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ARTICLES

Development and Validation of a Rapid Derivative Spectrophotometric Method for Simultaneous Determination of Acetaminophen, Ibuprofen and Caffeine[1]

Effat Souri*, Seyed Adel Mousavi Nasab, Massoud Amanlou, and Maliheh Barazandeh Tehrani

Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Research Center, Tehran University of Medical Sciences Tehran, (14155-6451) Iran

**e-mail: souri@sina.tums.ac.ir*

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Abstract—A zero-crossing derivative spectrophotometric method was used for the simultaneous determination of acetaminophen, ibuprofen and caffeine. The derivative spectra of standard solutions of each compound were obtained at different orders to find out the suitable zero-crossing points. Under the optimized conditions, determination of acetaminophen was performed at wavelengths of 311 and 270 nm using the third order ($\Delta\lambda = 24.5$) and fourth order ($\Delta\lambda = 12.0$) derivative spectra, respectively. Ibuprofen and caffeine were determined at wavelengths of 235 and 300 nm using the second order ($\Delta\lambda = 21.0$) and fourth order ($\Delta\lambda = 27.0$) derivative spectra. The method was found to be linear ($r^2 > 0.998$) in the range of (μg/mL) 5–50 for acetaminophen, 5–30 for ibuprofen and 1–7 for caffeine in the presence of other compounds. The within-day and between-day precision and accuracy of the proposed method were acceptable (CV < 3% and error < 2%) for all three components and the proposed method was satisfactory for quality control purposes. The method was successfully applied to the simultaneous determination of acetaminophen, ibuprofen and caffeine in pharmaceutical dosage forms without any interference from excipients and there was no need to any prior separation before analysis.

Keywords: acetaminophen, ibuprofen, caffeine, derivative spectrophotometry, dosage form

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Acetaminophen, N-(4-hydroxyphenyl) acetamide, is a common antipyretic-analgesic agent which is available in different dosage forms. Acetaminophen is used for pain and fever relief [1]. Ibuprofen, 2-[4-(2-methylpropyl)phenyl] propionic acid, is a non-steroidal anti-inflammatory drug with analgesic, anti-inflammatory and antipyretic effects. Ibuprofen is used for the treatment of pain in dysmenorrhea, osteoarthritis and rheumatoid arthritis [1]. Caffeine is a central nervous system stimulant which is used in combination with other analgesics to improve their efficacy [2, 3]. A combination of acetaminophen, ibuprofen and caffeine is used as an effective pain killer.

Various HPLC [4–9] and spectrophotometric [10–15] methods have been reported for determination of these drugs alone or in combination with other drugs in different dosage forms. There are only two reports for simultaneous determination of these drugs in pharmaceutical dosage forms using UV-visible spectrophotometry based on chemometrics [16] and also the combination of double divisor-ratio spectra derivative and H-point standard addition method [17]. The chemometric methods are relatively time-consuming and also need specialized person to perform the tests. Derivative spectrophotometry is a useful technique for simultaneous determination of a mixture of compounds with overlapping absorption.

In continuation to our previous reports [18–23], in this study, a simple and fast zero-crossing derivative spectrophotomertic method is proposed for simultaneous determination of the ternary mixture of acetaminophen, ibuprofen and caffeine. The proposed method does not require prior separation or sample preparation for determination of drugs in dosage forms and also there is no need for special computational techniques such as chemometrics.

EXPERIMENTAL

Instrumentation. A Shimadzu Model 160 double-beam UV-visible spectrophotometer (Kyoto, Japan) with a fixed band width of 2 nm and l0 mm quartz cells were used for spectrophotomertic measurements. The zero order spectra were obtained in the range of 200–350 nm. The derivative spectra were recorded in the same wavelength range at different slit widths ($\Delta\lambda$).

Chemicals. Ibuprofen was from Hubei Granules-biocause Pharmaceutical Company Ltd., China (Batch No. C100-0910136M). Acetaminophen was from Temad Co, Mashhad, Iran (Batch No. Ac 903304). Caf-

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feine was from BASF, Germany (Batch No. 967991Ax10). All drugs were kindly provided by Kish Medipharm Pharmaceutical Co., Kish, Iran. Methanol was of analytical grade and purchased from Merck (Darmstadt, Germany). Novafen capsules (325 mg acetaminophen, 200 mg ibuprofen and 40 mg caffeine) was from ALHAVI, Tehran, Iran (Batch No. 1871091).

Standard solutions. Stock standard solutions of acetaminophen, ibuprofen and caffeine were prepared in methanol at 325, 200 and 40 μg/mL, respectively. Working solutions of these drugs were prepared after proper dilution with methanol to obtain the zero order and derivative spectra.

To prepare the calibration solutions, accurate volumes of the standard solutions of acetaminophen (100 μg/mL, 0.5–5 mL), ibuprofen (50 μg/mL, 1–6 mL) and caffeine (20 μg/mL, 0.5–3.5 mL) were transferred into three sets of 10 mL calibrated flasks and completed to volume with methanol. The first set contained constant concentrations of ibuprofen (10 μg/mL) and caffeine (4 μg/mL) and varied concentrations of acetaminophen (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 μg/mL). The second set contained constant concentrations of acetaminophen (20 μg/mL) and caffeine (4 μg/mL) and varied concentrations of ibuprofen (5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, 25 and 30 μg/mL). The third set contained constant concentrations of acetaminophen (20 μg/mL) and ibuprofen (10 μg/mL) and varied concentrations of caffeine (1, 2, 3, 4, 5, 6 and 7 μg/mL).

Spectrophotometric measurement. The zero order spectra of standard solutions of acetaminophen, ibuprofen and caffeine were recorded in the range of 200–350 nm in the presence of methanol as blank. The first order to fourth order spectra of these solutions were also recorded in the same wavelength range. The values of ^3D ($\Delta\lambda = 24.5$) and ^4D ($\Delta\lambda = 12.0$) amplitudes for acetaminophen at 311 and 270 nm, (zero-crossing of ibuprofen and caffeine), ^2D ($\Delta\lambda = 21.0$) amplitude for ibuprofen at 235 nm (zero-crossing of acetaminophen and caffeine) and ^4D ($\Delta\lambda = 27.0$) amplitude for caffeine at 300 nm (zero-crossing of acetaminophen and ibuprofen) were used for spectrophotometric measurements.

Validation of the method. Calibration standard solutions (six series) of each component in the presence of constant concentrations of the two other compounds were analyzed at the above mentioned wavelengths and the absorbance was constructed over the concentration and statistical analysis performed.

To evaluate the precision and accuracy of the method, three synthetic mixtures of drugs were used. The first series contained 5, 20 and 50 μg/mL acetaminophen in the presence of 10 μg/mL of ibuprofen and 4 μg/mL of caffeine. The second series contained 5, 15 and 30 μg/mL ibuprofen in the presence of 20 μg/mL of acetaminophen and 4 μg/mL of caffeine. The third series contained 1, 4 and 7 μg/mL caffeine in the presence of 20 μg/mL of acetaminophen and 10 μg/mL of ibuprofen.

The absorbance value of the solutions was measured at the mentioned wavelengths and the concentration of each component was measured using their corresponding calibration curves. Each series were analyzed for three times in one day and also three consecutive days to find out the within-day and between-day precision and accuracy.

Application of the method. The content of twenty capsules of Novafen were weighed and combined thoroughly. An accurately weighed amount of the powder equivalent to one fourth of one capsule transferred to a 100 mL volumetric flask and 70 mL of methanol was added. After 20 min sonication, methanol was added to reach the volume. After filtration through a 0.45 μm membrane filter (Millipore), 3 mL of the solution was transferred to a 100 mL volumetric flask and made up to the volume with methanol. The concentration of acetaminophen, ibuprofen and caffeine were determined according to the general procedure by comparison with a standard solution at the same concentration level. Standard HPLC methods for determination of acetaminophen [24], ibuprofen [25] and caffeine [24] were also used to find out the accuracy and precision of the proposed method.

RESULTS AND DISCUSSION

Spectrophotometric measurements. The zero order absorption spectra of acetaminophen, ibuprofen and caffeine are shown in Fig. 1. Due to the extensive overlap of the spectral bands, conventional spectrophotometry could not be used for simultaneous determination of these drugs in mixtures. The derivative spectra of each compounds were obtained at different orders (1–4) and varied $\Delta\lambda$ values.

All the resulted spectra were compared to find out the zero-crossing points of two compounds where the third one showed absorption. The selection of optimum wavelength for determination of each compound was based on the fact that the absorbance value could have a linear response to the concentration and also is not affected by the concentration of other compounds. Accordingly, the third order (Fig. 2) and fourth order derivative spectra traced with $\Delta\lambda = 24.5$ and $\Delta\lambda = 12.0$ nm showed zero-crossing points for ibuprofen and caffeine at 311 and 270 nm which could be used for determination of acetaminophen in the presence of other compounds. Suitable wavelengths for ibuprofen and caffeine were 235 and 300 nm using the second order ($\Delta\lambda = 21.0$) (Fig. 3) and fourth order ($\Delta\lambda = 27.0$) (Fig. 4) derivative spectra.

Validation. The results of calibration curves for each component in the presence of constant concentration of the other compounds showed linear response to the analyte concentration over the range of 5–50, 5–30 and 1–7 μg/mL for acetaminophen, ibuprofen and

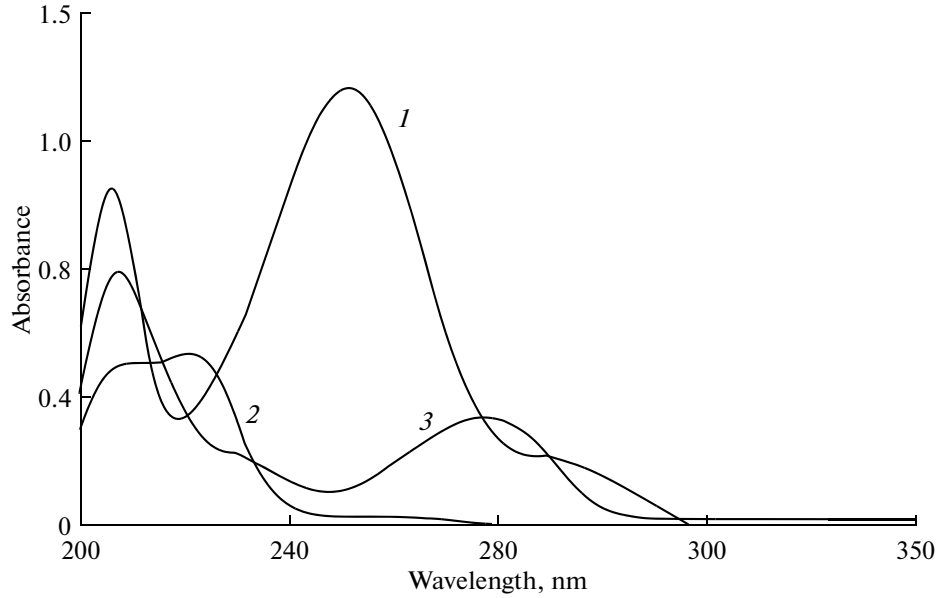

Fig. 1. Zero order spectra of 20 μg/mL acetaminophen (*1*), 10 μg/mL ibuprofen (*2*) and 7 μg/mL caffeine (*3*).

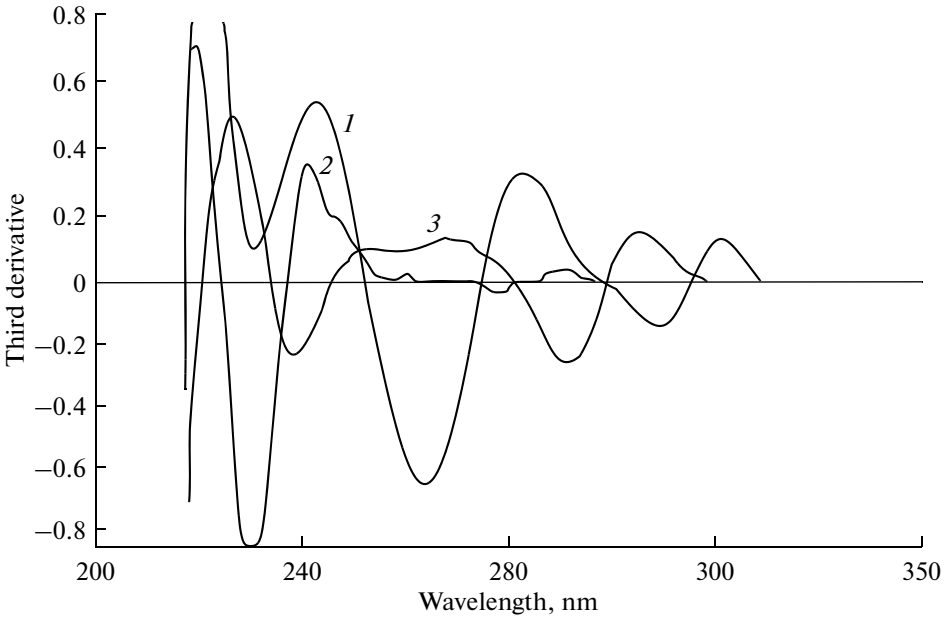

Fig. 2. Third order derivative spectra of 20 μg/mL acetaminophen (*1*), 10 μg/mL ibuprofen (*2*) and 7 μg/mL caffeine (*3*).

caffeine, respectively. The calculated statistical data for six calibration curves are presented in Table 1.

The within-day and between-day precision and accuracy of the method were acceptable (CV < 3% and error < 2%) for all three compounds in the presence of other components, as shown in Table 2.

The limits of detection (**LOD**) and quantification (**LOQ**) were calculated using the following equations [26]: LOQ = $10\sigma/s$ and LOD = $3.3\sigma/s$, where σ is the standard deviation of intercept and *s* is the slope of the calibration graph. According to the results obtained, LOQs were found to be 3.1, or 5, 2.1 and 0.7 μg/mL for acetaminophen, ibuprofen and caffeine, respectively. The LODs were found to be 1.0, or 1.7, 0.7 and 0.2 μg/mL for acetaminophen, ibuprofen and caffeine, respectively.

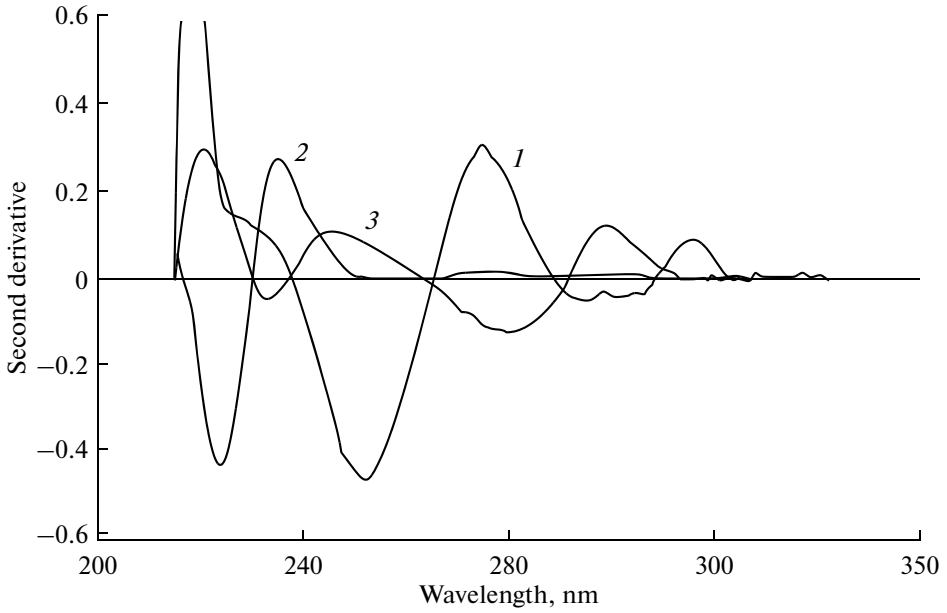

Fig. 3. Second order derivative spectra of 20 μg/mL acetaminophen (*1*), 10 μg/mL ibuprofen (*2*) and 7 μg/mL caffeine (*3*).

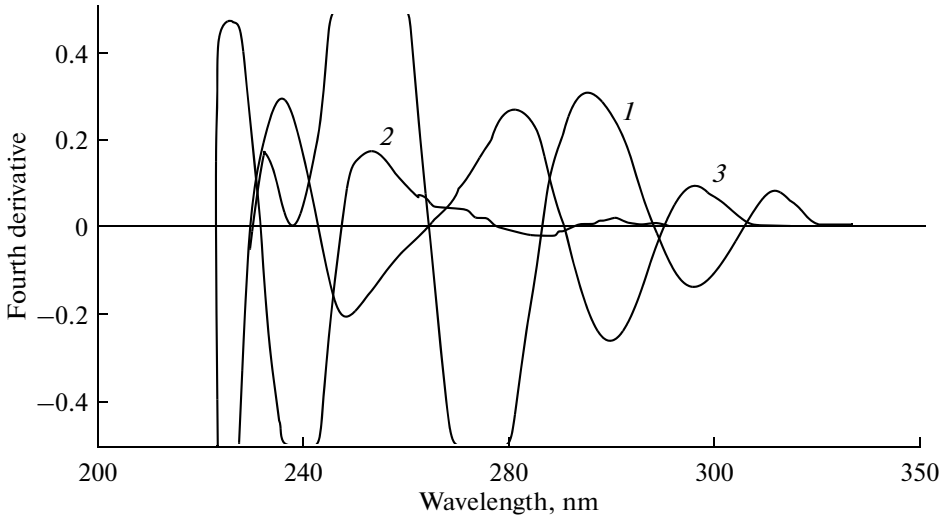

Fig. 4. Fourth order derivative spectra of 20 μg/mL acetaminophen (*1*), 10 μg/mL ibuprofen (*2*) and 7 μg/mL caffeine (*3*).

Application of the method. The proposed method alongside with a reference method was used for the determination of acetaminophen, ibuprofen and caffeine in Novafen capsules. The results are shown in Table 3 which is in good agreement with the label claim.

The statistical analysis of the obtained results from the proposed method and the reference HPLC method was performed using Student's paired *t*-test and the variance ratio *F*-test. As shown in Table 3, the calculated *t* and *F* values at 95% confidence level are less than the theoretical *t* (2.78) and *F* (19.0) values, which confirms absence of significant difference between the proposed reference methods.

* * *

Application of derivative spectrophotometry and selection of zero-crossing wavelengths offers us a simple and rapid technique for simultaneous determination of acetaminophen, ibuprofen and caffeine in pharmaceutical dosage forms.

Table 1. Statistical data of calibration curves of acetaminophen, ibuprofen and caffeine

Parameter	Acetaminophen[a]		Ibuprofen[b]	Caffeine[c]
	$^3D_{311}$ ($\Delta\lambda = 24.5$)	$^4D_{270}$ ($\Delta\lambda = 12.0$)	$^2D_{235}$ ($\Delta\lambda = 21.0$)	$^4D_{300}$ ($\Delta\lambda = 27.0$)
Linearity range, μg/mL	5–50	5–50	5–30	1–7
Regression equation	$Y = 6.1 \times 10^{-3}X + 0.01$	$Y = 4.8 \times 10^{-3}X - 0.02$	$Y = 1.3 \times 10^{-2}X - 0.06$	$Y = 1.5 \times 10^{-2}X - 0.01$
SD of slope	8.37×10^{-5}	8.94×10^{-5}	1.4×10^{-4}	2.4×10^{-4}
RSD of slope, %	1.38	1.86	1.11	1.58
SD of intercept	1.9×10^{-3}	2.4×10^{-3}	2.7×10^{-3}	1.1×10^{-3}
Correlation coefficient	0.998	0.997	0.998	0.998

[a] In the presence of 10 μg/mL ibuprofen and 4 μg/mL caffeine.
[b] In the presence of 20 μg/mL acetaminophen and 4 μg/mL caffeine.
[c] In the presence of 20 μg/mL acetaminophen and 10 μg/mL ibuprofen.

Table 2. Accuracy and precision data for simultaneous determination of acetaminophen[a], ibuprofen[b] and caffeine[c] (three sets for 3 days) by derivative spectrophotometry

Added, μg/mL	Within-day ($n = 3$)			Between-day ($n = 9$)		
	found, μg/mL	CV, %	error, %	found, μg/mL	CV, %	error, %
Acetaminophen $^3D_{311}$ ($\Delta\lambda = 24.5$)						
5.00	5.05 ± 0.08	1.67	1.00	4.98 ± 0.11	2.20	–0.40
20.0	20.4 ± 0.4	1.96	2.00	20.2 ± 0.4	1.98	1.00
50.0	51.0 ± 0.4	0.78	2.00	50.7 ± 0.6	1.18	1.40
Acetaminophen $^4D_{270}$ ($\Delta\lambda = 12.0$)						
5.00	5.03 ± 0.12	2.86	0.60	5.04 ± 0.12	2.31	0.80
20.0	19.7 ± 0.2	1.02	–1.45	20.0 ± 0.4	2.02	–0.20
50.0	50.1 ± 0.4	0.85	0.22	49.9 ± 0.6	1.19	–0.12
Ibuprofen $^2D_{235}$ ($\Delta\lambda = 21.0$)						
5.00	4.91 ± 0.07	1.44	–1.80	5.01 ± 0.13	2.60	0.20
15.0	15.1 ± 0.2	1.32	0.67	15.2 ± 0.3	1.97	1.33
30.0	30.2 ± 0.6	1.99	0.67	30.1 ± 0.6	1.99	0.33
Caffeine $^4D_{300}$ ($\Delta\lambda = 27.0$)						
1.00	1.01 ± 0.02	1.74	1.00	1.01 ± 0.02	1.96	1.00
4.00	4.00 ± 0.04	0.92	0.00	3.98 ± 0.05	1.21	–0.50
7.00	6.94 ± 0.06	0.91	0.86	7.01 ± 0.09	1.27	0.14

[a] In the presence of 10 μg/mL ibuprofen and 4 μg/mL caffeine.
[b] In the presence of 20 μg/mL acetaminophen and 4 μg/mL caffeine.
[c] In the presence of 20 μg/mL acetaminophen and 10 μg/mL ibuprofen.

Table 3. Comparison of the developed method with the reference method for the analysis of Novafen capsules

Compound	Label claim, mg	Found, mean ± SD, mg		Statistical tests[a]
		proposed method	HPLC	
Acetaminophen	325	322 ± 3	323 ± 2	$t = 0.70$, $F = 0.91$
Ibuprofen	200	201 ± 3	202 ± 3	$t = 0.60$, $F = 0.86$
Caffeine	40.0	39.7 ± 1.1	40.5 ± 1.0	$t = 0.39$, $F = 0.84$

[a] Theoretical values of t and F at $p = 0.05$ are 2.78 and 19.0, respectively.

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