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Determination of Paracetamol in the Presence of Caffeine and Acetylsalicylic Acid in Analgesic Formulations by the Linear Absorbances Method and the Derivative Spectroscopy Technique

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The simultaneous use of the linear absorbances method and the derivative spectroscopy technique allows paracetamol to be spectrometrically determined in the presence of two interfering drugs that are generally associated with paracetamol in analgesic formulations. The proposed procedure does not need standard solutions of the interfering drugs; only a test solution of acetylsalicylic acid (ASA) is needed. The ASA contents can be also determined with good accuracy by routine and control analysis. The procedure is applied to two commercial pharmaceutical samples. © 1994 Academic Press, Inc.

The removal of interferences in molecular absorption spectroscopy is the object of much research. Derivative spectroscopy has brought about major advances (*1*, *2*), since this technique allows the analytical signal of the analyte to be isolated from other molecules by "zero-crossing method."

However, as the number of interfering bands increases, the probability of finding a wavelength in which the bands do not interfere decreases. In many cases, it is necessary to use high-order-derivative spectra.

Different mathematical methods have been developed to remove interferences; the H-point method (*3–5*), the absorbance ratio method (*6*), and the apparent contents curve method (*7*) provide good results for binary mixtures. The recently developed linear absorbances method (LAM) allows one component of a binary mixture to be determined, avoiding the use of standard solutions of interferent (*8*).

In this work we apply the linear absorbances method to derivative spectroscopy. The combination of the zero-crossing method and LAM allows paracetamol to be determined in the presence of caffeine and acetylsalicylic acid in pharmaceutical formulations. Therefore, the use of *n*-order-derivative spectra is avoided and thus the sensitivity and precision are improved.

Ultraviolet–violet molecular absorption spectroscopy is widely used in pharmaceutical analysis due to its speed and applicability to many drugs and their derivative compounds. Because of this there are many paracetamol determination reports that use different experimental procedures and mathematical methods.

Sala and co-workers (*9*) compare three computational programs, MA, SIMPLEX, and MULTIC (relying on multiple regression analysis), to resolve

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mixtures of paracetamol, caffeine, and acetylsalicylic acid in commercial analgesic formulations (Fiorinal and Actron). They obtained the best results when MULTIC was used.

Chatterjee and co-workers (*10*) carried out simultaneous determinations of paracetamol and chlorzoxazone in combined dosage forms by the absorbance ratio method and difference spectrometry. They concluded that both methods are suitable for routine and control analysis.

The derivative spectroscopy technique has been used by Fornay and co-workers (*11*) to determine paracetamol in the presence of its degradation product. Eboka and co-workers (*12*) used the technique to determine paracetamol in the presence of some of the most common excipients in suspension form.

Other determinations of paracetamol have made use of the derivative oxidation products (*13–15*). Fluorimetric, chromatographic, immunoassay, and enzymatic methods have been reported also (*16*).

THEORETICAL BASES

As described in a previous paper, the spectrophotometric determination of one compound (A) in the presence of another (B) that has an interfering band can be carried out by the use of the linear absorbances method (*8*).

Therefore, if we consider a ternary mixture of absorbent species (A, B, and C) and if Beer's law is obeyed for the three compounds over the whole wavelength range, the absorbance of the solution at two wavelengths (using a cell path length of 1 cm) can be written as follows:

$$A^s_1 = C^A\epsilon^A_1 + C^B\epsilon^B_1 + C^C\epsilon^C_1 \quad (1)$$

$$A^s_2 = C^A\epsilon^A_2 + C^B\epsilon^B_2 + C^C\epsilon^C_2. \quad (2)$$

On the other hand, signals obtained at the same wavelengths by derivative spectroscopy will be

$$D^s_1 = C^AR^A_1 + C^BR^B_1 + C^CR^C_1 \quad (3)$$

$$D^s_2 = C^AR^A_2 + C^BR^B_2 + C^CR^C_2, \quad (4)$$

where

D_i is the value of the derivative signal

R_i is the coefficient of response, that is to say, the factor that relates the derivative signal with the concentration: $R = d\epsilon/d\lambda$.

If λ_1 and λ_2 correspond with the zero-crossing wavelengths of the derivative band of C, then Eqs. (3) and (4) are simplified to

$$D^s_1 = C^AR^A_1 + C^B_1R^B_1 \quad (5)$$

$$D^s_2 = C^AR^A_2 + C^B_2R^B_2. \quad (6)$$

Thus, the ternary mixture can be considered a binary mixture for these wavelengths.

Under these conditions, the LAM can be applied and, therefore, the content of A can be obtained without the standard solutions of B. With this information, a straight line connecting the two experimental points D^s_1,R^A_1 and D^s_2,R^A_2 can be established. Since the behavior of the system on this interval can not be linear, it

is obvious that this line only represents the real signal of the sample for points 1 and 2 (see Figs. 1a and 1b).

However, this equation allows C^A to be obtained without knowing the $R^B{}_i$ values at λ_1 and λ_2, as can be seen in the following.

Taking into account the established relation

$$D^S{}_i = n^S + m^S R^A{}_i, \tag{7}$$

we can write

$$D^S{}_1 = n^S + m^S R^A{}_1 \tag{8}$$
$$D^S{}_2 = n^S + m^S R^A{}_2, \tag{9}$$

and considering that

$$D^S{}_1 = C^A R^A{}_1 + C^B R^B{}_1 \tag{10}$$
$$D^S{}_2 = C^A R^A{}_2 + C^B R^B{}_2 \tag{11}$$

it is evident that, if expression (7) is correct, a relation between $R^B{}_i$ and $R^A{}_i$ (i = 1 or 2) must be linear also.

The mathematical expression for this linear function is

$$R^A{}_i = R^B{}_0 + [(R^A{}_1 - R^A{}_2)/(R^B{}_1 - R^B{}_2)]R^B{}_i$$
$$= R^B{}_0 + f^B R^B i, \tag{12}$$

where f^B is the slope and $R^B{}_0$ is the y-intercept of the straight line that connects $R^B{}_1$,$R^A{}_1$ with $R^B{}_2$,$R^A{}_2$.

Analogously to Eq. (7), this expression is a nonreal function, only true for points 1 and 2.

If $R^B{}_i$ is taken from Eq. (12) and substituted into Eqs. (10) and (11), the following equations are obtained:

$$D^s{}_1 = C^B(R^A{}_1 - R^B{}_0)/f^B + C^A R^A{}_1 \tag{13}$$
$$D^s{}_2 = C^B(R^A{}_2 - R^B{}_0)/f^B + C^A R^A{}_2. \tag{14}$$

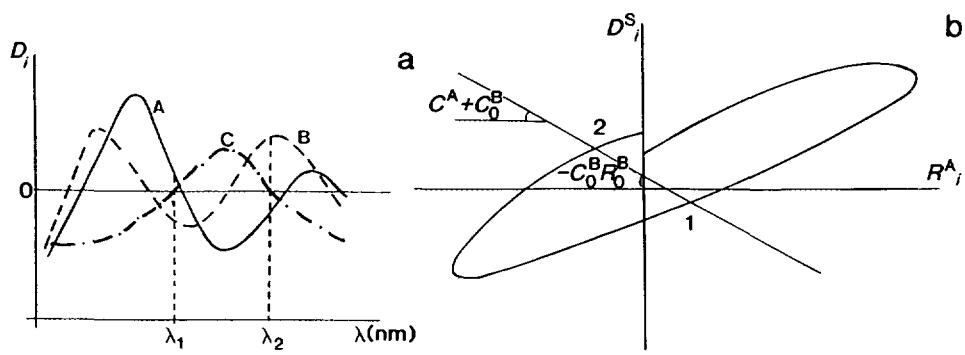

FIG. 1. (a) Simulated first-derivative spectra of A, B, and C species. (b) $D^s{}_i$ vs $R^A{}_i$ for the whole wavelength range of the simulated first-derivative spectrum of a mixture solution of A, B, and C. The straight line is plotted from Eq. (14) for λ_1 and λ_2. $D^s{}_i$ is the first-derivative signal of a mixture solution of A, B, and C species. $R^A{}_i$ is the coefficient of response of A.

Calling $C^B_0 = C^B/f^B$ and regrouping,

$$D^s_1 = -C^B_0 R^B_0 + (C^A + C^B_0)R^A_1 \quad (15)$$
$$D^s_2 = -C^B_0 R^B_0 + (C^A + C^B_0)R^A_2. \quad (16)$$

Thus, the linear relationship above established between the derivative signals of the sample and the coefficients of response of the analyte at the two working wavelengths are

$$D^s_i = -C^B_0 R^B_0 + (C^A + C^B_0)R^A_i = n^s + m^s R^A_i \quad (17)$$

and, subsequently,

$$n^S = -C^B_0 R^B_0 \quad \text{and} \quad m^S = C^A + C^B_0.$$

If the R^B_0 value is known, the C^A value can be obtained as follows:

$$C^A = m^s + n^s/R^B_0. \quad (18)$$

R^B_0 can be found as follows:

R^B_0 does not depend on the concentration of A and B in the sample [Eq. (12)], and its value can be obtained using a solution of B of an unknown concentration (called test solution and labeled with the t superscript).

Thus, if the equation of the straight line connecting $D^t_1 R^A_2$ and $D^t_2 R^A_2$ is obtained, we can write

$$D^t_i = (C^B_0)^t R^B_0 + (C^B_0)^t R^A_i = n^t + m^t R^A_i. \quad (19)$$

Therefore, the R^B_0 value is the x-intercept of this line (Fig. 2a). The $-R^B_0$ value also can be obtained from different test solutions (labeled by the j subscript) like the slope of the plot $(n^t)_j$ versus $(m^t)_j$ (Fig. 2b).

Therefore, the C^A value can be obtained also by the following equation:

$$C^A = m^s - n^s m^t/n^t. \quad (20)$$

The determination of C^B will be only possible if f^B is known ($C^B = f^B C^B_0$). f^B can be obtained as the slope of the straight line $(C^B)_k$ vs $(C^B_0)_k$, where k indicates

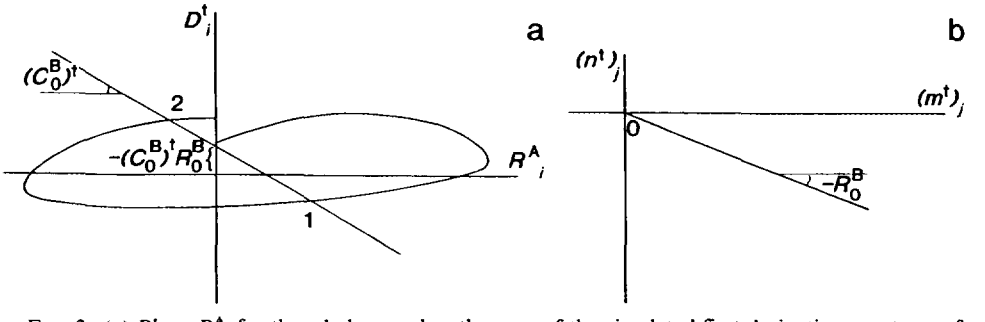

FIG. 2. (a) D^t_i vs R^A_i for the whole wavelength range of the simulated first-derivative spectrum of B. The straight line is plotted from Eq. (15) for λ_1 and λ_2. D^t_i is the first-derivative signal of a solution of B. (b) Plot of R^B_0 from a series of test solutions of B as the negative slope of the straight line.

different standard solutions of B (Fig. 3), or from the ratio $\Delta R^{A}{}_{1-2}/\Delta R^{B}{}_{1-2}$. In both cases it would be necessary to use standard solutions of B.

However, since f^{B} is a ratio of increments of R it is less affected by instrumental conditions than R, and so it will be possible to use a previously tabulated value. Thus, the C^{B} value can be obtained without the use of standard solutions of B.

EXPERIMENTAL

For the experimental stage Shimadzu UV-240 and Hewlett–Packard 8452A diode array spectrophotometers were indiscriminately used.

As can be seen in Fig. 4a, in alkaline solutions, paracetamol, caffeine, and acetylsalicylic acid (ASA) present overlapping absorption bands. However, the first-derivative spectrum of caffeine presents two zero-crossing wavelengths, whereas, at the same wavelengths, paracetamol and ASA show a suitable signal intensity (Fig. 4b).

Thus, using the zero-crossing method and the linear absorbances method, it will be possible to determine paracetamol in the presence of caffeine and ASA. In addition, it will be possible also to determine the ASA content using a previously tabulated f^{B} value.

The instrument parameters were chosen to enhance reproducibility and sensitivity and to lessen noise. They were slit width, 2 nm; $\Delta\lambda$ = 4 nm; and wavelengths measuring 244.5 and 272.5 nm.

Three calibration graphs of paracetamol were obtained (for the two working wavelengths) in order to: (a) establish the interval of linear response, (b) check for noninteraction between paracetamol and ASA or paracetamol and caffeine, and (c) test the correct selection of the wavelength.

For the first calibration graph, solutions containing paracetamol from 0 to 45 ppm and 0.1 M NaOH were prepared. The second calibration graph was obtained from solutions containing 5 ppm caffeine and 0–45 ppm paracetamol. In the last calibration 15 ppm ASA and 0–45 ppm paracetamol were used.

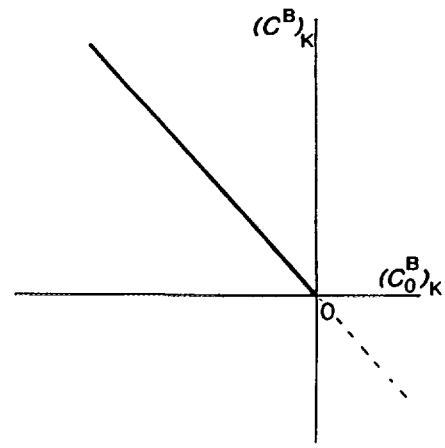

FIG. 3. Plot of f^{B} from a series of standard solutions of B as the slope of the straight line.

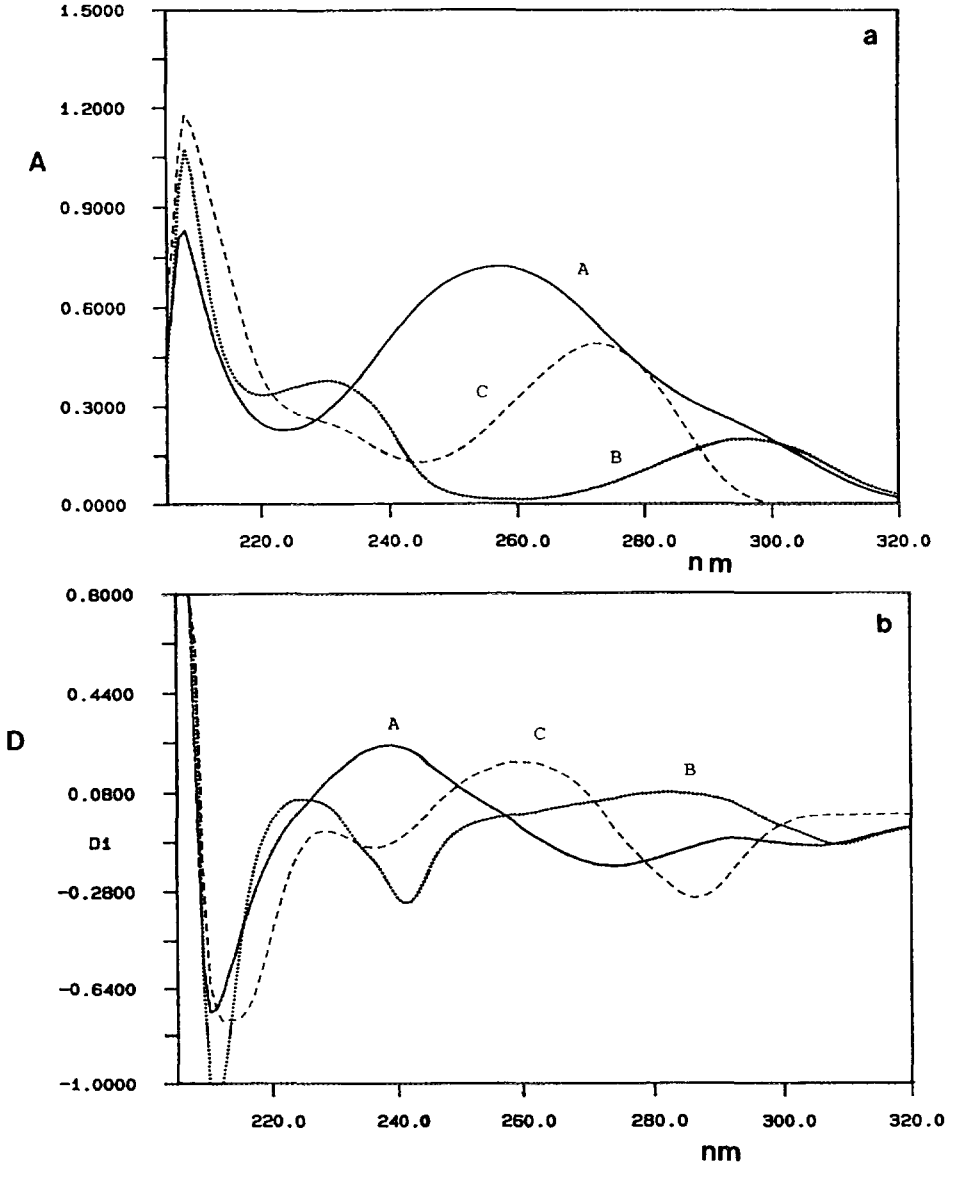

FIG. 4. (a) Absorption spectra of: (A) paracetamol, (B) ASA, and (C) caffeine in 0.01 *M* NaOH. (b) Zero-order-derivative spectra of: (A) paracetamol, (B) ASA, and (C) caffeine in 0.01 *M* NaOH.

The linear range was up to 35 ppm paracetamol. In addition, the statistical test (*18*) showed that (for each wavelength) the three slopes were significatively identical and the *y*-intercept of the first and second calibrations were significatively zero. Thus, the interference of caffeine was correctly eliminated and there was no interaction between paracetamol and caffeine or ASA.

As shown in Table 1 the f^B value was obtained from 18 standard solutions of

TABLE I
Values of f^B Obtained on Different Working Days and from Different ASA Solutions

C^B (ppm)	C^B_0 (ppm)	$C^B = f(C^B_0)$	f^B
5.00	−3.698		
10.01	−7.096	$C^B = 0.034 - 1.3753C^B_0$	−1.38
15.01	−10.964		
5.00	−3.754		
10.01	−7.096	$C^B = 0.077 - 1.3399C^B_0$	−1.34
15.01	−10.964		
4.98	−3.783		
9.96	−7.310	$C^B = -0.187 - 1.3769C^B_0$	−1.38
14.94	−11.015		
4.98	−3.717		
9.96	−7.537	$C^B = 0.084 - 1.3138C^B_0$	−1.31
14.94	−11.298		
4.98	−3.806		
9.96	−7.577	$C^B = 0.169 - 1.2783C^B_0$	−1.28
14.94	−11.595		
5.00	−3.674		
10.01	−7.175	$C^B = -0.262 - 1.4324C^B_0$	−1.43
15.01	−10.661		

ASA (from three different concentrations) prepared and measured on 6 different days. The average value found was

$$f^B = -1.35 \pm 0.02.$$

This f^B value was used to determine the amount of ASA along the whole process, and it is considered the correct value for routine analysis.

Application to Synthetic Samples

Once the f^B value was obtained, the accuracy of the proposed procedure was evaluated. For this purpose a series of synthetic samples containing different amounts of paracetamol, caffeine, and ASA were prepared (see Tables 2a and 2b).

Five standard solutions of paracetamol and three test solutions of ASA (of unknown concentration) were also prepared.

The first-derivative signals of each one of these solutions, at the two working wavelengths, were measured and the results obtained using the proposed procedure are shown in Tables 2a and 2b.

From the data of Tables 2a and 2b, three graphs were plotted in order to check the accuracy of the method:

(i) In the first graph the concentration of paracetamol found is plotted against the concentration of paracetamol present, for the samples with a constant caffeine concentration (5 ppm).

(ii) In the second graph, the concentration of paracetamol found was plotted against the concentration of paracetamol present for the samples with a constant ASA concentration (15 ppm).

TABLE 2a
Results Obtained from Prepared Samples Containing Different Paracetamol and ASA Concentrations and a Constant Caffeine Concentration[a]

Paracetamol (ppm)		ASA (ppm)	
Present	Found[b]	Present	Found
15.06	15.08 ± 0.06	5.02	5.22
		10.05	10.36
		20.10	20.30
		25.12	24.94
9.96	9.86 ± 0.10	5.00	4.76
		10.01	9.71
		15.01	15.21
		20.02	19.34
		25.02	24.83
4.98	5.06 ± 0.03	5.00	5.09
		10.01	9.90
		15.01	15.30
		20.02	19.80
		25.02	24.71
1.99	1.87 ± 0.02	4.98	5.05
		9.96	9.73
		14.94	15.07
		19.92	19.26
		24.90	24.02
1.00	1.57 ± 0.09	4.98	5.54
		9.96	10.51
		14.94	15.90
		19.92	20.54
		24.90	25.74

[a] All samples contained 5.02 ppm caffeine.
[b] Average value of five replicates.

(iii) The last graph shows the concentration of ASA found plotted against the concentration of ASA present for the samples with 5 ppm caffeine.

Linear graphs were obtained in the three cases and the mathematical expressions of the linear regression were

$$\text{(i)}\quad C_{\text{found}} = 0.049 + 0.994\, C_{\text{present}} \qquad (r = 0.9990)$$
$$\text{(ii)}\quad C_{\text{found}} = 0.043 + 0.993\, C_{\text{present}} \qquad (r = 0.9986)$$
$$\text{(iii)}\quad C_{\text{found}} = 0.268 + 0.985\, C_{\text{present}} \qquad (r = 0.9998).$$

The statistical model of Youden (*18*) indicates, for a 95% probability level, that the method does not present either constant or proportional systematic errors. In addition, the determination of ASA can be carried out with suitable accuracy using the f^B value of -1.35.

TABLE 2b
Results Obtained from Prepared Samples Containing Different Paracetamol and Caffeine Concentrations and a Constant ASA Concentration

Caffeine present (ppm)	Paracetamol (ppm)		ASA (ppm)	
	Present	Found[a]	Present	Found[a]
3.00 5.00 10.01 15.01	15.06	15.11 ± 0.06	10.00	10.45 ± 0.09
3.01 5.02 10.05 15.07	9.96	9.71 ± 0.11		9.85 ± 0.03
3.01 5.02 10.05 15.07	4.98	5.12 ± 0.04		10.09 ± 0.10
3.01 5.02 10.05 15.07	1.99	1.89 ± 0.03		9.78 ± 0.04
3.01 5.02 10.05 15.07	1.00	1.40 ± 0.07		10.59 ± 0.04

[a] Average value of four replicates.

Application to Real Samples

Two commercially available pharmaceutical formulations were selected to be analyzed by the proposed method. The label composition of the samples are

Fiorinal, from Sandoz Pharma, S.A. (capsules):

Acetylsalicylic acid, 200 mg
Paracetamol, 300 mg
Caffeine, 40 mg
Excipient, c.s.

Meridol, from Merrill Dow Pharmaceuticals, Inc. (tablets):

Aluminum acetylsalicylate, 300 mg
Paracetamol, 200 mg
Caffeine, 36 mg
Excipient, c.s.

Once the samples (tablets or capsules) were weighed and powdered, a known portion was dissolved in hot water or hot NaOH (about 150 ml 0.005 *M*); the excipient not dissolved was filtered and the solution was made up to 250 ml.

The solutions to be measured were prepared from this solution by dilution with 0.01 *M* NaOH. Five replicates for each sample were carried out, and different samples were analyzed.

The experimental procedure used was identical to that employed in the analysis of synthetic samples, and the results are shown in Tables 3 and 4.

Samples were also analyzed by a multiwavelength linear regression method, and the results were taken as reference values (Tables 3 and 4).

As can be seen, the results of both methods agree with each other and with the label contents. Perhaps the results from Meridol are low in both methods, but the ratio of Al-ASA/paracetamol found (1.19) is in agreement with the label value of 1.20.

Proposed Experimental Procedure

(a) Weigh and powder the sample; take a suitable portion and dissolve it in hot water or hot diluted NaOH with constant stirring. Filter the excipient and make the solution up to the mark in a 250-ml volumetric flask.

The measured solutions (A solutions) must contain 2–30 ppm paracetamol and 2–30 ppm ASA (the overall concentration must not exceed 20–25 ppm); they are prepared by dilution of the above solution with 0.01 *M* NaOH.

(b) Prepare standard solutions of paracetamol containing 2–35 ppm in 0.01 *M* NaOH (B solutions).

(c) Prepare three solutions of ASA in 0.01 *M* NaOH with different concentrations (<30 ppm); it is not necessary to know these concentrations (C solutions). If $R^{B}{}_{0}$ is calculated as $-n^{t}/m^{t}$, then only a test solution of ASA is needed.

(d) Record the absorption spectra of all the solutions and obtain the derivative signals at 244.5 and 272.5 nm.

(e) From the data of the B solutions obtain the R^{A} values at 244.5 and 274.5 nm.

(f) From the data of the C solutions, and the R^{A} values, obtain n^{t} and m^{t} and calculate $R^{B}{}_{0}$.

(g) From the data of the A solutions, obtain n^{s} and m^{s}.

TABLE 3
Results Obtained for Fiorinal Samples from the Proposed (LAM) and the Reference (MLR) Method

	LAM		MLR	
Weight capsule (g)	Paracetamol (mg)	ASA (mg)	Paracetamol (mg)	ASA (mg)
0.6015	299.7	198.7	298.8	199.1
0.6160	300.1	209.6	297.2	210.3
0.6005	293.3	208.5	292.7	213.9
0.6022	311.3	196.7	310.3	200.6
0.6037	304.3	213.8	303.4	218.8
0.6085	315.2	203.0	315.1	208.7
Average weight/capsule	Average (mg) paracetamol/capsule		Average (mg) ASA/capsule	
0.6054g	304 ± 3	205 ± 3	303 ± 3	209 ± 3

TABLE 4
Results Obtained for Meridol from the Proposed (LAM) and Reference (MLR) Methods

	LAM		MLR	
Weight (g)/ tablet	Paracetamol (mg)	Al-ASA (mg)	Paracetamol (mg)	Al-ASA (mg)
0.7909	243.8	274.7	242.7	279.3
0.8350	251.4	294.0	250.4	298.3
0.7853	243.7	296.3	239.8	288.5
0.7928	328.2	277.1	228.4	277.1
0.7880	240.8	291.2	234.6	289.2
Average weight/tablet	Average (mg) paracetamol/tablet		Average (mg) Al-ASA tablet	
0.7984g	242 ± 4	287 ± 5	239 ± 4	287 ± 4

(h) Finally, obtain the C^A value using Eqs. (*18*) or (*20*). The C^B value can be obtained as $C^B{}_0 \times f^B$.

Alternative Procedure

If the determination of ASA must be more accurate than that of paracetamol and only the ASA standard is available, the former procedure can be identically applied by reciprocating paracetamol and ASA.

That is to say, the R^A will be the ASA coefficient of response and the $R^B{}_0$ will correspond to paracetamol. Then, the C^A value will be the ASA concentration and the C^B value can be obtained since the f^B for paracetamol will be $-1/1.35 = -0.74\ (\pm 0.01_5)$.

CONCLUSIONS

(i) The LAM can be applied in derivative spectroscopy without modifications.

(ii) The combination of derivative spectroscopy and LAM allows the concentration of one compound in the presence of two interfering compounds to be determined if one of them has two zero-crossing wavelengths.

(iii) The proposed experimental procedure is suitable for determination of paracetamol in the presence of caffeine and ASA in pharmaceutical formulations.

(iv) The ASA contents can be obtained using the f^B value of -1.35.

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