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# Spectrofluorometric determination of alpha-aminocephalosporins in biological fluids and pharmaceutical preparations

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**Abstract**

A selective and highly sensitive fluorometric method was developed for the determination of four alpha-aminocephalosporins, namely cefaclor, cefadroxil, cephalexin and cephradine. The method involves the reaction of the target compounds with fluorescamine at a specific pH, ranging from 7.8 to 8.4. The produced derivatives exhibit maximum fluorescence intensities at 472–478 nm after excitation at 370–372 nm. The method is highly specific because other alpha-aminocephalosporins whose alpha amino group was blocked do not react similarly and hence do not interfere. At the specific pH range of the reaction where no degradation may occur with that medium the proposed method can be utilised as a stability-indicating assay. The different experimental parameters affecting the derivatisation reaction were carefully studied and incorporated into the procedure. Under the described conditions, the proposed method is linear over the concentration range of 0.05–1 μg $ml^{-1}$ for both cefaclor and cephalexin, and 0.05–0.65 and 0.025–0.5 μg $ml^{-1}$ for cefadroxil and cepharadine, respectively and the coefficients of determination were greater than 0.999 ($n = 3$). The recoveries of the title compounds from spiked serum ranged from 88.6 to 89.7% and from spiked urine from 92.2 to 93.3% with a limit of quantitation (LOQ) of 25–50 ng $ml^{-1}$ and limit of detection (LOD) of 5 ng $ml^{-1}$ (S/N = 2) for all drugs. The mechanism of the fluorometric reaction is proposed and the advantages of the proposed method are discussed. 

*Keywords:* Aminocephalosporins; Fluorescamine; Pharmaceutical preparations; Biological fluids; Fluorimetry

## 1. Introduction

Cefaclor, cefadroxil, cephalexine and cephradine are semisynthetic β-lactam antibiotics widely used in clinical chemotherapy. Many analytical methods have been reported for their determination in pharmaceutical preparations and in biological fluids. These methods include spectrophotometry [1–4], TLC [5], HPLC [6,7], capillary electrophoresis [8] and polarographic method [9,10]. The USP XXIII [11] recommends the hydroxylamine colorimetric method for the evalua-

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Table 1
Performance data for the fluorimetric determination of pure sample of the studied alpha-aminocephalosporin[a]

| Compound | Wavelength (nm) | | pH | Conc. range (μg/ml$^{-1}$) | RSD (%) | Regression data | | |
|---|---|---|---|---|---|---|---|---|
| | Excitation | Emission | | | | *r* | *a* | *b* |
| Cefaclor | 372 | 472 | 8.4 | 0.050–1.00 | 0.591 | 0.999 | 0.0101 | −0.001 |
| Cefadroxil | 370 | 472 | 8.0 | 0.050–0.65 | 0.528 | 0.999 | 0.0066 | 0.001 |
| Cephalexin | 372 | 478 | 7.8 | 0.050–1.00 | 0.421 | 0.999 | 0.0099 | −0.001 |
| Cephradine | 372 | 478 | 8.2 | 0.025–0.50 | 0.618 | 0.999 | 0.0049 | −0.001 |

[a] Where *r* is correlation coefficient, *a* is the slope and *b* is the intercept.

tion of cefaclor and cephradine in pure form and in their pharmaceutical formulations, and a microbial method for the determination of cephalexine in pure form and in its formulations. The British Pharmacopoeia [12] recommends an iodometric method for the determination of cephradine in pure form and in pharmaceutical preparations. Fluorometrically, cephalosporins were determined after either acid hydrolysis [13,14] or alkaline hydrolysis [15–17]. Thus the degraded cephalosporins will react similarly and therefore these methods are not stability indicating. It is thus clear that there is a need for a sensitive fluorometric method that measures the intact drug. The present method meets this requirement. It involves the reaction with fluoroscamine at pH range 7.8–8.4, where no degradation may occur with that medium. The proposed method has been applied to the determination of these drugs in biological fluids and formulations. The results obtained compare favourably with those obtained by the reference method [13].

## 2. Experimental

### 2.1. Apparatus

An Amino-Bowman model $J_4$-8960 Spectrofluorimeter was used with the excitation and emission slit controls set at 5 nm. The measurements were performed using a 1-cm quartz cell.

### 2.2. Materials

All the chemicals were of analytical reagent grade, and the solvents were of spectroscopic grade. Pure drug samples were kindly provided by different pharmaceutical companies: cephalexine monohydrate (Amoun Pharmaceutical Industrial Company, Cairo, Egypt), cephradine (Squibb and Sons, Cairo, Egypt), cefaclor and cefadroxil (Eli-Lilly, Switzerland). Fluorescamine was purchased from Aldrich (Milwaukee, WI, USA). Pharmaceutical preparations containing the studied compounds were obtained from commercial sources. Blood serum was kindly supplied by Mansoura University Hospital, Mansoura, Egypt.

### 2.3. Reagents

Stock solutions containing 1 mg ml$^{-1}$ of the aminocephalosporins were prepared in deionised water and serial dilutions with the same solvent were made to cover the working range (Table 1). Fluorescamine solution (10 mg in 100 ml) was prepared in acetone; the solution was kept in a refrigerator. Borate buffer (pH 7.8–8.4) of 0.02 M and trichloroacetic acid (10% v/v) aqueous solution were prepared.

Table 2
Application of the proposed method for the determination of alpha-aminocephalosporins in raw materials

| Compound | % Found ± S.D.[a] | |
|---|---|---|
| | Proposed method | Reference method [13] |
| Cefaclor | 99.9 ± 0.59 | 100.1 ± 1.08 |
| Cefadroxil | 100.2 ± 0.53 | 100.6 ± 0.47 |
| Cephalexin | 99.7 ± 0.42 | 100.4 ± 0.44 |
| Cephradine | 100.2 ± 0.62 | 99.6 ± 0.57 |

[a] Each result is the average of nine separate determinations.

Table 3
Application of the proposed method for the determination of alpha-aminocephalosporin in dosage forms

| Preparation | % Found ± S.D.[a] | |
|---|---|---|
| | Proposed method | Reported method [13] |
| Cefaclor capsules[b] (250 mg of cefaclor/ capsule) | 99.4 ± 0.88 | 99.6 ± 0.75 |
| Cefadroxil syrup[c] (250 mg of cefadroxil/5 ml) | 100.6 ± 0.55 | 99.4 ± 0.63 |
| Cephalexin capsules[c] (500 mg of cephalexin/capsule) | 101.2 ± 0.63 | 101.4 ± 0.55 |
| Velosef vials[a,d] (500 mg of cephradine/ vial) | 100.2 ± 0.53 | 100.3 ± 0.48 |
| Velosef capsules[a,d] (250 mg of cephradine/capsule) | 100.5 ± 0.82 | 100.5 ± 0.66 |

[a] Average of at least six triplicate determinations.
[b] Al-Kahra Pharmaceutical Chemical Company, Cairo, Egypt under license from Eli-Lilly, Switzerland.
[c] Amoun Pharmaceutical Industrial Company, Cairo, Egypt.
[d] Squibb and Sons, Cairo, Egypt.

### 2.4. Procedure

#### 2.4.1. Construction of calibration graph

Aliquot volumes of the final solution were transferred to a series of 10-ml calibrated flasks to produce solutions having the concentration ranges cited in Table 1. A total of 5.0 ml of borate buffer (pH 7.8–8.4) was added to each flask. After thorough mixing, 1.0 ml of fluorescamine solution was added and the solution was diluted to 10 ml with borate buffer. The fluorescence intensity was measured at the appropriate wavelength (Table 1). The percentage relative intensity was plotted against the final concentration of the title compounds to obtain the calibration graph. Alternatively, the corresponding regression equations were derived.

#### 2.4.2. Procedure for dosage forms

An accurately weighed amount of the powder equivalent to 100 mg of each drug was transferred into a 100-ml calibrated flask. The volume was brought to the mark with deionised water then sonicated for 5 min and filtered if necessary. The procedure was completed as described under calibration graph. The nominal content of the drugs in each solution was calculated either from linear regression equation (Table 1) or from the previously plotted calibration graphs.

#### 2.4.3. Assay of drugs in spiked human serum

To 5.0 ml of human serum one of the studied compounds within the concentration range cited in Table 4 was added. Then 5.0 ml of 10% (v/v) trichloroacetic acid was added for deproteination. The mixture was blended in a vortex mixer and centrifuged at 3000 rpm for 15 min before 1.0 of the protein-free supernatant was transferred into a 10-ml calibrated flask and brought to volume with deionised water. The procedure was completed as described under calibration graph.

#### 2.4.4. Assay of drugs in spiked human urine

To 5 ml of human urine, one of the studied compounds within the concentration range cited in Table 4 was added. Then 5.0 ml of methanol was added and the mixture was blended in a vortex and centrifuged at 1500 rpm for 3 min, before 1.0 ml of the supernatant was transferred into a 10-ml calibrated flask and brought to volume with deionised water. The procedure was completed as described under calibration graph.

## 3. Results and discussion

The use of fluoroscamine for the TLC detection of alpha aminocephalosporins [5] initiated the present study. The experimental conditions affecting the development and stability of the fluorophores produced were carefully studied. It was found that maximum fluorescence intensity was obtained at a definite pH corresponding to each compound (Table 1). In a fraction of a second, at room temperature, the reaction was completed, and in less than 1 min the excess reagent was destroyed. The fluorophores are stable for several hours which permits the convenient application of the proposed method. At the wave-

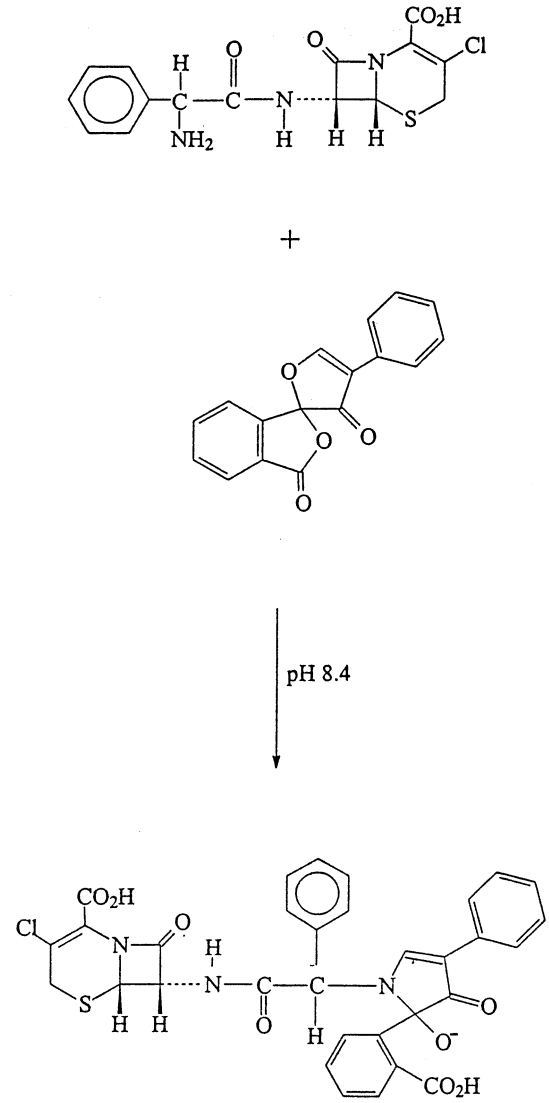
lengths of maximum excitation at 472–478 nm and emission at 370–372 nm, neither the drug nor fluorescamine fluoresces. The reaction pathway is suggested to proceed as shown in Scheme 1 by analogy to previously studied compounds [18]. Table 1 summarizes the performance data for the determination of the compounds studied by the proposed method.

To test the validity of the method, it was applied to the determination of pure samples of the

Table 4
Determination of the alpha-aminocephalosporins in serum and urine samples

| Compound | Concentration taken (μg/ml) | % Found ± S.D.* | |
|---|---|---|---|
| | | Serum | Urine |
| Cefaclor | 0.05 | 90.0 | 90.0 |
| | 0.10 | 85.0 | 89.5 |
| | 0.20 | 87.5 | 92.5 |
| | 0.40 | 88.7 | 93.8 |
| | 0.60 | 91.7 | 95.1 |
| | 0.80 | 90.0 | 94.9 |
| | 1.00 | 90.9 | 94.1 |
| $X \pm$ S.D. | | 89.1 ± 2.11 | 92.8 ± 2.11 |
| Cefadroxil | 0.05 | 86.6 | 93.3 |
| | 0.10 | 86.9 | 93.3 |
| | 0.20 | 90.0 | 94.8 |
| | 0.30 | 88.8 | 91.1 |
| | 0.40 | 90.0 | 94.9 |
| | 0.50 | 89.3 | 92.3 |
| | 0.60 | 88.8 | 93.8 |
| $X \pm$ S.D. | | 88.6 ± 1.27 | 93.4 ± 1.25 |
| Cephalexin | 0.05 | 90.0 | 90.0 |
| | 0.10 | 85.0 | 94.7 |
| | 0.20 | 90.0 | 92.7 |
| | 0.40 | 87.5 | 93.3 |
| | 0.60 | 89.9 | 93.9 |
| | 0.80 | 89.5 | 93.2 |
| | 1.00 | 90.9 | 93.9 |
| $X \pm$ S.D. | | 89.0 ± 1.89 | 93.8 ± 1.4 |
| Cephradine | 0.025 | 88.8 | 88.9 |
| | 0.05 | 89.9 | 90.0 |
| | 0.10 | 88.7 | 92.5 |
| | 0.20 | 89.6 | 93.2 |
| | 0.30 | 91.5 | 93.3 |
| | 0.40 | 90.0 | 92.5 |
| | 0.50 | 89.8 | 94.9 |
| $X \pm$ S.D. | | 89.8 ± 86 | 92.2 ± 1.91 |

* Mean ± S.D. ($n = 4$).




Scheme 1. Proposed reaction between flouresamine and cefaclor.

title compounds. Statistical analysis [19] of the results summarised in Table 2 obtained by the proposed and reported methods [13] shows no significant difference between the two methods as regards accuracy ($t$-test) and precision ($F$-test). The method was further applied to the determination of the studied drugs in dosage forms (Table 3). Excipients commonly co-formulated with the studied compounds such as magnesium stearate, lactose, starch, talc powder and flavouring agents,

did not interfere with the determination, indicating the high selectivity of the procedure. Alpha-aminocephalosporins whose alpha amino group was blocked, such as cefotaxime, ceftazidine, cefazoline, ceftizoxime, cefuroxime and cefixime, did not interfere with the determination indicating the high specificity of the proposed method.

Analysis of human serum and urine samples spiked with the title compounds was conducted using the proposed method. The studied drugs do not appear to undergo metabolism in human, as 85% of cefaclor and cefadroxil doses [20], 80% of cephalexin and 90% of cephradine doses [21] are excreted in the urine unchanged. Doses of 250–500 mg of the title compounds given by mouth produce peak plasma concentration of 7–19 μg $ml^{-1}$ after 1 h. Thus the proposed method is sufficient for real-life applications and routine estimation of the drugs in human serum and urine. For serum only a deproteination process was carried out using trichloracetic acid as a sample pre-treatment; an extraction procedure was not necessary. Antibiotic-free urine sample gave a relatively high fluorescence intensity compared with control aqueous solution. A dilution of the 1:1 (v/v) water with methanol was required to minimize this interference. The validation of the proposed assay was performed by evaluating the limit of detection, limit of quantitation and linearity of detector response. The limit of detection (LOD) was 5 ng $ml^{-1}$ for all drugs and the limit of quantitation (LOQ) was 25 ng $ml^{-1}$ for cephradine and 50 ng $ml^{-1}$ for cefaclor, cephlexin and cefadroxil, respectively. The linear calibration curves showed good linearity in the range of 0.05–1 μg $ml^{-1}$ for cefaclor and cephlalexin, and 0.05–65 and 0.025–0.5 μg $ml^{-1}$ for cefadroxil and cephradine, respectively (Table 4). The coefficient of determination was greater than 0.999 ($n = 3$). Representative linear regression equations obtained for the studied compounds were $Y = 0.0101X - 0.001$, $Y = 0.0066X + 0.001$, $Y = 0.0099X - 0.001$ and $Y = 0.0049X - 0.001$ for cefaclor, cefadroxil, cephalexin and cephradine, respectively.

To obtain excellent repeatability and reproducibility, nine preparations of pure studied drugs were prepared, analysed and successfully analysed in different laboratories, using a different spectrofluorometer (Sequoia-Tumer, Model 450, Fluorometer) on different days. A precision (%RSD) of 0.539 was obtained for response factors.

In conclusion a simple selective and highly specific method has been developed for determination of alpha-aminocephalosporin in dosage forms and in biological fluids. The method is sensitive to 5 ng $ml^{-1}$ of each compound and could be used for routine analysis of alpha-aminocephalosporins.

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