

Determination of Amoxicillin and Cephalexin in Mixtures by Second-derivative Spectrophotometry

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A method for the simultaneous determination of amoxicillin and cephalexin in mixtures by second-derivative spectrophotometry is described, using zero-crossing measurements. The procedure does not require any separation step. The calibration graphs are linear up to $60.0 \mu\text{g ml}^{-1}$ of cephalexin at 267.5 nm and up to $390 \mu\text{g ml}^{-1}$ of amoxicillin at 276 nm in the presence of each other.

Keywords: *Amoxicillin determination; cephalexin determination; β -lactam antibiotic; derivative spectrophotometry; simultaneous determination*

In derivative ultraviolet (UV) - visible spectroscopy, the information contained in the spectrum is presented in a potentially more useful form, greatly increasing the versatility of the technique¹⁻⁴ and offering a convenient solution to a number of well defined analytical problems, such as the resolution of multi-component systems, removal of sample turbidity, matrix background and enhancement of spectral details.⁵ Although the use of derivative spectra is not new, it has only become practical in recent years, with the development of microcomputer technology, which allows the almost instant generation of derivative spectra.

Most reports concerning derivative spectrophotometry have featured the application of this technique to multi-component resolution, demonstrating the importance of this approach. Derivative spectrophotometry has been used for the simultaneous determination of inorganic ions, *e.g.*, beryllium and magnesium⁶ and cobalt and nickel,⁷ in biological and biochemical samples, particularly amino acids⁸⁻¹⁰ and proteins,¹⁰⁻¹³ and has been applied to pharmaceutical and clinical systems such as the determination of heroin and morphine in mixtures,¹⁴ chlorpromazine and its sulphoxide¹⁵ and pirbuterol and butorphanol in mixtures.¹⁶

The methods used to determine cephalosporins and penicillins include indophenol derivatisation with and without alkaline degradation to ammonia,¹⁷ alkaline degradation to hydrogen sulphide and the formation of methylene blue^{18,19} and measurement of the absorbance of the blue species formed when cephalosporins and penicillins react with ammonium molybdate in a sulphuric acid medium.^{20,21} The non-specificity of these methods, however, renders them unsuitable for the analysis of mixtures of different antibiotics, and the separation of the individual antibiotics prior to determination is usually necessary.

Recently, derivative spectrophotometric procedures for the analysis of two-component mixtures of antibiotics, *viz.*, cephacetrile and ceftazidime,²² ampicillin and cloxacillin²³ and ampicillin and dicloxacillin,²⁴ have been developed. In this paper, the derivative technique is proposed for the simultaneous determination of amoxicillin and cephalexin, which are semi-synthetic β -lactam antibiotics of the penicillin and cephalosporin groups, respectively, without separation.

Amoxicillin and cephalexin have very similar absorbance spectra without prominent peaks, which could be used for reliable direct absorbance measurements. The aim of this work was to demonstrate the ease with which the derivative method circumvents the problem of overlapping spectral bands, so allowing the simultaneous determination of these antibiotics without prior separation. The method yields accurate and reproducible results.

Experimental

Reagents and Equipment

Stock reference solutions (2.0 mg ml^{-1}) were freshly prepared from pure samples of cephalexin sodium and amoxicillin sodium (Antibioticos, León, Spain).

A 0.5 M phosphate buffer solution (pH 8.2) was prepared using sodium dihydrogen phosphate and disodium hydrogen phosphate.

Five different Pipetman micropipettes were used to measure volumes in the range 2–1000 μl .

A Shimadzu UV - visible recording spectrophotometer (Model UV-160) and 1.0-cm quartz cells were used for the normal and first- and second-derivative spectrophotometric measurements.

Procedure

Mix suitable volumes of the 2.0 mg ml^{-1} antibiotic stock solutions, containing up to 60.0 or up to $390 \mu\text{g ml}^{-1}$ (see later) of antibiotics and add 5.0 ml of phosphate buffer solution (pH 8.2) in a 25-ml calibrated flask. Dilute to volume with distilled water and record the second-derivative spectra of the mixture against a reagent blank. Determine the amoxicillin content from the second-derivative spectrum by measuring the second-derivative signal at the zero-crossing point for cephalexin (276 nm) and comparing the value with an appropriate calibration graph. Determine the cephalexin content by measuring the second-derivative signal at the zero-crossing point for amoxicillin (267.5 nm) and comparing the value with the appropriate calibration graph. The wavelengths of the zero-crossing points quoted are those obtained in this work, but these values should be established independently for other instruments as part of the calibration procedure.

Results and Discussion

Spectrophotometric Measurements

The influence of pH on the absorption spectra of amoxicillin and cephalexin and the first and second derivatives was studied. The best results for analytical purposes were obtained at pH 8.2 (phosphate buffer solution).

The zero-order spectra of amoxicillin and cephalexin and a mixture of both in the 200–300-nm wavelength region are shown in Fig. 1. It can be seen that the absorption spectra of amoxicillin and cephalexin are very similar and that neither spectrum shows prominent peaks that can be used for reliable direct absorbance measurements. Incorrect results were

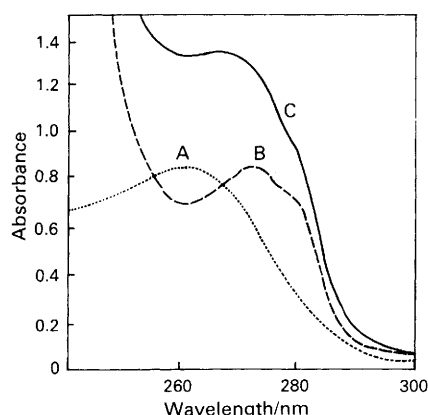


Fig. 1. Absorption spectra of A, amoxicillin; B, cephalixin; and C, a mixture of amoxicillin and cephalixin; [amoxicillin] = $254.0 \mu\text{g ml}^{-1}$; [cephalexin] = $35.2 \mu\text{g ml}^{-1}$; reference, reagent blank

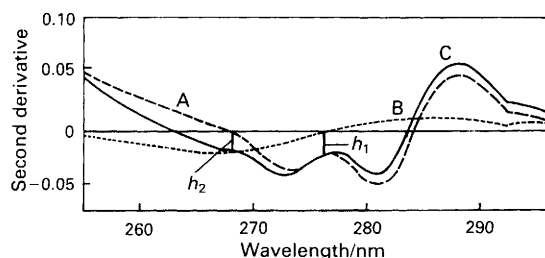


Fig. 2. Second-derivative spectra of A, amoxicillin; B, cephalixin; and C, a mixture of amoxicillin and cephalixin. [Amoxicillin] = $126.0 \mu\text{g ml}^{-1}$; [cephalexin] = $19.2 \mu\text{g ml}^{-1}$; reference, reagent blank

obtained by solving simultaneous equations for the two wavelengths of maximum absorption of both antibiotics.

The commonest procedures for the preparation of analytical calibration graphs are "peak-to-peak" and "base line" measurements (generally called graphical measurements) and "zero-crossing" measurements. In practice, the measurement selected is that which exhibits the best linear response, gives a zero or near-zero intercept on the ordinate of the calibration graph, and is least affected by the concentration of any other component. The shape of the first-derivative spectra is not adequate for the application of the above methods.

Fig. 2 shows the second-derivative absorption spectra of amoxicillin, cephalixin and of a mixture of both. It can be seen that, because of the closeness of the two overlapping spectra of these antibiotics, they are not sufficiently resolved to give two distinct peaks or shoulders in the second-derivative spectrum of the mixture. In this instance, the zero-crossing method is the most appropriate for resolving mixtures of these compounds and it was used in this work with satisfactory results.

The zero-crossing method involves measurement 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 only. The best zero-crossing wavelengths are 276 and 267.5 nm for cephalixin and amoxicillin, respectively. Fig. 3 shows a typical set of second-derivative spectra of $19.2 \mu\text{g ml}^{-1}$ of cephalixin plus increasing amounts of amoxicillin (from 59.4 to $390.6 \mu\text{g ml}^{-1}$). Analogously, Fig. 4 shows a series of second-derivative spectra of mixtures of $127 \mu\text{g ml}^{-1}$ of amoxicillin plus increasing amounts of cephalixin (from 8.0 to $54.4 \mu\text{g ml}^{-1}$).

Preliminary experiments showed that the height, h_1 , of the second-derivative at 276 nm (working zero-crossing wavelength of cephalixin) was proportional to the amoxicillin concentration. Linear calibration graphs with small intercepts on the ordinate were obtained and the variation of h_1 was

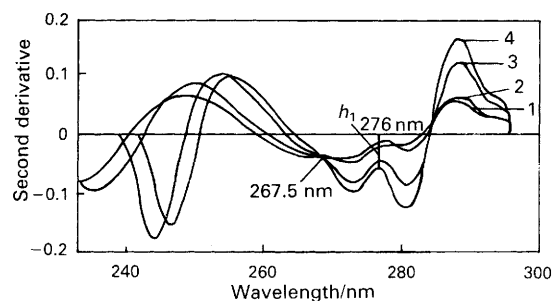


Fig. 3. Second-derivative spectra of mixtures of amoxicillin and cephalixin. Cephalixin, $19.2 \mu\text{g ml}^{-1}$, with 1, 93.4; 2, 127.0; 3, 288.7; and 4, $390.6 \mu\text{g ml}^{-1}$ of amoxicillin

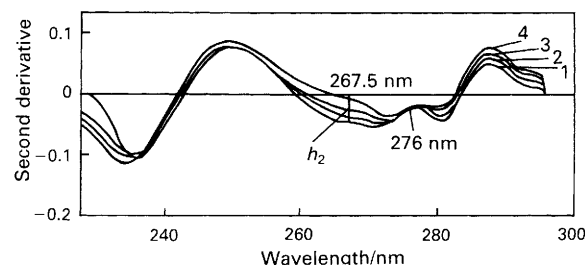


Fig. 4. Second-derivative spectra of mixtures of cephalixin and amoxicillin. Amoxicillin, $127.0 \mu\text{g ml}^{-1}$, with 1, 11.2; 2, 27.2; 3, 43.2; and 4, $54.4 \mu\text{g ml}^{-1}$ of cephalixin

hardly affected by the presence of cephalixin for any ratio of amoxicillin to cephalixin over the full range of concentrations investigated.

The height, h_2 , at 267.5 nm (zero-crossing wavelength of amoxicillin) was independent of the amount of amoxicillin. Of course, all the curves in Figs. 3 and 4 converge to the abscissa values selected as the working wavelengths, *i.e.*, 267.5 and 276.0 nm, respectively.

Selection of Optimum Instrumental Conditions

The main instrumental parameters that affect the shape of the derivative spectra are the wavelength scanning speed, the wavelength increment over which the derivative is obtained ($\Delta\lambda$) and the response time. These parameters need to be optimised to give a well resolved large peak, *i.e.*, to give good selectivity and higher sensitivity in the determination.

In our spectrophotometer, the derivative spectra are obtained by digital differentiation (convolution method) with 17–25 data points around each wavelength.

Generally, the noise level decreases with an increase in $\Delta\lambda$, thus decreasing the fluctuations in the derivative spectrum. However, if the value of $\Delta\lambda$ is too large, the spectral resolution deteriorates. Therefore, the optimum value of $\Delta\lambda$ should be determined by taking into account the noise level, the resolution of the spectrum and the sample concentration. Various values of $\Delta\lambda$ were tested and 3.2 nm was selected as the optimum in order to give a satisfactory signal to noise ratio.

The scanning speed of the monochromator has virtually no effect on the derivative signal obtained digitally. Hence a fast wavelength scanning speed (*ca.* 2400 nm min^{-1}) was selected. The response time is automatically selected by the spectrophotometer in accordance with the optical energy and the scanning speed.

Statistical Analysis of Results

In order to test the mutual independence of the analytical signals of cephalixin and amoxicillin, *i.e.*, to show that h_1 and

Table 1. Statistical analysis of the determination of amoxicillin (59.4–390.6 $\mu\text{g ml}^{-1}$) and cephalixin (8.0–54.4 $\mu\text{g ml}^{-1}$) in mixtures by second-derivative spectrophotometry for standard specimens ($n = 11$)

Antibiotic determined	Other antibiotic present		Slope	Intercept	Correlation coefficient	Standard deviation/ $\mu\text{g ml}^{-1}$
	Antibiotic	Concentration/ $\mu\text{g ml}^{-1}$				
Amoxicillin	—	—	0.15	−0.22	0.9995	0.582
	Cephalixin	19.2	0.14	1.49	0.9990	0.787
		43.2	0.15	0.53	0.9985	1.049
Cephalixin	—	—	0.90	−0.90	0.9990	0.738
	Amoxicillin	127.0	0.87	1.47	0.9994	0.534
		254.0	0.85	1.02	0.9988	0.754

Table 2. Resolution of amoxicillin and cephalixin mixtures by second-derivative spectrophotometry

Amoxicillin present, p.p.m.	Cephalixin present, p.p.m.	Amoxicillin found, p.p.m.	Recovery, %	Cephalixin found, p.p.m.	Recovery, %
59.4	8.0	60.8	102.3	8.0	100.0
93.4	11.2	94.6	101.3	11.4	101.8
110.3	15.3	108.1	98.0	14.8	96.8
127.0	19.2	128.4	101.1	19.4	101.0
186.8	27.2	189.2	101.3	28.5	104.8
220.6	31.3	209.5	95.0	30.7	98.1
254.0	35.2	256.7	101.1	36.4	103.4
288.7	43.2	290.5	100.6	43.3	100.2

h_2 are independent of the cephalixin and amoxicillin concentrations, respectively, the following experiments were performed.

Three calibration graphs were constructed from second-derivative signals for standards containing between 8.0 and 54.4 $\mu\text{g ml}^{-1}$ of cephalixin, in the absence of amoxicillin and in the presence of 127.0 and 254.0 $\mu\text{g ml}^{-1}$ of amoxicillin. Similarly, three calibration graphs were prepared for standards containing between 59.4 and 390.6 $\mu\text{g ml}^{-1}$ of amoxicillin in the absence of cephalixin and in the presence of 19.2 and 43.2 $\mu\text{g ml}^{-1}$ of cephalixin.

A critical evaluation of the proposed method was performed by statistical analysis of the experimental data. The slope, intercept, correlation coefficient and standard deviation obtained are summarised in Table 1. The linearity of the calibration graphs and the adherence of the systems to Beer's law are validated by the high value for the correlation coefficient of the regression equation and by the value of the intercept on the ordinate, which is close to zero.

From Table 1, it can be seen that the slope of the calibration graph for cephalixin is virtually independent of the amoxicillin concentration. Similarly, the slope of the calibration graph for amoxicillin is independent of the cephalixin concentration. Therefore, it can be deduced that the amplitude of the derivative signal of the mixture, measured at the zero-crossing point of the derivative spectrum of one of the two components, is a function only of the concentration of the other component, in accordance with the theoretical predictions.

In order to check the validity of the proposed method, amoxicillin and cephalixin were determined in several synthetic mixtures. Table 2 gives the results of the determinations for different mixtures. Satisfactory results were obtained for the recovery of both compounds, indicating that the second-derivative spectrophotometric method is effective for the simultaneous determination of amoxicillin and cephalixin.

Conclusion

Derivative spectrophotometry permits the simple, rapid, sensitive and direct determination of two β -lactam antibiotics derivatives, amoxicillin and cephalixin, in aqueous binary mixtures without previous separation.

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