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A Rapid Spectrophotometric Method to Resolve Ternary Mixtures of Propyphenazone, Caffeine, and Acetaminophen in Tablets

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Summary. A simple and rapid derivative spectrophotometric assay procedure is described for the analysis of caffeine (**1**), acetaminophen (**2**), and propyphenazone (**3**) in tablet formulations. The concentration range of application is 5.0–25.0 $\mu g \cdot cm^{-3}$ for **2** and **3** and 1.0–5.0 $\mu g \cdot cm^{-3}$ for **1**. The method involves the extraction of the drugs from tablets with 0.1 *N* H_2SO_4, filtration, appropriate dilution, and measurement of the fourth derivative absorbance values at zero crossing wavelengths of 230.0, 263.2, and 256.6 nm for **1**, **2**, and **3**. As a reference method, a reversed phase HPLC procedure was developed. Commercially available tablets were analyzed; statistical comparison of the results with those obtained from the reference method showed good agreement. The derivative spectrophotometric method has the advantage of being simple, rapid, inexpensive, and easy to perform.

Keywords. Derivative spectrophotometry; Pharmaceuticals analysis; Simultaneous determination of drugs.

Introduction

Acetaminophen is widely used as an analgesic and antipyretic alone or in combinations with various drugs. Caffeine (**1**), acetaminophen (**2**), and propyphenazone (**3**) are frequently present together in commercial analgesic preparations. For the assay of these dosage forms HPLC, methods have been reported [1, 2] as official methods. For the simultaneous determination of **1** and **2**, HPLC and derivative spectrophotometric procedure have been described [3]. A first derivative UV/Vis spectrophotometric method has also been developed for the simultaneous determination of **1** and **3** in sugar-coated tablets [4]. There is no pharmacopoeial method for the simultaneous analysis of **1**, **2**, and **3**.

The quality control of dosage form preparations of drugs requires reliable and quick analytical methods. To resolve problems of spectral overlap, derivative spectrophotometry has been widely used for the determination of drugs in mixtures [5, 6]. In previous papers we have described derivative spectrophotometric

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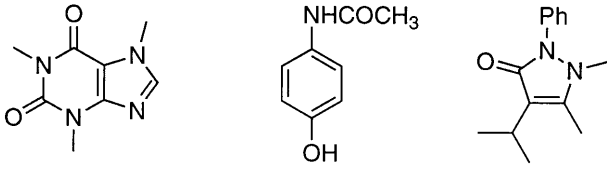
procedures for the determination of binary mixtures [7–10]. Recently, derivative spectrophotometric methods have been developed in order to resolve ternary mixtures of food dyes [11–14]. In the present study, derivative spectrophotometry is used for the simultaneous determination of **1**, **2**, and **3** in tablets.



1 **2** **3**

Results and Discussion

In Fig. 1 the absorption spectra of **1**–**3** are shown in the 190–350 nm wavelength range. It can be seen that the absorption spectra of the three drugs are strongly overlapped. In order to resolve this ternary mixture we have applied the proposed method on the basis of zero-crossing measurements in the fourth derivative spectra.

Figure 2 shows the fourth derivative spectra of **1**–**3**. As can be seen, **2** can be determined by measuring at 263.2 nm (zero-crossing points for **1** and **3**), **1** at 230.0 nm (zero-crossing points for **2** and **3**), and **3** at 256.6 nm (zero-crossing points for **2** and **1**). By measuring the fourth derivative absorbances ($d^4A/d\lambda^4$, in the following denoted D^4) of standard solutions of **1**–**3** at selected wavelengths, calibration curves were established; concentration ranges were 5.0–25.0 $\mu g \cdot cm^{-3}$ for **2** and **3** and 1.0–5.0 $\mu g \cdot cm^{-3}$ for **1**. Linear regression analysis of the calibration curves was performed using the method of least squares. The statistical

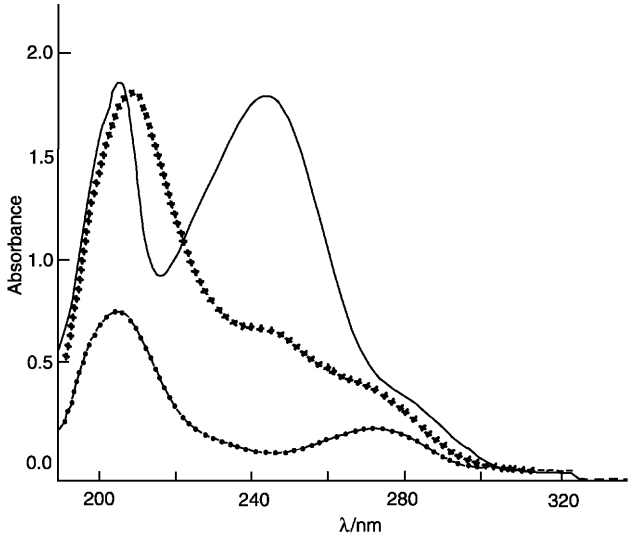


Fig. 1. Absorption spectra of 3 $\mu g \cdot cm^{-3}$ **1** (– · –), 30 $\mu g \cdot cm^{-3}$ **2** (—), and 15 $\mu g \cdot cm^{-3}$ **3** (+++)

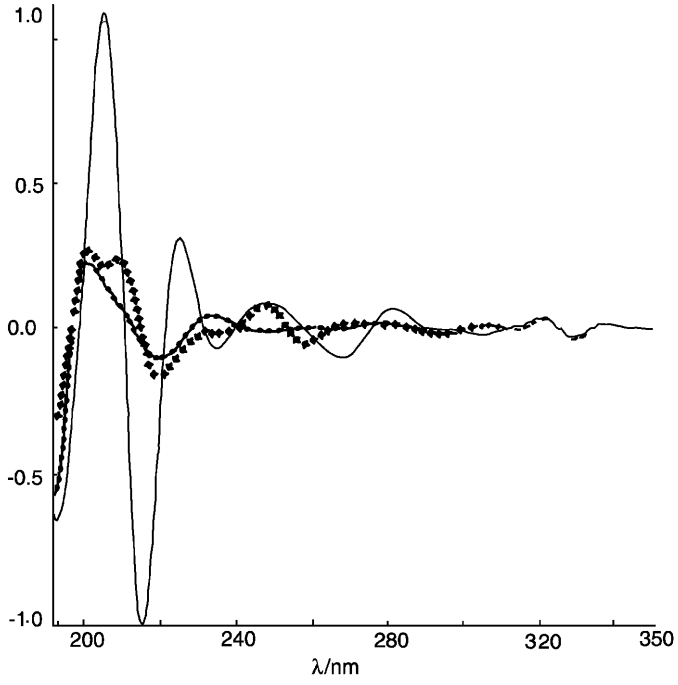


Fig. 2. Fourth derivative spectra of $3\,\mu g \cdot cm^{-3}$ **1** (– · –), $30\,\mu g \cdot cm^{-3}$ **2** (—), and $15\,\mu g \cdot cm^{-3}$ **3** (+++)

Table 1. Calibration equations for derivative spectrophotometry; C_1–C_3: concentrations of **1**–**3**

Drug	Linearity range (mg/dm^3)	Regression equation	Correlation coefficient
1	1.0–5.0	${}^4D_{230.0} = 3.00 \cdot 10^{-3} c_1 + 8.0 \cdot 10^{-3}$	0.9990
2	5.0–25.0	${}^4D_{263.2} = 1.91 \cdot 10^{-3} c_2 + 9.0 \cdot 10^{-4}$	0.9992
3	5.0–25.0	${}^4D_{256.6} = 3.78 \cdot 10^{-3} c_3 + 5.4 \cdot 10^{-4}$	0.9998

data of calibration obtained for each drug are summarised in Table 1. In all cases, good correlation coefficients were obtained.

Commercially available tablets containing 300 mg of **2**, 30 mg of **1**, and 150 mg of **3** were successfully analyzed by the proposed method. Since there is no pharmacopoeial method for the analysis of ternary mixtures of **1**, **2**, and **3**, a reversed phase HPLC procedure was developed as a reference method. In the chromatograms obtained by this procedure the retention times of **2**, **1**, and **3** were 3.27, 4.19, and 12.91 minutes. As an internal standard, antipyrine was used (retention time: 4.58 min). The tablets were also assayed by the HPLC method. The assay results obtained by both methods were statistically compared at 95% confidence level. As shown in Table 2 there was no significant difference between the mean values and precisions of the two methods.

In conclusion, the derivative spectrophotometric method is simpler than the HPLC method and does not require expensive solvents. It shows as good a

Table 2. Assay result of commercial tablets containing 30 mg **1**, 300 mg **2**, and 150 mg **3**

	Mean $\pm t \cdot s/\sqrt{n}$ (mg/tablet)		
	1	**2**	**3**
Derivative spectrophotometry	29.6 ± 0.8	298.7 ± 2.0	149.7 ± 1.7
HPLC	28.9 ± 0.7	297.8 ± 1.8	150.0 ± 1.6
t-Test of significance[a]	0.69	0.68	0.15
F-Test of significance[a]	1.68	1.22	1.13

[a] $t = 2.23$, $F = 5.05$ for $p = 0.05$ and $n_1 = n_2 = 6$

precision and accuracy as the HPLC method; therefore, the fourth derivative spectrophotometric method is more suitable for routine quality control analysis of tablets containing a mixture of **1**, **2**, and **3**. There was no interference from the commonly present tablet additives.

Experimental

Materials

Pharmaceutical grade samples of **1–3** and antipyrine (internal standard; Doğu, Turkey) were used as received. HPLC grade MeOH and other analytical grade chemicals were purchased from E. Merck. Mill Q water was used.

Instrumentation

For derivative spectrophotometric measurements a Philips 8740 UV/Vis spectrophotometer and 10 mm quartz cells were used. All spectra were recorded from 190 nm to 350 nm with 1 nm slit width, $250\,\text{nm} \cdot \text{min}^{-1}$ scan speed, and very high smoothing. Chromatographic analyses were performed using a Waters HPLC system consisting of a model 510 delivery system, a model 481 variable wavelength spectrophotometer (at 270 nm), and a computer. A 10 μm Bondapak C_{18} (3.9 mm × 30 cm) RP column and an isocratic mobile phase (MeOH: $H_2O = 3{:}7$) were used. The flow rate was $1.5\,\text{cm}^3 \cdot \text{min}^{-1}$ (PSI 2300). Quantitative analysis was accomplished by internal standard calibration.

Standard solution for derivative spectrophotometry

Stock solutions of **1–3** ($200\,\mu\text{g} \cdot \text{cm}^{-3}$ in 0.1 *N* H_2SO_4) were prepared daily. Standard solutions for the preparation of the calibration curve were obtained by diluting the stock solutions appropriately.

Standard preparation for HPLC

$2\,\text{cm}^3$ of **1** ($80.3\,\text{mg} \cdot \text{cm}^{-3}$), $2\,\text{cm}^3$ of **2** ($3\,\text{mg} \cdot \text{cm}^{-3}$), $2\,\text{cm}^3$ of **3** ($1.5\,\text{mg} \cdot \text{cm}^{-3}$), and $2\,\text{cm}^3$ ($0.3\,\text{mg} \cdot \text{cm}^{-3}$) of internal standard solutions were combined and the volume was completed to $10\,\text{cm}^3$ with H_2O.

Calibration procedure for derivative spectrophotometry

Three solutions were prepared by diluting the sample solutions (*i*: 1.0–$5.0\,\mu\text{g} \cdot \text{cm}^{-3}$ **1**; *ii*: 5.0–$25.0\,\mu\text{g} \cdot \text{cm}^{-3}$ **2**; *iii*: 5.0–$25.0\,\mu\text{g} \cdot \text{cm}^{-3}$ **3**). Fourth derivative spectra of the three solutions were

recorded against 0.1 *N* H_2SO_4. Peak-to-zero values of the spectra at 263.0, 230.0, and 256.6 nm were measured for the determination of **1**–**3**. The quantities of the drugs were calculated using the regression equations of the corresponding calibration curves prepared by the corresponding peak-to-zero values of the spectra of standard drug solutions.

Sample preparation

Twenty tablets were weighed and powdered. To an accurately weighed amount of the powder equivalent to approximately 2 mg of **1**, 20 mg of **2**, and 10 mg of **3** in a 100 cm^3 calibrated flask, about 60 cm^3 of 0.1 *N* H_2SO_4 was added. The mixture was shaken for 20 min, diluted to volume with 0.1 *N* H_2SO_4, and filtrated. The filtrate was used for derivative spectrophotometric and HPLC determinations after appropriate dilutions.

HPLC method

A 10 cm^3 aliquot of the sample preparation was transferred into a 10 cm^3 calibrated flask, 2 cm^3 of internal standard (1 $mg \cdot cm^{-3}$) were added, and the volume was completed with H_2O. After filtration and sonication, 10 mm^3 of the solution were injected into the HPLC apparatus.

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