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First-derivative spectrophotometric and LC determination of cefuroxime and cefadroxil in urine

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Abstract

Two methods are presented for the determination of cefuroxime and cefadroxil in human urine using first (^{1}D) derivative spectrophotometry and high-performance liquid chromatography. Cefuroxime and cefadroxil were determined by measurement of their first-derivative amplitude in 0.1 N sodium hydroxide at 292.5 and 267.3 nm, respectively in the concentration range of 2–10 μg ml^{-1} for each drug. The HPLC method depends upon using a LiChrospher 100 RP-18 (5 μm) column at ambient temperature for cefuroxime and 35°C for cefadroxil with mobile phases consisting of water–acetonitrile–acetic acid (85:15:0.1 v/v) at a flow rate of 1.5 ml min^{-1} for cefuroxime; and 0.02 M potassium dihydrogen phosphate–acetonitrile (95:5 v/v) containing 0.003% (w/v) hexanesulphonic acid sodium salt and adjusted to apparent pH 3 with phosphoric acid at a flow rate of 2 ml min^{-1} for cefadroxil. Quantitation was achieved with UV detection at 275 and 260 nm for cefuroxime and cefadroxil, respectively, based on peak area with linear calibration curves at the concentration ranges of 2–10 μg ml^{-1} for cefuroxime and 5–20 μg ml^{-1} for cefadroxil. The proposed methods were applied to the determination of dissolution rate for tablets and capsules containing each drug. The urinary excretion patterns as the cumulative amounts excreted have been calculated for each drug using the proposed methods.

Keywords: Cefadroxil; Cefuroxime; First-derivative spectrophotometry; HPLC; Urine

1. Introduction

Cefuroxime is a second generation cephalosporin antibiotic. Cefuroxime axetil is an ester prodrug of cefuroxime, which is rendered more lipophilic by esterification of the carboxyl group of the molecule by the racemic 1-acetoxyethyl bromide, thus enhancing absorption. The absorbed ester is hydrolyzed in the intestinal mucosa and in the portal circulation. Products of the de-esterification are active cefuroxime, acetaldehyde and acetic acid. Cefuroxime axetil itself is not detected in the systemic circulation [1].

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Cefuroxime is not metabolized and is excreted unchanged primarily in urine by both glomerular filtration and tubular secretion [2]. Several analytical methods have been reported for the determination of cefuroxime in urine, including HPLC [3–7] and adsorptive stripping voltammetric methods [8].

Cefadroxil is a first generation cephalosporin

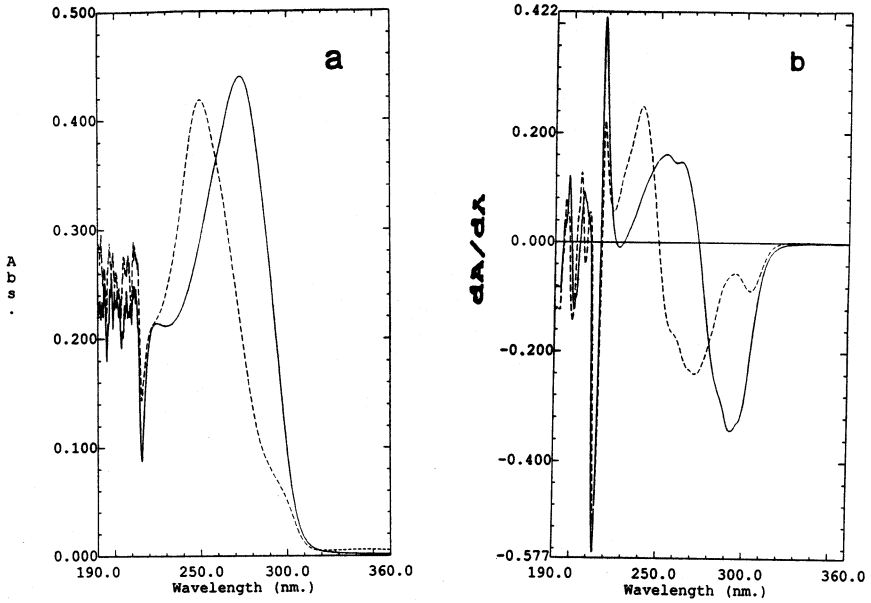

Fig. 1. UV absorption spectra (a) and first-derivative spectra (b) of 10 μg ml^{-1} of cefuroxime (solid line) and cefadroxil (dot–dash line) in 0.1 N sodium hydroxide.

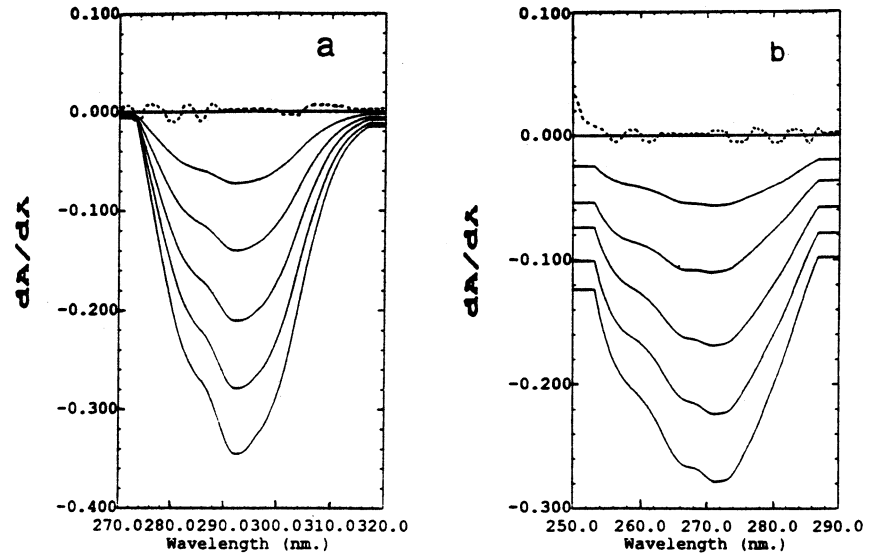

Fig. 2. First-derivative spectra of cefuroxime (a) and cefadroxil (b) (solid line) in the concentration range 2–10 μg ml^{-1} (in 2 μg ml^{-1} steps) spiked in urine and unspiked urine (dot–dash line) after dilution with 0.1 N sodium hydroxide.

Table 1
Statistical analysis of the calibration graphs[a] of cefuroxime using first-derivative spectrophotometry for $n = 5$ specimens

Day no.		Intercept a ($\pm$S.D.)	Slope b ($\pm$S.D.)	Correlation coefficient (r)
First day	Without urine	2.10×10^{-3} ($\pm6.35\times10^{-3}$)	34.65×10^{-3} ($\pm9.57\times10^{-5}$)	0.9999
	With urine	9.00×10^{-4} ($\pm9.95\times10^{-4}$)	34.75×10^{-3} ($\pm1.50\times10^{-4}$)	0.9999
Second day	Without urine	7.00×10^{-4} ($\pm1.36\times10^{-3}$)	34.95×10^{-3} ($\pm2.06\times10^{-4}$)	0.9999
	With urine	4.00×10^{-4} ($\pm9.38\times10^{-4}$)	34.90×10^{-3} ($\pm1.41\times10^{-4}$)	0.9999
Third day	Without urine	1.10×10^{-3} ($\pm8.35\times10^{-4}$)	34.85×10^{-3} ($\pm1.26\times10^{-4}$)	0.9999
	With urine	-2.00×10^{-4} ($\pm1.01\times10^{-3}$)	35.00×10^{-3} ($\pm1.53\times10^{-4}$)	0.9999
Fourth day	Without urine	-5.00×10^{-4} ($\pm1.56\times10^{-3}$)	35.05×10^{-3} ($\pm2.36\times10^{-4}$)	0.9999
	With urine	1.30×10^{-3} ($\pm3.32\times10^{-3}$)	34.75×10^{-3} ($\pm5.00\times10^{-5}$)	0.9999
Fifth day	Without urine	1.20×10^{-3} ($\pm5.42\times10^{-4}$)	34.90×10^{-3} ($\pm8.16\times10^{-5}$)	0.9999
	With urine	5.00×10^{-4} ($\pm9.18\times10^{-4}$)	34.85×10^{-3} ($\pm1.38\times10^{-4}$)	0.9999

[a] Regression equation: $^1D = a + bC$ where C is the concentration in μg ml^{-1} and ^1D is the first-derivative amplitude at 292.5 nm.

Table 2
Statistical analysis of the calibration graphs[a] of cefadroxil using first-derivative spectrophotometry for $n = 5$ specimens

Day no.		Intercept a ($\pm$S.D.)	Slope b ($\pm$S.D.)	Correlation coefficient (r)
First day	Without urine	1.20×10^{-3} ($\pm1.15\times10^{-3}$)	26.70×10^{-3} ($\pm1.73\times10^{-4}$)	0.9999
	With urine	1.00×10^{-4} ($\pm1.06\times10^{-3}$)	26.15×10^{-3} ($\pm1.61\times10^{-4}$)	0.9999
Second day	Without urine	1.00×10^{-3} ($\pm6.63\times10^{-4}$)	26.80×10^{-3} ($\pm1.00\times10^{-4}$)	0.9999
	With urine	8.00×10^{-4} ($\pm1.01\times10^{-3}$)	26.00×10^{-3} ($\pm1.53\times10^{-4}$)	0.9999
Third day	Without urine	1.00×10^{-4} ($\pm9.95\times10^{-4}$)	26.95×10^{-3} ($\pm1.50\times10^{-4}$)	0.9999
	With urine	2.00×10^{-4} ($\pm7.66\times10^{-4}$)	26.20×10^{-3} ($\pm1.15\times10^{-4}$)	0.9999
Fourth day	Without urine	1.00×10^{-3} ($\pm1.38\times10^{-3}$)	26.80×10^{-3} ($\pm2.08\times10^{-4}$)	0.9999
	With urine	1.00×10^{-4} ($\pm9.95\times10^{-4}$)	26.25×10^{-3} ($\pm1.50\times10^{-4}$)	0.9999
Fifth day	Without urine	6.00×10^{-4} ($\pm1.15\times10^{-3}$)	26.80×10^{-3} ($\pm1.73\times10^{-4}$)	0.9999
	With urine	-3.00×10^{-4} ($\pm1.20\times10^{-3}$)	26.35×10^{-3} ($\pm1.80\times10^{-4}$)	0.9999

[a] Regression equation: $^1D = a + bC$ where C is the concentration in μg ml^{-1} and ^1D is the first-derivative amplitude at 267.3 nm.

antibiotic that is excreted unchanged in the urine by glomerular filtration and tubular secretion [9]. Different HPLC methods have been reported for the determination of cefadroxil using Spherisorb S5-ODS2 C18 reversed-phase in rat urine [10], Excalibar amino columns in human urine [11] and post-column derivatization with fluorescamine [12].

Monitoring of the antibiotics concentrations in biological fluids, such as urine, is important for pharmacokinetic studies. Direct spectrophotometric methods are nonspecific because they are subjected strong interference by biological endogenous components. Derivative spectrophotometry has proved advantageous in eliminating spectral interferences. Cephalexin and cephradine were analyzed in urine using first-derivative spectrophotometry [13]. At the same time, second-derivative, first-derivative difference and second-derivative difference spectrophotometric methods have been described for the assay of amoxycillin in urine [14]. The present work presents two methods for determining cefuroxime and cefadroxil in urine using first-derivative spectrophotometry and high performance liquid chromatography.

2. Experimental

2.1. Instrumentation

A double-beam Shimadzu (Japan) UV–Visible spectrophotometer, model UV-1601PC, connected to a Promax computer fitted with UVPC personal spectroscopy software version 3.7 (Shimadzu) and a Hewlett-Packard DeskJet 600 printer were used. The spectral bandwidth was 2 nm and the wavelength scanning speed was 2800 nm min^{-1}. The first-derivative curves ($dA/d\lambda$) of the spectra of test and reference solutions were recorded in 1-cm quartz cells over the range of 320–270 nm for cefuroxime and 290–250 nm for cefadroxil with $\Delta\lambda = 4$ nm and scaling factor = 20. The first-derivative curves were obtained by digital first-order differentiation of the experimental absorption curves.

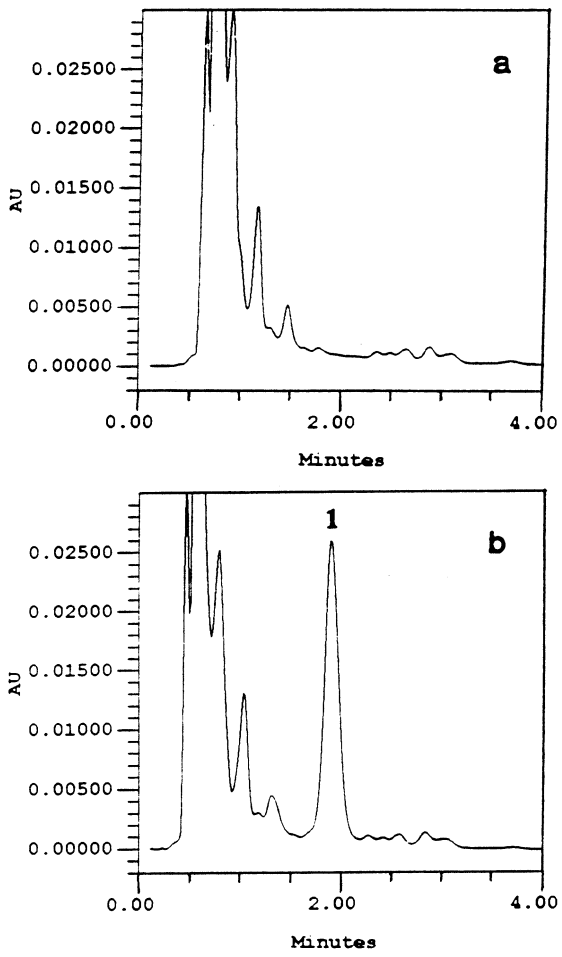

Fig. 3. HPLC chromatograms of urine without cefuroxime treatment (a) and urine spiked with 10 μg ml^{-1} cefuroxime (1) (b).

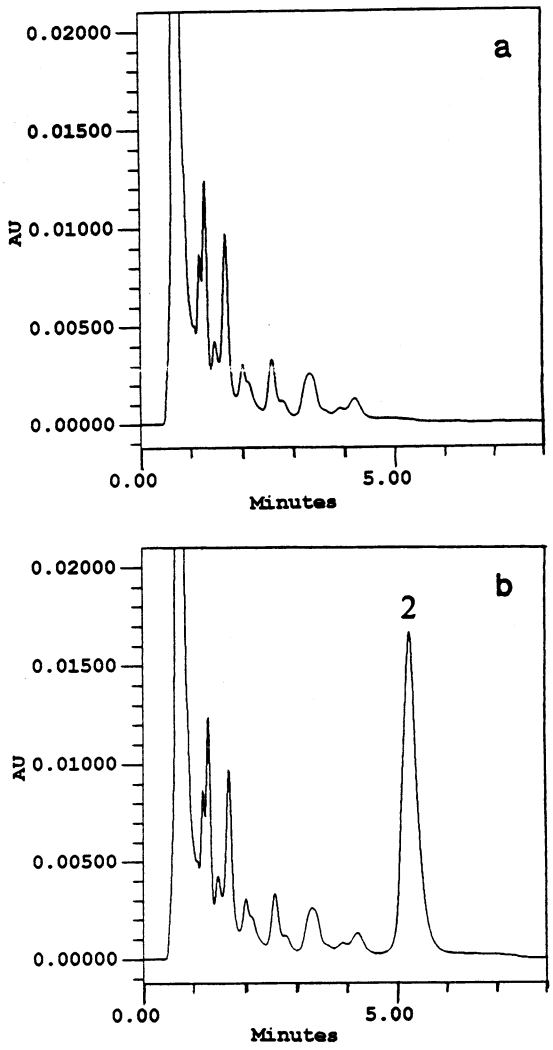

Fig. 4. HPLC chromatograms of urine without cefadroxil treatment (a) and urine spiked with 20 μg ml^{-1} cefadroxil (2) (b).

The HPLC (Waters Associates, Milford, MA) instrument was equipped with a model 600 pump, Rheodyne injector with a 20-μl loop and model 996 photodiode array detector. Separation and

quantitation were made on a 125 × 4 mm LiChrospher 100RP-18 column (5 μm particle size) (Merck, Darmstadt, Germany). Detection was made at 275 nm for cefuroxime and 260 nm for cefadroxil. Peak data handling was performed with Waters Millennium 2010 Chromatography Manager Software (version 2.15.01) and a Hewlett-Packard Laser Jet 5L printer.

Erweka DT dissolution test apparatus USP 23, Erweka, Heusenstamm, Germany, was used with paddle at 55 rev. min^{-1} for cefuroxime axetil tablets or with basket at 100 rev. min^{-1} for cefadroxil monohydrate capsules.

2.2. Materials and reagents

Cefuroxime sodium and cefadroxil monohydrate were kindly supplied by Glaxo Wellcome (Cairo, Egypt) and Pharco Pharmaceutical (Alexandria, Egypt) with a purity of 99.3 and 99.1%, respectively. The water for HPLC was prepared by double glass distillation and filtration through a 0.45-μm membrane filter. The acetonitrile used for the chromatographic separation was HPLC grade (Romil Chem, UK). All other reagents were analytical grade.

The commercial cefuroxime axetil tablets equivalent to 250 mg cefuroxime and three brands of cefadroxil monohydrate capsules equivalent to 500 mg cefadroxil were purchased from a local market.

Table 3
Analytical data and regression characteristic of cefuroxime and cefadroxil using HPLC method

Parameter	Cefuroxime	Cefadroxil
Linearity range (μg ml^{-1})	2–10	5–20
Detection limit (μg ml^{-1})	0.07	0.24
Regression equation (Y)[a]: slope (b)	2.51×10^4	11.61×10^3
S.D. of the slope (S_b)	1.04×10^2	0.918×10^2
R.S.D. of the slope (%)	0.41	0.79
Confidence limit of the slope (95% confidence limit)	2.48×10^4 -2.54×10^4	11.32×10^3 -11.90×10^3
Intercept (a)	9.58×10^2	5.81×10^2
S.D. of the intercept (S_a)	6.89×10^2	1.23×10^3
Confidence limit of the intercept (95% confidence limit)	(-1.23×10^3) -3.15×10^3	(-3.32×10^3) -4.48×10^3
Correlation coefficient (r)	0.9999	0.9999

[a] $Y = a + bC$ where C is the concentration in μg ml^{-1} and Y is the peak area.

2.3. HPLC conditions

The mobile phase of cefuroxime was prepared by mixing water, acetonitrile and acetic acid in a ratio of 85:15:0.1 v/v, while the mobile phase of cefadroxil was prepared by mixing 0.02 M potassium dihydrogen phosphate and acetonitrile in a ratio of 95:5 v/v, followed by addition of 0.03 g hexane sulphonic acid sodium salt to every 1000 ml mobile phase mixture, and the apparent pH was adjusted to 3.0 using phosphoric acid. The mobile phases were filtered using a 0.45-μm membrane filter (Millipore, Milford, MA) and degassed by vacuum prior to use. The flow rates were 1.5 and 2 ml min^{-1} for cefuroxime and cefadroxil, respectively. All determinations were performed at ambient temperature for cefuroxime and at 35°C for cefadroxil. The injection volume was 20 μl. The samples were also filtered using 0.45-μm disposable filters.

2.4. Standard solutions and calibration graphs (spiked urine)

Stock standard solutions of each of cefuroxime and cefadroxil were prepared by dissolving an accurate weight of cefuroxime sodium equivalent to 10 mg cefuroxime or cefadroxil monohydrate equivalent to 10 mg cefadroxil in 100 ml water. Fresh stock standard solutions were prepared every day.

2.4.1. First-derivative method

Aliquots of 1–5 ml (in 1-ml increments) of each of the stock standard solution were transferred to

Table 4
Within-day precision of the assay of cefuroxime and cefadroxil in urine using ^{1}D and HPLC methods

Concentration (μg ml^{-1})	^{1}D		HPLC	
	Mean measured[a] concentration ± S.D.	CV%	Mean measured[a] concentration ± S.D.	CV%
Cefuroxime				
2	1.99 ± 0.01	0.50	1.99 ± 0.01	0.50
4	3.99 ± 0.03	0.75	3.99 ± 0.02	0.50
6	6.03 ± 0.05	0.83	6.01 ± 0.04	0.67
8	7.97 ± 0.05	0.63	7.99 ± 0.03	0.38
10	10.04 ± 0.08	0.80	9.98 ± 0.06	0.60
Cefadroxil				
5	4.92 ± 0.03	0.61	4.99 ± 0.03	0.60
6	5.93 ± 0.05	0.84	5.98 ± 0.02	0.33
7	6.93 ± 0.06	0.87	6.98 ± 0.04	0.57
8	7.89 ± 0.03	0.38	8.03 ± 0.05	0.62
10	9.89 ± 0.05	0.51	10.02 ± 0.05	0.50

[a] Mean of five urine samples for each concentration.

Table 5
Between-day precision of the assay of cefuroxime and cefadroxil in urine using ^{1}D and HPLC methods

Concentration (μg ml^{-1})	^{1}D		HPLC	
	Mean measured[a] concentration ± S.D.	CV%	Mean measured[a] concentration ± S.D.	CV%
Cefuroxime				
2	2.01 ± 0.01	0.50	1.99 ± 0.01	0.50
4	3.99 ± 0.03	0.75	4.03 ± 0.03	0.74
6	5.99 ± 0.02	0.33	5.99 ± 0.03	0.50
8	7.98 ± 0.03	0.38	7.97 ± 0.05	0.63
10	10.03 ± 0.06	0.60	10.02 ± 0.06	0.60
Cefadroxil				
5	4.94 ± 0.02	0.40	5.01 ± 0.03	0.60
6	5.94 ± 0.04	0.67	6.02 ± 0.02	0.33
7	6.88 ± 0.03	0.44	6.99 ± 0.04	0.57
8	7.93 ± 0.04	0.50	8.03 ± 0.03	0.37
10	10.04 ± 0.06	0.60	10.04 ± 0.05	0.50

[a] Mean of 5 days results for each concentration.

50-ml volumetric flasks. To each flask, 2 ml of blank urine were added and the solutions were diluted to 50 ml with 0.1 N sodium hydroxide to obtain a concentration range of 2–10 μg ml^{-1} for both cefuroxime and cefadroxil. The ^{1}D curves were scanned in the range of 320–270 nm for cefuroxime and 290–250 nm for cefadroxil against a blank of 2 ml of urine diluted to 50 ml with 0.1 N sodium hydroxide. The values of the ^{1}D amplitudes at 292.5 nm for cefuroxime and 267.3 nm for cefadroxil were measured, and the concentrations versus their absolute first-derivative amplitudes were plotted in order to obtain the calibration graph.

2.4.2. HPLC method

Different volumes of each of the stock standard solutions were transferred to 50-ml volumetric flasks. To each flask, 2 ml of blank urine were added and the solutions were diluted to 50 ml with corresponding mobile phase to obtain concentration ranges of 2–10 and 5–20 μg ml^{-1} for cefuroxime and cefadroxil, respectively. The solutions were filtered through 0.45-μm membrane filters. Triplicate 20-μl injections were made for each concentration and chromatographed under the conditions described above. The peak area of each concentration was plotted against the corresponding concentration to obtain the calibration graph for each compound.

2.5. Sample preparation (in vivo procedure)

The study was carried out on a normal, healthy, male, informed adult volunteer (37 years, 80 kg), with no past history of allergic reaction to cefuroxime or cefadroxil. The volunteer was instructed to abstain from all medications for 2 weeks before each administration and also during the study. Also, the volunteer was instructed to be sure of evacuating his bladder as thoroughly as possible exactly before the administration of one tablet of cefuroxime axetil equivalent to 250 mg cefuroxime or one capsule of each brand of cefadroxil monohydrate equivalent to 500 mg cefadroxil with about 250 ml water after food. The 0-h urine sample was collected as blank for ^1D method. Urine samples were collected after 0.5 h of administration and up to 17 h with complete empties of the bladder. The volume of the urine was measured and recorded after each collection.

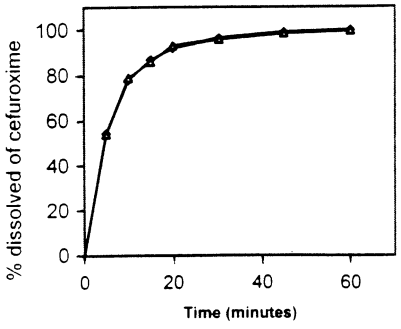

Fig. 5. Dissolution profile for tablet containing cefuroxime axetil equivalent to 250 mg cefuroxime, using first-derivative (◆) and HPLC (△) methods.

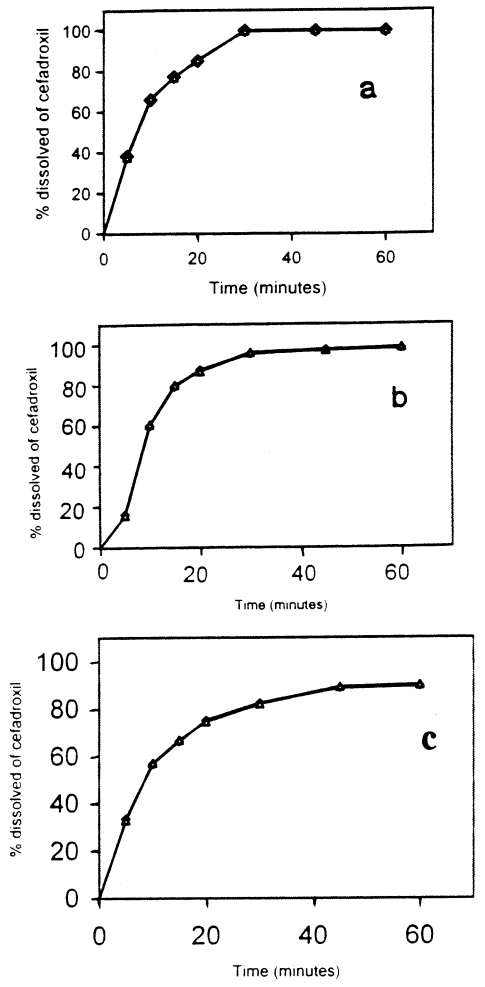

Fig. 6. Dissolution profiles for brands a, b and c of capsules containing cefadroxil monohydrate equivalent to 500 mg cefadroxil, using first-derivative (◆) and HPLC (△) methods.

2.5.1. First-derivative method

Two milliliters of the urine specimen from each sampling point was diluted to 50 ml with 0.1 N sodium hydroxide and analyzed for the cefuroxime or cefadroxil by recording the ^1D spectrum of each drug against blank urine prepared in the same manner as described under the calibration

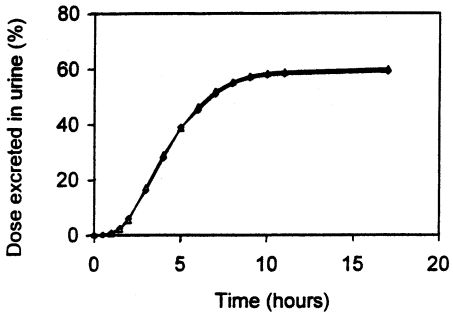

Fig. 7. Cumulative curve of percentage-dose excreted of cefuroxime in urine after oral administration of cefuroxime axetil tablet containing 250 mg cefuroxime, using first-derivative (◆) and HPLC (△) methods.

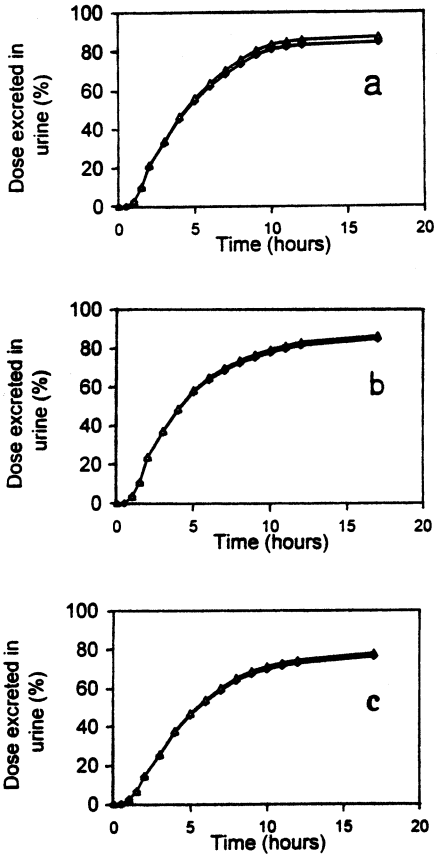

Fig. 8. Cumulative curves of percentage-dose excreted of cefadroxil in urine after administration of brands a, b and c of cefadroxil monohydrate capsules containing 500 mg cefadroxil, using first-derivative (◆) and HPLC (△) methods.

graph. The ^{1}D-amplitude values were measured at 292.5 nm for cefuroxime and 267.3 nm for cefadroxil and the percentage dose excreted in urine was calculated for each drug at each sampling point, using the corresponding calibration graph.

2.5.2. HPLC method

Two milliliters of the urine specimen from each sampling point was diluted to 50 ml with the corresponding mobile phase. The solutions were filtered through 0.45-μm membrane filters. A 20-μl volume was injected into the HPLC, in triplicate for each solution and chromatographed under the conditions described above. The peak area was used for determination of each drug at each sampling point and the percentage dose excreted in urine was calculated, using the corresponding calibration graph.

2.6. Dissolution rate study

Using USP 23 dissolution apparatus: one tablet of cefuroxime axetil was dissolved in 900 ml of 0.07 N hydrochloric acid with paddle at 55 rev. min^{-1} or one capsule of cefadroxil monohydrate was dissolved in 900 ml water with basket at 100 rev. min^{-1}. The dissolution was carried out at 37 ± 0.5°C and the dissolved amounts of cefuroxime or cefadroxil were determined at 5, 10, 15, 20, 30, 45 and 60 min using the two proposed methods.

2.6.1. First-derivative method

After each specified time, a portion of the solution under test was filtered and suitably (neutralized for cefuroxime and) diluted with 0.1 N sodium hydroxide. The values of ^{1}D amplitudes at 292.5 nm for cefuroxime and 267.3 nm for cefadroxil were measured. The dissolved amount of each drug at each specified time was determined using the corresponding calibration graph.

2.6.2. HPLC method

After each specified time, a portion of the solution under test was filtered and suitably diluted with corresponding mobile phase. A 20-μl volume was injected into the HPLC, in triplicate for each solution and chromatographed under the condi-

tions described above. The peak area was used for determination of each dissolved drug at each specified time using the corresponding calibration graph.

3. Results and discussion

The aim of this work was to develop a rapid, simple and sensitive assay for monitoring each of the excreted intact cefuroxime and cefadroxil in human urine after administration of the drug.

The first-derivative spectrophotometric method has been reported for the determination of either cephalexin or cephradine in urine by measuring the peak amplitude at 268 nm [13]. The method was valid in the range of 5–30 μg ml^{-1} for cephalexin and 5–40 μg ml^{-1} for cephradine in aqueous urine solutions. The recoveries ranged from 98.90 to 104.00% for cephalexin and from 97.85 to 105.10% for cephradine. The within-day coefficient of variation varied from 3.27 to 6.45%. However, no derivative spectrophotometric method has been reported for the determination of cefuroxime and cefadroxil in urine. In the present work, the first-derivative spectrophotometric method has been developed for the determination of cefuroxime and cefadroxil in human urine. The solutions of cefuroxime and cefadroxil in 0.1 N sodium hydroxide exhibited maximum absorbance at 272.6 and 249.3 nm, respectively (Fig. 1). These maxima were distorted in the presence of urine due to the interference of biological endogenous components, which displayed strong absorbances in the range 200–250 nm [13].

The zero-order spectrum did not permit the determination of cefuroxime and cefadroxil in human urine owing to lack of sensitivity and to interference from the urine matrix. Application of the first-derivative spectrophotometry was found to correct for the urine matrix interference and to enhance the sensitivity. By measuring the values of the ^1D amplitudes at 292.5 nm for cefuroxime and 267.3 nm for cefadroxil, the concentration of each drug can be directly calculated since the first-derivative measurement cancels the irrelevant absorbance due to the urine matrix at these wavelengths. The measurement of the ^1D amplitude of each drug at the specified wavelength was carried out within 10 min of addition of 0.1 N sodium hydroxide to avoid alkaline hydrolysis of each drug. Fig. 2a,b shows first-derivative spectra of cefuroxime and cefadroxil, respectively, in the concentration range 2–10 μg ml^{-1} of spiked urine diluted with 0.1 N sodium hydroxide.

In order to test the independence of the analytical signal for each compound on the presence or absence of urine, the following experiments were performed. The calibration graphs were constructed by plotting the absolute values of the first-derivative at 292.5 and 267.3 nm against concentration of cefuroxime and cefadroxil, respectively, in the absence and presence of urine, evaluated for 5 days. Linear relationships were obtained in the concentration range of 2–10 μg ml^{-1} for each drug. Tables 1 and 2 summarize the statistical analysis of the experimental data: the regression equations calculated from the calibration graphs, along with the S.D. of the slopes and the intercepts. From Tables 1 and 2, it can be seen that the slopes of the calibration graphs of each drug were virtually independent of the presence or absence of urine. Therefore, it can be deduced that amplitudes of the first-derivative signals measured at 292.5 and 267.3 nm were only a function of cefuroxime and cefadroxil concentrations, respectively. The linearity of the calibration graphs, the adherence of the system to Beer's law and the negligible scatter of the experimental points were validated by the values of the correlation coefficients of the regression equations and the values of the intercepts, which were close to zero (Tables 1 and 2). The intercept value was not statistically ($P < 0.05$) different from zero for each calibration graph.

The detection limits (DL) were calculated from the calibration data by means of the following equation [15]:

$$\mathrm{DL} = \frac{t_p}{b}\sqrt{S_0^2\left(\frac{n-2}{n-1}\right)}$$

where n is the number of standards, t_p the Student's coefficient at the selected level of significance, b the slope of the regression line and S_0^2 the variance characterising the scatter of the points with respect to the regression line, composed of

the variance characterising the reproducibility error and the variance characterising the non-linearity of the graph; it is defined by the relationship

$$S_0^2 = \frac{\sum(y - y_{calc})^2}{n-2}$$

where y is the experimental value on the ordinate and y_{calc} the value calculated from the regression equation.

The detection limits of the first-derivative spectrophotometric method were calculated at $t_{0.95}$ and was found to be 0.10 and 0.11 for cefuroxime and cefadroxil, respectively.

Several HPLC methods have been reported for the determination of cefuroxime in deproteinized urine using reversed-phase column with different mobile phases, including acetate buffer solution containing methanol or acetonitrile [3], 9% ethanol containing 0.2% of ammonium acetate [4], methanol and 1 mM phosphoric acid with mean recovery of 96% and coefficient of variation of 1.9% [5], 0.01 M acetate buffer pH 4.8 containing 15% methanol or acetonitrile [6], and 0.05 M phosphate buffer and acetonitrile [7].

The influence of probencid on the rat's renal excretion mechanisms of cefadroxil was studied using HPLC analytical method with Spherisorb S5-ODS2 C18 reversed phase column and 0.1 M acetate buffer pH 3–methanol (87:13 v/v) mobile phase. The coefficient of variation of the analytical methods was $< 5\%$ and the detection limit was 0.3 μg ml^{-1}[10]. Two HPLC methods have been reported for the determination of cefadroxil in human urine. The first method was based on using the Excalibar amino column with acetonitrile and 0.02 M sodium phosphate (4:1) as mobile phase. The coefficients of variation were from 2.0 to 5.4% for within-day and day-to-day analysis. The calibration graph was rectilinear from 10 to 600 μg ml^{-1} and the detection limit was 20 μg ml^{-1} [11]. The second method depends on post column derivatization with fluorescamine after separation on Lichrosorb RP18 column with acetonitrile and 25 mM phosphate buffer pH 7 (5:95) as a mobile phase [12].

In the present work, a new, simpler, more accurate, reproducible and sensitive HPLC method has been developed for the determination of cefuroxime and cefadroxil in human urine. A satisfactory separation of each drug from biological endogenous components in urine was obtained. Separation and quantitation were carried out at ambient temperature for cefuroxime and at 35°C for cefadroxil. The addition of 0.003% (w/v) hexane sulphonic acid sodium salt to the mobile phase and elevation of the column temperature were found to be essential to improve the sharpness and thinness of the cefadroxil peak. The specificity of the HPLC method is illustrated in Figs. 3 and 4 where complete separation of each of cefuroxime and cefadroxil from biological endogenous components in the urine was noticed and no interfering peaks at the retention times of cefuroxime or cefadroxil peaks were observed in the blank urine. The average retention time ± S.D. for cefuroxime and cefadroxil were found to be 1.90 ± 0.005 and 5.30 ± 0.009 min, respectively, for 10 replicates.

To determine the linearity of the HPLC detector response, calibration standard solutions of cefuroxime and cefadroxil were prepared as described in the text. Linear correlation was obtained between peak area versus concentration for each drug. Each measurement represented the average of three replicates. For the HPLC method: linearity range, detection limit calculated at $t_{0.95}$ [15], regression equation and correlation coefficient obtained by least-squares treatment of these results are given in Table 3. The intercept value was not statistically ($P < 0.05$) different from zero for each drug.

The interference of other cephalosporins with the HPLC method was studied. Cefoxitin and cephalothin were found to be interfering with the HPLC method for cefuroxime, while cephalexin and cefaclor were found to be interfering with the HPLC method for cefadroxil.

Absolute and relative recovery studies were carried out on urine samples spiked with known concentrations of either cefuroxime or cefadroxil. Absolute recovery was calculated by comparing the peak amplitudes (for the first-derivative method) or peak area (for HPLC method) of the antibiotic in urine and without urine. The mean absolute recoveries ± S.D. were found to be

100.6 ± 0.84 and 100.3 ± 0.61 for cefuroxime and 100.4 ± 0.71 and 99.1 ± 0.63 for cefadroxil using first-derivative and HPLC methods, respectively. The relative recoveries were computed by comparing the calculated concentrations of each antibiotic from drug-supplemented urine samples (using the calibration graph) with the actual added amounts. The mean relative recoveries $\pm$ S.D. were found to be 99.9 ± 0.54 and 99.8 ± 0.39 for cefuroxime and 98.9 ± 0.50 and 99.9 ± 0.49 for cefadroxil using the first-derivative and HPLC methods, respectively.

The within-day precision was evaluated by replicate analysis of urine samples spiked with known concentrations of cefuroxime or cefadroxil (Table 4), determined by first-derivative and HPLC methods. The between-day precision was similarly evaluated on several days up to 5 days (Table 5). Every day, a calibration graph was constructed and the results are calculated in comparison with the calibration graph. The results in both cases indicated high precision, as the CV% did not exceed 1%.

The concentrations of cefuroxime and cefadroxil in cefuroxime axetil tablets and cefadroxil monohydrate capsules were determined according to the USP methods of assay [16]. The percentage contents were found to be 99.9% for cefuroxime axetil tablets and 100.0, 99.7 and 99.5% for the three brands (a, b and c, respectively) of cefadroxil monohydrate capsules. The proposed methods were applied for determination of the dissolved amount of cefuroxime and cefadroxil from cefuroxime axetil tablets and cefadroxil monohydrate capsules, respectively. The amounts of cefuroxime dissolved in 15 and 45 min were found to be 86.9 and 99.1% (^{1}D method) and 86.1 and 98.5% (HPLC method) of the labeled amount of cefuroxime from cefuroxime axetil tablets, respectively (Fig. 5), while the amounts of cefadroxil dissolved in 30 min were found to 99.9, 95.5 and 82.3% (^{1}D method) and 99.7, 96.1 and 82.1% (HPLC method) of the labeled amount of cefadroxil from three brands (a, b and c, respectively) of cefadroxil monohydrate capsules (Fig. 6). The relatively low percentage of the dissolved amount of cefadroxil from brand c of cefadroxil monohydrate capsule affects the cumulative amount of cefadroxil excreted in urine after 17 h following oral administration of this brand.

Since cefuroxime and cefadroxil are excreted almost entirely in the urine, measurement of urinary cefuroxime and cefadroxil output will reflect their gastrointestinal absorption. The oral administration of cefuroxime axetil tablets and cefadroxil monohydrate capsules in in vivo study were carried out after food because the gastrointestinal absorption of cefuroxime axetil is enhanced in the presence of food which may be due to the effects of food on drug absorption, gastric emptying, gut motility, neutralization of gastric acidity, inhibition of luminal esterases, or the surfactant properties of the food or of bile salts [1]. The gastrointestinal absorption of cefadroxil is not affected by food [17].

The proposed analytical methods were applied for determination of the cumulative amounts of cefuroxime and cefadroxil excreted in urine after oral administration. The cumulative amount of cefuroxime excreted in urine was found to be 59.1% (^{1}D method) and 60.0% (HPLC method) of the dose after 17 h following oral administration of cefuroxime axetil tablet containing 250 mg cefuroxime (Fig. 7), while the cumulative amounts of cefadroxil excreted in urine were found to be 85.1, 84.8 and 76.2% (^{1}D method) and 86.8, 86.1 and 77.4% (HPLC method) of the dose after 17 h following oral administration of three brands (a, b and c, respectively) of cefadroxil monohydrate capsules containing 500 mg cefadroxil (Fig. 8).

4. Conclusion

The proposed ^{1}D and HPLC methods provide simple, accurate, sensitive and direct quantitative analysis for the assay of cefuroxime and cefadroxil in urine. The proposed methods can be used for determination of dissolution profiles of tablets and capsules containing cefuroxime axetil and cefadroxil monohydrate. The HPLC method was found to be more selective than the ^{1}D method, while the ^{1}D method has the advantages of low cost and speed.

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