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Spectrophotometric methods for the determination of cephradine or ceftazidine in human urine using batch and flow-injection procedures

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Abstract

Sensitive and fast spectrophotometric methods for the determination of cephradine or ceftazidine in human urine, based on the formation of compounds between these drugs and Pd(II), are described. In the batch procedures the calibration graphs resulting from the measurement of the absorbance at 330 nm is linear over the range 5.0–60.0 μg ml^{-1} for cephradine and 3.0–60.0 μg ml^{-1} for ceftazidine. The methods were successfully adapted to FI-systems, the peak heights being proportional to cephalosporin concentration over the range 5.0–60.0 μg ml^{-1} for cephradine and 3.0–60.0 μg ml^{-1} for ceftazidine. The sampling frequency was 60 h^{-1} with a sample injection of 72 μl. © 1997 Published by Elsevier Science B.V.

Keywords: Cephradine; Ceftazidine; Spectrophotometric; Flow-injection; Human urine

1. Introduction

The cephalosporins, cephradine (CP) and ceftazidine (CF), have in their structure a β-lactam ring fused to a dihydrothiazine and different chain substituents in C_3 and C_7. Generally β-lactam antibiotics have been described using the sulphur ring as position 1.

Substituents at the C-3 and C-7 positions are acknowledged as being important in the antibacterial activity and stability of cephalosporins [1] and the chemical reactivity, especially that of the β-lactam ring, is linked to antimicrobial activity and bacterial resistance [2]. Commercially available cephalosporins may be classified as oral (CP) and parenteral (CP and CF) drugs [3].

Cephradine and ceftazidine are semisynthetic cephalosporins of the first and third generation with high antibacterial activity, widely used in the treatment of commonly-occurring bacterial infections. The chemistry of cephalosporins has been widely explored because of their extensive medical applications.

The normal cephradine dose for adults is 2.0–4.0 g per day with a maximum of 8.0 g per day, the corresponding doses of ceftazidine being 2.0–6.0 g per day with a maximum of 12.0 g per day. Both drugs are rapidly and almost completely

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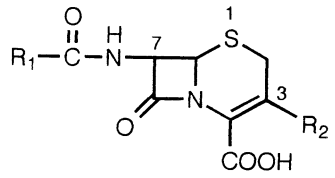

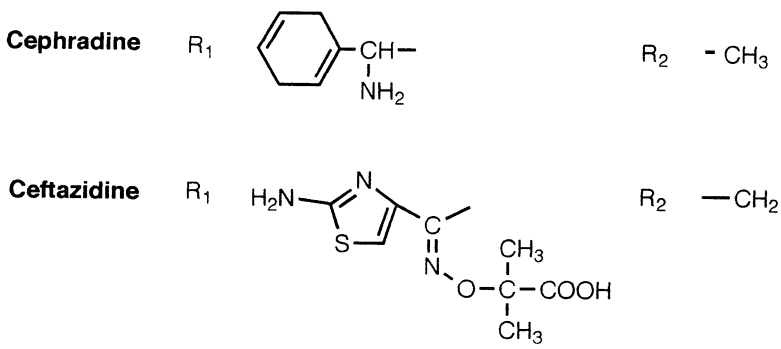

absorbed and as these cephalosporins are not metabolized in the human organism, they are excreted unaltered in active form in urine by glomerular filtration. In the urine passed during 24 h between 95 and 100% of CP or 92 and 95% of CF of the dose administered are excreted [4].

Several methods have been described in the bibliography for determining these cephalosporins in urine, including spectrofluorimetric [5], polarographic [6] or HPLC [7–10] for cephradine, and fluorescence ELISA [11] or HPLC [12,13] for ceftazidine. However, there are no reports involving batch spectrophotometric or FI methods for such determination.

Flow injection (FI) is an easy and inexpensive way of automating analytical determinations and can be applied in several situations to reduce reagent consumption, and to increase the repeatability, selectivity and accuracy of determinations.

This paper describes quick and simple methods for use in the routine determination of cephradine or ceftazidine in urine. The proposed methods are based on the reaction between these drugs and Pd(II) and the measurement of the absorbance of the compounds thus, formed. The procedures are carried out using either batch or flow-injection procedures.

2. Experimental

2.1. Apparatus

A Shimadzu UV/VIS 240 (Tokyo, Japan) spectrophotometer equipped with a plotter unit and a 1×1 cm quartz cell was used for recording the spectra and carrying out absorbance measurements.

The FI system comprised a Gilson HP4 peristaltic pump (Worthington, OH,USA), an Omnifit injection valve (NY, USA), a Hellma 18 μl flow cell (Jamaica, NY, USA) and a Philips PU 8625 UV/VIS spectrophotometer (Cambridge, UK) as the detector. Connecting tubing (0.5 mm bore) poly (tetrafluoroethylene) (PTFE) tubing and various end-fittings and connectors (Omnifit) were used. A Colora Ultra-Thermostat V5 (Lorch, Würt, Germany) was used.

2.2. Reagents

All chemicals were of analytical reagent grade and the solutions were prepared with doubly-distilled water.

2.2.1. Palladium dichloride standard solution (10^{-2} M)

The standard solution was prepared by dissolving 0.1773 g of Pd Cl_2 (Merck) in 5 ml of water, to which 1.0 ml of concentrated HCl had been added and warming the mixture in a water bath. The solution was cooled and diluted with water in a 100 ml calibrated flask.

More diluted solutions were obtained by appropriate dilution with Britton-Robinson buffer.

2.2.2. Stock cephradine solution (500.0 μg ml^{-1})

The stock solution was prepared by dissolving 0.0500 g of cephradine (Sigma, St. Louis, MO, USA) in 100 ml of distilled water; the stock solution was kept at 4°C.

2.2.3. Stock ceftazidine solution (500.0 μg ml^{-1})

The stock solution was prepared by dissolving 0.0500 g of ceftazidine (Sigma, St. Louis, MO, USA) in 100 ml of water and stored at 4°C.

Working solutions were made daily by suitable dilution of the stock solution.

2.3. Britton-Robinson buffer solutions

These covered the pH range 3.0–6.5 and were prepared by adding suitable volumes of 1 M NaOH to 100 ml of 0.2 M in phosphoric acid, acetic acid and boric acid and diluting to 1000 ml with distilled water.

2.4. Procedure for determination of cephradine or ceftazidine in urine samples

Urine samples before the ingestion of cephalosporins (blank) were collected, their creatinine content was determined and appropriate dilution was made to obtain the same level of creatinine in all urine samples. Calibration graphs were made following the batch or FI procedures described below. The recovery of CP or CF was obtained by adding different known amounts of cephlosporin to blank urine samples, wich were then treated in the same form as the calibration standadrs.

Creatinine was determined by the creatinine PAP method (Boehringer Mannheim) based on the enzymatic degradation of creatinine to creatine by creatininase, of creatine to sarcosine by creatinase and of sarcosine to formaldehyde, glycine, and hydrogen peroxide by sarcosine oxidase. Hydrogen peroxide is then quantified by a reaction with 2,4,6-tribromo-3-hydroxybenzoic acid, 4-aminoantipyrine and peroxidase to yield a chinone(purple) dye [14].

2.5. Batch procedure

Calibration graphs were prepared by adding 0–600 μl of 500.0 μg ml^{-1} CP or CF to 25 μl blank urine samples. Then 0.5 ml 10^{-2} M $PdCl_2$ and 2.0 ml BR buffer pH 6.0 were added and the mixture was diluted in a 5.0 ml calibrated flask with doubly distilled water. The absorbances were measured at 330 nm against the reagent blank for both drugs, after 5 min.

2.6. FI-procedure

Fig. 1 shows the flow-injection system. In order to obtain the calibration graphs appropriate volumes (0–600 μl) of 500.0 μg ml^{-1} of CP or CF were added to 25 μl of blank urine samples and diluted with distilled water to 5.0 ml in calibrated flask. Aliquots of 72 μl of these solutions were injected in triplicate into an inert carrier stream, which then joined the reagent stream 10^{-3} M $PdCl_2$ at pH 6.0. The peak height was measured at 330 nm for both drugs. A calibration graph was prepared by plotting absorbance of the peak(A) versus CP or CF concentration.

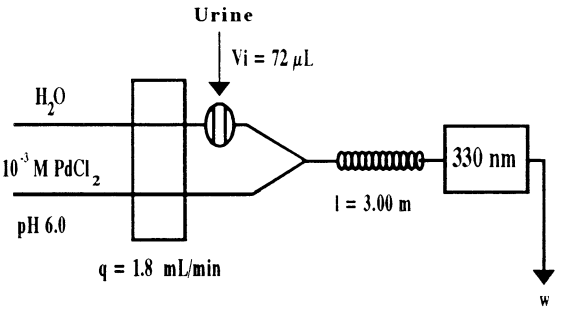

Fig. 1. FI manifold for the determination of cephradine or ceftazidine in urine samples.

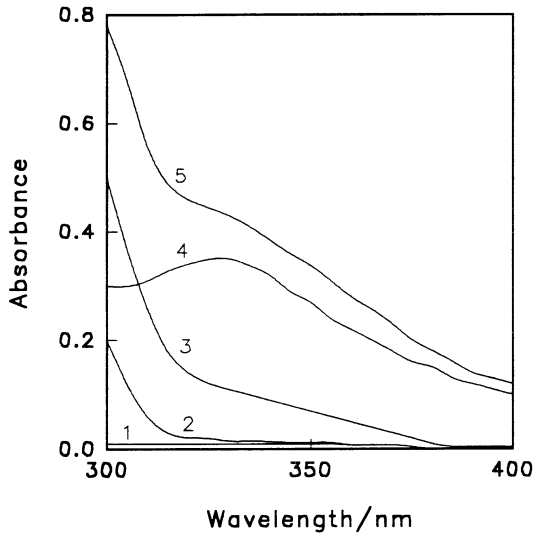

Fig. 2. Absorption spectra at pH 5.5 of (1) 20.0 μg ml^{-1} cephradine; (2) diluted urine sample; (3) diluted urine sample and 10^{-3} M Pd(II); (4) 20 μg ml^{-1} cephradine and 10^{-3} M Pd(II); (5) diluted urine, 20 μg ml^{-1} cephradine and 10^{-3} M Pd(II).

3. Results and discussion

In a previous paper [15] it was shown that cephradine and ceftazidine react with palladium(II) in neutral or weakly acid medium to produce compounds that present an absorption maximum at 330–340 nm. CP or CF do not absorb at this wavelength and palladium(II) chloride has a low absorbance under the same experimental conditions. In this study, the above mentioned reactions are applied to the determination of cephradine or ceftazidine in human urine by spectrophotometric batch or FI methods.

3.1. Batch method

Fig. 2 shows the absorption spectra of 20.0 μg ml^{-1} cephradine in water, (curve 1); diluted urine sample(1:200) without (curve 2) or with 10^{-3} M Pd(II) (curve 3); 20 μg ml^{-1} cephradine, 10^{-3} M Pd(II) without (curve 4) or with diluted urine sample (curve 5). All experiments were carried out at pH 5.5 (B-R buffer) and recorded min after the preparation of the sample. Curves 3,4 and 5 were performed against the reagent blank.

As can be seen, the compound cephradine-Pd(II) showed a well defined absorption maximum at 330 nm (curve 4). The diluted urine sample to which Pd(II) was added had a low absorbance at 330 nm (curve 3) and the presence of cephradine in very low concentration caused an increase in the absorbance (curve 5). Ceftazidine presented a similar spectrophotometric behaviour. Consequently, all subsequent experiments were carried out at 330 nm against reagent blank, in both cases.

Because the absorbance at 330 nm of the urine sample-Pd(II) mixture is a critical factor, the urine samples were treated to obtain a constant analytical signal in all the urine blanks.

For that purpose 0.5 ml 10^{-2} M $PdCl_2$ and 2.0 ml BR pH 5.5 were added to suitable volumes 25–200 μl of different blank urine samples, the mixture was diluted to 5.00 ml and their absorbances were measured at 330 nm against a blank reagent. The lowest variation coefficients were obtained for dilutions 1:200. To obtain more constant analytical signals for the blank urine samples, the creatinine content was determined. The absorbance of the blank urine samples-Pd(II) showed a linear increase with increasing levels of creatinine. Accordingly, all urine samples were first taken to the same level of creatinine by appropriate dilution and following the same procedure, it was found that the coefficients of variation were now lower than those obtained previously.

The effect of pH was studied with samples containing diluted urine, 10^{-3} M Pd(II) and 20 μg ml^{-1} of cephradine or ceftazidine, the pH being adjusted over the range 4.0–6.5 by adding Britton-Robinson buffer. Maximum and constant absorbance was obtained between pH 5.5 and 6.0; pH 5.5 was selected in both cases.

The influence of the concentration of Pd(II) was studied in the range 5×10^{-4}–2×10^{-3} M in the same experimental conditions. Maximum and constant absorbance was obtained with a Pd(II) concentration of 10^{-3} M and above and so this concentration was used.

It was found that a time of 5 min after the preparation of the samples was sufficient for constant absorbances to be obtained.

Table 1
Recovery of cephalosporins from human urine samples by batch methods

Urine	Cephradine		Ceftazidine	
	Found[a] (μg ml^{-1} ± S.D.)	Recovery (%)	Found[a] (μg ml^{-1} ± S.D.)	Recovery (%)
1	9.97 ± 0.15[b]	99.7	9.81 ± 0.08[b]	98.1
	19.59 ± 0.25[c]	98.0	19.52 ± 0.25[c]	97.6
2	10.38 ± 0.17[b]	103.8	10.09 ± 0.10[b]	100.9
	19.68 ± 0.20[c]	98.4	19.64 ± 0.18[c]	98.2
3	9.89 ± 0.10[b]	98.9	9.64 ± 0.12[b]	96.4
	19.93 ± 0.17[c]	99.7	19.46 ± 0.23[c]	97.3
4	10.40 ± 0.14[b]	104.9	10.23 ± 0.10[b]	102.3
	20.10 ± 0.18[c]	100.5	20.28 ± 0.26[c]	101.4

[a]Average of five determinations.
[b]10 μg ml^{-1} added.
[c]20 μg ml^{-1} added.

3.2. Features of the batch methods

The calibration graphs were prepared by the procedure described in Experimental. Beer's law was valid over the concentration range 5.0–60.0 μg ml^{-1} for cephradine or 3.0–60.0 μg ml^{-1} for ceftazidine. The regression equations (A = Intercepted ± S.E. + Slope ± S.E. (Analyte)) found was $A = 0.256 \pm 2.4 \times 10^{-3} + 1.42 \times 10^{-2} \pm 9.4 \times 10^{-5}$ (CP) and $A = 0.260 \pm 2.9 \times 10^{-3} + 1.92 \times 10^{-2} \pm 1.4 \times 10^{-4}$ (CF) where the concentration of cephalosporin was expressed in μg ml^{-1}. The correlation coefficients were 0.9994 for CP and 0.9995 for CF.

The precision of the methods was tested by analysing 10 replicate urine samples containing 10.0 or 20.0 μg ml^{-1} of cephalosporin, the variation coefficients were ± 0.6% or 0.8% for CP and 0.5% or 0.8% for CF. The detection limits were 2.0 μg ml^{-1} for cephradine and 1.5 μg ml^{-1} for ceftazidine.

In order to evaluate the validity of the proposed methods, recovery studies were carried out on different diluted human urine samples to which known amounts of CP or CF (10.0 or 20.0 μg ml^{-1}) were added. The results are summarized in Table 1; as can be seen, the percentages of recovery ranged from 96.4 to 104.9%.

3.3. Spectrophotometric FI methods

Preliminary experiments in continuous-flow were carried out in order to test the manifold configuration and the approximate ranges of the tested parameters. The design of the manifold selected is shown in Fig. 1. A two channel FI assembly was adopted in which the urine sample, previously diluted, was injected into the water stream since injection of the sample into the reagent stream led to negative peaks. The acidity of the carrier-palladium(II) reagent solution was adjusted to pH 6.0 (BR buffer) for CP and CF. The reagents and the carrier stream of water were pumped at the same flow rate in order to achieve effective mixing of the urine sample and reagent solutions. Pd(II) reacted with the CP or CF contained in the urine and formed the respective compounds, the absorbance was measured at 330 nm in the detector previously adjusted to zero with the Pd(II) carrier solution. The presence of cephradine or ceftazidine in the urine caused an increase in the analytical signal, proportional to its concentration.

FI methods for determining cephradine or ceftazidine depend on optimization of the system to achieve maximum height, with low residence time and minimum dispersion. As a consequence, different FI variables (sample volume, reaction

Table 2
Recovery of cephalosporins from human urine samples by FI methods

Urine	Cephradine		Ceftazidine	
	Found[a] (μg ml^{-1} ± S.D.)	Recovery (%)	Found[a] (μg ml^{-1} ± S.D.)	Recovery (%)
1	9.70 ± 0.49[b]	97.0	9.54 ± 0.30[b]	95.4
	19.52 ± 0.50[c]	97.6	19.23 ± 0.29[c]	96.2
2	9.58 ± 0.50[b]	95.8	9.82 ± 0.36[b]	98.2
	19.64 ± 0.54[c]	98.2	19.65 ± 0.29[c]	98.3
3	10.30 ± 0.42[b]	103.0	10.09 ± 0.34[b]	100.9
	20.12 ± 0.50[c]	100.6	19.91 ± 0.34[c]	99.6

[a]Average of five determinations.
[b]10 μg ml^{-1} added.
[c]20 μg ml^{-1} added.

coil length and flow rate) chemical variables (acidity and Pd(II) concentration) were selected by the univariate method in the continuous flow procedure with diluted urine samples containing a fixed concentration of the CP or CF 20 μg ml^{-1}.

The peak height increased with loop size in the range studied, 40–120 μl. A loop size of 72 μl was chosen since such a sample volume provided sufficient sensitivity without excessive waste of sample.

The influence of reactor length was studied from the minimum distance possible between the injection valve and detector up to 4 m. The peak height remained practically constant in the range studied. A reactor length of 3 m (0.5 mm i.d.) was selected in both cases as this provided a high sampling frequency and reproducibility.

The effect of flow rate on peak height was studied over the range 0.5–2.5 ml min^{-1}; for both CP and CF a flow rate of 1.8 ml min^{-1} was selected as a compromise between sensitivity and sampling rate.

The influence of the pH on the peak height was studied in the range 4.0–6.5 (Britton-Robinson buffers). Maximum peak height was obtained in both cases at pH 6.0, this being the pH selected.

The influence of Pd(II) concentration was studied in the range 5×10^{-2}–2×10^{-3} M. A concentration of 10^{-3} M $PdCl_2$ was selected in both cases.

3.4. Features of the FI methods

With the described design and under the selected experimental conditions (10^{-3} M $PdCl_2$, pH 6.0) a series of standard solutions of CP or CF were added to urine samples and injected in triplicate to test the linearity. The calibration graphs were found to be linear from 5.0 to 60.0 μg ml^{-1} for CP and 3.0–60.0 μ ml^{-1} for CF. The regression equations were $A = 0.020 \pm 6.59 \times 10^{-4} + 1.84 \times 10^{-3} \pm 2.13 \times 10^{-5}$ (CP) and $A' = 0.021 \pm 5.71 \times 10^{-4} + 3.44 \times 10^{-3} \pm 1.85 \times 10^{-5}$ (CF), where A is the absorbance peak, the drug concentrations being expressed in μg ml^{-1}; the correlation coefficients were 0.9990 and 0.9998, respectively. The detection limits were 2.0 and 1.1 μg ml^{-1} for CP and CF, respectively.The flow system selected provided a sampling frequency of 60 samples h^{-1}.

A study of the reproducibility of the methods for 10.0 or 20.0 μg ml^{-1} of CP or CF added to the same urine sample showed that the variation coefficient of the absorbance peak was 1.4 or 1.1% for CP and 2.4 or 1.8% for CF ($n = 10$).

These methods were validated by applying the standard addition method. Different urine samples, to which known amounts of CP or CF had been added were analyzed by the proposed methods. results are shown in Table 2; in all cases recoveries were in the range 95.4–100.9%.

4. Conclusions

The batch and FI procedures developed for cephradine and ceftazidine allow their determination in human urine samples in the physiological concentration range obtained after the usual therapeutic dose of CP or CF has been administered. These methods showed good accuracy and reproducibility and are useful for the routine control of CP or CF in urine samples.

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