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Simultaneous determination of clopamide–pindolol combination in tablets by zero-crossing derivative spectrophotometry

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Abstract: A first-derivative spectrophotometric method, using a 'zero-crossing' technique of measurement has been used for determining clopamide–pindolol mixture in tablets. In the first-derivative mode the zero-crossing points of clopamide and pindolol occur at 272.6 and 262.4 nm, respectively. The relative ease offered by this technique for the quantification of these drugs with closely overlapping bands was demonstrated. The linearity of the calibration curves was satisfactory (r = 0.9998) and the precision (RSD%) better than 1.89. Detection limits were 0.50 and 0.44 μg ml^{-1} for pindolol and clopamide, respectively. No spectral interferences from tablet excipients were found. Applications are given for the assay of commercial tablets and content uniformity test. The procedures proved to be suitable for rapid and reliable quality control.

Keywords: *Clopamide; pindolol; derivative spectrophotometry.*

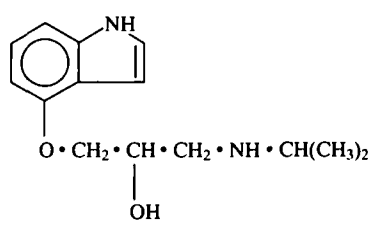
Introduction

Pindolol (1-(indo-4-yloxy)-3-isopropylamino-propan-2-ol), is a non-cardioselective β-adrenoreceptor blocking agent with intrinsic sympathomimetic activity and is used in the treatment of hypertension and angina pectoris. Its combination with clopamide (4-chloro-N-(2,6-dimethyl-piperidino)-3-sulphamoylbenz-amide), a diuretic that reduces the re-absorption of electrolytes from renal tubules, increases the antihypertensive effects. Their structures are shown in Scheme 1.

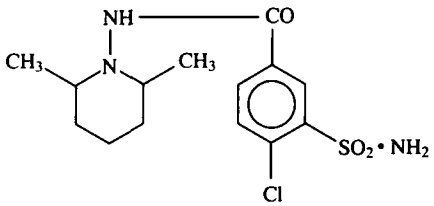
Several analytical methods have been reported for assaying pindolol; these include HPLC [1, 2], thin-layer chromatography [3, 4], GC–MS [5] and GLC [6, 7]. HPLC [8], derivative spectrophotometry [9], and GC–MS [10], have been described for clopamide determination. However, no method has been reported for their simultaneous quantification in two component mixtures.

Derivative spectrophotometry [11–16] is an analytical technique of great utility for extracting both qualitative and quantitative information from spectra composed of unresolved bands. It tends to emphasize subtle spectral features by presenting them in a new and visually more accessible way, allowing the resolution of multi-component elements, and reducing the effect of spectral background interferences.

In pharmaceutical application, derivative spectrophotometry has led to significant developments in the analysis of drugs in the presence of their degradation products or in



Pindolol



Clopamide

Scheme 1

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multicomponent mixtures [17–23]. The method has also been applied for the determination of drugs in biological fluids [24–25].

The objective of this work was to demonstrate the ease with which the proposed derivative method (first-derivative spectrophotometry using a 'zero-crossing' technique of measurements [26]) circumvents the problem of overlapping spectral bands, allowing the simultaneous determination of clopamide and pindolol in laboratory mixtures without the need for prior separation.

Experimental

Materials

Methanol and hydrochloric acid were of analytical-reagent grade. Clopamide and pindolol, of pharmaceutical grade, were kindly provided by Sandoz (Greece) and were used without further purification. Several excipients, used for the interference study, were obtained from commercial sources.

Tablets of Viskaldix (Sandoz, Greece), labelled to contain 10 mg of pindolol and 5 mg of clopamide per tablet, were used.

Apparatus

Spectrophotometric measurements were performed on a double beam UV–vis spectrophotometer (Perkin–Elmer, Lambda 7), capable of derivative mode. All measurements were carried out in 1-cm matched quartz cells. Suitable settings were: wavelength range, 250–280 nm; response time, 2 s; scan speed, 60 nm min^{-1}; spectral slit width, 2 nm; delta wavelength, 6 nm; ordinate maximum and minimum, ±20.0.

Procedure

Calibration procedure

Working standard solutions of clopamide–pindolol mixtures in 0.1 M HCl (containing 40.0 μg ml^{-1} of pindolol and increasing concentrations of clopamide ranging from 6.0 to 50.0 μg ml^{-1}) were prepared daily from stock solutions of clopamide (0.5 mg ml^{-1}) and pindolol (1.0 mg ml^{-1}) in methanol. The first-order derivative spectra (D_1) of these solutions were recorded over the wavelength range 250–280 nm, against 0.1 M HCl as blank. The derivative values at 262.4 nm $D_{1(262.4)}$ were measured for the determination of clopamide in presence of pindolol.

Working standard solutions of clopamide–pindolol mixtures in 0.1 M HCl (containing 20.0 μg ml^{-1} of clopamide and increasing concentrations of pindolol ranging from 8.0 to 100.0 μg ml^{-1}) were prepared daily using the same stock solutions. The D_1 spectra of these solutions were also recorded between 250 and 280 nm and the derivative values at 272.6 nm $D_{1(272.6)}$ were measured for the determination of pindolol in presence of clopamide.

Assay of tablets

Twenty tablets were weighed and pulverized. An accurately weighed amount of powder equivalent to 10 mg of pindolol and 5 mg of clopamide was placed in a 25 ml volumetric flask with methanol. The mixture was sonicated for 1 min and diluted to volume with methanol. A portion of this solution was centrifuged at 4000 rpm for 15 min. A 0.50 ml aliquot was transferred in a tube containing 4.5 ml of 0.1 M HCl. The D_1 spectrum was recorded from 250 to 280 nm and the absolute derivative values at 262.4 and 272.6 nm were measured. The concentrations of clopamide and pindolol in the sample solution were obtained by interpolating the corresponding calibration curves. For the content uniformity test, the same procedure was followed (using one tablet as a sample), except that the mixture was sonicated for 30 min.

Results and Discussion

Spectrophotometric measurements

Figure 1 shows the absorption zero-order UV spectra of clopamide with a maximum at 243 nm, of pindolol with a maximum at 264 nm and a shoulder at 286 nm and clopamide–pindolol mixtures (1:2, v/v) with a maximum at 255 nm and a shoulder at 286 nm.

Due to the extensive overlap of the spectral bands of the two drugs, conventional UV spectrophotometry cannot be used for their individual determination in a binary mixture. However, zero-crossing first-order derivative spectrophotometry permits a more selective identification and determination of the two drugs in a mixture. The zero-crossing method, involves measurements of the absolute value of the total derivative spectrum at an abscissa value corresponding to the zero-crossing wavelengths of the derivative spectra of the individual component.

Figure 2 shows the D_1 spectra of clopamide

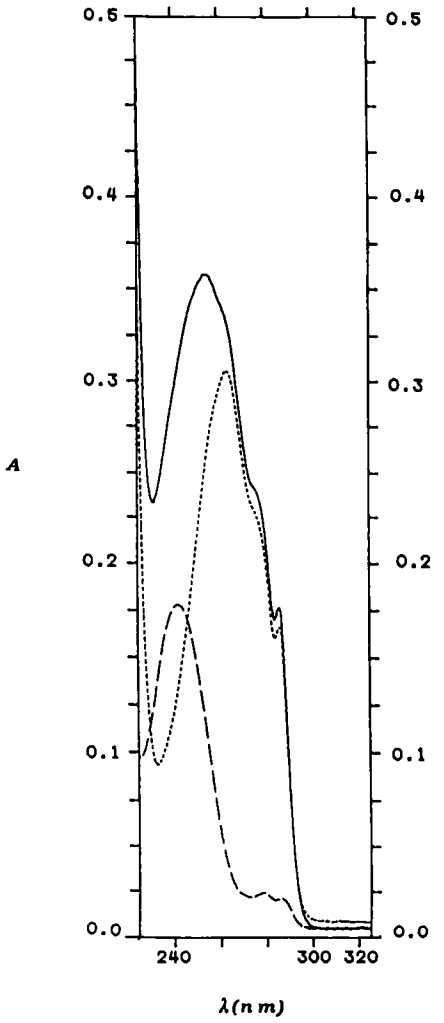

Figure 1
Absorption (zero-order) UV spectra of 10.0 μg ml^{-1} clopamide (----), 20.0 μg ml^{-1} pindolol (·····), and its binary mixture 1:2, v/v (———), in 0.1 M HCl.

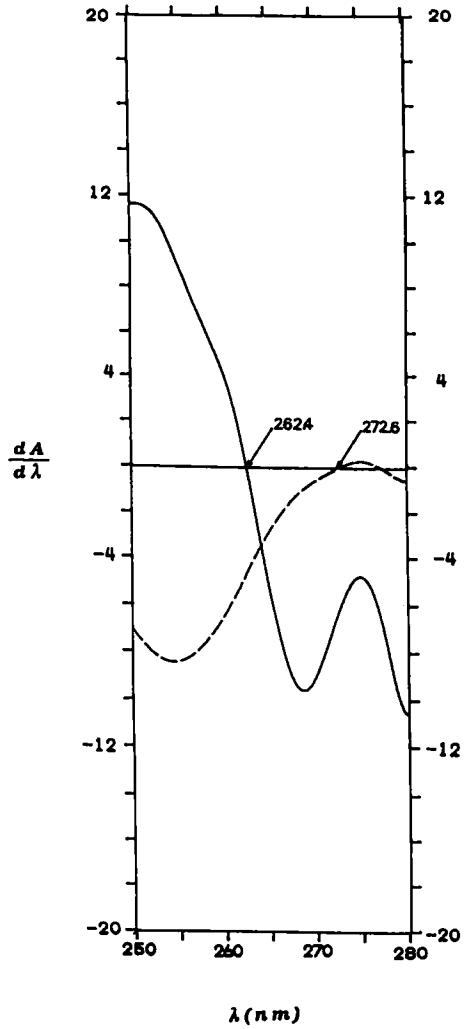

Figure 2
First-order derivative spectra of 20.0 μg ml^{-1} clopamide (-----), and of 40.0 μg ml^{-1} pindolol (———), in 0.1 M HCl.

and pindolol (the zero-crossing wavelength points are indicated). The selection of the optimum wavelength is based on the fact that the absolute value of the total derivative spectrum at the selected wavelength has the best linear response to the analyte concentration, it is not affected by the concentration of any other component and gives a near-zero intercept on the ordinate axis of the calibration curve. Therefore, 262.4 nm (zero-crossing wavelength point of pindolol) and 272.6 nm (zero-crossing wavelength point of clopamide) were chosen as optimum working wavelengths for the simultaneous determination of clopamide and pindolol in a binary mixture, respectively. Measurements of the absolute values of the total derivative spectrum taken at these wavelengths gave the best linear response to the analyte concentration.

Figure 3 shows a typical set of D_1 spectra of 40.0 μg ml^{-1} pindolol plus increasing amounts of clopamide (10.0–50.0 μg ml^{-1}) and the D_1 spectrum of pindolol alone (40.0 μg ml^{-1}). Analogously, Fig. 4 exhibits a series of D_1 spectra of mixtures of 20.0 μg ml^{-1} clopamide plus increasing amounts of pindolol (20.0–100.0 μg ml^{-1}) and the D_1 spectrum of clopamide alone (20.0 μg ml^{-1}). It is interesting to note the distinct isosbestic points in Fig. 3 at 272.6 nm (zero-crossing wavelength points of clopamide) and in Fig. 4 at 262.4 nm (zero-crossing wavelength points of pindolol) irrespective of their concentration.

Statistical analysis of data

Under the experimental conditions described above, linear relationships between

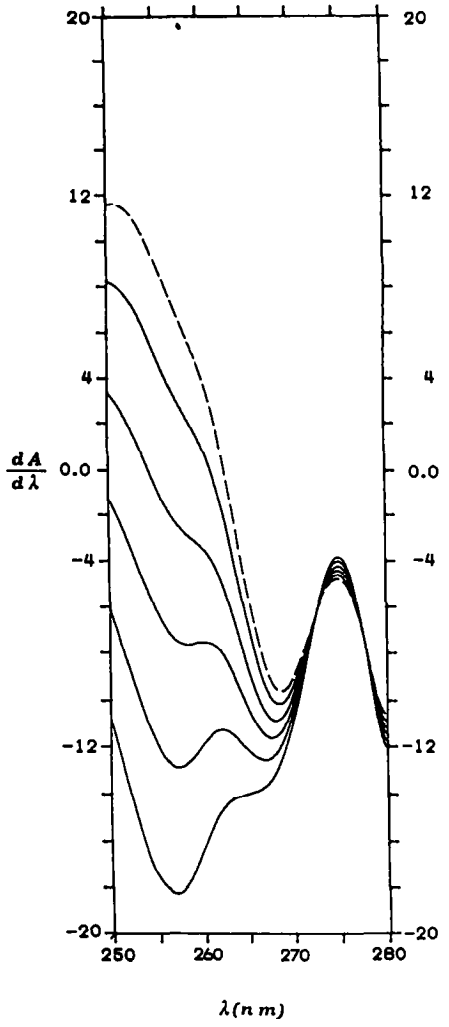

Figure 3
First-order derivative spectra of mixtures containing 40.0 μg ml^{-1} pindolol plus increasing amounts of clopamide ranging from 10.0 to 50.0 μg ml^{-1} (———), and first-order derivative spectrum of 40.0 μg ml^{-1} pindolol (-----), in 0.1 M HCl.

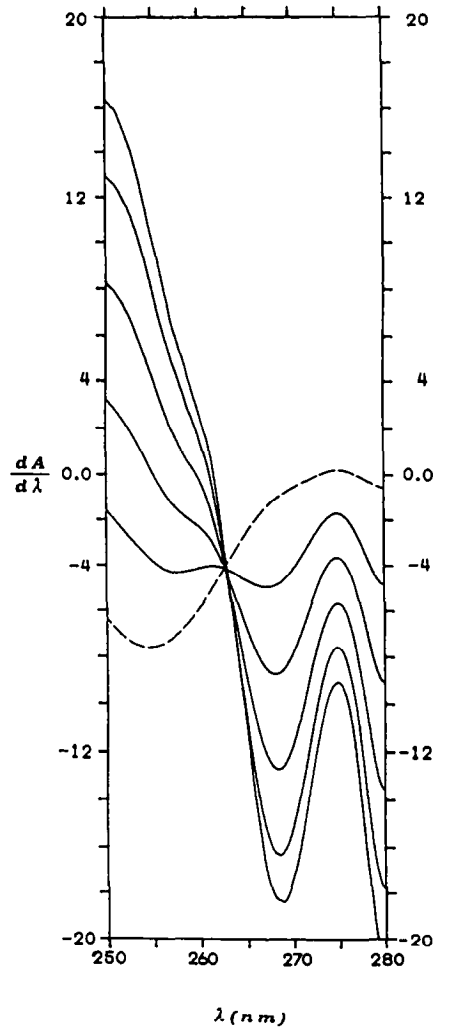

Figure 4
First-order derivative spectra of mixtures containing 20.0 μg ml^{-1} clopamide plus increasing amounts of pindolol ranging from 20.0 to 100.0 μg ml^{-1} (———), and first-order derivative spectrum of 20.0 μg ml^{-1} clopamide (-----), in 0.1 M HCl.

Table 1
Analytical data of the calibration graphs for the determination of clopamide and pindolol by first-order derivative spectrophotometry

Compound	λ (nm)	Linearity range (μg ml^{-1})	Calibration equation*	r†
Pindolol	272.6	8–100	$D_1 = (0.119 \pm 0.010)C + (0.041 \pm 0.056)$	0.9999
Clopamide	262.4	6–50	$D_1 = (0.223 \pm 0.002)C + (0.047 \pm 0.092)$	0.9998

* Derivative value of the first-order derivative spectrum versus concentration of each drug in μg ml^{-1}; six standards.
† Correlation coefficient.
$t = 2.78$ for $n = 6$ at 95% confidence level.

selected derivative values from D_1 spectra of drugs tested and their concentrations were observed as shown in Table 1.

The detection limits [27], defined by IUPAC as $C_{L(k=3)} = kS_B/b$, where b is the slope of the corresponding calibration curve, S_B is the standard deviation of the blank signal and $k = 3$ was 0.50 μg ml^{-1} and 0.44 μg ml^{-1} for pindolol and clopamide, respectively.

A Student's t-test was performed to determine whether the experimental intercepts (a) of the above mentioned regression lines were

significantly different from the theoretical zero value. The test is based on the calculation of the quantities $t = a/S_a$, where a is the intercept of the regression lines and S_a is the standard deviation of a, and their comparison with tabulated data for the t-distribution. The values calculated for t are 2.05 for pindolol and 1.42 for clopamide (these values do not exceed the 95% criterion of $t_P = 2.78$ for six samples), so the intercepts are not significantly different from zero.

The mutual interference between the two drugs was also investigated. A series of six working standard solutions containing 8.0–100.0 μg ml^{-1} of pindolol in 0.1 M HCl and a series of six working standard solutions containing 6.0–50.0 μg ml^{-1} of clopamide in 0.1 M HCl were analysed by the proposed method. The derivative values at 262.4 nm, $D_{1(262.4)}$, and at 272.6 nm, $D_{1(272.6)}$, were measured for the determination of clopamide and pindolol, respectively. The following linear equations were obtained through regression analysis of data; the bracketed values represent the confidence intervals on the slopes and intercepts that were calculated at 95% confidence level for $n = 6$:

$$D_{1(262.4)} = (0.223 \pm 6.50 \times 10^{-3})C_{cl} + (0.032 \pm 0.119),\ r = 0.9998;$$

$$D_{1(272.6)} = (0.119 \pm 2.17 \times 10^{-3})C_{pd} + (0.090 \pm 0.081),\ r = 0.9999.$$

The slopes and intercepts do not differ significantly from those obtained from the analysis of mixed standard solutions shown in Table 1, and therefore we can conclude that no interference occur in the determination of each substance in the presence of the other.

In order to assess the precision (RSD%) and the accuracy (E_r%) of the proposed method 10 replicate determinations were carried out on a clopamide–pindolol working standard solution of 20.0 μg ml^{-1} clopamide and 40.0 μg ml^{-1} of pindolol. The data shown in Table 2 indicate good accuracy and precision.

In order to examine the effect of common excipients used in the formulation of tablets on the D_1 determination of clopamide–pindolol combinations, recovery experiments were carried out on the synthetic standard solutions in 0.1 M HCl containing 20.0 μg ml^{-1} of clopamide and 40.0 μg ml^{-1} of pindolol plus various excipients in excess. From the results

Table 2
Precision and accuracy for the determination of clopamide and pindolol by first-order derivative spectrophotometry

Drug	Added (μg ml^{-1})	Found (μg ml^{-1}) mean ± SD ($n = 10$)	RSD(%)*	E_r(%)†
Clopamide	20.0	20.15 ± 0.18	0.91	0.75
Pindolol	40.0	39.90 ± 0.20	0.51	−0.25

* Relative standard deviation.
† Percentage relative error.

Table 3
Effect of tablet additives on the first-order derivative spectrophotometric determination of clopamide–pindolol mixtures

Additive	Concentration ratio		Recovery % ($n = 3$)	
	Addit./clop.	Addit./pind.	Clopamide	Pindolol
Lactose	16	8	102.9	99.4
Gelatine	4	2	102.0	98.8
Starch	16	8	101.9	98.7
Carbowax 4000*	6	3	99.9	99.2
Sodium lauryl sulphate	2	1	100.2	99.5
Mg stearate	2	1	102.0	98.5
CAHP†	6	3	101.2	98.6
Carbopol‡	9	4.5	100.8	98.8
HPMC§	12	6	100.4	100.1

* Polyethylene glycol 4000.
† Cellulose acetate hydroxypthalate.
‡ Carboxypolymethylene.
§ Hydroxypropyl methyl cellulose.

Table 4
Determination of clopamide–pindolol combination in tablets by first-order derivative spectrophotometry (D_1) and HPLC

	Clopamide (mg tablet^{-1})		Pindolol (mg tablet^{-1})	
	HPLC	D_1	HPLC	D_1
Mean* ± SD	4.98 ± 0.01	5.09 ± 0.02	9.90 ± 0.09	9.96 ± 0.13
RSD (%)	0.20	0.39	0.90	1.3
E_r(%)	−0.4	1.8	−1.0	−0.4

*n = 5.

shown in Table 3, it is evident that the D_1 spectrophotometric determination does not suffer from spectral interference of the excipients and the direct determination of clopamide–pindolol mixtures in tablets is successful without isolation of the analyte.

A further comparison of the proposed first-order derivative spectrophotometric method vs an HPLC procedure (Poulou and Panderi, unpublished data) was made analysing commercial tablets of Viskaldix (containing 5 mg of clopamide and 10 mg of pindolol). The results obtained by both methods are demonstrated in Table 4.

Assay of tablets — content uniformity

The proposed method was evaluated in the assay of commercial tablets of the clopamide–pindolol combination (Viskaldix, 10 mg of pindolol and 5 mg of clopamide). Ten replicate determinations were carried out on an accurately weighed amount of pulverized tablets equivalent to 5 mg of clopamide and 10 mg of pindolol, giving a mean value of 5.06 ± 0.05 with a RSD% = 1.04 and percentage relative error E_r% = 1.2 for clopamide, and a mean value of 9.92 ± 0.11, a RSD% = 1.00 and a E_r% = −0.8 for pindolol.

The advantages of the proposed D_1 method such as short analysis time, unnecessary sample pre-treatment, good precision and accuracy make the method suitable in content uniformity tests, where a large number of individual tablet assays are required. For this purpose 10 tablets of Viskadix were analysed. The results for all the tablets gave a mean value of 5.08 ± 0.10 with a RSD% = 1.89 and a E_r% = 1.6 for clopamide; and 9.89 ± 0.18 with RSD% = 1.83 and E_r% = −1.1 for pindolol.

In conclusion, the proposed analytical procedure offers the advantage of increased resolution and decreased spectral interferences and could be used for rapid and reliable quality control of commercial formulations containing clopamide and pindolol in combination.

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