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Determination of binary mixtures of analgesic and spasmolytic drugs in pure and dosage forms by derivative spectrophotometry

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Abstract

Binary mixtures of dipyrone and pitophenone hydrochloride are assayed by zero-crossing second- and third-derivative spectrophotometry and by ratio-spectra first- and second-derivative spectrophotometry. In the first method, calibration plots are linear at 266.5 and 302.5 nm (dipyrone, second derivative), and 257 and 286 nm (pitophenone second derivative) and 242 and 278.3 nm (dipyrone third derivative), and 228.5 and 300 nm (pitophenone, third-derivative). By the second method, lines of regression are linear at 235 and 262 nm (dipyrone, first derivative), and 229.5 and 288.5 nm (pitophenone, first-derivative), and 249.7 and 268 nm (dipyrone, second derivative), and 280.5 and 300 nm (pitophenone, second-derivative). In all methods calibration curves follow the Beer's law up to 40 μg/ml of each drug. LOD and LOQ values were calculated. The developed derivative spectrophotometric methods were applied to laboratory mixtures and to vials for these drugs. The procedures are simple, rapid, and did not require any preliminary separation or treatment of the samples.

Keywords: Dipyrone; Pitophenone hydrochloride; Analgesic drugs; Spasmolytic drugs; Ratio-spectra derivative spectrophotometry; Zero-crossing derivative spectrophotometry

1. Introduction

Dipyrone, N-methhyl-N-(2,3-dimethyl-5-oxo-1-phenyl-3-pyrazolone-4 yl) aminomethane sulphonate sodium salt or noramidopyrine methanesulphonate sodium salt and pitophenone, methyl 2-[4-(2-piperidinoetoxy) benzoyl] benzoate, are substances often found associated in the therapy of some diseases.

Dipyrone exerts both an analgesic and antispasmodic action. This spasmolytic effect is re-inforced by pitophenone hydrochloride, a papaverine analogue. The combination of these drugs develops a synergetic effect very effective, for example, in the renal and biliary colic. In contrast with their therapeutic effectiveness, the above drugs could develop, in hypertensive patients, collateral effects,

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such as gastro-enteric and renal diseases and, rarely, anaphylactic shock.

In conclusion, dipyrone and pitophenone are drugs of clinical and analytical interest, hence we deemed useful to propose novel methods for the simultaneous determination of these substances in admixture. The procedures are rapid, simple and non-destructive, and require simple spectrophotometer, i.e. instrumentation commonly available in all laboratories. They can be a useful tool for the assay of dosage forms for these drugs and are suitable for quality control laboratories, where economy and time are essential.

For the above considerations, for the importance of the two drugs in clinical and pharmacological field coupled with the risk of side effects sometimes developed in the course of the therapy and, finally, for lack of other methods or methods easier than the present for the simultaneous determination of dipyrone and pitophenone, we consider the present work of interest for the readers of journals devoted to biomedical and pharmaceutical analysis.

Dipyrone and pitophenone hydrochloride (D and P, respectively, from this point for brevity) have closely overlapping absorption spectra. Derivative UV-spectroscopy has been successfully applied in pharmaceutical and environmental analysis for the simultaneous determination of drugs in multi-component systems. Recent examples are in [1–9]. The present work is a continuation of the author's research on the possibility of the application of derivative spectrophotometry in the analysis of multi-components formulations [10–14], allowing an increase in the selectivity of spectrophotometric determinations. The aim of this work was to develop reliable spectrophotometric procedures for the determination of D and P, either in laboratory samples, or in dosage forms for these drugs.

Various orders of derivative and different kinds of measurements were assayed, i.e. zero-crossing second- and third-derivative, and ratio-spectra first- and second-derivative. Satisfactory results were obtained with all the methods. An attempt to a brief comparison between the usefulness of the two methods was made.

The fundamental principles and convection of derivative spectrophotometry, and the different kinds of measurements, e.g. zero-crossing [15–21] and ratio-spectra [22–27], are described in literature, hence we retain superfluous to report a detailed account of the techniques used.

2. Experimental

2.1. Reagents

Reference materials. 0.2 mg/ml freshly prepared stock solutions in water of dipyrone and pitophenone hydrochloride analytical-reagent grade.

2.1.1. Pharmaceuticals

Mixtures of pharmaceutical samples of dipyrone and pitophenone hydrochloride, and 2-ml vials of Baralgina, labelled to contain 1 g dipyrone and 4 mg pitophenone hydrochloride per vial. Additive, fenpiverine bromide 0.04 mg per vial. Other potentially interfering substances like excipients or diluents were absent.

All pharmaceuticals were provided by Hoechst Italia, Milan.

2.2. Apparatus

A Perkin–Elmer Lambda 3A spectrophotometer was used, coupled with a PC running spectrophotometric software PECSS. Settings: slit width, 1 nm; scan speed, 60 nm/min; wavelength interval, 0.5 nm.

2.3. Procedures

Suitable volumes of D and P, reference materials, expected to contain up to 40 μg/ml for each drug were mixed in a 5-ml calibrated flask and diluted to volume with distilled water. All reagent were tested for stability in solution and in the course of the actual analysis. The behaviour of the analytes remained unchanged up to 48 h from their preparation. Further tests of stability (i.e. over 48 h) were found unnecessary and were not made. Before use, the samples were stored in stoppered vessels in a refrigerator. All measurements were

made at room temperature. The main instrumental parameter affecting the shape of derivative spectra is $\Delta\lambda$. This parameter needs to be optimised to give a good selectivity and a higher sensitivity and an adequate signal-to-noise ratio. Various values of $\Delta\lambda$ were tested: a value of $\Delta\lambda = 6$ was found optimal in connection with both slit width and wavelength interval. In these conditions, a smoothing function was not necessary. A part of this, no particular expedients were found necessary to optimise the procedure.

2.3.1. Zero-crossing method

In the second-derivative mode, the second-derivative spectra of mixtures were recorded against water and values of derivative were measured at 266.5 and 302.5 nm (zero-crossing wavelengths of second-derivative of P) for the determination of D, and at 257 and 286 nm (zero-crossing of second-derivative of D) to determine P.

In the third-derivative mode, the values of the third-derivative spectra of mixture were measured at 242 and 278.3 nm (zero-crossing points of P) for the determination of D, and at 228.5 and 300 nm (zero-crossing of D) for the determination of P.

The calibration graphs for each drug, were obtained with samples at constant concentration of one of the components and variable concentration of the other.

The concentrations of D and P in a binary mixture of pharmaceuticals samples were computed from the corresponding calibration graphs.

2.3.2. Ratio-spectra method

According to the theory [22–27], the absorption spectrum of the mixture was divided, wavelength by wavelength, by a standard spectrum of P (P°, 7.5 μg/ml) for determining D and by a standard spectrum of D (D°, 7.5 μg/ml) for determining P. Then, the first- and second-derivative of the above ratio-spectra were recorded and the values of derivatives were measured at suitably selected wavelengths.

In particular, the concentration of D was proportional to the value of first-derivative of the ratio-spectra at 235 and 262 nm, and to the value of the second-derivative at 249.7 and 268 nm.

The concentration of P was proportional to the value of first-derivative at 229.5 and 288.5 nm, and to the value of second-derivative at 280.5 and 300 nm.

The calibration graphs for D and P were obtained as described under Section 3 (Results and discussion, ratio spectra method), and the concentration of D and P in mixtures of pharmaceuticals was computed.

2.4. Procedure for injections

To minimise a possible variation in the composition of the injections, the mixed contents of five vials was transferred in a 50-ml calibrated flask and diluted to volume with distilled water. Then, samples were prepared as follows. Appropriate volumes of the pharmaceutical (i.e. in the range of linearity of calibration graphs) were accurately measured and transferred in a 5-ml volumetric flask and diluted to the mark with water. The assay was completed as described under Section 2.3 (Procedures for reference materials). The spectrophotometric measurements were made at a time of 2–20 min, approximately. The percentage recovery of D and P in injections was calculated from the corresponding regression equations.

3. Results and discussion

Fig. 1 shows the zero-order spectra of D and P which closely overlap.

3.1. Zero-crossing method

Preliminary tests to optimise the method concerned, in particular, the selection of the more convenient order of derivative and working wavelengths. The basic requirements of the present procedure were that neither shape of derivative spectra nor location of the zero-crossing wavelengths were dependent on the composition of binary mixtures. Both, second- and third-derivative proved to be optimal, with minimal differences.

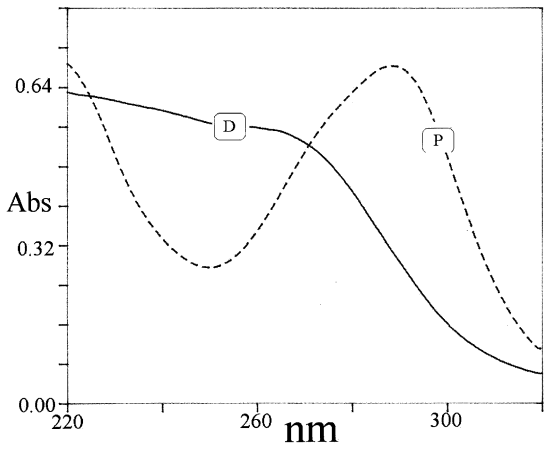

Fig. 1. Absorption spectra of dipyrone (20 μg/ml, continuous line) and pitophenone (15 μg/ml, dashed line). Reference, water.

The higher resolution of the third-derivative spectra allowed to select the optimal working wavelengths from a larger number of zero-crossing wavelengths, i.e. those which exhibited the best linear response to analyte concentration and higher sensitivity.

In Fig. 2 are shown the second- (a) and third-derivative spectra (b) of D (continuous lines) and P (dashed lines) to individuate the zero-crossing wavelengths. Among these, in the second-derivative mode, 266.5 and 302.5 nm for determination of D, and 257 and 286 nm for P were selected as working wavelengths, in that measurements of the absolute value of the total derivative spectra taken at these wavelengths gave the best response to the analyte concentration. Analogously, 242 and 278.3 nm for determination of D, and 228.5 and 300 nm for P, were found optimal in the third-derivative mode. The working wavelengths are indicated in the figure.

Note that two spectra at different concentration for each one of the two drugs are reported, in order to evidence the repeatability of the values of the zero-points.

In Fig. 3 are presented typical sets of second- (a) and third-derivative spectra (b) of mixtures of 5 μg/ml of P and increasing concentrations of D (continuous curves, left ordinate) and 5 μg/ml of D and increasing concentrations of P (dashed lines, right ordinate). Curves of this kind are required to

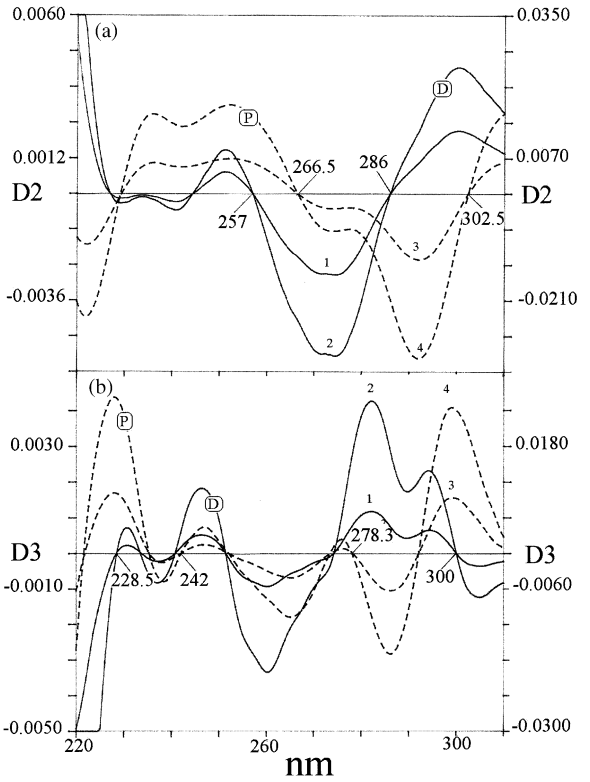

Fig. 2. (a) Second-derivative of dipyrone, (1) 10 μg/ml and (2) 20 μg/ml (continuous lines, left ordinate), and pitophenone, (3) 10 μg/ml and (4) 25 μg/ml (dashed lines, right ordinate). (b) third-derivative of dipyrone, (1) 5 μg/ml and (2) 18 μg/ml (continuous lines, left ordinate), and pitophenone, (3) 7.5 μg/ml and (4) 20 μg/ml (dashed lines, right ordinate). The zero-crossing working wavelengths are marked.

calculate the calibration graphs for D and P, obtained by plotting the values of derivative at the selected wavelengths, against the variable concentrations of D and P, respectively. It is important to note that all curves converge, as expected, to distinct isosbestic points, corresponding to zero-crossing wavelengths of D and P, i.e. irrespective of the concentration of D and P, respectively.

The equations for calibration curves are compiled in Table 1, together with statistical data, including the standard deviations for the slope and the intercept and the detection limits, (*d.l.*), calculated by a statistical treatment of calibration data [28,29] at $P = 0.05$ level of significance. Correlation coefficients, r, evidence for both

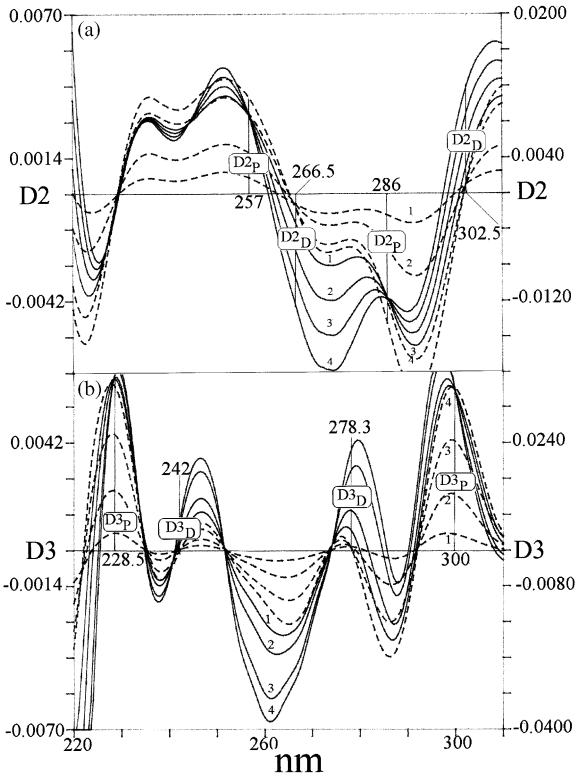

Fig. 3. (a) Continuous curves, left ordinate: second-derivative spectra of mixtures with constant concentration of pitophenone (5 μg/ml) and increasing concentration of dipyrone (5, 10, 15 and 20 μg/ml, curves 1–4). Dashed curves, right ordinate: second-derivative spectra of mixture with constant concentration of dipyrone (5 μg/ml) and increasing concentration of pitophenone (3, 7.5, 15 and 18 μg/ml, curves 1–4). (b) Continuous curves, left ordinate: third-derivative spectra of mixtures with constant concentration of pitophenone (5 μg/ml) and increasing concentration of dipyrone (5, 10, 20 and 25 μg/ml, curves 1–4). Dashed curves, right ordinate: third-derivative spectra of mixtures with constant concentration of dipyrone (5 μg/ml) and increasing concentration of pitophenone (3, 10, 20 and 30 μg/ml, curves 1–4). The working wavelengths are indicated.

linearity of regression lines and negligible scatter of experimental points. LOD and LOQ values are also reported. LOD is the lowest concentration of analyte that the analytical process can reliably detect, defined as the analyte concentration leading to a signal three times the standard deviation σ of the blank in ten successive scans. Hence the limit of detection, LOD, is located 3σ above the measured average blank [30]. It is interesting to

Table 1
Statistical data for the calibration graphs of D and P by zero-crossing second and third-derivative spectrophotometry[a]

Drug	Regression equations	λ (nm)	r	Standard deviations		Detection limit (μg/ml)	LOD (μg/ml)	LOQ (μg/ml)	$t = a/S_a$ [b]
				Intercept S_a	Slope S_b				
Second-derivative									
D	$D2_D = -5.36E-05 - 2.10E-04C_D$	266.5	0.9998	2.87E−05	1.67E−06	0.45	0.40	1.33	1.86
D	$D2_D = 3.11E-05 + 1.98E-04C_D$	302.5	0.9998	2.73E−05	1.91E−06	0.45	0.48	1.60	1.13
P	$D2_P = -3.56E-05 + 6.04E-04C_P$	257	0.9997	1.09E−04	5.31E−06	0.67	0.61	2.03	0.32
P	$D2_P = 2.77E-04 - 8.51E-04C_P$	286	0.9994	2.08E−04	1.00E−05	0.91	0.88	2.93	1.33
Third-derivative									
D	$D3_D = 2.41E-05 + 3.46E-05C_D$	242	0.9974	1.51E−05	1.02E−06	1.29	0.99	3.30	1.59
D	$D3_D = -2.49E-05 + 1.72E-04C_D$	278.3	0.9994	3.79E−05	2.20E−06	0.72	0.75	2.50	0.66
P	$D3_P = -2.11E-04 + 1.33E-03C_P$	228.5	0.9996	2.64E−04	1.36E−05	0.72	0.70	2.33	0.80
P	$D3_P = 2.39E-04 + 1.19E-03C_P$	300	0.9997	2.18E−04	1.05E−05	0.68	0.73	2.43	1.10

[a] D, dipyrone (noramidopyrine methanesufonate sodium salt); P, pitophenone hydrochloride. C_D and C_P, concentration of drugs (μg/ml). Number of samples, n = 10.
[b] Theoretical value of t at $P = 0.05$ level of significance, 2.31.

observe the good agreement between these values and the values of *d.l.*, calculated in a different way.

Lines of regression are linear up to 40 μg/ml of each drug. In order to verify if the developed method was free from procedural errors depending on the simultaneous presence of more components, the experimental intercepts *a* of lines of regression were tested for the significance of the deviation from the expected value zero [11,20,28]. The values calculated for *t*, shown in the table, never exceed the 95% criterion, 2.31, which denotes that the intercepts of all regression lines are not significantly different from zero.

From an examination of Table 1, do not appear substantial differences between second- and third-derivative modes. A little superiority of second- over third-derivative, results by detection limits and correlation coefficients.

Accuracy and precision were tested by five replicate determinations of various mixtures of pharmaceutical samples of D and P, with variable D:P concentration ratio. The results shown in Table 2 indicate that both methods are very effective for the simultaneous determination of these drugs. The R.S.D. values range between 0.01 and 2.39%.

3.2. Ratio-spectra method

In a preliminary investigation, different concentrations of D and P as divisors were examined. An accurate choice of either standard divisors or working wavelengths is fundamental for several reasons [23,25,26]. For example, overlapping of the spectra in a certain region is actually desirable because in division of spectrum by another, the error increases when one of absorbances approaches to zero. By increasing or decreasing the concentration of divisor, the resulting derivative values and, hence, the slope of lines of regression are proportionately decreased or increased, with variation of both sensitivity and linearity range.

We found the best results in terms of signal-to-noise ratio, sensitivity, repeatability and range of validity of Beer's law, by using as divisors standard spectra of 7.5 μg/ml of D (D°) and 7.5 μg/ml of P

Table 2
Replicate determinations on mixtures of pharmaceutical samples of D and P, by zero-crossing second and third-derivative spectrophotometry[a]

Nominal value (μg/ml)		Found[b] (μg/ml)			
D	P	D, 266.5 nm	D, 302.5 nm	P, 257 nm	P, 286 nm
Second-derivative					
3.0	40.0	3.02±0.02 (0.66)	2.97±0.03 (1.01)	40.20±0.24 (0.60)	40.44±0.42 (1.04)
10.0	25.0	10.08±0.08 (0.80)	10.04±0.05 (0.50)	25.06±0.07 (0.28)	24.91±0.22 (0.88)
15.0	20.0	14.98±0.03 (0.20)	15.09±0.10 (0.66)	20.09±0.18 (0.89)	19.97±0.04 (0.20)
20.0	15.0	20.22±0.25 (1.25)	20.14±0.16 (0.79)	14.90±0.11 (0.74)	14.91±0.09 (0.01)
25.0	10.0	24.98±0.05 (0.25)	25.19±0.22 (0.75)	10.09±0.09 (0.89)	10.09±0.09 (0.01)
30.0	3.0	30.22±0.21 (0.71)	29.23±0.25 (0.85)	3.04±0.03 (0.98)	3.03±0.03 (0.99)
Nominal value (μg/ml)		Found[b] (μg/ml)			
D	P	D, 242 nm	D, 278.3 nm	P, 228.5 nm	P, 300 nm
Third-derivative					
3.0	40.0	3.05±0.05 (1.64)	3.04±0.04 (1.31)	40.50±0.48 (1.18)	39.63±0.43 (1.08)
25.0	10.0	25.27±0.30 (1.19)	24.70±0.27 (1.09)	10.12±0.11 (1.09)	10.13±0.14 (1.38)
30.0	3.0	29.60±0.41 (1.38)	29.65±0.35 (1.18)	2.94±0.05 (1.70)	2.92±0.07 (2.39)
20.0	15.0	19.80±0.22 (1.10)	20.05±0.06 (0.30)	14.90±0.10 (0.67)	14.82±0.19 (1.28)
20.0	7.5	20.22±0.23 (1.14)	19.88±0.14 (0.70)	7.60±0.09 (1.18)	7.56±0.07 (0.92)
18.0	18.0	17.78±0.23 (1.31)	17.73±0.25 (1.41)	17.83±0.16 (0.89)	17.77±0.23 (1.29)

[a] D, dipyrone (noramidopyrine methanesufonate sodium salt); P, pitophenone hydrochloride.
[b] Mean±standard deviation (μg/ml) for five determinations with R.S.D.% in parentheses.

(P°). Both, first- and second-derivative of the ratio-spectra were used and the results compared. Higher derivative orders gave less accurate and precise results.

In Fig. 4 are shown two series of ratio spectra of D/P° (continuous lines, left ordinate) and P/D° (dashed lines, right ordinate), from 3 to 40 μg/ml.

In Fig. 5 are shown examples of first- (a) and second-derivatives (b) of the ratio-spectra. For calibration graphs, we selected the wavelengths which exhibited the best linear response to the analyte concentration, i.e. in the first-derivative mode 235 and 262 nm to determine D, and 229.5 and 288.5 nm to determine P. In the second-derivative mode, 249.7 and 268 nm for D, and 280.5 and 300 nm for P. The working wavelengths are indicated in the figures.

The above wavelengths all correspond to maxima or minima of derivative spectra.

The calibration graphs for each drug in both derivative modes were obtained by plotting the values of first- and second-derivatives of the ratio-spectra D/P° and P/D°, with variable concentrations of D and P, at the above working wavelengths, against the concentration of D and P in the standards, respectively. Regression equations and statistical data are compiled in Table 3. Lines of regression are linear up to 40 μg/ml. Detection limits, *d.l.*, at $P = 0.05$ level of significance calculated by statistical treatment of calibration data [28,29] and LOD [30] are reported, showing minimal differences. LOQ values are also shown. Also in the present instance test of significance of the intercepts of regression lines showed that the value calculated for t (in the table) never exceed the 95% criterion, 2.31 at $P = 0.05$ level of significance, for $n-2 = 8$ degrees of freedom.

First- and second-derivative mode gave comparable findings. Despite of higher values of the slopes of first-derivative regression lines, a certain prevalence of second-derivative results from a comparison of detection limits.

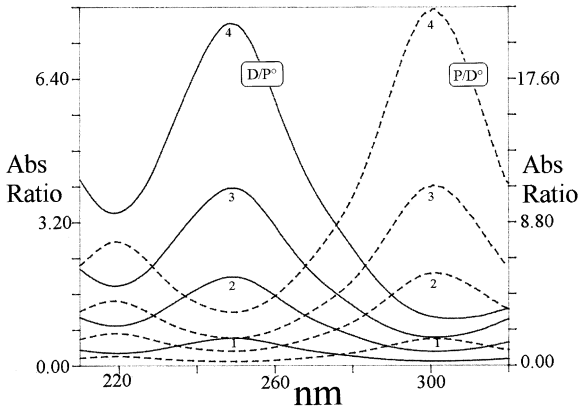

Fig. 4. Ratio-spectra for different concentrations of dipyrone (3, 10, 20 and 40 μg/ml, continuous curves 1–4, divisor P° 7.5 μg/ml, left ordinate scale) and pitophenone (3, 10, 20 and 40 μg/ml, dashed curves 1–4, divisor D° 7.5 μg/ml, right ordinate scale).

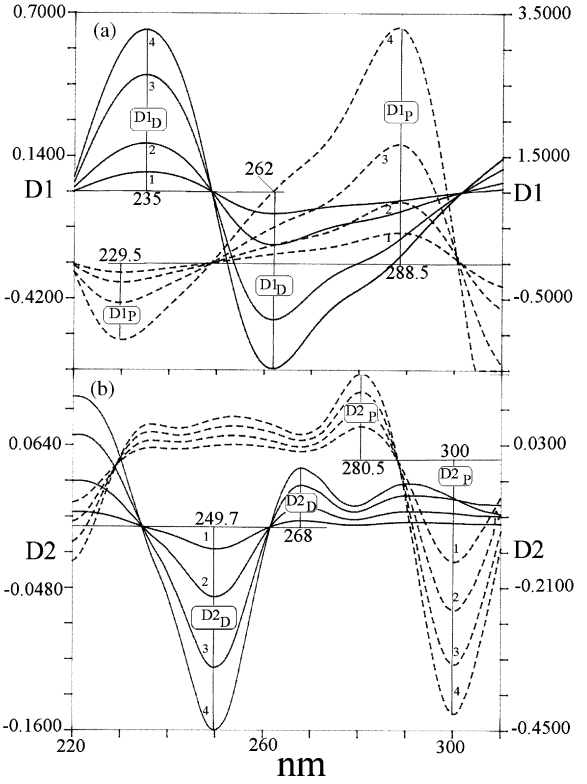

Fig. 5. (a) First-derivative spectra of the ratio spectra of dipyrone (3, 7.5, 18 and 25 μg/ml, continuous curves 1–4, left ordinate scale) and pitophenone (5, 10, 20 and 40 μg/ml, dashed curves, right ordinate scale). (b) Second-derivative spectra of the ratio-spectra of dipyrone (3, 10, 20 and 30 μg/ml, continuous curves 1–4, left ordinate scale) and pitophenone (10, 15, 20 and 25 μg/ml, dashed curves 1–4, right ordinate scale). The standard divisors are, respectively, P° and D°, both 7.5 μg/ml. The working wavelengths are indicated.

Table 3
Statistical data for the calibration graphs of D and P by ratio-spectra first and second-derivative spectrophotometry[a]

Drug	Regression equations	λ (nm)	r	Standard deviations		Detection limit (μg/ml)	LOD (μg/ml)	LOQ (μg/ml)	$t = a/S_a$ [b]
				Intercept S_a	Slope S_b				
First-derivative									
D	$D1_D = 9.53E-03+2.46E-02C_D$	235	0.9997	4.44E−03	2.15E−04	0.67	0.70	2.33	2.14
D	$D1_D = -1.76E-02-2.62E-02C_D$	262	0.9991	8.25E−03	3.99E−04	1.17	1.10	3.67	2.13
P	$D1_P = 1.71E-03-2.77E-02C_P$	229.5	0.9988	9.74E−03	4.72E−04	1.31	1.21	4.03	0.17
P	$D1_P = 1.90E-02+8.20E-02C_P$	288.5	0.9999	9.36E−03	4.53E−04	0.42	0.45	1.50	2.03
Second-derivative									
D	$D2_D = -2.00E-03-5.35E-03C_D$	249.7	0.9997	9.96E−04	4.83E−05	0.69	0.71	2.36	2.09
D	$D2_D = 1.44E-03+1.51E-03C_D$	268	0.9982	6.66E−04	3.33E−05	1.65	1.38	4.60	2.16
P	$D2_P = 1.70E-03-5.87E-03C_P$	280.5	0.9999	7.73E−04	3.34E−05	0.43	0.43	1.43	2.20
P	$D2_P = 6.15E-03-1.72E-02C_P$	300	0.9996	3.62E−03	1.75E−04	0.78	0.75	2.50	1.70

[a] D, dipyrone (noramidopyrine methanesufonate sodium salt); P, pitophenone hydrochloride. C_D and C_P, concentration of drugs (μg/ml). Number of samples, n = 10. Standard divisors, P°, 7.5 μg/ml and D°, 7.5 μg/ml.
[b] Theoretical value of t at $P = 0.05$ level of significance, 2.31.

According to the theory, the slope of calibration graphs should be inversely proportional to the concentration of the divisors. To assess this statement, various divisor concentrations, 5, 7.5 and 10 μg/ml, were tested. In all cases, using regression analysis, the equations for the calibration curves Slope versus 1/Divisor concentration, were obtained. The corresponding graphs, not shown for brevity, are straight lines with correlation coefficients, r, ranging from 0.9992 to 0.9999. Also the standard deviation values of intercept, S_a, and slope, S_b, were calculated. Examples of the regression equations follow.

P, 288.5 nm, first − derivative:

$$s = 1.30E-03 + 5.95E-01 \times 1/d;$$
$$S_a = 3.38E-03, \quad S_b = 2.25E-02$$

D, 262 nm, first − derivative:

$$s = 8.27E-05 + 1.96E-01 \times 1/d;$$
$$S_a = 4.48E-05, \quad S_b = 2.98E-04$$

P, 300 nm, second − derivative:

$$s = 3.46E-04 + 1.24E-01 \times 1/d;$$
$$S_a = 8.99E-04, \quad S_b = 5.88E-03$$

D, 249.7 nm, second − derivative:

$$s = 7.66E-05 + 3.89E-02 \times 1/d;$$
$$S_a = 2.25E-04, \quad S_b = 1.49E-03$$

where s is the slope and d is the divisor concentration (μg/ml). The remaining equations are not shown for sake of brevity. These results confirm the reliability of the ratio-spectra method in the present instance.

Five replicate determinations of mixtures of pharmaceutical samples of D and P, with variable D:P concentration ratio, were performed to test accuracy and precision. The results are reported in Table 4 and evidence that accuracy and precision are satisfactory. The R.S.D. values range between 0.10 and 2.18%.

Sensitivity of the assays results for ±0.5 nm shift in the wavelength scale has been tested. The percentage deviation at ±0.5 nm shift from the results obtained from the analysis of a few

Table 4
Replicate determinations on mixtures of pharmaceutical samples of D and P, by ratio-spectra first and second-derivative spectrophotometry[a]

Nominal value (μg/ml)		Found[b] (μg/ml)			
D	P	D, 235 nm	D, 262 nm	P, 229.5 nm	P, 288.5 nm
First-derivative					
9.3	25.0	9.12±0.17 (1.86)	9.32±0.03 (0.32)	25.30±0.32 (1.26)	25.32±0.28 (1.10)
14.5	20.0	14.30±0.20 (1.40)	14.61± 0.12 (0.82)	20.38±0.36 (1.76)	20.11±0.12 (0.60)
20.0	15.0	19.99±0.02 (0.10)	20.23±0.24 (1.18)	14.66±0.32 (2.18)	14.90±0.10 (0.67)
25.5	10.0	25.45±0.07 (0.27)	25.80±0.32 (1.24)	9.90±0.10 (1.01)	10.18±0.16 (1.57)
20.5	7.5	20.28±0.22 (1.08)	20.51±0.02 (0.10)	7.44±0.07 (0.94)	7.58±0.07 (0.92)
18.0	18.0	17.93±0.08 (0.44)	18.35±0.33 (1.79)	17.67±0.30 (1.70)	17.89±0.12 (0.67)
Nominal value (μg/ml)		Found[b] (μg/ml)			
D	P	D, 249.7 nm	D, 268 nm	P, 280.5 nm	P, 300 nm
Second-derivative					
9.3	25.0	9.20±0.10 (1.09)	9.26±0.05 (0.54)	25.26±0.22 (0.87)	24.88±0.13 (0.52)
14.5	20.0	14.37±0.13 (0.90)	14.64±0.16 (1.09)	20.05±0.06 (0.29)	19.82±0.19 (0.96)
20.0	15.0	19.91±0.08 (0.40)	20.17±0.18 (0.88)	15.02±0.03 (0.20)	14.80±0.21 (1.42)
25.5	10.0	25.44±0.07 (0.27)	25.30±0.21 (0.83)	10.17±0.15 (1.47)	9.99±0.02 (0.20)
20.5	7.5	20.30±0.22 (1.08)	20.70±0.23 (1.11)	7.56±0.07 (0.92)	7.58±0.09 (1.18)
18.0	18.0	17.94±0.06 (0.33)	17.97±0.20 (1.11)	18.00±0.03 (0.17)	17.73±0.28 (1.57)

[a] D, dipyrone (noramidopyrine methanesufonate sodium salt); P, pitophenone hydrochloride.
[b] Mean±standard deviation (μg/ml) for five determinations with R.S.D.% in parentheses.

mixtures of pharmaceutical samples using the ratio-spectra method were measured. The experimental findings showed that the method is robust to ±0.5 nm shift in the wavelength scale, as expected, also because the measurements were made in correspondence of peaks. Analogously the results proved to be robust to ±0.5 nm shift for the zero-crossing third-derivative method at 228.5 and 300 nm. On the contrary, the zero-crossing method at the other wavelengths and derivative order is less robust, when the ±0.5 nm shift has been studied.

3.3. Assay of injections

As example of application to a real sample, the methods were applied to the recovery of D and P in injections as described under Section 2.4 (Procedure for injections). For the absence of excipients or other interfering substances, no preliminary treatment of the samples was necessary.

The mean recoveries of five replicate determinations, expressed as percentage of the content are reported in Table 5 for all procedures tested. The level of precision and accuracy are adequate for the quality control analysis of pharmaceutical preparations. Either zero-crossing or ratio-spectra procedures yield good recoveries with no substantial differences. The recoveries obtained were between 99.2 and 102.3% (zero-crossing), and between 99.5 and 102.1% (ratio-spectra). From the experimental findings, it is evident that the additive fenpiverine bromide does not interfere, in the present instance, principally for two reasons: low concentration in the vials and low absorbance in the working wavelengths interval (its absorption maximum lies around 180 nm).

4. Conclusions

The purpose of this work was to investigate if two different applications of derivative spectrophotometry, zero-crossing and ratio-spectra, were

Table 5
Recovery of D and P from injections[a]

	D, 266.5 nm	D, 302.5 nm	P, 257 nm	P, 286 nm
Zero-crossing, second-derivative				
Batch no. 1	99.2±0.81	101.8±0.98	102.0±0.99	101.8±0.85
Batch no. 2	99.8±0.81	101.5±0.95	100.6±0.89	102.1±0.91
	D, 242 nm	D, 278.3 nm	P, 228.5 nm	P, 300 nm
Zero-crossing, third-derivative				
Batch no. 1	99.9±0.86	101.4±0.78	102.3±1.01	101.5±0.91
Batch no. 2	101.0±0.96	101.0±1.10	101.9±0.94	101.8±0.93
	D, 235 nm	D, 262 nm	P, 229.5 nm	P, 288.5 nm
Ratio-spectra, first-derivative				
Batch no. 1	101.2±0.91	100.9±0.88	100.5±0.78	101.8±0.94
Batch no. 2	101.4±0.92	102.1±0.91	101.1±0.88	101.5±0.92
	D, 249.7 nm	D, 268 nm	P, 280.5 nm	P, 300 nm
Ratio-spectra, second-derivative				
Batch no. 1	100.9±1.01	101.4±0.95	98.9±0.81	102.1±0.86
Batch no. 2	101.0±0.98	102.0±0.90	99.5±0.91	101.9±0.93

Mean±standard deviation for five determinations, given as percentage of the nominal content. The label claim of injections and the firm are under Section 2.

[a] D, dipyrone (noramidopyrine methanesufonate sodium salt); P, pitophenone hydrochloride.

suitable for resolving mixtures of D and P, drugs with strictly overlapping spectra, and for a "quality control" of commercial injections for these drugs, and to remark possible differences in the experimental findings. From a comparison of the results obtained with the two methods, they do not appear to have substantial differences. A little prevalence of the zero-crossing over the ratio spectra results from an observation of detection limits and tests of significance of the intercepts (t values), generally a little smaller in the zero-crossing. On the other hand, the slopes of lines of regression of the ratio-spectra are higher than the corresponding zero-crossing values, and this represents an advantage. The main advantage of the ratio-spectra method may be the chance of doing measurements in correspondence of peaks, hence a potential greater sensitivity and accuracy. On the contrary, disadvantages of the zero-crossing method are the risk of small drifts of the cross-over points and the fact that the working wavelengths do not fall in correspondence of maxima or minima of derivative spectra. Finally, the zero-crossing method is less tedious and lengthy in respect to the ratio-spectra, which requires an accurate investigation about the influence of the variable (in particular, the choice of standard divisors) and a greater number of measures.

However, all procedures presented in the present paper, enable the quantitation of mixtures of D and P with good accuracy and precision in laboratory samples. As real application, the method was profitably used for the determination of these drugs in commercial dosage forms.

The procedures described allow the simultaneous determination of the two drugs in admixture. This represents a considerable advantage over methods which enable the determination of the analgesic drugs in question one at time and this is highly desirable in particular to support formulations assay. A comparison of the present data with an official reference procedure was not possible because of the absence of an official method for this binary mixture of substances. However, the experimental results previously displayed give full evidence for the usefulness, robustness, reliability and repeatability of the methods, which have been validated to be precise, sensitive and accurate. Satisfactory recovery was found from injections.

We cannot exclude that other techniques, (HPLC, electrophoresis, etc.), would also give good results. Unfortunately, the above techniques generally need sophisticated and more expensive instrumentation in respect to spectrophotometry, which offers the chance of using instrumentation commonly available in all research and analysis laboratories. Furthermore, the proposed methods did not require the elaboration of treatment and procedures, which are usually associated with chromatographic methods. Hence they are generally fast and economical in comparison to the more time-consuming chromatographic techniques, often used for the assay of formulations. As concerns the choice of dipyrone and pitophenone, we emphasise that these drugs have been selected for their importance in therapeutic field and the potential toxic effects.

For the above reasons, coupled with analysis time, ease of operation, and widespread availability of commercial instruments with derivative capability, the described procedures offer a distinct advantage over other techniques and confirm their suitability for routine analysis of mixtures of dipyrone and pitophenone and for control purposes of pharmaceutical dosage forms for these drugs.

In our opinion, the above considerations, the importance of the two substances in pharmaceutical and clinical field, as described under Section 1 (Introduction), and the need of analytical procedures for their determination, would make the present work of interest for the readers of journals like JPBA.

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