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Spectrophotometric determination of selected cephalosporins in drug formulations using flow injection analysis

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Abstract

A sensitive, accurate and rapid flow injection analysis (FIA) method for the determination of cefotaxime, cefuroxime, ceftriaxone, cefaclor, cefixime, ceftizoxime, and cephalexin is proposed. Aliquots of each cephalosporin were hydrolyzed for 15 min with 0.1 M NaOH at 80°C and then oxidized with Fe^{3+} in sulfuric acid medium to produce Fe^{2+}. The produced Fe^{2+} is then complexed by *o*-phenanthroline (*o*-phen) in citrate buffer at pH 4.2 to form the red complex, $Fe(o\text{-phen})_3^{2+}$, which exhibits an absorption maximum at 510 nm. Variables such as acidity, reagent concentrations, flow rate of reagents and other FI parameters were optimized to produce the most sensitive and reproducible results. The method was successfully applied to the analysis of pharmaceutical preparations. The results have been compared with those obtained using the official methods. Excellent agreement between the results of the proposed method and the official methods was obtained.

Keywords: Cephalosporins; Spectrophotometric; Flow injection analysis; Pharmaceutical analysis

1. Introduction

Cephalosporins are referred to as the β-lactam antibiotics, which are among the oldest and most frequently prescribed of naturally occurring antimicrobial agents. The key intermediate for semisynthetic production of a large number of cephalosporins is 7-aminocephalosporanic acid, which is formed by hydrolysis of cephalosporin C produced by fermentation [1]. Cephalosporins are penicillinase-resistant antibiotics with significant activity against both gram-positive and gram-negative bacteria.

Many analytical procedures have been adopted for the determination of cephalosporins. These methods use high-performance liquid chromatography [2–4], liquid chromatography with amperometric detection [5], polarography [6–9], fluorometry [10] and spectrophotometry [11–18]. Most of these methods are time consuming and large sample volumes are required for some of them.

The spectrophotometric methods used to determine cephalosporins include nickel(II)-hydroxylamine [11], chlorobenzotriazole and sodium hypochlorite [12], oxidation with Ce(IV) [13], ammonium molybdate [14], precipitate formation with excess Pb(II) and the determination of the remaining Pb(II) by atomic absorption spectrometry [15], derivative spectrophotometry [16], deriva-

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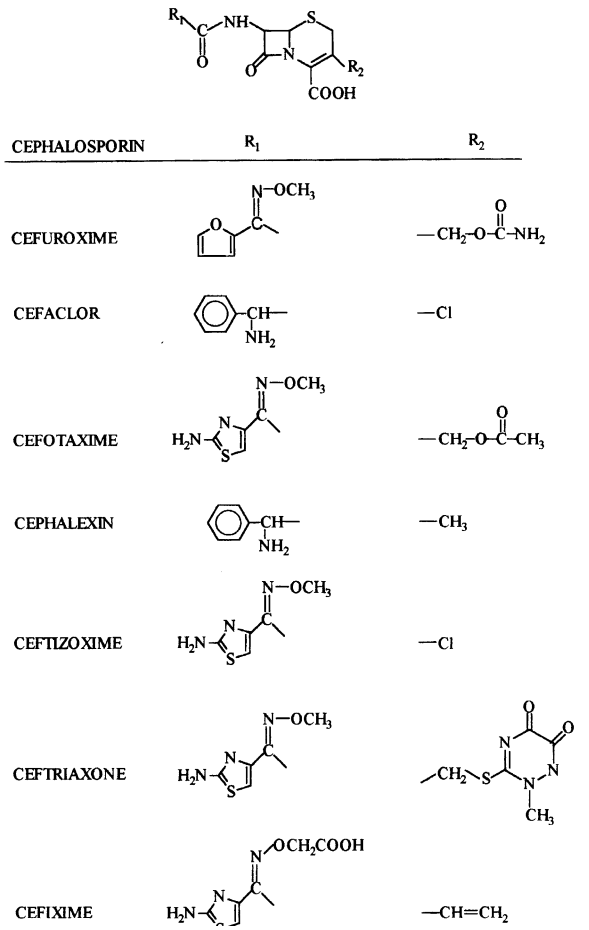

Fig. 1. Structures of cephalosporins tested.

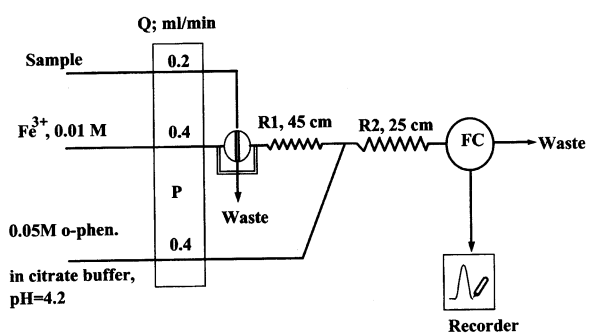

Fig. 2. Flow injection manifold for cephalosporins determination, P, pump; Q, flow rate; FC, flow cell ($\lambda = 510$ nm); R1 and R2, reaction coils.

tization with 1,2-naphthoquinone 4-sulphonate followed by solid-phase extraction and absorbance measurements [17] and alkaline degradation to hydrogen sulfide and the formation of methylene blue [18].

These methods are time consuming because they require long reaction times (30–60 min), and most require heating. In most of these methods, absorbance measurements for both samples and standards must be done either at a constant, fixed time after addition of the colorimetric reagent or waiting for the reaction to proceed to completion in order to attain the required reproducibility.

In this work, we have demonstrated the possibility of using flow injection analysis (FIA) to overcome these difficulties. In FIA, reaction completion is not necessary because measurements for all samples and standards are subjected to the same timing sequence in a precise, automatic manner. FIA technique has found recently wide applications mainly due to reduction of the analysis time and reagents consumption compared with conventional manual procedures. Moreover, FIA is scarcely reported for the determination of cephalosporins.

2. Experimental

2.1. Reagents

Analytical-reagent grade chemicals and deionized water were used to prepare all solutions. Cephalosporins and excipients usually used in pharmaceutical formulations were all supplied by Al-Hikma Pharmaceuticals, Amman, Jordan. The structure of the cephalosporins tested are given in Fig. 1. Citric acid, ferric nitrate, ($Fe(NO_3)_3 \cdot 9H_2O$), sodium hydroxide, *o*-phenanthroline (*o*-phen) were all obtained from Fisher Scientific (USA).

2.2. Solutions

2.2.1. Citrate buffer

Citric acid (1.910 g, 0.10 M citric acid) was dissolved in about 900 ml distilled water. The pH of the solution is then adjusted to 4.2 by 0.5 M NaOH and completed to 1.0 l by distilled water.

Table 1
Effect of various reaction conditions on the analytical signal of cefotaxim

H_2SO_4	Absorbance	Fe^{3+}	Absorbance	*O*-phen	Absorbance
0.30	0.067	0.001	0.060	0.01	0.044
0.60	0.091	0.005	0.083	0.02	0.078
0.80	0.099	0.010	0.095	0.03	0.089
1.00	0.097	0.025	0.078	0.04	0.099
1.20	0.093	0.050	0.052	0.05	0.095
1.40	0.085	0.100	0.033	0.08	0.088

Table 2
Statistical analysis of calibration graphs for cephalosporins tested by the proposed FI method

Parameter	Cefixime	Cefaclor	Cefotaxime	Cefuroxime	Cephalexin	Ceftriaxone	Ceftizaxime
Linearity range (mg/ml)	0.08–2.00	0.04–0.40	0.20–2.50	0.30–2.50	0.04–0.50	0.10–1.50	0.25–2.50
Regression equation							
(*Y*). Intercept (b)[a]	8.1×10^{-3}	0.2×10^{-3}	-1.3×10^{-3}	-6.0×10^{-3}	8.0×10^{-3}	2.6×10^{-3}	-1.1×10^{-3}
S.E. of (b)	3.6×10^{-3}	3.3×10^{-3}	9.4×10^{-4}	7.2×10^{-3}	2.0×10^{-3}	2.2×10^{-3}	1.8×10^{-4}
Slope (a)	1.8×10^{-2}	2.13×10^{-1}	5.3×10^{-2}	3.0×10^{-2}	2.23×10^{-1}	4.3×10^{-2}	4.9×10^{-2}
S.E. of (a)	4.6×10^{-4}	8.5×10^{-3}	9.0×10^{-4}	1.2×10^{-3}	6.7×10^{-3}	0.6×10^{-3}	1.1×10^{-3}
Correlation coefficient (*r*)	0.999	0.993	0.999	0.995	0.999	0.996	0.998
LOD (μg/ml)	60	30	150	200	30	80	150
LOQ (μg/ml)	200	100	500	660	100	260	500

[a] $Y = aC + b$ where C is the concentration in mg/ml and Y is the absorbance.

Table 3
Analytical recovery of cephalosporins in synthetic mixtures

Cephalosporin	Taken (μg/ml)	Percent found ± R.S.D.		$t_{Experimental}$[c]	$F_{experimental}$[d]
		Proposed method[a]	Official method [1][b]		
Cefixime	800	103.9 ± 2.7	101.8 ± 1.4	0.591	1.83
Cefaclor	100	102.3 ± 3.0	100.7 ± 2.1	1.733	2.54
Cefuroxime	600	98.6 ± 1.2	99.5 ± 0.7	0.471	1.66
Cephalexin	500	99.7 ± 1.8	98.7 ± 1.6	0.633	1.57
Ceftriaxone	400	97.4 ± 3.8	98.8 ± 2.2	1.140	1.92
Ceftizoxime	500	99.1 ± 0.9	100.6 ± 0.6	1.365	2.13
Cefotaxime	600	97.6 ± 1.8			

[a] Percent R.S.D. for six determinations.
[b] Percent R.S.D. for three determinations.
[c] Theoretical value for $t = 2.31$ at $P = 0.05$.
[d] Theoretical value for $F = 9.01$ at $P = 0.05$.

2.2.2. O-phenanthroline solution

A stock solution of 0.02 M was prepared by dissolving 3.965 g of *o*-phen in 1.0 l of citrate buffer, pH 4.2.

2.2.3. Fe(III) solution

A stock solution of 0.01 M Fe(III) solution was prepared by dissolving 4.040 g of $Fe(NO_3)_3 \cdot 9H_2O$ in 1.0 l of 0.8 M sulfuric acid.

2.2.4. Standard solutions of antibiotics

These were prepared by dissolving 500 mg of each antibiotic in 50 ml of 0.1 M NaOH. Solutions containing 0.60–2.50 mg/ml of each antibiotic were transferred into a test-tube. A 10 ml of 0.1 M NaOH solution were added to each test-tube. All test tubes were heated in a water bath at 80°C for 15 min. This time was found appropriate for complete hydrolysis. The contents of each test tube was transferred to a 50 ml calibrated volumetric flask and completed to volume by 0.1 M NaOH.

2.2.5. Capsules and tablets

The contents of ten capsules (or tablets) were weighed and mixed. An accurately weighed portion of the powder equivalent to 250 mg of the antibiotic under investigation was transferred into a test-tube. A 10 ml of 0.1 M NaOH solution was added to the test-tube and heated in a water bath at 80°C for 15 min. The contents of the test-tube was transferred to a 50 ml calibrated volumetric flask and completed to volume by 0.1 M NaOH. The solution was filtered (if necessary) and 10 ml of the filtrate was transferred to 25 ml volumetric flask and then completed to volume with 0.1 M NaOH.

2.2.6. Suspension

The oral suspension of the antibiotics investigated was prepared by diluting with distilled water as directed by the manufacturer. An accurately measured volume of the oral suspension — equivalent to 250 mg of the antibiotic under investigation — was transferred into a test-tube and the procedure is completed as described under capsules and tablets.

Table 4
Determination of cephalosporins in pharmaceutical preparations using the proposed method and official methods [1]

Cephalosporin	Trade name	Recovery (%)		*t*-Values[i]	*F*-values[j]
		Proposed method[g]	Official methods[h]		
Ceftriaxone (injection)	Roxcef[a]	98.9 ± 2.3	99.7 ± 0.8	1.63	1.92
Cefuroxime (tablets)	Zinnat[b]	98.2 ± 2.2	100.3 ± 0.5	0.95	2.43
Cefuroxime (injection)	Maxil-750[c]	98.0 ± 1.8	98.7 ± 1.1	0.73	1.35
Cefuroxime (injection)	Maxil-1500[c]	99.6 ± 1.3	101.2 ± 0.9	1.62	1.60
Cefaclor (suspension)	Cloracef[d]	103.3 ± 2.7	101.7 ± 1.3	0.35	1.44
Cefixime (suspension)	Suprax-100[c]	103.6 ± 3.2	101.3 ± 0.8	0.82	2.11
Cephalexin (capsule)	Ultrasporin[e]	99.5 ± 0.9	100.4 ± 0.6	0.64	1.23
Cefotaxime (injection)	Claforn[f]	100.5 ± 1.4			
Cefotaxime (injection)	Ceftax[c]	98.4 ± 1.9			

[a] APM, Jordan.
[b] Glaxo, UK.
[c] Hikma, Jordan.
[d] Dar Al Dawa, Jordan.
[e] Midpharma, Jordan.
[f] Roussel, France.
[g] Mean ± R.S.D. of six determinations.
[h] Mean ± R.S.D. of three determinations.
[i] Tabulated *t*-value for $P = 0.05$ and five degrees of freedom is 2.31.
[j] Tabulated *F*-value for $P = 0.05$ is 9.01.

2.3. Apparatus

All measurements were performed with a Varain DMS-100 ultra violet (UV)–visible spectrophotometer connected to a linear 1200 recorder. The wavelength was adjusted at 510 nm. Teflon tubing of 0.51 mm i.d. was used in the flow system. A rheodyne 4-way injection valve was used to introduce the sample into the carrier stream. The reaction coil was 45 cm long and a homemade confluence point was used to ensure rapid mixing of sample mixture with *o*-phen. A sample injection volume of 40 μl was used. The manifold is shown in Fig. 2.

2.4. General procedure

A volume of 40 μl of prepared sample solution was injected into the sample loop by means of a syringe. Samples were injected into Fe^{3+} stream pumped at a rate of 0.4 ml/min. The *o*-phen solution was added at a rate of 0.4 ml/min in a confluence manner down stream to ensure rapid and adequate mixing of the sample with mixed reagent. After injection, the valve was returned to the load position when the maximum change in absorbance value has been reached. The absorbance was monitored at 510 nm at which maximum absorption occurs, and the increase in absorbance was registered using a chart recorder, at 0.5 mV and with a chart speed of 30 cm/h. When the base line was reached, another slug of sample was injected. The height of the absorbance peak was used for calibration.

3. Results and discussion

The different parameters affecting the oxidation reaction and hence the subsequent determination of antibiotics are optimized. These include, the acidity of the medium, concentrations of Fe^{3+} and *o*-phen solutions, flow rate, sample volume, reaction coil length and the stability of the analytes in solution.

The equilibrium constant for the complex $Fe(o\text{-phen})_3^{2+}$ is 2.5×10^6 at 25°C. Quantitative formation of the complex could be achieved in the pH range between 2 and 9. Different pH values in the range of 3–5 were tried. At pH > 5 the solubility of the *o*-phen was incomplete. Maximum analytical signal was obtained at pH 4.2. Therefore, the pH of the *o*-phen was adjusted to 4.2.

The effect of the acidity of Fe^{3+} solution on the absorbance was examined. The effects of changing the concentration of H_2SO_4 (0.3–1.4 M) on the absorbance of the complex formed are presented in Table 1 for cefotaxim. The absorbance was found to increase with increasing the acidity of the Fe^{3+} solution until it reached a maxima at 0.8 M H_2SO_4. The influence of Fe^{3+} concentration on the analytical signal was also investigated. Different concentrations of Fe^{3+} were prepared in the range of 1.0×10^{-3}–1.0×10^{-1} M in 0.8 M H_2SO_4. Maximum absorbance was obtained at 0.01 M Fe^{3+} (Table 1). Therefore, a concentration of 0.01 M Fe^{3+} solution was used in all the analysis.

Different concentrations of *o*-phen in the range of 0.01–0.08 M in citrate buffer at pH 4.2 were tested (Table 1). At low concentrations, a splitting of the peak was observed which might be due to the presence of unreacted sample zone. As the concentration of the *o*-phen increases, the absorbance increases, until maximum absorbance was reached at 0.04 M *o*-phen. Therefore, a concentration of 0.04 M *o*-phen was chosen as a compromise.

The flow rate of the carrier and the reagent streams were optimized with *o*-phen solution at pH 4.2 and Fe^{3+} solution in 0.8 M H_2SO_4. The sample volume was 40 μl containing 40 μg of the analyte. The distance between the mixing point and the detector was 25 cm. The flow rates investigated were 0.3, 0.5, 0.7, 0.8, 1.0, and 1.2 ml/min. The highest signals were obtained at a flow rate of a 0.4 ml/min for each reagent. At higher flow rates, the signals were lower because the reaction did not proceed to completion, which also caused irreproducible results. Lengthening the coil between the injection port and the mixing point (R1) and between the mixing point and the detector (R2) did not improve the results for high flow rates. The signal obtained at 0.8 ml/min was not significantly affected when the distance R1 was changed between 10 and 30 cm, but greater length

caused peak broadening because of sample dispersion. Peak broadening was also observed at flow rates less than 0.8 ml/min. At this flow rate about 75 s are needed for one determination which means that the average sampling rate using a 40 μl sample injections is about 45 samples per h.

The effect of the reaction coil (R1) length on the analytical signal was investigated. A slight increase in the analytical signal was observed when the reaction coil length increased from 10 to 45 cm. Lengthening the coil caused peak broadening due to sample dispersion. Also the effect of the length of line linking the mixing point with the flow cell was studied but it was found to have no effect on the analytical signal over the range 10–30 cm.

The effect of injection volume on peak height is also investigated. As expected, an increase in the volume of injected sample solution leads to an increase in peak height and consequently the sensitivity of measurement but results in increasing the peak width and time for the signal to retain to the baseline. So a 40 μl volume was chosen which produces reasonable sensitivity and sampling rate. In case more sensitivity is needed a larger volume can be used.

The stability of the hydrolyzed cephalosporins in solution was also investigated. Known amounts of each of the cephalosporins studied were injected into the manifold shown in Fig. 2, at different time intervals using the optimum experimental conditions. All solutions were stored in the refrigerator until the time of analysis. At the beginning, solutions were injected every 2 h and then they were performed every day. No significant changes in the results were observed during the 5 days of this study.

3.1. Evaluation of the method

Table 2 shows the results of the statistical analysis of the experimental data for all cephalosporins tested. The regression equations calculated from the calibration graphs, along with S.D. of the slopes and intercepts are given. The linearity of the calibration graphs and the conformity of the systems to Beer's low are proven by the high values of correlation coefficients of regression equations. The limits of detection (LOD) were determined as the concentration of analyte leading to a signal that is three times the blank standard deviation. Similarly, the limits of quantification (LOQ) were determined as the concentration of analyte leading to a signal that is ten times the blank S.D. The LOD and LOQ values for the drugs studied are listed in Table 2.

The intra-day (within-day) precision was evaluated by replicate analysis of two different concentrations of cefotaxime within the linearity range at different time intervals. The inter-day (different days) precision was similarly evaluated on several days up to 5 days. Every day, a new calibration graph was constructed. The results in both cases indicated high precision, as the percent R.S.D. did not exceed 4%. The precision of the measurements ranged from a R.S.D. of 0.85–3.75% ($n = 6$).

In order to examine the applicability of the proposed FI method to routine pharmaceutical analysis, the effect of common excipients normally used in pharmaceutical formulations was studied. Synthetic mixtures containing different concentrations of the studied compounds in the presence of more than 100 folds of common additives (magnesium stearate, sodium benzoate, xanthan gum, natural and artificial flavors, starch and sucrose) were prepared. The undissolved material was filtered off before injection. No significant changes were observed on the results and recoveries in the range of 103.9–97.4% were obtained in all cases (Table 3). The accuracy of the proposed method is further tested by analyzing the same samples by official methods [1]. The results obtained by the proposed methods were in excellent agreement with those obtained by official methods (Table 3). No interferences were observed from the additives normally present in commercially available products.

The performance of the method was assessed by calculation of the t-values and F-values compared with the official methods [1]. The results showed that the calculated t-values and F-values did not exceed the theoretical values at 95% confidence limits for the five degrees of freedom (Table 3). These results indicate that the proposed method does not differ significantly from official methods. However, the method must be consid-

ered non-specific regard to differentiate between cited drugs. This shortcoming does not affect the applicability of the method in routine analysis of these drugs as they singly prescribed. The proposed method can be used as alternative method to the reported ones for the routine determination of the cited compounds in pure form and in pharmaceutical formulations.

Analyzing some of the commercially available pharmaceutical preparations performed a further evaluation of the proposed FI method in pharmaceutical analysis. As shown in Table 4, the recoveries are excellent (98.0–103.3%) for all cephalosporins tested, proving the potential of this method in pharmaceutical analysis. Statistical analysis of the results obtained by the proposed method and the official methods using the *t*-test and the *F*-test showed no significant difference between the performance of these methods regarding accuracy and precision.

In conclusion, the proposed FI procedure can be used for the analysis of pharmaceutical preparations containing cephalosporins presented in Fig. 1. The method is simple, using minimum number of reagents and reaction sequence. The speed of analysis and the precision make this method also suitable for the quality control of formulations containing these drugs replacing tedious, expensive, and slow official and chromatographic methods.

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