetic mixtures prepared in our laboratory are illustrated in Table 1. Also, Beer's law compliance for both compounds, the regression equations and correlation coefficients were summarized in Table 2. Mean recoveries and R.S.D.s of the method were found satisfactory.

Divisor concentration is main instrumental parameter. The standard spectra of 12.0 µg/ml of CAF and 24.0 µg/ml of CP was considered as suitable for the determination of CP and CAF, respectively, as divisor. The  $\Delta\lambda$  found as optimum for the first derivative of their ratio spectra was 8 nm.

A critical evaluation of all the proposed methods was performed by statistical analysis of the data, where slopes, intercepts and correlation coefficients were shown in Table 2.

Summary of the assay results for commercial preparation were shown in Table 6. The results of two chemometric methods and ratio spectra derivative spectrophotometry developed by us for the same commercial formulation were compared by Student's *t*-test. The calculated (experimental) *t*-values did not exceed the tabulated (theoretical) values in the test, indicating that there was no significant difference between the methods compared.

#### 4.2. Chemometric techniques

Fig. 1 shows the zero-order absorption spectra for CP and CAF and their binary mixture in 0.1 M HCl. For two techniques, the absorbance data

matrix for the training set concentration matrix (Table 3) were obtained by the measurements of absorbances between 225.0 and 285.0 nm in the intervals with  $\Delta\lambda = 5$  nm at 13 wavelengths in the zero-order absorption spectra. In the techniques, calibration or regression was obtained by using the absorbance data matrix and concentration data matrix for prediction of the unknown concentrations of CP and CAF in their binary mixtures and pharmaceutical formulations.

The predictive ability of a model can be defined in various ways. The most general expression is the standard error of prediction (SEP) which is given the following equation:

$$SEP = \sqrt{\frac{\sum_{i=1}^N (C_i^{\text{Added}} - C_i^{\text{Found}})^2}{n}}$$

where  $C_i^{\text{Added}}$  is the added concentration of drug,

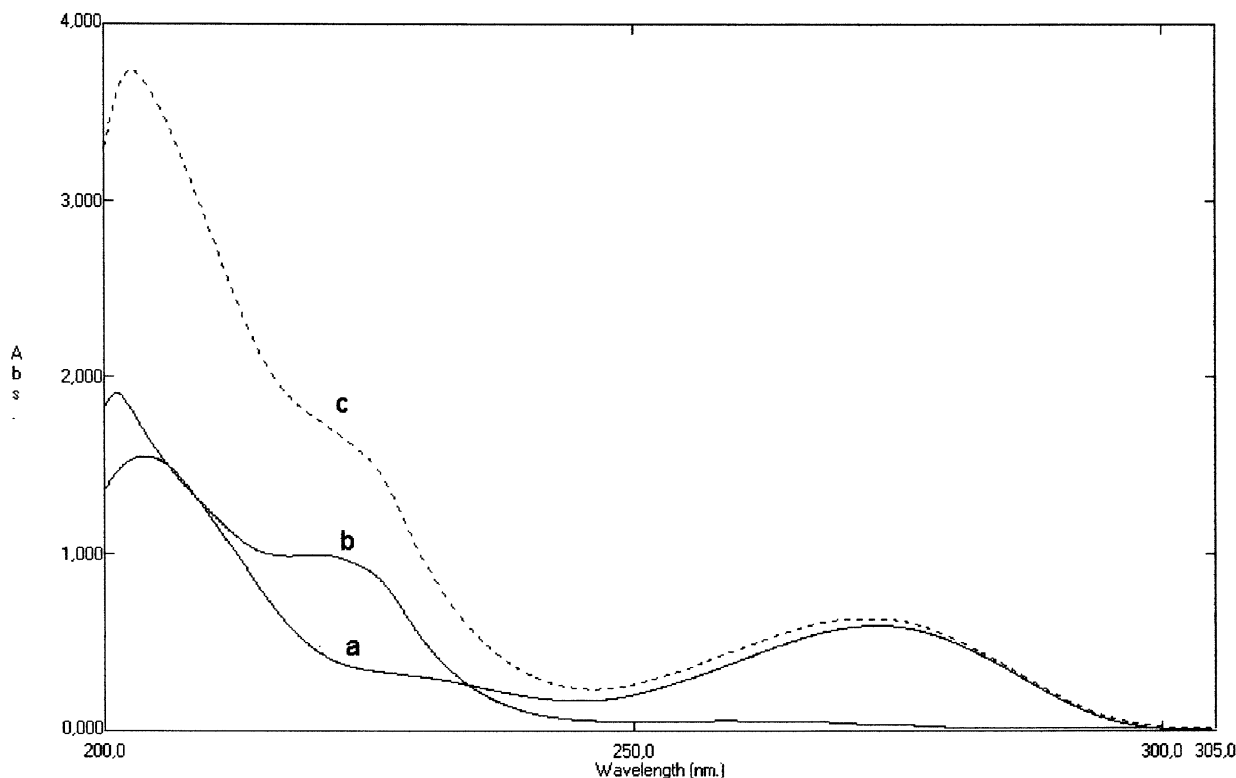


Fig. 1. Zero-order absorption spectra of (a) 24 µg/ml solution of chlorphenoxamine hydrochloride, (b) 20 µg/ml solution of caffeine in 0.1 M HCl, (c) their mixture.

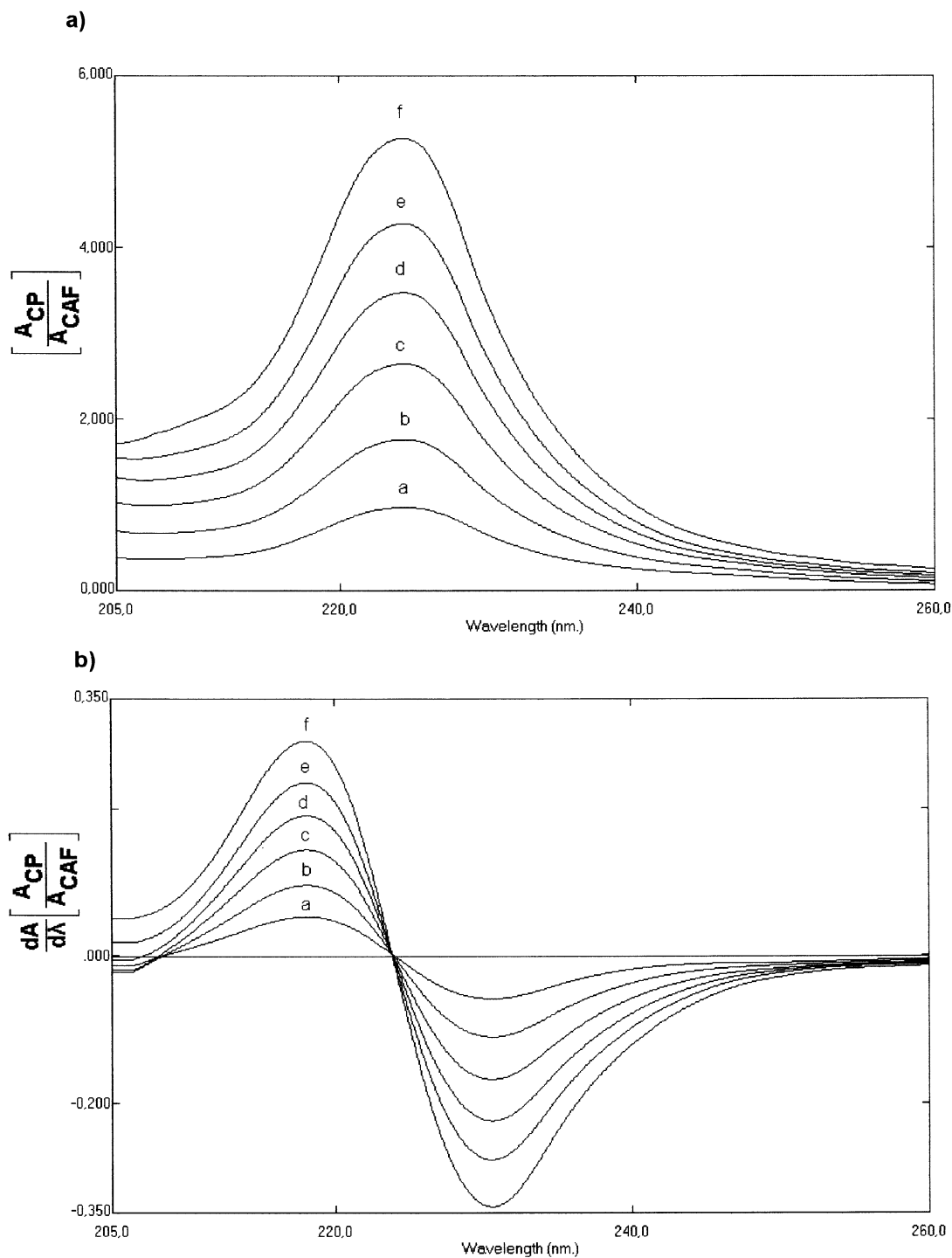


Fig. 2. Ratio spectra (a) and first derivative of the ratio spectra (b) of (a) 8  $\mu\text{g/ml}$ , (b) 16  $\mu\text{g/ml}$ , (c) 24  $\mu\text{g/ml}$ , (d) 32  $\mu\text{g/ml}$ , (e) 40  $\mu\text{g/ml}$ , (f) 48  $\mu\text{g/ml}$  solution of chlorphenoxamine hydrochloride in 0.1 M HCl when 12  $\mu\text{g/ml}$  solution of caffeine in 0.1 M HCl used as divisor ( $\Delta\lambda = 8$  nm).

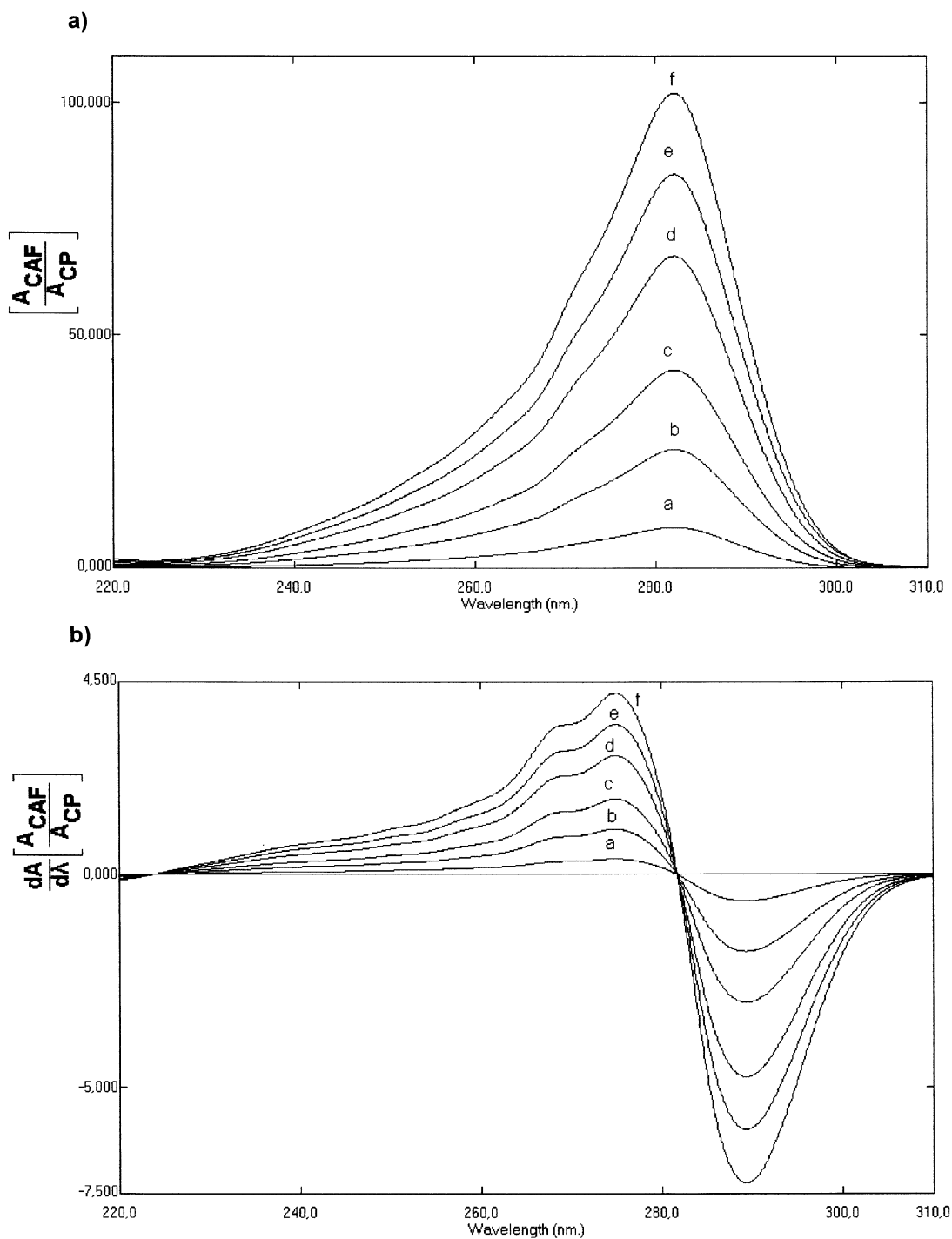


Table 1  
Recovery results for CP and CAF in synthetic mixtures by ratio spectra first derivative spectrophotometry

Mixture number	CP			CAF			
	Added ( $\mu\text{g}$ )	Recovery (%)		Added ( $\mu\text{g}$ )	Recovery (%)		
		218.3 nm	229.1 nm		272.2 nm	278.5 nm	288.5 nm
1	8	102.3	98.5	20	100.7	101.8	100.8
2	16	100.0	101.7	20	103.0	102.0	98.3
3	20	104.8	98.5	20	102.0	98.8	97.9
4	24	96.0	98.5	20	102.0	102.5	100.8
5	28	100.1	101.9	20	101.9	98.9	98.8
6	32	100.1	99.3	20	102.5	101.9	100.0
7	40	101.4	100.9	20	100.9	103.7	100.8
8	48	99.8	98.5	20	98.5	102.0	98.3
9	12	103.8	97.5	8	102.5	102.5	103.1
10	12	102.3	102.0	16	101.9	98.9	101.2
11	12	98.0	99.0	20	100.0	103.0	97.5
12	12	102.4	99.0	24	102.5	101.8	98.5
13	12	103.8	98.8	28	101.9	101.9	100.4
14	12	96.0	97.5	32	97.4	98.4	99.1
15	12	102.3	102.0	40	102.5	101.8	100.3
16	12	99.7	99.0	48	101.0	102.0	99.0
$n = 16$	$\bar{x}$	100.8	99.5		101.3	101.4	99.7
	R.S.D.	2.60	1.60		1.53	1.62	1.49

R.S.D., relative standard deviation.

Table 2  
Beer's law data and statistical analysis for the calibration graphs of CP and CAF using ratio spectra derivative spectrophotometric procedures

Compounds	$\lambda$ (nm)	Regression equations		$r$	Concentration range ( $\mu\text{g}/\text{ml}$ )
		$a$ (S.E.)	$b$ (S.E.)		
CP	218.3	$4.80 \times 10^{-2}$ ( $6.00 \times 10^{-4}$ )	$4.48 \times 10^{-3}$ ( $2.00 \times 10^{-4}$ )	0.9996	8.0–48.0
CP	229.1	$-4.45 \times 10^{-3}$ ( $9.60 \times 10^{-4}$ )	$3.73 \times 10^{-4}$ ( $3.26 \times 10^{-5}$ )	0.9996	8.0–48.0
CAF	272.2	$9.21 \times 10^{-2}$ ( $1.80 \times 10^{-3}$ )	$-1.95 \times 10^{-2}$ ( $3.60 \times 10^{-3}$ )	0.9990	8.0–48.0
CAF	278.5	$1.10 \times 10^{-1}$ ( $4.90 \times 10^{-2}$ )	$1.58 \times 10^{-1}$ ( $9.90 \times 10^{-3}$ )	0.9994	8.0–48.0
CAF	288.5	$-1.67 \times 10^{-1}$ ( $4.90 \times 10^{-3}$ )	$3.80 \times 10^{-2}$ ( $9.90 \times 10^{-3}$ )	0.9999	8.0–48.0

$a$ , slope;  $b$ , intercept;  $r$ , correlation coefficient; S.E., standard error.

$C_i^{\text{Found}}$  is the predicted concentration of drug and  $n$  is the total number of synthetic mixtures.

In order to test the proposed techniques, the sets of synthetic mixtures containing the two drugs in variable composition were prepared. The results obtained in the application of CLS and ILS methods to the same binary mixture are indicated in

Tables 3 and 4. The SEP were completely acceptable (0.28 and 0.59 for CP and 0.48 and 0.40 for CAF, respectively, for CLS and ILS methods) (Table 4).

In Table 4,  $r$  is defined as the correlation between constituent concentrations and shows the absorbance effects relating to the constituent of

interest. The obtained  $r$  values in the methods are close to 1, this means no interference is coming from the other constituents in this set of synthetic mixtures.

Another statistical value is the standard error of calibration (SEC) and the calculation of this value was realized by using following equation:

$$SEC = \sqrt{\frac{\sum_{i=1}^N (C_i^{\text{Added}} - C_i^{\text{Found}})^2}{n - p - 1}}$$

where  $C_i^{\text{Added}}$  is the added concentration of drug,

$C_i^{\text{Found}}$  is the predicted concentration of drug and  $n$  is the total number of synthetic mixtures,  $p$  is the number of components in the mixtures.

The SEC were found acceptable in CLS and ILS methods (0.31 and 0.65 for CP and 0.53 and 0.44 for CAF), respectively, (Table 4) in the synthetic mixtures containing these two drugs in variable compositions prepared as indicated in Table 3.

Mean recoveries and R.S.D.s for the CLS and ILS techniques were found as 101.4, 1.40, 101.8 and 1.79%, for CP and 99.1, 1.68%, 99.2 and 1.56% for CAF, respectively, in the synthetic mixtures of both drugs (Table 3).

Table 3

Results obtained for the determination of CP and CAF in synthetic mixtures by using classical least-squares and inverse least-squares techniques

Mixture		Classical least-squares technique				Inverse least-squares technique			
Added ( $\mu\text{g}$ )		Found ( $\mu\text{g}$ )		Recovery (%)		Found ( $\mu\text{g}$ )		Recovery (%)	
CAF	CP	CAF	CP	CAF	CP	CAF	CP	CAF	CP
4.0	12.0	4.0	12.0	100.0	100.0	4.0	12.6	100.0	105.0
12.0	12.0	12.0	11.7	100.0	97.5	12.0	11.7	100.0	97.5
16.0	12.0	15.9	12.3	99.4	102.5	15.9	12.3	99.4	102.5
20.0	12.0	19.4	12.2	97.0	101.7	19.4	12.2	97.0	101.6
24.0	12.0	23.8	12.1	98.1	100.8	23.8	12.0	99.2	100.0
28.0	12.0	27.4	12.2	97.9	101.7	27.4	12.2	99.9	101.6
32.0	12.0	33.0	12.1	103.1	100.8	32.3	12.1	101.0	100.8
40.0	12.0	39.1	12.2	97.8	101.7	39.1	12.2	97.8	101.6
20.0	8.0	19.4	8.2	97.0	102.5	19.4	8.1	97.0	101.0
20.0	16.0	19.6	16.4	98.0	102.5	19.6	16.4	98.0	102.5
20.0	20.0	19.6	20.7	98.0	103.5	19.6	20.7	98.8	103.5
20.0	24.0	20.3	24.2	101.5	101.2	20.3	24.2	101.5	101.0
20.0	28.0	19.6	28.6	98.0	102.1	19.7	28.6	98.5	102.5
20.0	32.0	20.1	32.8	100.5	102.5	20.2	33.3	101.0	104.0
20.0	40.0	20.0	40.1	100.0	100.2	20.1	41.2	100.5	103.0
20.0	48.0	19.7	49.0	98.5	102.1	19.8	48.9	99.0	102.0
			$\bar{x}$	99.1	101.4			99.2	101.8
			R.S.D.	1.68	1.40			1.56	1.79

Table 4

Summary of statistics in CLS and ILS methods for CP and CAF in the mixture

	SEP		SEC		$r$		Intercept		Slope	
	CLS	ILS	CLS	ILS	CLS	ILS	CLS	ILS	CLS	ILS
CP	0.28	0.59	0.31	0.65	0.9998	0.9997	-0.043	-0.150	1.017	0.9997
CAF	0.48	0.40	0.53	0.44	0.9984	0.9991	-0.136	-0.148	0.9914	0.9828



Table 5  
ANOVA for the proposed methods

Parameters	Classical least-Squares		Inverse least-squares		Ratio spectra derivative spectrophotometry	
	CAF	CP	CAF	CP	CAF	CP
Between-days variance	0.038	0.208	0.064	0.368	0.069	0.228
Within-days variance	0.106	0.610	0.160	0.365	0.088	0.299
<i>F</i> ratio	0.32	2.33	0.34	2.26	0.68	2.15
Mean value	19.84	12.16	19.95	12.08	19.83	12.39
Between-days R.S.D. (%)	1.73	1.72	1.71	1.82	1.48	1.92
Within-days R.S.D. (%)	1.64	2.10	1.88	1.58	1.49	1.69

Between-day and within-day degrees of freedom 2 and 27, respectively. The critical *F* ratio value for 2 and 27 degrees of freedom and a confidence level of 95% is 3.35.

Table 6  
Assay results of commercial preparation marketed in Turkey (mg/sugar-coated tablet)

Methods	CP (Label claim = 20 mg per tablet)		CAF (Label claim = 50 mg per tablet)	
	Mean ± S.D.**	<i>t</i> values	Mean ± S.D.	<i>t</i> values
Classical least-squares (CLS)	20.0 ± 0.3	CLS – ILS = 0.35	49.9 ± 0.4	CLS – ILS = 0.16
Inverse least-Squares (ILS)	20.0 ± 0.4	ILS – <sup>1</sup> DD = 1.25	49.9 ± 0.4	ILS – <sup>1</sup> DD = 0.99
Ratio spectra derivative spectrophotometry ( <sup>1</sup> DD)	20.1 ± 0.4	<sup>1</sup> DD – CLS = 1.65	49.9 ± 0.7	DD – CLS = 0.99
First derivative spectrophotometry ( <sup>1</sup> D) (4)****	20.0 ± 0.4	<sup>1</sup> D – ILS = 1.37 <sup>1</sup> D – <sup>1</sup> DD = 1.51 <sup>1</sup> D – CLS = 1.08	50.1 ± 0.6	<sup>1</sup> D – ILS = 1.55 <sup>1</sup> D – <sup>1</sup> DD = 1.55 <sup>1</sup> D – CLS = 1.15

\*, Obtained results are average of ten tablets for three techniques; \*\*, S.D., standard deviation; \*\*\*, theoretical value for *t* at *P*: 0.05 level = 2.26; \*\*\*\*, literature method.

Linearity range was 8–48 µg/ml for CP and 4–40 µg/ml for CAF in both chemometric methods.

#### 4.3. Precision

The precision was determined by means of a one-way analysis of variance (ANOVA) including ten replicates carried out on three successive days using ratio spectra derivative spectrophotometry and two chemometric methods (CLS and ILS) for synthetic mixtures. Snedecor *F* values below the tabulated levels were obtained in all cases (*F* = 3.35, *n*<sub>1</sub> = 2, *n*<sub>2</sub> = 27; Table 5).

#### 4.4. Applications

Comparison of the spectra of CP and CAF in standard and drug formulation solutions showed that the wavelength of maximum absorbances in the zero-order spectra did not changed. It has been decided that excipients placed in the commercial preparations selected (lactose, starch, avicel, povidon, sodium dodecylsulfate, aerosil, magnesium stearate and titan dioxide) did not interfere the quantitation of CP and CAF in these methods. All the results obtained by using the methods described above were compared with each other and no significant difference was observed between the amount of drugs found as

theoretical values for  $t$  at  $P = 0.05$  level for commercial formulation (Table 6).

The assay results were also compared with those obtained by using first derivative spectrophotometric method ( $^1D$ ) developed by us[4], for the same pharmaceutical preparation by the fact that not existence of any official method for the analysis of CAF + CP mixture. No significant difference was observed between the methods proposed as  $t$  values calculated (Table 6).

## 5. Conclusion

The proposed methods, ratio spectra derivative spectrophotometry and two chemometric methods could be applied with great success for the simultaneous determination of CP and CAF in mixtures and the pharmaceutical formulation selected containing its binary mixture without interference of each other. Easy measurements on the separate peaks, higher values of analytical signals and no need to work only at zero-crossing points (sometimes co-existing compounds have no maximum or minimum at these wavelengths) is an advantage for ratio spectra derivative spectrophotometry in comparison with the derivative spectrophotometry [2,4]. Derivative ratio method is also an advantageous method by not needing any additional mathematical calculations, not working in different mediums and different measurements in comparison with the methods explained in literatures (derivative spectrophotometry and ion-pair extraction spectrophotometry [1–4]). Satisfactory results were obtained by using chemometric methods but they need a software for the mathematical calculations. Using only zero-order spectra in the procedures and no need for any other mode, such as derivative mode, in the instruments are an advantages for the chemometric methods. These three methods were found suitable for simple and precise routine analysis of the pharmaceutical preparation selected.

Good agreement was seen in the assay results of pharmaceutical preparation, sugar-coated tablet, for all the methods proposed.

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