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Simultaneous Determination of Caffeine and Paracetamol by Zero-Crossing Second Derivative Spectrophotometry in Pharmaceutical Preparations

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A second-derivative spectrophotometric method is described for simultaneous determination of caffeine and paracetamol. All measurements for made at the zero-crossing wavelengths at 260.0 nm for paracetamol and 288.0 nm for caffeine. Calibration graphs were linear over the range 0.1-30.0 mg/L for paracetamol and 0.1-20.0 mg/L for caffeine. The detection limits achieved were 0.095 mg/L for caffeine and 0.090 mg/L for paracetamol. The method is able to determine caffeine to paracetamol ratio 40:1 to 1:12 (w/w), accurately. Accuracy and reproducibility of determination method for the various known amounts of caffeine and paracetamol in their binary mixtures were tested. The proposed methods were suitably applied to assay of pharmaceutical preparations.

Key Words: Derivative spectrophotometry, Caffeine, Paracetamol.

INTRODUCTION

Paracetamol [4-acetamidophenol] (PAR), is an effective and safe analgesic agent used worldwide for the relief of mild to moderate pain associated with headache, backache and also used for the reduction of fever of bacterial or viral origin[1]. Caffeine (1,3,7-trimethylxanthine) (CAF), is mainly ingested by drinking coffee, cola-beverages and tea to act both as diuretic and as stimulant the central nervous and to the cardiovascular systems[2].

The mixture of paracetamol and caffeine is used in analgesic pharmaceutical preparations. In the literature one or two of these compounds has been determined in their mixtures with different methods, for example: flow injection-solid phase spectrometry using C18 silica gel as a sensing support[3], Flow-injection spectrophotometric determination in tablets and oral solutions[4], reverse phase high performance liquid chromatography[5], high performance liquid chromatography[6], spectrofluorimetric determination in solid-phase using partial least squares multivariate calibration[7] and H-point standard addition method[8] have been reported.

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Moreover, these methods are necessary to the use of difficult procedure or instrument. The direct UV-Vis spectrophotometry is a simple method but presents a severe problem in that the spectral band of CAF and PAR overlap, which makes the simultaneous determination of these drugs impossible by this method. Derivative spectrophotometry in the UV-Vis region is a useful technique in extracting qualitative and quantitative information from overlapping bands of the analyte and interferents due to incompletely resolved peaks. Several papers on the theoretical aspects of derivative spectrophotometry have been reported[9-16]. Derivative spectrophotometry has been used advantageously in the determination of binary mixtures of drugs[17-29].

This paper describes a simple, sensitive and selective method involving second-derivative spectrophotometry for simultaneous determination of caffeine and paracetamol and its application to pharmaceutical preparations.

EXPERIMENTAL

All chemicals and solvents were of analytical reagent grade and were used without further purification. A standard stock solution of PAR (1000.0 mg/L) was prepared by dissolving 0.1g of paracetamol (Merck) in water and diluting with distilled water to 100.0 mL in volumetric flask. A standard stock solution of CAF (1000.0 mg/L) was prepared by dissolving 0.1 g of caffeine (Merck) in water and diluting with distilled water to 100.0 mL in volumetric flask.

Titrazol buffers (Merck) were used at different pHs for this study.

Pharmaceutical products: A commercial tablet product (Remidon® tablet, Deva Pharm. Ind., Turkey, Batch no. 706-1770) is containing 65 mg caffeine and 500 mg paracetamol per tablet, was studied. The other commercial product that was studied (Novafen capsule, Brown & Burk Ind., UK, Batch no. NVF34E3) is containing 325 mg Paracetamol and 40 mg caffeine and 200 mg Ibuprofen per capsule.

All spectral measurements and treatment of data were carried out in 1 cm quartz cells using a Perkin-Elmer Lambada 2 double beam spectrophotometer. Measurements of pH were made using a Jenway Model 3510 pH-meter equipped with a glass-saturated calomel combined electrode.

Procedure: Different volumes of stock solutions of PAR and CAF (1000.0 mg/L) were used simultaneously and placed in a 10 mL calibrated flask and diluted with distilled water to give final concentration between 2.0 and 60.0 mg/L of paracetamol and caffeine.

Second derivative spectrums of sample solutions were recorded against its blank in the wavelength rang of 200-350 nm with interval ($\Delta\lambda$ = 2 nm) using scan speed of 960 nm/min and spectral slit width 2 nm. The second derivative analytical signals were at zero-crossing wavelength of 260 nm and 288 nm, respectively, for paracetamol and caffeine determination.

Analysis of pharmaceutical formulations: (1) For preparation of Remidon sample, 20 tablets (Remidon®) were accurately weighed and powdered in a mortar.

A mass corresponding to a tablet was dissolved in 0.1 M HCl in 100 mL calibrated flask. After 0.5 h of mechanically shaking, the solution was filtrated in a 100 mL calibrated flask through Whatmann filter paper no. 40. The residue was washed 3 times with 10 mL solvent then the volume was completed to 100 mL with 0.1 M HCl and then was diluted 1:500 with water. (2) For preparation of Novafen sample 8 capsules of Novafen were accurately opened and weighed. A mass corresponding to a capsule (565 mg), was dissolved in water in 100 mL calibrated flask. After 20 min of mechanically shaking, the solution was filtrated in a 100 mL calibrated flask through Whatmann filter paper no. 40. The residue was washed four times with distilled water then the volume was completed to 100 mL with distilled water. The clear solutions obtained were diluted with water to give concentration within the linear range of both drugs.

RESULTS AND DISCUSSION

In Fig. 1 the zero-order spectra of 3.0 mg/L CAF (A), 4.0 mg/L of PAR (B) or both mixed drugs (C), in the wavelength range of 200-350 nm are shown. Caffeine exhibits an absorbance maximum at 275 nm and for paracetamol at 245 nm. As can be seen, there is a clear overlapping of two spectra, which prevents the simultaneous determination of the both compounds by direct UV-Vis absorbance measurement. The pH of the solutions of CAF and PAR varied. The results showed that the λ_{max} and sensitivity is not dependent on the pH range between 1.0 to 10.0.

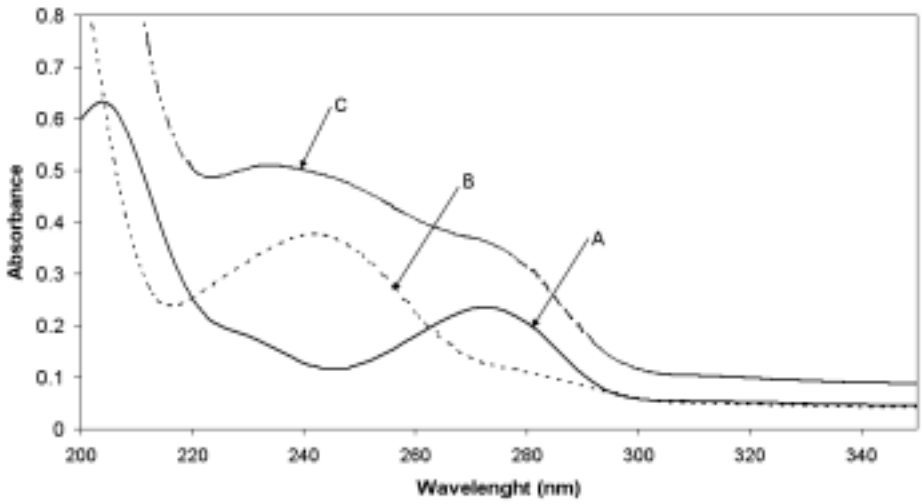

Fig. 1. Absorption (zero-order) spectra of (A) 3.0 mg/L of CAF; (B) 4.0 mg/L of PAR; (C) 3.0 mg/L of CAF and 4.0 mg/L of PAR

A suitable technique for overcoming the problem is derivative spectrophotometry with the zero-crossing method being the most common procedure for preparation of analytical calibration graphs. In the zero-crossing derivative method, the measurements

selected are those which exhibit the best linear response, give a zero or near zero intercept on the ordinate of the calibration graphs and it is necessary that zero-crossing wavelength do not change by varying concentration of related species. Zero-crossing method can not be used for determination of species which their spectra are shifted with change of concentration.

Fig. 2 shows the second UV-Vis spectra of the same solution of Fig. 1, respectively. The suitable zero-crossing wavelength applicable to sensitive second derivative simultaneous determination of caffeine and paracetamol are 288 and 260 nm, respectively.

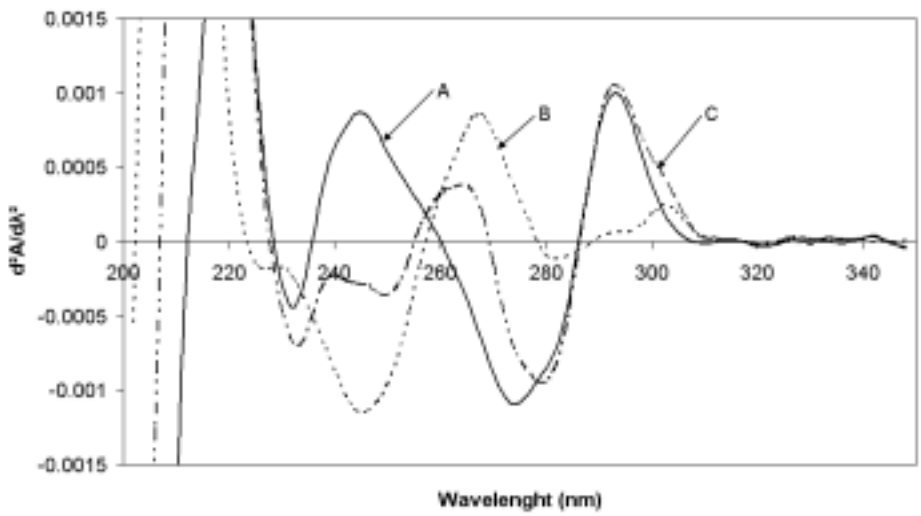

Fig. 2. Second derivative spectra ($\Delta\lambda$ = 2 nm) of; (A) 3.0 mg/L of CAF; (B) 4.0mg/L of PAR; (C) 3.0 mg/L of CAF and 4.0 mg/L PAR

Linear plot (calibration curve): Second-order spectra of solutions containing fixed concentration of PAR (1 mg/L) and different concentration of CAF (2-5 mg/L) are shown in Fig. 3 and second-order spectra of solutions containing fixed concentration of CAF (0.7 mg/l) and different concentration of PAR (2-5 mg/L) are shown in Fig. 4.

As shown in Figs. 3 and 4, the zero-crossing wavelengths do not change by varying concentration of the related species.

Two calibration graphs were constructed at the zero-crossing wavelengths (260 nm for PAR and 288 nm for CAF) for the simultaneous determination of PAR and CAF. In Table-1 calibration data of second derivative zero-crossing method for simultaneous determination of CAF and PAR were shown.

The linear range of CAF and PAR are 0.1-20 and 0.1-30 mg/L, respectively and detection limit were obtained 0.095 and 0.090 mg/L for caffeine and paracetamol, respectively.

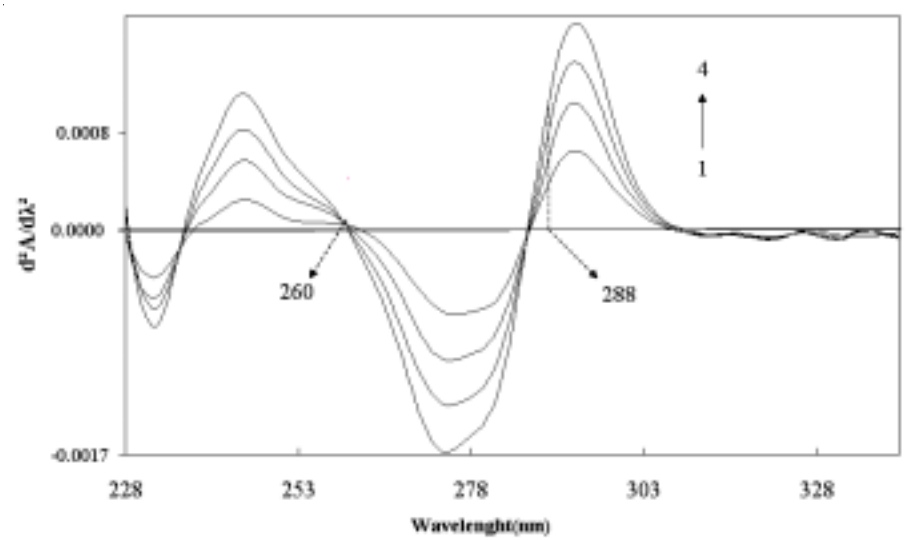

Fig. 3. Second derivative spectra of solution containing fixed 1 mg/L PAR and CAF concentrations (1)2.0 mg/L (2) 3.0 mg/L (3) 4.0 mg/L (4) 5.0 mg/L

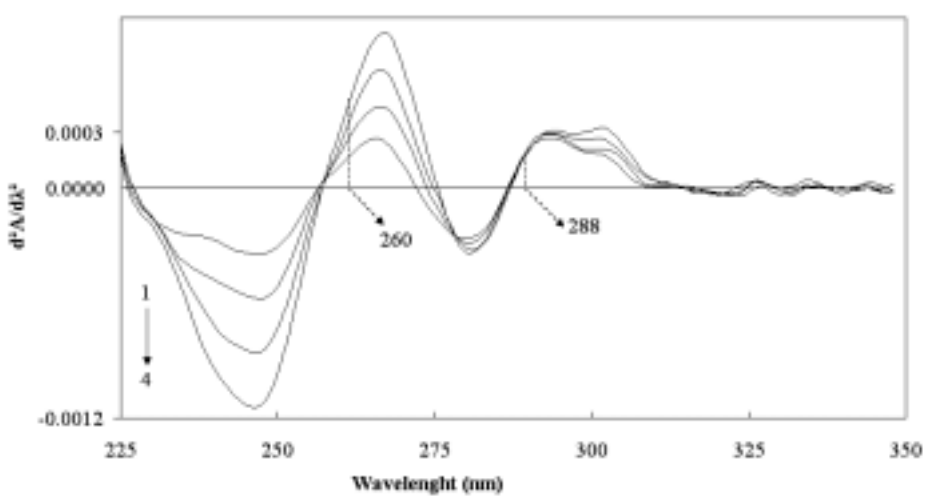

Fig. 4. Second derivative of solution containing fixed 0.7 mg/L CAF and PAR concentrations of (1) 2.0 mg/L (2) 3.0 mg/L (3) 4.0 mg/L (4) 5.0 mg/L

Application

Recoveries and precision of caffeine and paracetamol in their binary mixtures: Known amounts of caffeine and paracetamol were determined to access the proposed method. The recoveries and precision are given in Table-2. Based on the results, good precision (RSD < 2) and recovery of the method for the simultaneous determination of paracetamol and caffeine in binary mixtures were obtained.

TABLE-1
CALIBRATION DATA FOR THE DETERMINATION OF CAFFEINE AND PARACETAMOL

Sample	Regression equation	λ (nm)	r	Linear range (mg/L)	*DL (mg/L)
CAF	$d^2A/d\lambda^2 = -3 \times 10^{-5} + 1 \times 10^{-3} C_{CAF}$	288	0.9994	0.1-20	0.095
PAR	$d^2A/d\lambda^2 = -7 \times 10^{-6} + 6 \times 10^{-5} C_{PAR}$	260	0.9994	0.1-30	0.090

[a]Detection limit (DL) = 3 sb/m

TABLE-2
SECOND-DERIVATIVE SIMULTANEOUS DETERMINATION OF CAFFEINE AND PARACETAMOL IN SOME THEIR BINARY MIXTURES

Sample	Paracetamol (mg/L)			Caffeine (mg/L)		
	Taken	Found*	Recovery (%)	Taken	Found*	Recovery (%)
1	1.5	1.480 ± 0.020 (1.35)	98.70	2.0	1.970 ± 0.01 (0.50)	98.50
2	0.5	0.505 ± 0.010 (1.98)	101.00	3.0	3.045 ± 0.05 (1.70)	101.50
3	3.0	2.980 ± 0.050 (1.70)	99.30	1.0	1.010 ± 0.01 (0.10)	101.00
4	4.0	3.950 ± 0.010 (0.28)	98.75	4.0	3.960 ± 0.01 (0.25)	99.00
5	5.0	5.050 ± 0.020 (0.40)	101.00	20.0	19.800 ± 0.10 (0.50)	99.00
6	12.0	12.100 ± 0.200 (1.60)	100.80	3.0	3.040 ± 0.04 (1.30)	101.00
7	20.0	20.300 ± 0.200 (0.98)	101.50	10.0	10.100 ± 0.05 (0.49)	101.00
8	6.0	6.070 ± 0.025 (0.40)	101.20	18.0	17.800 ± 0.15 (0.84)	99.00
9	15.0	14.700 ± 0.100 (0.68)	98.00	5.0	5.060 ± 0.02 (0.36)	101.20
10	2.0	1.980 ± 0.015 (0.76)	99.00	16.0	16.200 ± 0.20 (1.20)	101.30

*Mean ± Standard deviation (mg/L) for four determination with RSD % in parentheses.

Applicable ratio of paracetamol to caffeine (paracetamol/caffeine) and *vice versa* (caffeine/paracetamol): For this purpose the fixed concentration of PAR (0.5 mg/L) and various concentrations of CAF (0.5-20.0 mg/L) and fixed concentration of CAF (0.5 mg/L) and various concentration of PAR (0.5-12.0 mg/L) were used for obtain the ratio of (PAR/CAF) and (CAF/PAR). The results were shown in Tables 3 and 4, which give applicability of proposed method.

TABLE-3
DETERMINING RATIO OF PARACETAMOL TO CAFFEINE

Sample	Paracetamol (PAR)		Caffeine (CAF)		(PAR/CAF)
	Taken (mg/L)	Found (mg/L)	Taken (mg/L)	Found (mg/L)	
1	0.500	0.505	0.500	0.504	1/1
2	0.500	0.503	3.00	3.02	1/6
3	0.500	0.495	6.00	6.05	1/12
4	0.500	0.501	10.0	9.90	1/20
5	0.500	0.496	15.0	14.9	1/30
6	0.500	0.504	20.0	20.03	1/40

TABLE-4
DETERMINING RATIO OF CAFFEINE TO PARACETAMOL

Sample	Caffeine (CAF)		Paracetamol (PAR)		(CAF/PAR)
	Taken (mg/L)	Found (mg/L)	Taken (mg/L)	Found (mg/L)	
1	0.500	0.502	0.50	0.503	1/1
2	0.500	0.497	1.50	1.520	1/3
3	0.500	0.501	3.00	2.970	1/6
4	0.500	0.495	4.00	3.960	1/8
5	0.500	0.504	5.00	5.080	1/10
6	0.500	0.502	6.00	5.970	1/12

Analysis of commercial preparations: To confirm the usefulness of the proposed derivative spectrophotometric methods, it has been applied to the simultaneous determination of paracetamol and caffeine in different samples commercial pharmaceutical where excellent agreement between reported and obtained results was achieved (Table-5).

TABLE-5
SIMULTANEOUS DETERMINATION OF PARACETAMOL AND CAFFEINE IN REAL SAMPLES

Sample	Reported		Obtained[a,d]		RSD (%)	
	Paracetamol	Caffeine	Paracetamol	Caffeine	Paracetamol	Caffeine
Remidon[b]	500.0	65.0	504.0 ± 1.50	65.70 ± 0.16	0.30	0.24
Novafen[c]	325.0	40.0	327.0 ± 1.20	40.50 ± 0.14	0.36	0.35

[a]Mean ± standard deviation (mg) for four determination.
[b]Remidon® tablet, Deva Pharm.Ind., Turkey, Batch no. 706-1770.
[c]Novafen capsule, Brown & Burk Ind., UK, Batch no. NVF34E3.
[d]After dilution and determination by the proposed method.

Conclusion

The proposed second-derivative method is suitable for simultaneous determination of paracetamol and caffeine without requiring a separation procedure. It is a simple, accurate and precise method which can be used, for rapid and reliable study of paracetamol and caffeine simultaneously in pharmaceuticals products and can be used in the routine analysis of the compounds.

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