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Short Communication

First and second derivative spectrophotometric assay of mixtures of cefuroxime and cephalexin

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Keywords: *Cefuroxime; cephalexin; cephalosporins determination; derivative spectrophotometry.*

Introduction

Derivative spectrophotometry offers a convenient solution to a number of well defined analytical problems, such as resolution of multicomponent systems, elimination of interference from sample turbidity and matrix background, and enhancement of spectral details [1–3].

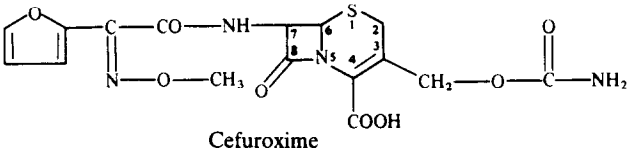
Fell [4] discussed the use of the derivative technique for resolution enhancement in HPLC. The technique involves differentiating the output of the detector, which may be based on UV absorbance, fluorescence, etc., with respect to time to give the second or fourth derivative.

One of the classical analytical problems in the field of UV–visible spectrophotometry is the resolution of a number of components mixture with overlapping bands. A number of papers have appeared describing applications of derivative spectrophotometry in the analysis of mixtures of metals. For example, satisfactory derivative methods for the simultaneous determination of mixtures of bismuth(III)–copper(II) [5], iron(III)–copper(II) [6] and ruthenium(III)–palladium(II) [7], using 2-thiobarbituric acid as the colorimetric reagent and osmium(VIII)–palladium(II) [8], using allyl thiourea as the reagent. First derivative spectrophotometry has recently been employed for the determination of mixtures of iron(III)–bissmuth(II) using EDTA as the reagent [9].

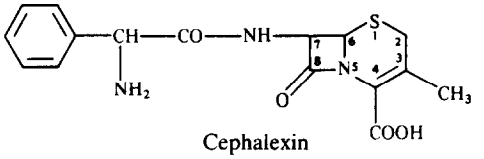
Derivative spectrophotometry has also proved to be useful in the assay of many drugs. Fell has discussed the relative ease of second derivative UV–visible spectrophotometry for the analysis of pharmaceutical dosage forms [10] and the rationale for developing bioanalytical procedures based on higher derivative methods [11]. First and second derivative spectrophotometry recently have been employed for the determination of certain drugs, e.g. acepifylline and phenobarbitone [12], some cephalosporins [13] and pirbuterol and butorphanol [14].

For the determination of substances of clinical interest [15–19], first and second derivative spectrophotometry has been employed for the simultaneous determination of cefuroxime and cephalexin, which are cephalosporins with closely overlapping absorption spectra. The chemical structures of these antibiotics are shown in Table 1. It is the purpose of this work to demonstrate the ease with which the method overcomes the problem due to overlapping spectral bands and to prove that it is a very simple and useful procedure for determining the two cephalosporins in a mixture without tedious and time-consuming separation procedures.

Table 1
Chemical structures of cefuroxime and cephalexin



Cefuroxime



Cephalexin

Materials and Methods

Reagents

Solutions of cefuroxime sodium and cephalexin sodium (0.2 mg ml^{-1}) in water were freshly prepared.

Apparatus

A Perkin–Elmer 555 double-beam UV–visible spectrophotometer was used.

The optimisation of derivative spectra requires the selection of a suitable slit width, response time and scan speed of the monochromator. A spectral bandwidth of 1 nm, a response time of 4 s and a scan speed of 120 nm min^{-1} were found to be optimum for both 1st and 2nd derivative spectra. The recorder scale expansion was also optimised to facilitate readings on the recorder tracing.

Procedure

A few μl of the cephalosporins solutions were mixed in a 5-ml calibrated flask and diluted to volume with distilled water. The spectrophotometric measurements were performed against water in 1-cm quartz cells.

Results and Discussion

Spectrophotometric measurements

Figure 1 shows the absorption spectra of: (a) cephalexin sodium (20 μg ml^{-1}) with a maximum at 261 nm; (b) cefuroxime sodium (15 μg ml^{-1}) with a maximum at 275 nm and; (c) a mixture of cephalexin and cefuroxime (20 μg and 15 μg ml^{-1}, respectively) with a maximum located at 265 nm, i.e. between the absorption maxima of the two

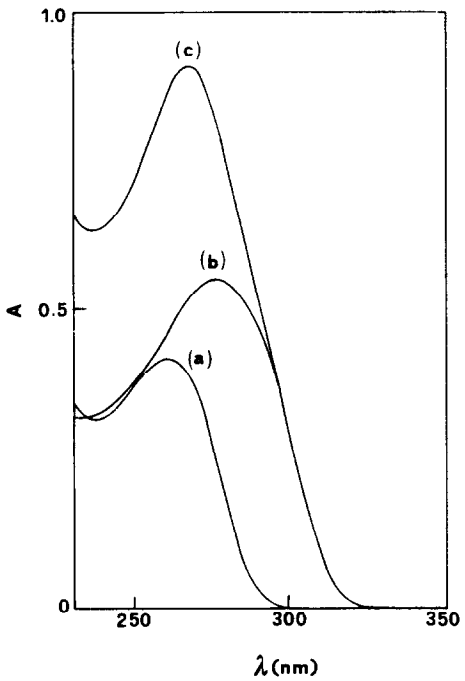


Figure 1
Zero-order spectra of: (a) cephalexin sodium; (b) cefuroxime sodium; and (c) cephalexin sodium plus cefuroxime Na. cephalexin sodium, 20 μg ml $^{-1}$, cefuroxime sodium, 15 μg ml $^{-1}$; reference, water.

components. Thus the overlap of the spectra of cefuroxime and cephalexin result in the absence in the zero-order spectrum of the mixture of any spectral feature (e.g. shoulder) that can be used to assay the two substances. Moreover, the traditional Vierordt's method used for assaying binary mixtures which involves the use of two simultaneous equations, and its variant, the modified Vierordt's method, are time-consuming and tedious, and lack precision when the λ_{max} are close together [10, 12–14].

These difficulties were overcome by the use of derivative spectrophotometry. Figure 2 is the first derivative spectrum of the zero-order spectrum of the mixture shown in Fig. 1. Figure 3 shows a typical second derivative spectrum of another mixture (30 μg ml^{-1} of cefuroxime and 20 μg ml^{-1} of cephalexin). In contrast to the zero-order spectrum, the first derivative spectrum gives separate peaks at 296 and 276 nm. The second derivative spectrum exhibits two distinct maxima at 303 and 280 nm and a minimum at 262 nm.

The choice of suitable wavelengths at which to take measurements that are proportional to the cefuroxime and cephalexin concentrations was made after an inspection of the derivative spectra. The measurements made were "peak-to-baseline" and "peak-to-peak" measurements, generally referred to as "graphical measurements" [1].

First derivative mode. The height of the peak at 296 nm (Fig. 2) is denoted by h_1 (i.e. the distance between the maximum and the baseline), and the distance between the maximum at 276 nm and the intersection of the ordinate through this maximum with the tangent drawn to the left side of the peak at 296 nm is denoted by h_2. Preliminary experiments showed that h_1 and h_2 were proportional to cefuroxime and cephalexin concentrations, respectively. Moreover, the values of h_1 or h_2 were not affected by the presence of the other cephalosporin. For example, the graph [11] in Fig. 4, shows that

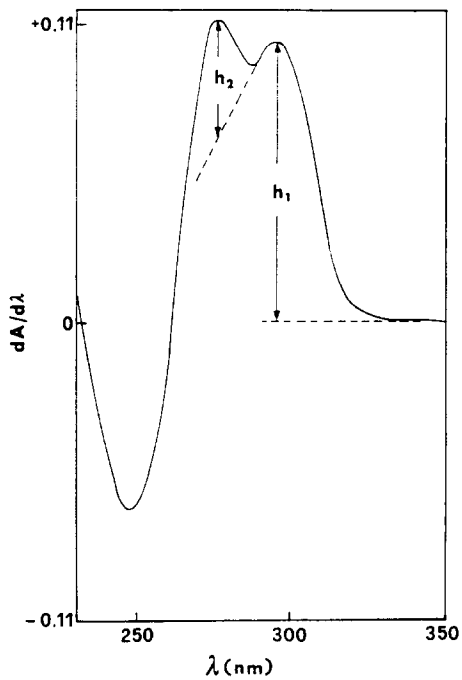


Figure 2
First derivative spectrum of a mixture of cefuroxime sodium (15 μg ml^{-1}) and cephalexin sodium (20 μg ml^{-1}).

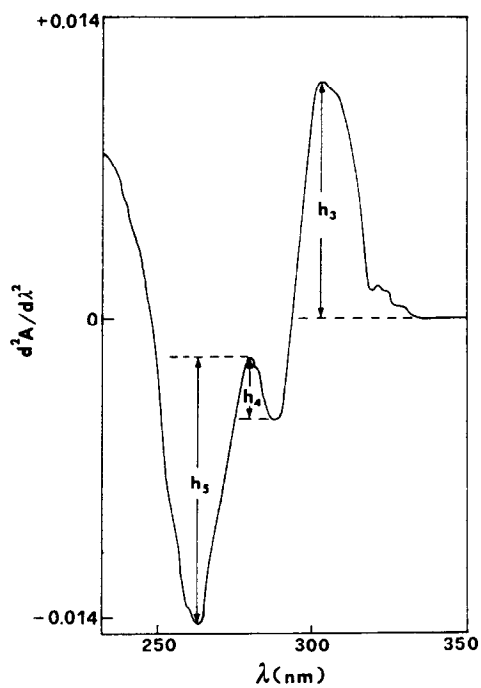


Figure 3
Second derivative spectrum of a mixture of cefuroxime sodium (30 μg ml^{-1}) and cephalexin sodium (20 μg ml^{-1}).

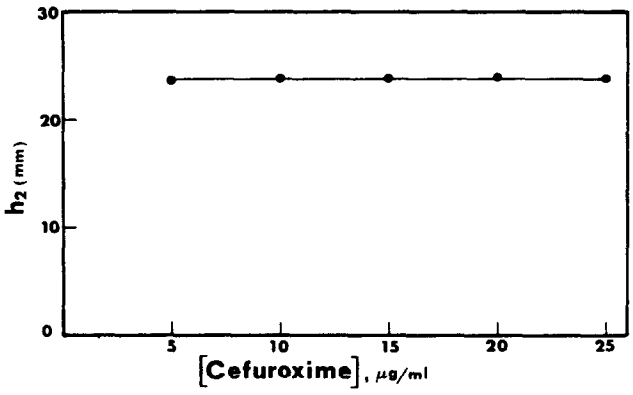


Figure 4
Interaction graph for cephalexin in mixture with cefuroxime (4 μg ml $^{-1}$) by first derivative method (h_2 measurements).

when the cephalexin concentration is constant (4 μg ml^{-1}) and the cefuroxime concentration is varied from 5 to 25 μg ml^{-1}, the h_2 value is unaltered.

Second derivative mode. The height h_3 at 303 nm in Fig. 3 (peak-to-baseline measurement) was found to be proportional to the concentration of cefuroxime; the heights h_4 at 280 nm and h_5, distance between the maximum at 280 nm and the minimum at 262 nm (peak-to-peak measurement) were found to be proportional to the concentration of cephalexin. Also, the height h_3 and the heights h_4 and h_5 are independent of cephalexin and cefuroxime concentrations, respectively.

Calibration graphs and statistical analysis of results

The derivative measurements may be used as easily as ordinary absorbance measurements for the determination of concentration. The linear regression equations calculated for mixtures of cefuroxime sodium and cephalexin sodium in both first and second derivative modes are shown in Table 2, together with the correlation coefficients, the variances and the detection limits, at a level of significance $P = 0.05$ for 10 standard solutions. The range of concentrations investigated was from 4 to 30 μg ml^{-1} of each cephalosporin and three determinations of each measured value were made.

The ordinate values of the lines of regression (H) were calculated from the measurements of h (mm) read from the chart recordings of the spectra as follows [5–7]: H = recorder divisions (h mm) × scale expansion/100 mm full scale.

Beer's Law obtains for concentrations up to 20 and 28 μg ml^{-1} of cephalexin in the first and second derivative modes, respectively, and up to 30 μg ml^{-1} of cefuroxime in both first and second derivative modes.

Tests of significance of the intercept of the lines of regression, $H = a + bc$, were performed to establish whether the experimental intercept (a) differed significantly from the theoretical value, zero. A simplified method of estimating the differences a − 0 is based on the determination of the quantities $t = a/s_a$ [20] and their comparison with the corresponding tabular data for t − distribution: s_a is defined by the expression:

$$s_a^2 = s_0^2 \Sigma c^2 / n \Sigma c^2 - (\Sigma c)^2$$

Table 2
Statistical analysis of the determination of cefuroxime and cephalexin in mixtures by 1st and 2nd derivative spectrophometry

Antibiotic	λ (nm)	Regression equation	Correlation coefficient	Variance (s_0^2)	Detection limit (μg ml^{-1})
Cefuroxime	296	H_1 = 9.70E-04 + 6.78E-03c	0.9999	1.02E-06	0.32
Cephalexin	276	H_2 = 3.20E-04 + 2.31E-03c	0.9996	1.36E-07	0.34
Cefuroxime	303	H_3 = −1.21E-04 + 3.61E-04c	0.9991	1.99E-08	0.84
Cephalexin	280	H_4 = 1.69E-05 + 1.61E-04c	0.9987	3.88E-09	0.84
Cephalexin	280/262	H_5 = −2.01E-04 + 6.32E-04c	0.9991	4.05E-08	0.69

c: concentration of the drug, μg ml^{-1}.
Number of standard specimens, n = 10; level of significance, P = 0.05.

Table 3
Estimate of significance of the intercept in the calibration data*

Measurement	Antibiotic	(a/s_a)	t_p†
1st deriv. (h_1)	Cefuroxime	1.33	2.31
1st deriv. (h_2)	Cephalexin	1.06	2.31
2nd deriv. (h_3)	Cefuroxime	1.19	2.31
2nd deriv. (h_4)	Cephalexin	0.34	2.31
2nd deriv. (h_5)	Cephalexin	1.24	2.31

*a = experimental value of intercept of lines of regression.
†Theoretical value of t at P = 0.05 level of significance; no. of degrees of freedom, f = 8.

in which s_0^2 = variance and c = concentration of the samples. The results of these calculations are reported in Table 3. The values calculated for t do not exceed the 95% criterion, t_p = 2.31 (t_p is Student's coefficient at the selected level of significance), which indicates that the intercepts of all lines of regression are not significantly different from zero and, hence, the proposed methods for determining cefuroxime and cephalexin in mixture are free from errors due to the presence of the other component. However, it should be noted that tests of significance on the estimated slopes and intercepts, when carried out independently of each other, ignore the strong correlation that exists between them. Hence, a more rigorous approach, which requires the construction of a joint confidence region for the possible values of the two parameters, has the further advantage of allowing the evaluation of the calibration graph as a whole.

The 95% joint confidence regions for slopes and intercepts of the regression equations reported in Table 2 are shown in Fig. 5 (first derivative method) and Fig. 6 (second derivative method), constructed by the method of Mandel and Linning [20–21]. These confidence regions are bounded by an ellipse which has the point of best fit as centre (i.e. coordinates a and b). The boundary is dependent on the magnitude of the experimental errors and on the level of significance (0.05, in the present instance) of the probability that the real point lies in the interior of the ellipse.

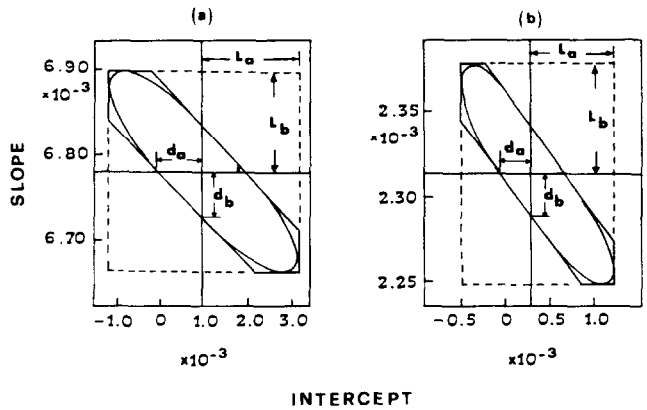


Figure 5
Joint confidence regions for slope and intercept of regression equations, by first derivative method. (a) cefuroxime sodium (h_1 measurements); (b) cephalexin sodium (h_2 measurements); level of significance $P = 0.05$; number of samples, $n = 10$.

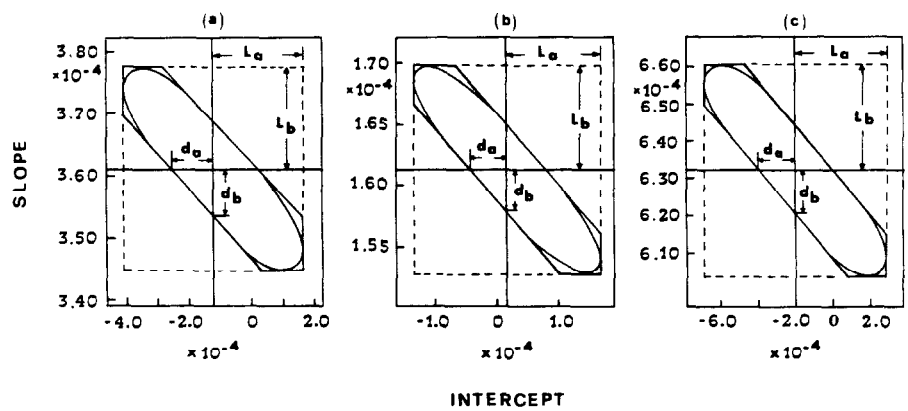


Figure 6
Joint confidence regions for slope and intercept of regression equations, by second derivative method. (a) Cefuroxime sodium (h_3 measurements); (b) Cephalexin sodium (h_4 measurements); (c) Cephalexin sodium (h_5 measurements); level of significance, $P = 0.05$; number of samples, $n = 10$.

The confidence ellipses were used to confirm the hypothesis $a = 0$ in the determination of the mixtures of cefuroxime and cephalexin considered above. The question is answered by determining whether the ellipses in Figs 5 and 6 contain points for which the intercept is zero. Such points are on a vertical line through abscissa = 0 and it is evident that they fall well inside the ellipses, thus confirming the hypothesis that $a = 0$.

The histograms in Fig. 7 (1st derivative method) and Fig. 8 (2nd derivative method) show the confidence limits [20] for the determination of cefuroxime and cephalexin in a mixture, at $P = 0.05$ level of significance. These are plotted from calibration data in a particular way [6–8, 15–17, 22, 23], i.e. as uncertainty (%) on concentration (relative error) ($t_p s_c/c$,%) against the concentration of cefuroxime or cephalexin, respectively. Hence they are a guide to the level of precision that may be expected in the full range of concentrations tested.

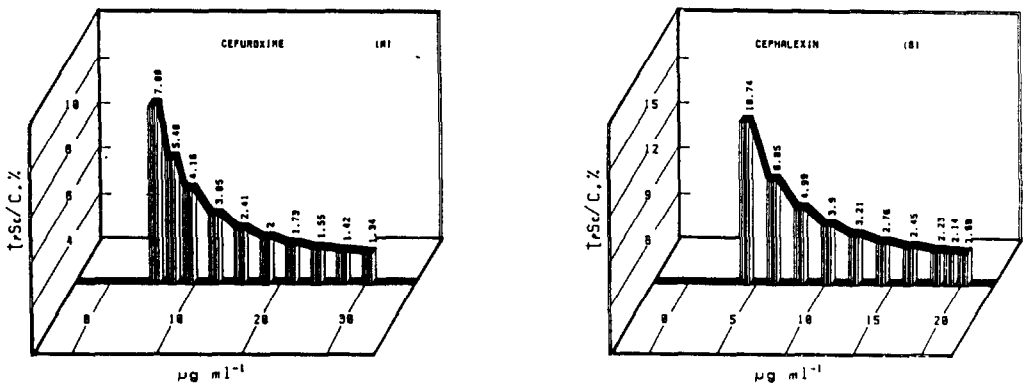


Figure 7
Histograms of the variation of confidence limits at $P = 0.05$ level of significance, in the form of uncertainty per cent on the concentration, by first derivative method. (a) cefuroxime sodium (h_1 measurements); (b) cephalexin sodium (h_2 measurements).

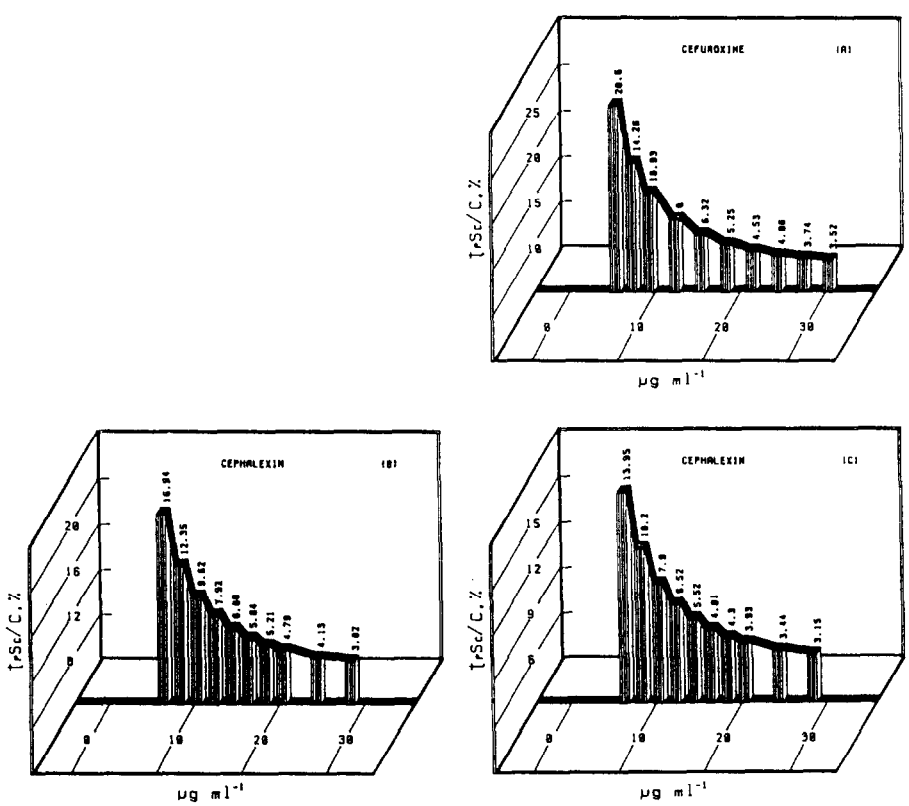


Figure 8
Histograms of the variation of confidence limits at $P = 0.05$ level of significance, in the form of uncertainty percent on the concentration, by second derivative method. (a) Cefuroxime sodium (h_3 measurements); (b) Cephalexin sodium (h_4 measurements); (c) Cephalexin sodium (h_5 measurements).

Table 4
Replicate determinations on synthetic mixtures of cefuroxime and cephalexin

Measurement	Nominal value (μg ml^{-1})		Mean value* (μg ml^{-1})		Standard deviation		Relative standard deviation (%)	
	Cefuroxime	Cephalexin	Cefuroxime	Cephalexin	Cefuroxime	Cephalexin	Cefuroxine	Cephalexin
1st deriv. (h_1 and h_2)	18.0	12.0	18.12	11.99	0.0158	0.0016	0.087	0.013
2nd deriv. (h_3 and h_4)	9.0	18.0	8.91	17.86	0.0223	0.0270	0.250	0.151
2nd deriv. (h_3 and h_5)	12.0	16.0	11.96	16.14	0.0286	0.0230	0.239	0.142

*Mean of five determinations.

To test accuracy and precision of all methods proposed, five successive determinations on synthetic mixtures of cefuroxime and cephalexin were carried out. The results reported in Table 4 show that the accuracy and precision were satisfactory.

Recovery of cefuroxime and cephalexin in pharmaceuticals

As cefuroxime and cephalexin do not occur together in pharmaceutical preparations, the methods were tested with mixtures of two commercial pharmaceuticals. First and second derivative measurements were applied to the determination of film-coated Ceporex tablets (1 g of cephalexin sodium per tablet) and Kesint injectable powder (1 g of cefuroxime sodium per vial).

Extraction of cephalexin from powdered tablets with methanol (as described in a previous paper [15]) eliminates interference from the tablet excipients. Methanolic extraction, rather than aqueous extraction, was used because the poor solubility of some of the excipients in water caused slight opalescence in the samples and less reproducible results. However, injectable cefuroxime is in a pure form without excipients and consequently prior extraction was unnecessary.

Mixtures of the cephalosporins were prepared by dissolving appropriate amount of the compounds in water. The assays were carried out as described under "Procedure". Table 5 shows the results of five replicate determinations on mixtures of Ceporex and Kesint. The results were in good agreement with the stated content of each cephalosporin.

The method is very simple and illustrates the usefulness of derivative spectrophotometric methods for the rapid, precise and sensitive analysis of mixtures of drugs.

Table 5
Results of the assay of cefuroxime and cephalexin in mixtures of pharmaceuticals*

Measurement	Mean recovery %† Cefuroxime	Cephalexin
1st deriv. (h_1 and h_2)	100.25 ± 0.0710	99.90 ± 0.0178
2nd deriv. (h_3 and h_4)	99.94 ± 0.0961	99.46 ± 0.0226
2nd deriv. (h_4 and h_5)	99.93 ± 0.0903	100.63 ± 0.0254

*Ceporex tablets, 1 g cephalexin Na/tablet; supplier, Glaxo S.p.A. (Italy). Kesint powder for injections, 1 g cefuroxime Na/vial; supplier, Proter S.p.A. (Italy).

†Mean of five determinations ± standard deviation; assay as percentage of label claim.

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