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SIMULTANEOUS DETERMINATION OF ACTIVE INGREDIENTS IN BINARY MIXTURES CONTAINING CAFFEINE USING LIQUID CHROMATOGRAPHIC AND SPECTROPHOTOMETRIC METHODS

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Abstract

Two new spectrophotometric methods are described for the simultaneous determination of caffeine, aspirin and propyphenazone in binary mixtures containing caffeine without prior separation procedure. In the first method, ratio spectra derivative spectrophotometry, the signals were measured at 254.3 nm for caffeine and at 220.0 nm for aspirin in caffeine - aspirin mixture and at 215.6 nm for caffeine and at 250.6 nm for propyphenazone in caffeine - propyphenazone mixture, in the first derivative of their ratio spectra. In the second method, matrix resolution method, the amounts of the drugs in their binary mixtures containing caffeine were found by the solution of the equations prepared by using A_1^1 (%1, 1 cm) values calculated at selected wavelengths for caffeine, aspirin and proyphenazone in their zero-order spectra and matrix inversion procedure was used for the calculations. The results were compared with those obtained using HPLC method developed by us, for the same combinations. The methods were successfully applied to two pharmaceutical formulations marketed in Turkey.

***Key Words**: Ratio spectra derivative spectrophotometry, matrix resolution method, caffeine, aspirin, propyphenazone, pharmaceutical formulation*

Kafein içeren ikili karışımlardaki etken maddelerin sıvı kromatografisi ve spektrofotometrik yöntemlerle aynı anda miktar tayinleri

Bu çalışmada, kafein içeren ikili karışımlarda kafein, aspirin ve propifenazon' un hiçbir ayırma işlemi gerekmeksizin aynı anda miktar tayinleri için iki spektrofotometrik yöntem geliştirilmiştir. Birinci yöntemde, spektrum oranları türev spektrofotometri, analitik sinyaller karışımların spektrum oranlarının birinci türevlerinde kafein-aspirin karışımında kafein için 254.3 nm de ve aspirin için 220.0 nm de, kafein- propifenazon karışımında kafein için 215.6 nm de ve propifenazon için 250.6 nm de okunmuştur. İkinci yöntemde, matris çözümleme yöntemi, kafeinle ikili karışımlarında kafein, aspirin ve propifenazon'un miktar tayinleri için bu etken maddelerin 0.1M HCl içindeki çözeltilerinin sıfırıncı derece absorpsiyon spektrumlarında seçilen dalga boylarında hesaplanan A_1^1 (%1, 1 cm) değerlerine göre hazırlanan eşitliklerin çözümünden yararlanılmış ve bu hesaplamalarda matris inversiyon yöntemi kullanılmıştır. Elde edilen sonuçlar aynı karışımlar için uygulanan ve tarafımızdan geliştirilen bir HPLC yöntemiyle bulunanlarla karşılaştırılmıştır. Yöntemler Türkiye ilaç piyasasında bulunan farmasötik preparatlara başarıyla uygulanmıştır.

***Anahtar Kelimeler**: Spektrum oranları türev spektrofotometri, matris çözümleme yöntemi, kafein, aspirin, propifenazon, farmasötik preparat.*

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Introduction

The binary mixtures of caffeine (**CAF**) –aspirin (**ASP**) and caffeine (**CAF**) –proyphenazone (**PRO**) are widely used in analgesic pharmaceutical formulations. Quantitative analysis of the mixtures containing caffeine with various methods including spectrophometry (1-13), gas chromatography (14,15), HPLC (16-19), and polarography (20) have been used for several pharmaceutical preparations.

Salinas et al. (21) 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.(22-25) applied the same method to determine the active compounds in different mixtures.

Also, we used ratio spectra derivative spectrophotometry for the simultaneous determination of drugs in pseudoephedrine hydrochloride + acrivastine (26), oxfendazole + oxyclozanide (27) and, metamizol + paracetamol + caffeine (28) combinations in pharmaceutical dosage forms.

In this paper, ratio spectra derivative spectrophotometry, matrix resolution method and HPLC are proposed for the simultaneous determination of caffeine, aspirin and proyphenazone in their binary mixtures containing caffeine. These methods were applied to two pharmaceutical formulations containing these mixtures and the results were compared with each other .

Experimental

Apparatus

A Shimadzu 1601 double beam spectrophotometer with a fixed slit width (2 nm) connected to a computer loaded with Shimadzu UVPC Software and equipped with an HP 600 printer was used for all the absorbance measurements and treatment of data.

Zero-order spectra of references and test solutions were recorded in 1-cm quartz cells over the ranges 200.0 - 310.0 nm.

In ratio spectra derivative spectrophotometry, range was selected as 200.0 - 305.0 nm ($\Delta\lambda$ = 4 nm) for caffeine (**CAF**) + proyphenazone (**PRO**) and 205.0 - 310.0 nm for caffeine (**CAF**) + aspirin (**ASP)** mixture for reading the analytical signals. The ordinate maximum and minimum settings were (+0.25) - (-0.25) for **CAF** and (+0.3) - (-0.4) for **PRO** and, (+1.0) - (-0.80) for **CAF** and (+0.40) - (-0.40) for **ASP** determination respectively in their mixtures.

For HPLC, Jasco PU-980 model liquid chromatograph equipped with its pump and with programmable Jasco UV-975 model wavelength detector was used. The chromatograms were recorded and the peaks were quantitated using its automatic integrator. The separation was carried out at ambient temperature on 5 μm Hypersil BDS Column of 250 x 4.6 mm. The mobile phase for **CAF + PRO** mixture was methanol - water - triethanolamine (50:50:0.1, v/v) and for **CAF + ASP** mixture was % 0.05 CH_3COO-Na.$3H_2O$ (pH 3.45): acetonitrile (90:10, v/v). The wavelength of detection was 260 nm for **CAF + PRO** mixture and 293 nm for **CAF + ASP** mixture respectively. The flow rate was set at 1 ml/min with 20 μl as injection volume.

Pharmaceutical formulations

Two commercial product ASPIRIN® FORT tablet (produced by Bayer Pharm.Ind., Turkey, Batch no.604031, containing 500 mg aspirin and 50 mg caffeine per tablets) and OPTALIDON® sugar-coated tablet (produced by Sandoz Pharm. Ind., Turkey, Batch no. 349, containing 175 mg proyphenazone and 25 mg caffeine per sugar-coated tablet) were studied.

Aspirin, caffeine and proyphenazone were kindly donated by Bayer Pharm. Ind. and Sandoz Pharm.Ind. (Turkey).

All the solvent used in spectrophotometric analysis were of analytical reagent grade. HPLC grade solvent were used in HPLC procedures.

Standard solutions

The solutions of 100 mg/100 mL of **ASP**, **CAF** and **PRO** were prepared, individualy in 0.1 M HCl for spectrophotometric procedures. These solutions were used in preparation of calibration graphs and for spectra. In HPLC, 50 mg / 100 mL solution of **CAF**

and 100 mg / 100 mL solution of **ASP** in formic acid - acetonitrile (1:1, v/v) for **CAF + ASP** mixture and 50 mg / 100 mL solution of **CAF** and 100 mg / 100 mL solution of **PRO** in methanol - water (1:1, v/v) were used.

Procedures :

a) for spectrophotometric analysis : 20 tablet (from ASPIRIN FORT®) were accurately weighed and powdered in a mortar, an amount equivalent to one tablet was dissolved in 0.1 M HCL in a 100 mL calibrated flask. This solution was filtered through Whatman no 42 filter paper to a calibrated flask. The residue was washed three times with 10 mL of solvent then the volume was completed to 100 mL with 0.1 M HCl (***I***). Analogous procedure was applied to sugar-coated tablets of OPTALIDON® and the sample solution (***II***) was prepared. (***I***) and (***II***) were diluted 1:25 and 1:36 with the same solvent, respectively. All the methods were applied to the solutions thus prepared.

b) for HPLC procedure: 20 tablet (ASPIRIN FORT®) were accurately weighed and powdered in a mortar, an amount equivalent to one tablet, was dissolved in formic acid : acetonitrile (1 : 1, v/v) in 200 mL calibrated flasks. This solution was filtered through Whatman no 42 filter paper to a calibrated flask. 1 mL of this solution was diluted to 100 mL with the same solvent (***III***). Also, 20 sugar-coated tablets of OPTALİDON® were accurately weighed and powdered in a mortar, an amount equivalent to one sugar-coated tablet was dissolved in methanol : water (1 : 1, v/v) in 100 mL calibrated flasks. This solution was filtered through Whatman no 42 filter paper to a calibrated flask. 1.5 mL of this solution was diluted to 100 mL with the same solvent (***IV***). ***III*** and ***IV*** were injected to column in 20 L.

Result and discussion

Matrix resolution method :

For overlapping spectra the total absorbance at a given wavelength can be written as:

$$k_1C_1 + k_2C_2 + k_3C_3 + \ldots + k_nC_n = b$$

where,

k_i = is the reference sample coefficients at the given wavelength

C_i = is the relative concentration of the component in the mixture

b = is the total absorbance at the given wavelength

n = number of reference components in the mixture

By the measurements of absorbances at *n* different wavelengths one thus obtain a system of *n* equations in *n* unknowns which can be solved for the component concentrations.

In our study, $A\,{}^{1}_{1}$ (1%, 1 cm) values were used as k_i values. For a two component mixture following equations can be written:

$$A_1 = \alpha_1 \,.\, C_1 + \beta_1 \,.\, C_2$$

$$A_2 = \alpha_2 \,.\, C_1 + \beta_2 \,.\, C_2$$

where A_1 and A_2 denotes the absorbances of a mixture solutions and α and β represent the values of $A\,{}^{1}_{1}$ (absorbance value of the % 1 solution in 1-cm cell) calculated for these active ingredients , respectively, at λ_1 and λ_2. C_1 and C_2 are the concentrations of the ingredients, respectively, in g/100 ml.

Matrix notation greatly simplifies the matters and solves system of equations with two unknowns, easily as shown below:

$$\begin{vmatrix} A_1 \\ A_2 \end{vmatrix} = \begin{vmatrix} \alpha_1 \beta_1 \\ \alpha_2 \beta_2 \end{vmatrix} \begin{vmatrix} C_1 \\ C_2 \end{vmatrix}$$

In matrix form the system to be solved, AC = b and the solution of which is, $C = A^{-1}b$. A key advantage in using matrix inversion in solving such a system is the computational efficiency. This matrix was solved by means of the program "Matlab" in the computer and the concentrations of each compound in the mixture were determined.

As seen in Figure 1, the spectra of the two compounds, **CAF** and **ASP**, are overlapped at the region of 200.0 - 310.0 nm. But, **CAF** and **ASP** have absorption peaks that are well separated in terms of wavelength. In application of the matrix resolution method,

λ_{max} of **CAF** are chosen as λ_1 (226.6 nm), whilst λ_{max} of **ASP** is chosen as λ_2 (272.5 nm). A_1^1 values were calculated by using the absorbances measured at 226.6 nm for **CAF** and at 272.5 nm for **ASP** at zero-order spectra for each of the drugs in their binary mixture. By using matrix resolution method, the determination of these two drugs were realized by direct measurements of absorbances measured at 226.6 and 272.5 nm in the zero-order spectra of the solution of their mixture in 0.1N HCL.

In the method, the parameters used in the method were shown in Table 1. Beer's law was obeyed in the concentration range 2 - 28 μg/mL for **CAF** and 8 - 36 μg/mL for **ASP** in the synthetic mixture containing **CAF** and **ASP**. Mean recoveries and relative standard deviations of the method were found as 99.67 % and 0.74 % for **CAF**, 99.53 % and 0.63 % for **ASP**, recpectively, in the synthetic mixtures prepared by adding known amounts of **CAF** and **ASP** (Table 4).

LOD was found 0.6 g /mL for **CAF** and 2.0 μg /mL for **ASP** (determined as blank + 3SD), LOQ was found 2.0 μg /mL for **CAF** and 8.0 g /mL for **ASP** (determined as blank + 10SD) in the method.

In a similar manner, the mathematical explanation of procedure can be written for the binary mixture containing **CAF** and **PRO** where λ_1 (240.8 nm) for **CAF** and λ_2 (272.5 nm) for **PRO** in their binary mixture in 0.1N HCL (Figure 2). The parameters used and mean recoveries and relative standard deviations of the method in the synthetic mixtures prepared by adding known amounts of **CAF** and **PRO** are shown in Table 1 and 5.

LOD was found 0.5 g /mL for CAF and 1.1 μg /mL for PRO (determined as blank + 3SD), LOQ was found 2.0 μg /mL for CAF and 4.0 μg /mL for PRO (determined as blank + 10SD) in the method.

Ratio spectra derivative spectrophotometry

a) <u>For caffeine + aspirin mixture:</u> In this method, the absorption spectra of the mixture solutions prepared in different concentrations of **CAF** were recorded at the range 200.0 - 310.0 nm and divided by the spectrum of the standard solution of 20 μg/mL **ASP** in 0.1 M HCL. The ratio spectra thus obtained were smoothed with $\Delta\lambda$ = 4 nm intervals (Figure 3a) and their first derivatives were plotted with $\Delta\lambda$= 4 nm intervals (Figure 3b).

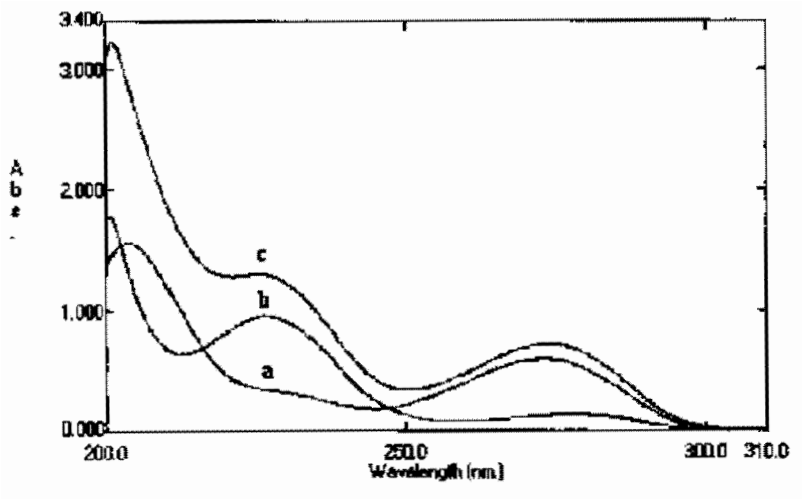

Figure 1. Zero-order spectra of a) 12 μg/ml caffein, b) 20 μg/ml aspirin, c) their binary mixture in 0.1 M HCl.

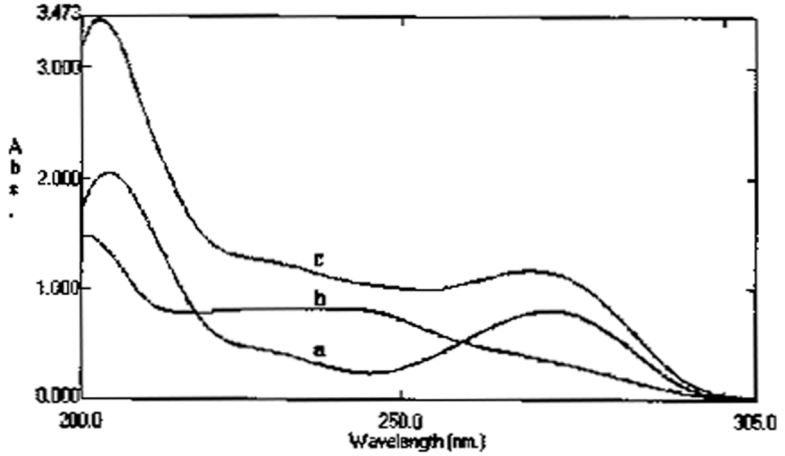

Figure 2. Zero-order spectra of a) 16 μg/ml caffeine, b) 20 μg/ml propyhenazone, c) their binary mixture in 0.1 M HCl.

TABLE 1. Exerimental parameters for the Matrix resolution method used for the si multaneous determination of ingredients in binary mixtures containing caffeine-aspirin and caffeine-proyphenazone

For caffeine – aspirin mixture	Caffeine		Aspirin	
λ (nm)	α_1	α_2	β_1	β_2
λ_1 = 226.6	483.6		302.6	
λ_2 = 272.5		67.9		515.8
linearity range (μg/mL)	2 – 28		8 – 36	
For caffeine – propyphenazone mixture	Caffeine		Propyphenazone	
λ (nm)	α_1	α_2	β_1	β_2
λ_1 = 240.8	172.4		416.9	
λ_2 = 272.5		515.8		187.7
linearity range (μg/mL)	2 – 28		4 – 32	

The amount of **CAF** was determined by measuring the amplitude at 254.3 nm corresponding to a maximum wavelength and at 297.6 nm corresponding to a minimum wavelength in the range 205.0 - 310.0 nm shown in Figure 3b. Various mixture compositions of **CAF** and **ASP** were prepared and tested and, the linearity range was found as 2 - 28 μg/mL of **CAF** in these binary mixtures (Table 6).

LOD was found 0.5 μg /mL for **CAF** and 2.0 μg /mL for **ASP** (determined as blank + 3SD), LOQ was found 2.0 μg /mL for **CAF** and 8.0 μg /mL for **ASP** (determined as blank + 10SD) in the method.

We selected 254.3 nm for the determination of **CAF** in the assay of pharmaceutical preparation, tablet, due to its lower RSD value and suitable mean recovery among the wavelengths mentioned (Table 2).

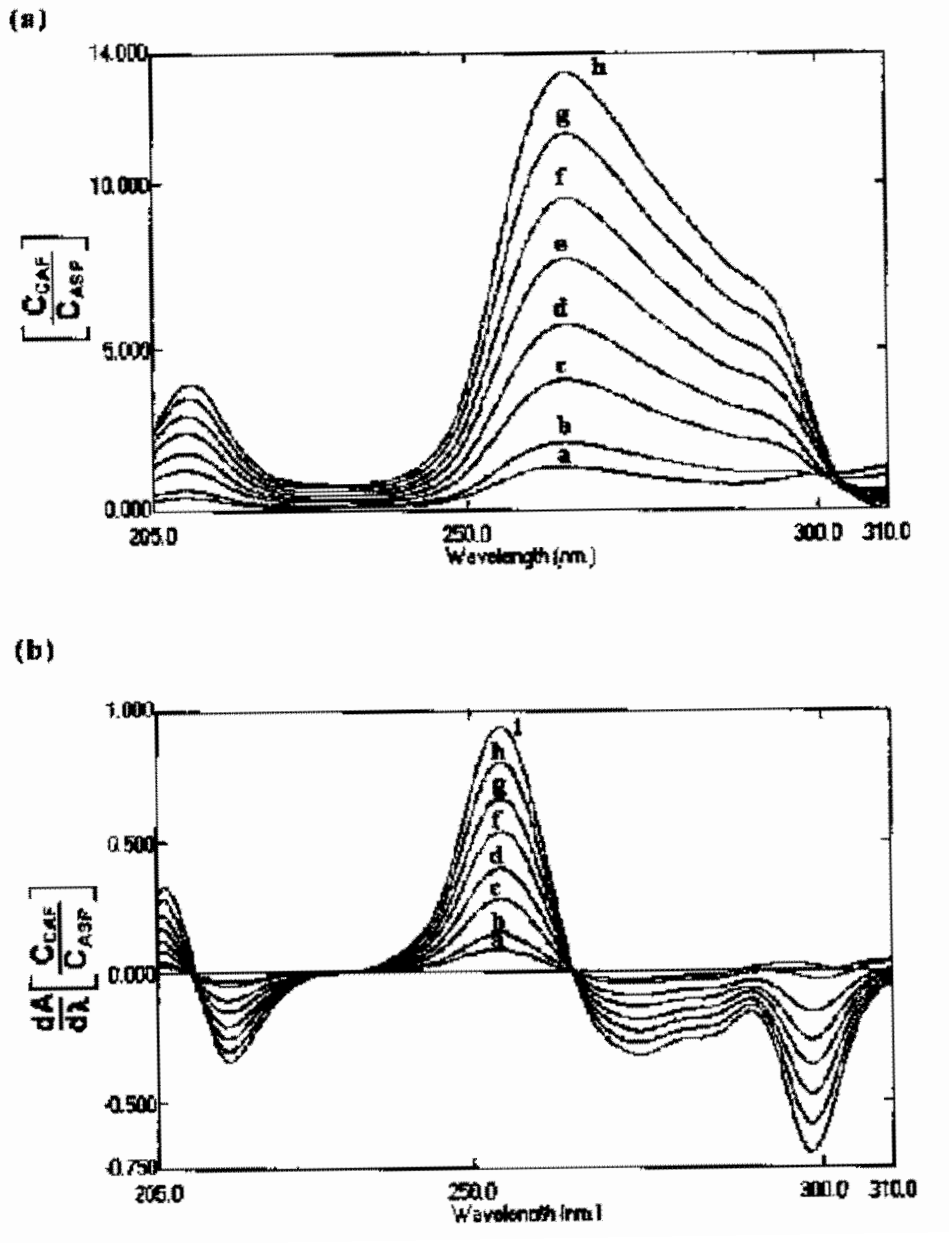

Figure 3. Ratio spectra of a) and first derivative of the ratio spectra b) of caffeine 2 g/ml, b) 4 μg/ml, c) 8 μg/ml, d) 12 μg/ml, e) 16 μg/ml, f) 20 μg/ml, g) 24 μg/ml, h) 28 μg/ml as divisor 20 μg/ml aspirin in 0.1 N HCl ($\Delta\lambda$= 4 nm)

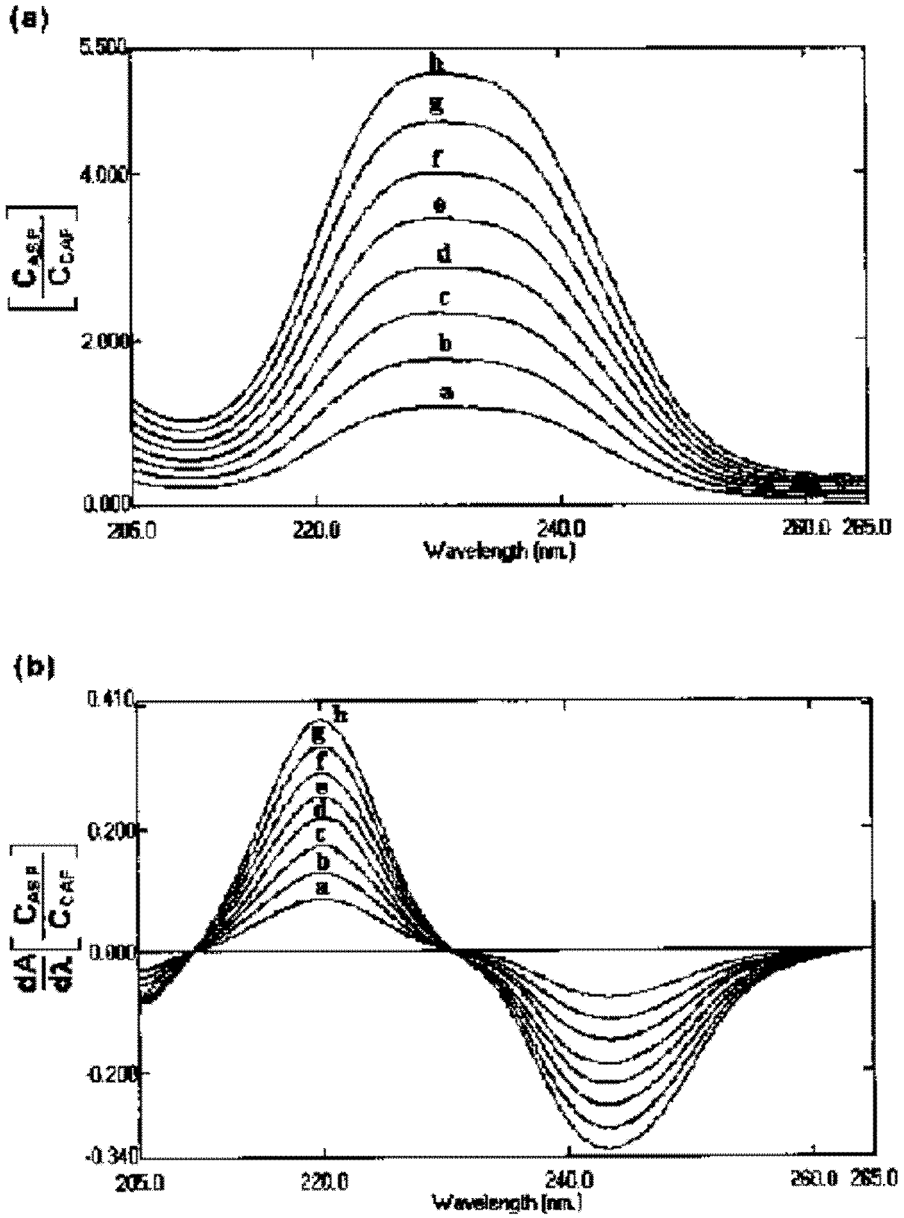

Figure 4. Ratio spectra of (a) and first derivative of the ratio spectra (b) of aspirin a) 8 μg/ml, b) 12 μg/ml, c) 16 μg/ml, d) 20 μg/ml, e) 24 μg/ml, f) 28 μg/ml, g) 32 μg/ml, h) 36 μg/ml, as divisor 12 μg/ml caffeine in 0.1 M HCl ($\Delta\lambda$ = 4 nm)

TABLE 2. Recovery results for CAF and ASP in synthetic mixtures by ratio spectra first derivative spectrophotometry

	CAF		ASP	
nm	254.3	297.6	220.0	243.4
Mean recovery % (± CI* for P=0.05)	100.26 (± 0.18)	98.2 (± 2.00)	100.04 (± 0.21)	99.0 (± 0.76)
RSD** %	0.32	1.93	0.38	1,69

**CI = confidence interval*

***RSD = Relative standard deviation*

TABLE 3. Recovery results for CAF and PRO in synthetic mixtures by ratio spectra first derivative spectrophotometry

	CAF			PRO		
nm	215.6	262.3	268.0	219.3	238.1	250.6
Mean recovery % (± CI* for P=0.05)	100.25 (± 0.37)	99.2 (± 1.91)	97.9 (± 1.79)	99.1 (± 1.06)	101.2 (± 0.91)	100.16 (± 0.35)
RSD** %	0.66	1.79	2.68	1.69	1.54	0.64

TABLE 4. Recovery data obtained for synthetic mixtures of caffeine-aspirin in the methods

	Matrix resolution (n=10)**		**Ratio spectra derivative spectrophotometry (**n=10)**		**HPLC (**n=10)**	
	CAF	**ASP**	**CAF** (254.3 nm)	**ASP** (220.0 nm)	**CAF**	**ASP**
Mean recovery % (±CI* for P=0.05)	99.67 (±0.41)	99.53 (±0.35)	100.26 (±0.18)	100.04 (±0.21)	100.38 (±0.42)	100.71 (±0.34)
RSD %	0.74	0.63	0.32	0.38	0.75	0.62

*CI = confidence interval
**n = number of mixed standard samples

TABLE 5. Recovery data obtained for synthetic mixtures of caffeine-propyphenazone in the methods

	Matrix resolution (n=10)**		**Ratio spectra derivative spectrophotometry (**n=10)**		**HPLC (**n=10)**	
	CAF	**ASP**	**CAF** (215.6 nm)	**ASP** (250.6 nm)	**CAF**	**ASP**
Mean recovery % (± *CI for P=0.05)	99.81 (±0.29)	99.92 (±0.36)	100.25 (±0.37)	100.16 (±0.35)	100.10 (±0.28)	100.12 (±0.52)
RSD %	0.53	0.65	0.66	0.64	0.51	0.93

In the same way, the absorption spectra of the mixture solutions prepared in different concentrations of **ASP** were recorded between 200.0 - 310.0 nm and divided by the spectrum of the standard solution of 12 μg/ml **CAF**. The ratio spectra of the result were smoothed with $\Delta\lambda$ = 4 nm intervals (Figure 4a) and their first derivatives were plotted with $\Delta\lambda$ = 4 nm intervals (Figure 4b). The content of **ASP** was determined by measuring the amplitudes at 220.0 nm and 243.4 nm corresponding to a maximum and a minimum wavelengths in the spectral region 205.0 - 265.0 nm (Figure 4b). Various mixture compositions of **CAF** and **ASP** were prepared and were tested and linearity range was found as 8 - 36 μg/mL for **ASP** in these binary mixtures (Table 6).

We selected 220.0 nm for the determination of **ASP** in the assay of pharmaceutical preparation, tablet , due to its lower RSD value and suitable mean recovery among the wavelengths mentioned (Table 2).

b) For caffeine + proypenazone mixture*:* The recorded absorption spectra of **CAF** in its binary mixture with **PRO**, were divided by the spectrum of the standard solution of **PRO** of 20 μg/mL in 0.1 N HCL and the ratio spectra were obtained in the spectral region 203.0 - 304.0 nm. These were smoothed with $\Delta\lambda$ = 4 nm intervals (Figure 5a) and the first derivative of the ratio spectra was plotted with $\Delta\lambda$ = 4 nm intervals in the range 205.0 - 285.0 nm (Figure 5b). In the mixture, **CAF** can be determined by measuring the first derivative signals at 215.6 nm, 262.3 nm and 268.0 nm corresponding to a minimum and two maximum, respectively, in the range mentioned above.

Following the similar procedure, the stored spectra of **PRO** were divided by the spectrum of a standard solution of **CAF** of 16 μg/ml and the ratio spectra was obtained (Figure 6a). These were smoothed with $\Delta\lambda$= 4 nm intervals. The first derivative of the result was plotted with $\Delta\lambda$= 4 nm intervals in 205.0 - 280.0 nm (Figure 6a).. The concentration of **PRO** was determined at 219.3 nm, 238.1 nm and 250.6 nm which corresponds to two maximum and a minimum wavelengths, respectively, in above mentioned spectral region.

Various mixture compositions of **CAF** and **PRO** were prepared and the linearity range was found as 2 - 28 μg/ml for **CAF** and 4 - 32 μg/ml for **PRO** in their binary mixtures (Table 6).

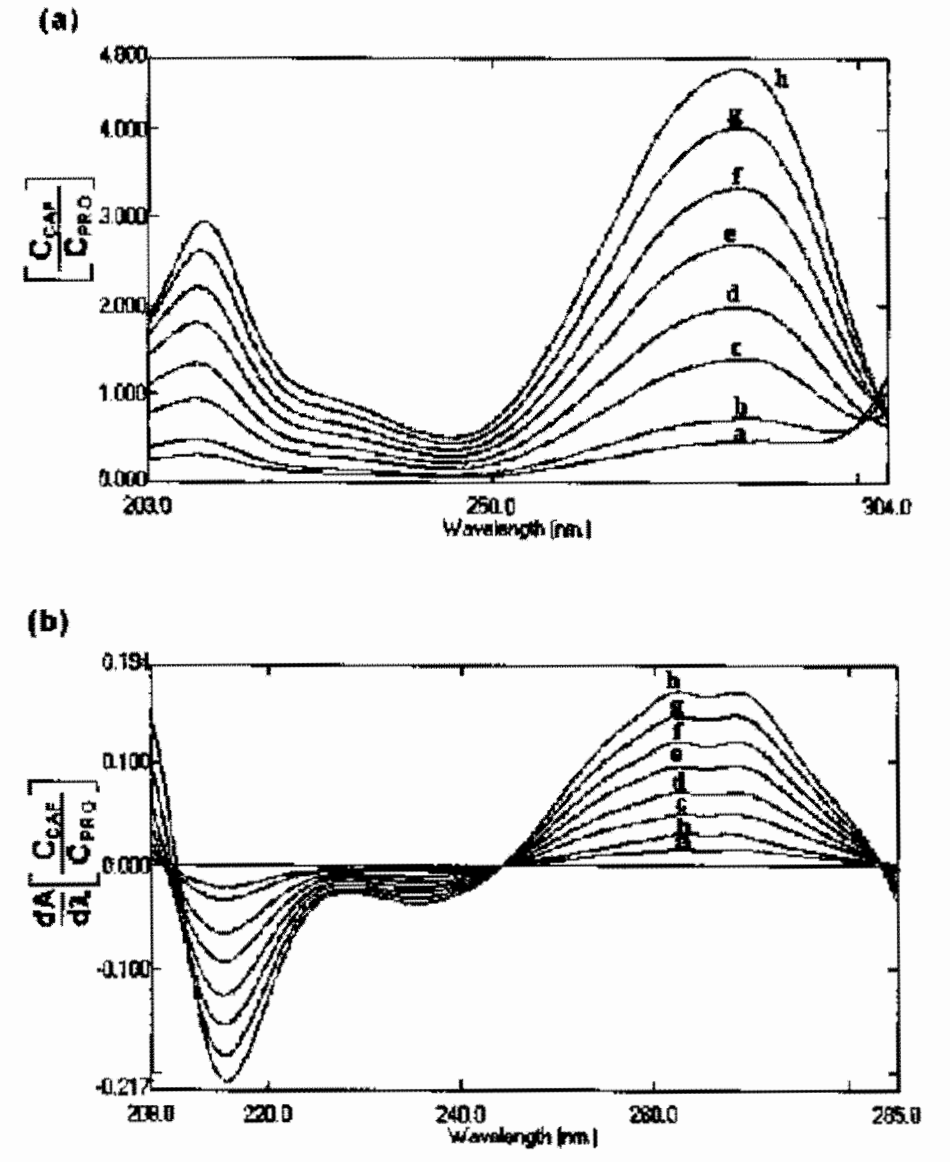

Figure 5. Ratio spectra (a) and first derivative of the ratio spectra (b) of caffeine a) 2 μg/ml b) 4 μg/ml c) 8 μg/ml d) 12 μg/ml e) 16 μg/ml f) 20 g/ml g) 24 μg/ml h) 28 μg/ml, as divisor 20 μg/ml propyhenazone in 0.1 M HCl ($\Delta\lambda$ = 4 nm).

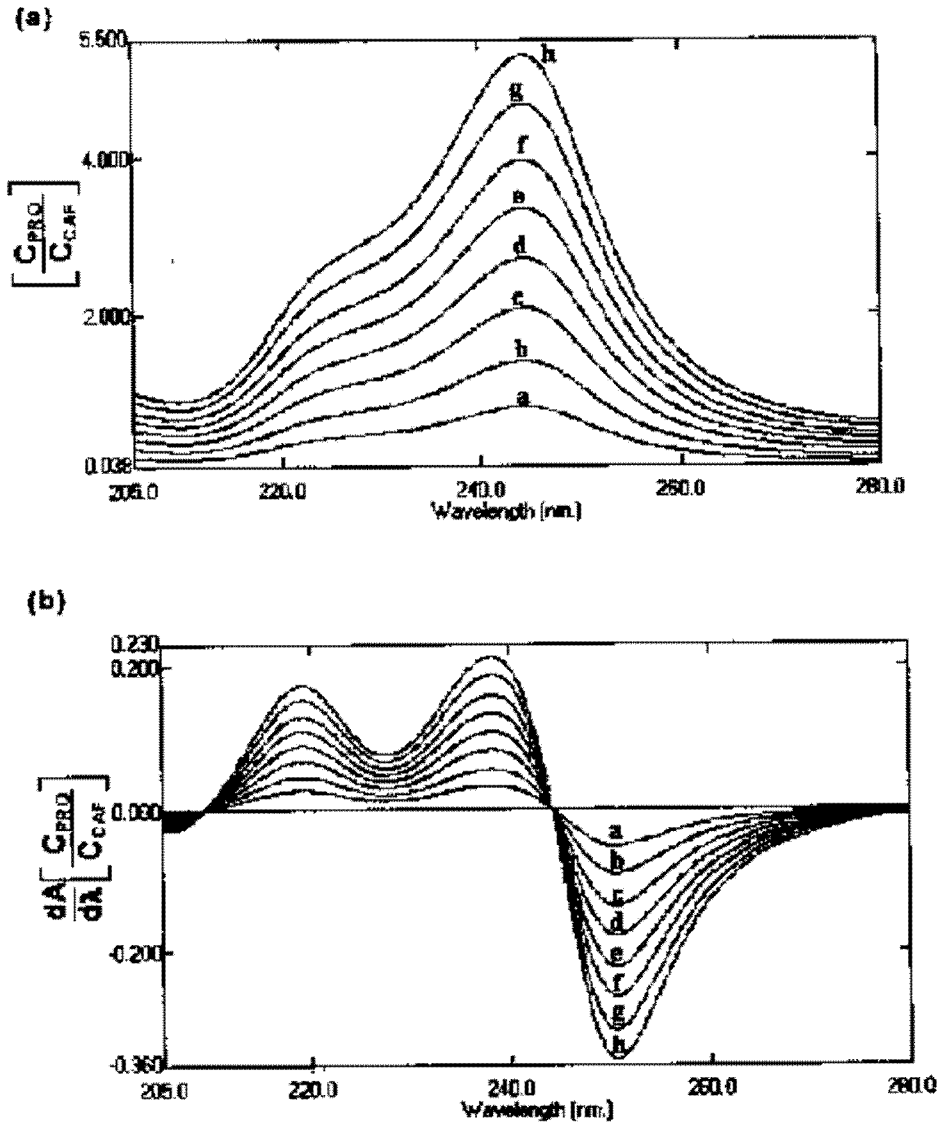

Figure 6. Ratio spectra (a) and first derivative of the ratio spectra (b) of propyhenazone of a) 4 μg/ml b) 8 μg/ml c) 12 μg/ml d) 16 μg/ml e) 20 μg/ml f) 24 μg/ml g) 28 μg/ml h) 32 μg/ml, as divisor 16 μg/ml caffeine in 0.1 M HCl ($\Delta\lambda$ = 4 nm)

TABLE 6. Calibration data in the determination of ingredients in mixtures containing caffeine and its binary mixture with aspirin, and proyphenazone using ratio spectra derivative spectrophotometry

a) For caffeine – aspirin mixture

λ (nm)	Linearity range μg/ml	Regression equations	Regression coefficient (r)
254.3	2 - 28	$Y = 3.3\ 10^{-2}\ C_{CAF} + 1.6\ \ 0^{-3}$	0.9998
220.0	8 - 36	$Y = 1.0\ 10^{-2}\ C_{ASP} + \ 5.7\ \ 0^{-3}$	0.9987
243.4	8 - 36	$Y = 8.9\ 10^{-2}\ C_{ASP} + 8.8\ \ 0^{-3}$	0.9998

b) For caffeine – propyphenazone mixture

λ (nm)	Linearity range μg/ml	Regression equations	Regression coefficient (r)
215.6	2 - 28	$Y = 7.4\ 10^{-3}\ C_{CAF} + 4.7\ \ 0^{-4}$	0.9996
268.4	2 - 28	$Y = 5.9\ 10^{-2}\ C_{CAF} + 1.9\ \ 0^{-3}$	0.9997
219.3	4 - 32	$Y = 5.3\ 10^{-3}\ C_{PRO} + 4.9\ \ 0^{-3}$	0.9998
238.1	4 - 32	$Y = 6.5\ 10^{-2}\ C_{PRO} + 4.9\ \ 0^{-3}$	0.9998
250.6	4 - 32	$Y = 1.1\ 10^{-2}\ C_{PRO} + 2.5\ \ 0^{-4}$	0.9999

C_{CAF} = μg/mL of caffeine, C_{ASP} = μg/mL of aspirin, C_{PRO} = μg/mL of proyphenazone

LOD was found 0.5 μg /mL for CAF and 1.1 μg /mL for PRO (determined as blank + 3SD), LOQ was found 2.0 μg /mL for CAF and 4.0 μg /mL for PRO (determined as blank + 10SD) in the method.

We selected 215.6 nm for the determination of **CAF** and 250.6 nm for the determination of **PRO** in the assay of pharmaceutical preparation, tablet, due to its lower RSD value and suitable mean recovery among the wavelengths mentioned (Table 3). Mean recoveries and the relative standard deviations of method were found as 100.26 % and 0.66 % for **CAF**, 100.16 % and 0.64 % for **PRO** in their synthetic binary mixtures when worked at this wavelength (Table 3 and 5).

Table 6 shows the regression coefficients and the linearity ranges of the calibration graphs for active ingredients at the suitable wavelengths for the determinations of **CAF**, **ASP** and **PRO** in their binary mixtures.

The main instrumental parameter conditions were optimized for a reliable determination of the subject compounds. For selecting the standard solution as divisor at an appropriate concentration, which is very important factor in practice, some divisor concentrations were tested in the determinations and the standard solution of 20 μg/mL **ASP** for determining **CAF** and of 12 μg/mL **CAF** for determining **ASP** in **CAF + ASP** mixture and, the standard solution of 12 μg/mL **PRO** for determining **CAF** and of 16 μg/ml **CAF** for determining **PRO** in **CAF + PRO** mixture were found suitable. The influence of the on the first derivative spectra and the smoothing function for the ratio spectra were tested and found very appropriate to use the values of $\Delta\lambda = 4$ for **CAF + ASP** and $\Delta\lambda = 4$ nm for **CAF + PRO** mixtures in the determination of these compounds.

HPLC procedure:

Although there exist HPLC procedures for the multicomponent combinations containing caffeine (16-19), we developed new procedures for the analysis of our mixtures selected. These procedures were tested for the determination of the drugs in **CAF + ASP** and **CAF + PRO** mixtures either in synthetic mixtures prepared in our laboratoy and in commercial preparations as explained in experimental section .

In the methods, linearity ranges were found 2 - 28 μg/ml for **CAF** and 8 - 36 μg/ml for **ASP** in **CAF + ASP** mixture and, 2 - 28 μg/ml for **CAF** and 4 - 32 μg/ml for **PRO** in **CAF + PRO** mixture. Mean recoveries and relative standard deviations of the methods found for the synthetic mixtures of these compounds were illustrated in Table 2 and 3.

The regression equations were calculated as: $y = 1.8\ 10^{1}\ x - 4.9\ 10^{-1}$ for **CAF** and $y = 2.9\ 10^{1}\ x - 2.5\ 10^{-1}$ for **PRO** in **CAF + PRO** mixture and, $y = 2.8\ 10^{1}\ x - 1.9\ 10^{-1}$ for **CAF** and $y = 1.9\ 10^{1}\ x - 4.7\ 10^{-1}$ for **ASP** in **CAF + ASP** mixture (where y is peak area and x is the concentration in g/mL).

LOD was found 0.4 μg /mL for **CAF** and 1.6 μg /mL for **ASP** (determined as blank + 3SD), LOQ was found 2.0 μg /mL for **CAF** and 8.0 μg /mL for **ASP** (determined as blank + 10SD) in **CAF + ASP** mixture in the method.

LOD was found 0.4 μg /mL for CAF and 1.0 μg /mL for PRO (determined as blank + 3SD), LOQ was found 2.0 μg /mL for CAF and 4.0 μg /mL for PRO (determined as blank + 10SD) in CAF + PRO mixture in the method.

In the method, t_R values were obtained as 6.5 min for **CAF** and 15.5 min for **ASP** in **CAF + ASP** mixture and, 3.6 min for **CAF** and 7.9 min for **PRO** in **CAF + PRO** mixture in the conditions explained in experimental section.

These methods were also applied to the commercial preparations selected. Results were shown in Table 7. A good coincides was observed for the assay results of the commercial preparations by application of the three methods in this paper (Table 7).

The results of two spectrophometric methods and also HPLC method 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 (Table 7).

Conclusion

For a comparative study of two spectrophotometric methods developed by us, the ratio spectra derivative spectrophotometry and matrix resolution method, have been applied for the determination of the active ingredients in two commercial preparations containing binary mixtures of caffeine with aspirin and proyphenazone and in all cases good results were obtained. Reading signals on separate peaks and not need to any other mathematical procedure gives an advantage for ratio spectra derivative spectrophotometry when compared with the matrix resolution method. For the determination of ingredients in the two different pharmaceutical preparation containing **CAF** and its binary mixtures with **PRO** and **ASP**, it was observed that the methods proposed in this paper were more simple than the methods mentioned in introduction chapter.

TABLE 7. Assay results for commercial preparations (mg)

	ASPIRIN® Fort			
Methods	Caffeine mean ± SD	*t* values	Aspirin mean ± SD	*t* values
Matrix resolution Method (MRM)	49.5 ± 0.5	MRM-^{1}DD= 0.42	499.2 ± 0.6	MRM-^{1}DD= 1.11
Ratio spectra derivative spectrophotometry (^{1}DD)	49.3 ± 0.5	MRM-HPLC= 0.23 ^{1}DD-HPLC= 0.28	498.8 ± 0.9	MRM-HPLC= 1.01 ^{1}DD-HPLC= 0.32
HPLC	49.4 ± 0.5		498.9 ± 1.0	
	OPTALIDON®			
	Caffeine mean ± SD	*t* values	Propyphenazone mean ± SD	*t* values
Matrix resolution Method (MRM)	24.7 ± 0.3	MRM-^{1}DD= 1.02	174.6 ± 0.4	MRM-^{1}DD= 1.12
Ratio spectra derivative spectrophotometry (^{1}DD)	25.2 ± 0.2	MRM-HPLC= 0.98 ^{1}DD-HPLC= 0.82	175.6 ± 0.4	MRM-HPLC= 0.63 ^{1}DD-HPLC= 0.79
HPLC	25.0 ± 0.5		175.0 ± 0.8	

* Results obtained are average of ten experiments for each

** SD = standard deviation

*** Theoretical value for *t* at P : 0.05 level = 2.26

In addition, all the results were compared with an alternative method developed by us, HPLC, and the first-derivative ratio spectra and matrix resolution methods used in this study were found more simple and less expensive and does not require sophisticated instrumentation and any prior separation step.

These three methods described in the text were found to be suitable for the routine analysis of active ingredients in two different pharmaceutical formulations selected containing **CAF** and its binary mixtures with **PRO** and **ASP** marketed in Turkey.

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