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Spectrofluorimetric analysis of cefuroxime in pharmaceutical dosage forms

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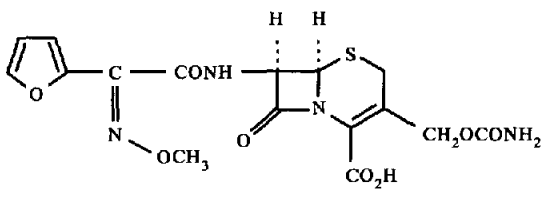
Abstract: A fluorimetric method has been developed for the quantitative analysis of cefuroxime, based upon the formation of a fluorescent derivative formed by alkaline hydrolysis with 1.0 M sodium hydroxide and heating at 100°C for 60 min. The fluorescent product gave excitation and emission maxima at 380 and 436 nm, respectively. The method was performed in aqueous solution adjusted to pH 10.5 by addition of phosphate buffer solution. The calibration curve was found to be linear in the range of concentrations 0.050–1.70 μg ml^{-1}. The lower limit of detection was 1.0×10^{-2} μg ml^{-1}. The method was applied to authentic pharmaceutical preparations containing cefuroxime sodium or cefuroxime axetil, the 1-(acetyloxy) ethyl ester of the drug, and was found to be satisfactory. Cefuroxime sodium was also determined in physiological solutions used to prepare intravenous infusions of this antibiotic.

Keywords: *Cefuroxime; spectrofluorimetry; pharmaceutical dosage forms.*

Introduction

Cefuroxime is a semisynthetic cephalosporin antibiotic. The major structural difference between cefuroxime and other commercially available cephalosporins is that cefuroxime contains a methoxyimino group at position 7 on the β-lactam ring and also contains a carbamate group at position 3 on the ring. The methoxyimino group confers stability against hydrolysis by many β-lactamases and the carbamate group confers metabolic stability [1–3].

Microbiological methods for determining cefuroxime have been reported [4] and have been applied in various pharmacokinetic and bioavailability studies [5, 6]. Recently, fluorimetric assays of other cephalosporins have been developed to give rapid and sensitive methods for the determination of these antibiotics [7–11].



Cefuroxime

A fluorimetric method was developed for the determination of cefuroxime [12]. This method involved double treatment based on the addition of hydrochloric acid, heating and cooling, followed by addition of sodium hydroxide and further heating at 100°C. The fluorescence intensity of the final solution was measured at an excitation wavelength of 375 nm and an emission wavelength of 440 nm and was related to the antibiotic concentration. The minimum detectable concentration of cefuroxime was 0.1 μg ml^{-1} for an aqueous solution of the drug.

The objective of the present work was to develop a method that was less tedious, more rapid and more sensitive than that previously reported [12].

Experimental

Reagents

Cefuroxime sodium was obtained from Sigma (Madrid, Spain). A stock solution containing 200 μg ml^{-1} of the drug in water filtered through a Milli-Q (Millipore) filter was prepared. This solution was stored protected from light at about 4°C. A 0.5 M buffer solution (pH 10.5) was prepared by mixing appropriate amounts of sodium dihydrogen

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phosphate with sodium hydroxide. Sodium hydroxide and hydrochloric acid were obtained from Merck (Barcelona, Spain). All the reagents used in the assay were of analytical grade.

Physiological solutions of 0.9% sodium chloride were supplied by Apiroserum (Instituto de Biología y Sueroterapia, Madrid, Spain) and Grifols (Laboratorios Grifols, Madrid, Spain). A physiological solution of 5% anhydrous glucose (dextrose) was supplied by Apiroserum (Instituto de Biología y Sueroterapia, Madrid, Spain).

Apparatus

Fluorimetric measurements were made on a Perkin–Elmer LS-50 spectrofluorimeter (Beaconsfield, Buckinghamshire, England) equipped with a xenon lamp, connected to an Ataio S 3000 ST 386 computer (West Germany) fitted with Perkin–Elmer FL Data Manager software (designed for handling fluorescence data on a personal computer) and an Epson FX-850 printer. A thermostatically controlled bath, Selecta Model Frigiterm (Barcelona, Spain), was used for temperature control. A pH-meter, Crison Model 2001, with a glass-saturated calomel combination electrode was also used.

General procedure

An aliquot of stock solution of cefuroxime was appropriately diluted, treated with 1.0 M sodium hydroxide and heated at 90°C for 60 min to produce a fluorescent product. After completion of the heat treatment, the solution was immediately cooled to room temperature using an ice-bath and was then adjusted to neutral pH with hydrochloric acid.

A suitable aliquot of the resulting solution was transferred to a 25-ml volumetric flask so that the resulting solution contained 0.050–1.70 μg ml^{-1} of the hydrolysed cephalosporin; 5.0 ml of buffer solution (pH 10.5) was added, and it was then diluted to volume with water. The fluorescence intensity was measured at 436 nm, with excitation at 380 nm, against a blank sample that had been similarly treated. The concentration of cefuroxime present in the sample was determined using a suitable calibration graph, obtained by application of the method to known concentrations of the antibiotic.

Procedure for determining cefuroxime in injections

The contents of the injection vial were placed in a 100-ml volumetric flask and diluted to volume with Milli-Q-filtered water. An aliquot of this solution containing 5×10^3 μg of the drug was diluted and submitted to high temperature alkaline treatment as described in the general procedure. The percentage of antibiotic in the injection was calculated using the linear regression equation obtained for the pure compound.

Procedure for determining cefuroxime in physiological solutions

The contents of the vial of cefuroxime injection were dissolved in different physiological solutions: sodium chloride intravenous infusion (Apiroserum and Grifols); and glucose intravenous infusion (Apiroserum). The concentration of cefuroxime in these solutions was that used clinically, that is 7.5 mg ml^{-1}. Suitable volumes of these solutions of cefuroxime in the different infusion fluids were diluted appropriately and submitted to the alkaline treatment described above in the general procedure, and the concentration of cefuroxime was determined in the same way. The percentage of antibiotic in the intravenous infusion was calculated using the linear regression equation obtained for the pure compound.

Procedure for determining cefuroxime in suspensions

A suitable amount of powder used for preparing suspensions equivalent to 0.025 mg of cefuroxime (expressed in terms of cefuroxime sodium) was transferred to a 250-ml volumetric flask. Cefuroxime axetil is soluble in sodium hydroxide [13] and thus it was possible to carry out the hydrolysis process described above to obtain the same fluorescent product from cefuroxime axetil. After cooling the hydrolysed solution, it was necessary to filter it through a paper filter designed for gravimetric analysis (Albet, 240). Finally the assay was completed as described under the general procedure.

Results and Discussion

Optimization of hydrolysis conditions

The fluorescence intensity of the sample can be optimized by choosing suitable conditions.

Thus, the reaction conditions that were varied in order to optimize fluorescence included the sodium hydroxide concentration, the reaction temperature and the reaction time.

The effect of sodium hydroxide concentration on fluorescence intensity was studied by using several concentrations of this alkaline solution (0.1 M–2.0 M) and maintaining a reaction temperature of 85°C for 60 min (Fig. 1). In all cases the same spectrum was obtained, that is, with the same excitation and emission maxima. No great differences between the fluorescence intensities of the different concentrations were observed; however, the optimum sodium hydroxide concentration was 1.0 M since a slight increase in fluorescence was achieved at this concentration.

Figure 2 shows the rate of formation of the fluorescent product from cefuroxime in 1.0 M sodium hydroxide at four different temperatures (45, 60, 75 and 90°C). It can be seen that the highest fluorescence intensity was obtained with the highest temperature; i.e. 90°C was the optimum reaction temperature. At lower reaction temperatures, even with much longer reaction times, the fluorescence intensity did not approach closely that obtained at 90°C. When the cefuroxime standard was treated with 1.0 M sodium hydroxide at 90°C, a constant fluorescence intensity was obtained for 50–150 min, indicating a fairly stable product. 60 min was considered to be a suitable reaction time.

In order to demonstrate that the fluorescent product was the same at all temperatures studied, the spectra obtained for each after 150 min of reaction were normalized (Fig. 3). As can be seen, the shape of all spectra are the same in the range of wavelengths studied. This means that the fluorescent product is indeed the same in all cases. The literature on cephalosporins gives no indication of possible fluorescent products under extremely alkaline conditions. Jusko [14] proposes the formation of a diketopiperazine as a product of acidic hydrolysis of ampicillin. A diketopiperazine product similar to the structure proposed by Jusko is indicated for the alkaline degradation studies on cephradine [15]. The presence of an α-aminobenzyl group in the penicillins or cephalosporins is necessary for the formation of this product. The first studies on hydrolysis of cephalosporins indicated the oxazolones could not be obtained [16]. However, Yu *et al.*

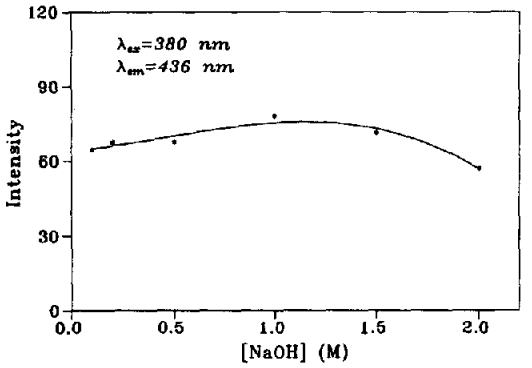

Figure 1
Effect of sodium hydroxide concentration on the fluorescence of cefuroxime standards, at a reaction temperature of 85°C for 60 min. [Cefuroxime] = 0.9 μg ml^{-1}.

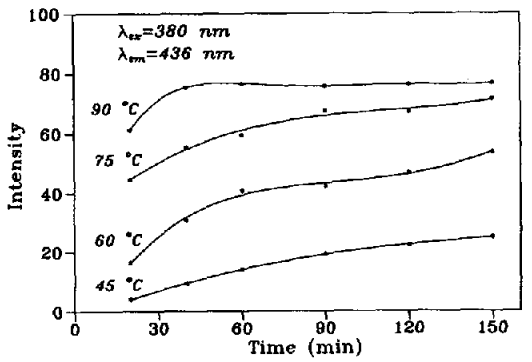

Figure 2
Effects of temperature and heating time on the fluorescence of cefuroxime standards treated with 1.0 M NaOH. [Cefuroxime] = 1.0 μg ml^{-1}.

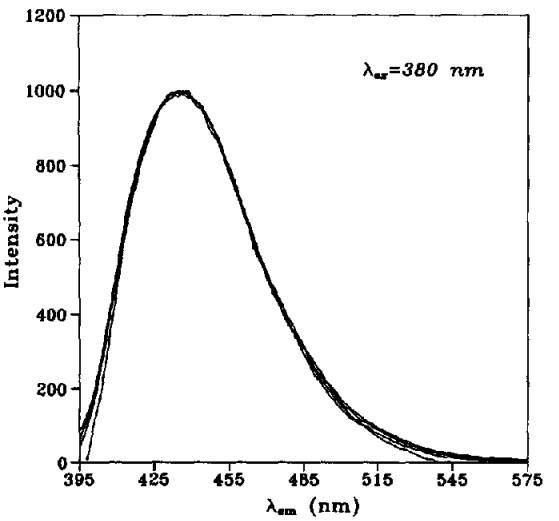

Figure 3
Normalized emission spectra of cefuroxime treated with 1.0 M NaOH at 45, 60, 75 and 90°C for 150 min. [Cefuroxime] = 0.9 μg ml^{-1}.

[17] proposed the formation of oxazolones as a fluorescent product if the hydrolysis is conducted at high temperatures and under highly alkaline conditions. The presence in the cefuroxime molecule of an oxime group in the position corresponding to the α-aminobenzyl group of the cephalosporins whose hydrolysis has been described [17] suggests that a similar fluorophor may be obtained with cefuroxime.

The stability of the solution containing the fluorescent product formed from cefuroxime was studied. The results showed that in a neutral medium, the fluorophor is stable at room temperature for at least 24 h and at 4°C for at least 30 days, whereas in strongly alkaline solution the fluorescence rapidly deteriorates. The solution is less stable in a highly acidic of basic medium. For this reason it is proposed that the pH of the solution should be adjusted to pH 7 after hydrolysis treatment before storage.

Effect of variables on fluorescence intensity

The dielectric constant and polarity of the medium may affect the fluorescent properties of a drug [18]. Because of this possibility studies on the influence of the ethanol content in the medium were carried out by preparing samples of fluorescent product from cefuroxime, varying the percentage of ethanol in water between 0 and 96%, v/v. The results suggested that measurements should be made in an aqueous medium, since the presence of ethanol has practically no effect on the fluorescence signal.

Of the parameters which can be varied in order to maximize fluorescence intensity, one of the most important is the pH of the medium. In order to choose the most suitable pH at which to perform the assay, the pH of samples was varied by adding hydrochloric acid or sodium hydroxide. In this study it was found that the excitation and emission maxima showed a slight displacement to lower wavelengths when the pH values were increased and the intensity of fluorescence was also greater at higher pH values. It was proposed to adjust the pH of the fluorescent solution to pH 10.5 prior to the fluorimetric determination. The fluorescent product was satisfactorily stable at pH 10.5, as judged by the small changes in fluorescence intensity over 180 min. The pH chosen for the determination of cefuroxime was obtained by addition of phosphate buffer solution at pH 10.5; the concentration of buffer had practically no effect on the fluorescence.

Dependence of the fluorescence intensity on temperature was critical, showing a decrease in fluorescence emission of 0.71% $°C^{-1}$ when the temperature was increased from 3–60°C. This effect can be explained by a higher internal conversion as the temperature increases, facilitating non-radiative deactivation of the excited singlet state [18]. It is therefore recommended that a temperature set at 20°C, that is about room temperature, be used.

Another factor to be considered is the effect of cefuroxime concentration on the fluorescence of the fluorophor derived from this drug. Inner filter quenching due to high fluorophor concentration, that is, deviation from the linear relationship between the concentration of the cefuroxime to be assayed and the observed fluorescence, becomes significant when the cefuroxime concentration exceeds 10 $\mu g\ ml^{-1}$.

Fluorescence spectral properties

An intensely fluorescent product was obtained from cefuroxime in alkaline solution after heating at 90°C for 60 min. The tridimensional spectra provide the best characterization of the fluorescence of the cefuroxime degradation product. These spectra were obtained with a suitable computer program [19] and presented as an isometric projection, where the excitation spectra at stepped increments of 4 nm emission wavelength were recorded and plotted. Figure 4 shows the tridimensional spectrum of the fluorophor where light scattering had been removed.

In the two-dimensional representation (Fig. 5) it can be observed that the cefuroxime fluorescent product displays two fluorescence maxima which are localized at different excitation wavelengths (270 and 380 nm) and the same emission wavelength (436 nm). All fluorescence measurements in subsequent studies were carried out at the excitation maximum of 380 nm and the emission maximum of 436 nm, where the highest intensity was obtained.

Spectrofluorimetric determination of cefuroxime

The above technique enabled a method to be developed for determining cefuroxime by the direct measurement of fluorescence intensity at

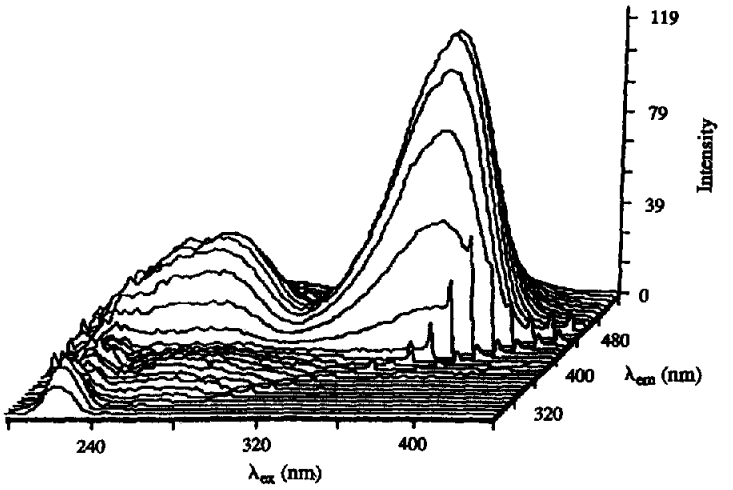

Figure 4
Isometric plot of the emission–excitation matrix (forward projection) of cefuroxime after reaction at 90°C for 60 min in 1.0 M NaOH. [Cefuroxime] = 1.6 μg ml^{-1}, pH = 10.5.

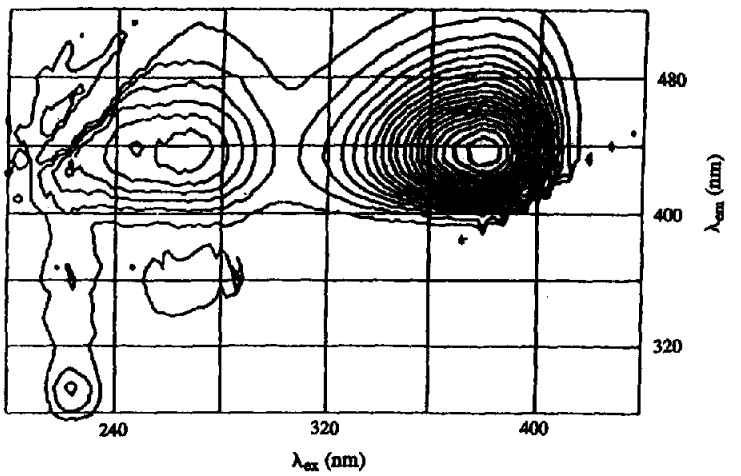

Figure 5
Contour plot of cefuroxime fluorescence after reaction at 90°C for 60 min in 1.0 M NaOH. [Cefuroxime] = 1.6 μg ml^{-1}, pH = 10.5.

an excitation wavelength of 380 nm and an emission wavelength of 436 nm.

A linear dependence of fluorescence intensity on cefuroxime concentration was obtained between 0.050 and 1.70 μg ml^{-1}. The linear regression equation, $y = a + mx$, resulting from the application of the least-squares method to the experimental data together with the statistical parameters are summarized in Table 1.

Table 1
Statistical parameters

Intercept on the y axis (a)	−0.77
Standard deviation of intercept	1.38
Slope (m)	223.32
Standard deviation of slope	1.52
Correlation coefficient	0.999
Theoretical t value	2.262
Experimental t value	0.557
New slope (m_o)	222.65

The significance of the intercept on the y-axis of the previously obtained regression line was evaluated by applying Student's t-test at a 95% confidence level and nine degrees of freedom [20]. The result of this study is shown in Table 1. The value calculated for t does not exceed the theoretical value; hence the intercept on the y axis is negligible, that is, the experimental intercept does not differ significantly from the theoretical value, zero. As a

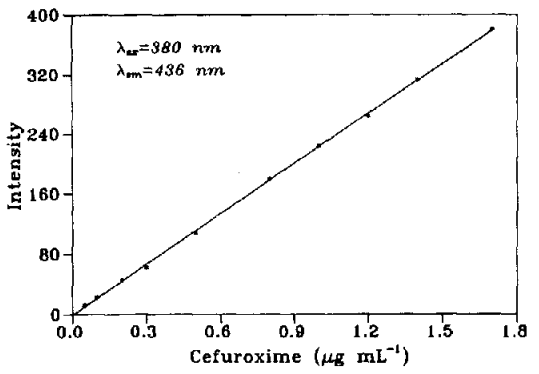

Figure 6
Fluorimetric standard curve for cefuroxime.

result, the experimental data were fitted to a function, $y = m_o x$, and the new value of the slope (m_o) of the linear calibration graph was calculated (Table 1).

The linearity of the standard curve (Fig. 6) in the useful concentration range and the negligible scatter of the experimental points is clearly demonstrated by the high value of the correlation coefficient. This together with the negligible value for the intercept on the *y*-axis showed the excellent linear relationship between fluorescence intensity and cefuroxime concentration.

In order to determine the accuracy and the precision of the recommended procedure, two series of 10 standard samples containing 0.40 and 1.20 μg ml^{-1} of the antibiotic were prepared and fluorescence measurements were carried out on each reaction product by the proposed method. A mean value ± standard deviation of 0.399 ± 2.77 × 10^{-3} and 1.221 ± 5.86 × 10^{-3}, respectively and a mean standard error (as a percentage) of 1.6 and 1.1% were found for each sample, at the 95% confidence level.

The determination and detection limits, calculated as defined in References [21, 22], were 0.034 and 0.010 μg ml^{-1}, respectively.

Determination of cefuroxime in pharmaceuticals

Cefuroxime is commercially available for parenteral administration as the sodium salt and for oral administration as cefuroxime axetil, the 1-(acetyloxy)ethyl ester of the drug. Cefuroxime axetil is a prodrug of cefuroxime and has little, if any, antibacterial activity until hydrolysed *in vivo* cefuroxime. Esterification of the carboxyl C-4 group of cefuroxime results in a more lipophilic form of the drug that is readily absorbable from the gastrointestinal tract.

The proposed method was applied to the determination of cefuroxime in the pharmaceutical formulations of this antibiotic commercially available in Spain. Curoxima injection (Laboratorio Glaxo, Burgos, Spain) contained 250 mg of cefuroxime sodium salt per vial. Zinnat (Laboratorio Glaxo, Burgos, Spain) and Nivador (Laboratorios Menarini, Barcelona, Spain) oral suspensions each contained 1500 mg in 60 ml of cefuroxime axetil.

The assays were carried out as described under the corresponding procedures. Cefuroxime axetil is soluble in sodium hydroxide. For this reason it is possible to conduct the hydrolysis procedure described earlier and to obtain the same fluorescent product from the axetil form of this antibiotic. Table 2 shows the assay results obtained in this experiment, expressed as a percentage of the content representing the mean of three determinations of three different vials or bottles of pharmaceutical products. These results concurred with the stated content of the cephalosporin.

Since cefuroxime sodium is administered intravenously in serious infections, the method was applied to the determination of the antibiotic in physiological solutions. These solutions were: 0.9% sodium chloride intravenous infusion (Apiroserum and Grifols); and 5% glucose intravenous infusion (Apiroserum).

Table 2
Determination of cefuroxime in pharmaceutical dosage forms

	Cefuroxime			
Pharmaceutical products	Taken (μg ml^{-1})	Found* (μg ml^{-1})	SD (μg ml^{-1})	Recovery (%)
Curoxima injection	0.80	0.81	1.2 × 10^{-2}	100.9
Zinnat oral suspension	0.80	0.80	2.3 × 10^{-2}	100.1
Nivador oral suspension	0.80	0.79	1.8 × 10^{-2}	99.0

*Mean of three determinations with three replicates of each.

Table 3
Determination of cefuroxime in physiological solutions

Medium	Pharmaceutical product Curoxima injection			
	Taken ($\mu g\ ml^{-1}$)	Found* ($\mu g\ ml^{-1}$)	SD ($\mu g\ ml^{-1}$)	Recovery (%)
Sodium chloride intravenous infusion (Apiroserum)	0.80	0.79	1.0×10^{-2}	98.8
Sodium chloride intravenous infusion (Grifols)	0.80	0.80	1.1×10^{-2}	99.7
Glucose intravenous infusion (Apiroserum)	0.80	0.79	0.6×10^{-2}	98.7

*Mean of three determinations with three replicates of each.

The pharmaceutical product was added to different solutions and was then determined according to the proposed method. The results are shown in Table 3. Other components of the pharmaceutical preparations tested showed no fluorescence in the range of wavelengths tested and it was concluded that the fluorescent product in the pharmaceutical dosage form was the degradation product from cefuroxime only. This made it possible to apply the proposed method to the determination of cefuroxime in the different pharmaceutical formulations.

Conclusions

The described method enabled the fluorimetric method to be used in the determination of cefuroxime in the concentration range 0.050–1.70 $\mu g\ ml^{-1}$. Measurements of the fluorophor product were carried out at 436 nm with excitation at 380 nm. These measurements were found to be more sensitive than measurements by a previously reported fluorimetric method. The lower limit of detection was 0.010 $\mu g\ ml^{-1}$, representing a 10-fold increase in sensitivity as compared to that of the assay described previously [12]. Moreover, the suggested method offered advantages in speed and simplicity over the existing method.

The method was applied to the determination of cefuroxime in pharmaceutical dosage forms and physiological solutions used as intravenous infusions. Satisfactory recoveries were found for each formulation.

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