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Flow-injection spectrophotometric determination of certain cephalosporins based on the formation of dyes

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Abstract

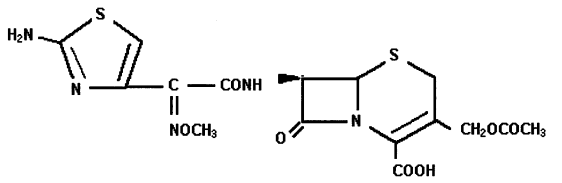
A flow-injection spectrophotometric method is described for the determination of cefadroxil (**I**) and cefotaxime (**II**). The method is based on the hydrolysis of the cephalosporin with sodium hydroxide whereby the sulfide ion is produced. The latter is allowed to react with *N*,*N*-diethyl-*p*-phenylenediamine sulfate (*N*,*N*-DPPD) and Fe (III), and the blue color produced is measured at 670 nm (method A). Linear calibration graphs are obtained in the range 36.34–109.2 and 95.48–477.4 μg ml^{-1} for **I** and **II**, respectively. The experimental limits of detection (three times the noise signal) are 0.036 and 0.048 μg ml^{-1} for **I** and **II**, respectively. The total flow-rate is 5.3 ml min^{-1} for both drugs. Alternately, the sulfide ion produced is allowed to react with *p*-phenylenediamine dihydrochloride (PPDD) and Fe (III), and the violet color produced is measured at 597 nm (method B). Linear calibration graphs are obtained in the range 0.5–400 and 0.5–450 μg ml^{-1} for **I** and **II**, respectively. The limits of detection are 0.4 and 0.2 μg ml^{-1} for **I** and **II**, respectively. The total flow-rate is 3 ml min^{-1} for both drugs. The methods have been successfully applied to the analysis of some pharmaceutical formulations, particularly of the injection and capsule types. The relative standard deviation (RSD) ($n = 10$) at the 50 and 100 μg ml^{-1} levels of I and **II** were 0.83–0.77 and 0.9–0.8% with *N*,*N*-DPPD and PPDD as reagents, respectively. Recoveries were quantitative; the results obtained agreed with those obtained by other reported methods.

Keywords: Cefadroxil; Cefotaxime determination; Flow-injection analysis; Spectrometry

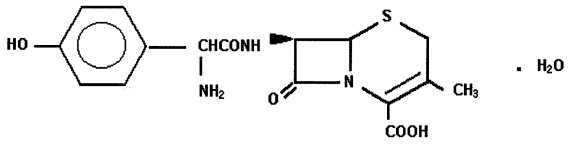
1. Introduction

Cefadroxil (**I**) and cefotaxime (**II**) are considered to be broad-spectrum antibiotics, primarily used to treat bacterial infections of the skin, soft tissues and the urinary tract. They belong to an important class of antibiotics, the cephalosporins. They are referred to as the β-lactam antibiotics, which are among the oldest and the most valuable clinical antimicrobial agents [1].

Chromatographic methods such as thin layer chromatography (TLC) [2] and high-performance liquid chromatography (HPLC) [3,4] are commonly used for their determination; all of them require lengthy treatments and tedious extraction procedures. UV spectrophotometric methods [5,6] have been proposed, but not all are specific. Many fluorimetric methods [7] have been used, some requiring long heating times and others requiring the use of sophisticated equipments. A chemiluminescence method is also mentioned [8]. Furthermore, a new anodic voltammetry procedure is used [9]. Different colorimetric methods, some based on



(I)



(II)

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reactions with molybdophosphoric acid [10], or with copper (II) and vandium (V) in sulfuric acid medium [11], and other methods [12–14] are described.

In this paper, the advantages of flow-injection analysis (FI) (simplicity, high precision, rapidity and low reagent consumption) are combined with the benefits of utilizing dye formation for the spectrophotometric determination of cefadroxil and cefotaxime. The chemical reaction variables were studied and the FI variables were optimized on the basis of sensitivity, sampling rate, temperature and reagent consumption.

2. Experimental

2.1. Materials

All chemicals were of analytical reagent grade and were used without further purification. Distilled de-ionized water was used throughout. Reference cefotaxime sodium and cefadroxil were kindly provided by Sigma Chemical Co. (Dorest, UK) and were used as received.

The procedures were applied on formulations that were purchased from the local market:

1. Claforan, 1 g of cefotaxime sodium, i.v. injection (Laboratories Roussel), lot/batch 130-189A.
2. Ultracef, 500 mg of cefadroxil per capsule (Bristol Laboratories, Division of Bristol-Myers Company, Syracuse, New York).

2.2. Reagents and solutions

2.2.1. Cefadroxil solution

A stock solution of 1×10^{-2} M was prepared by dissolving 0.1817 g in 0.5 M NaOH and then diluting to 50 ml with 0.5 M NaOH.

2.2.2. Cefotaxime solutions

A stock solution of 1×10^{-2} M was prepared by dissolving 0.2387 g in 0.5 M NaOH and then diluting to 50 ml with 0.5 M NaOH.

2.2.3. N,N-DPPD sulfate solution 0.5 M

Prepared by dissolving 6.5575 g of *N*,*N*-DPPD in 1 M sulfuric acid and diluting to 50 ml with 1 M sulfuric acid.

2.2.4. Ammonium iron (III) sulfate [Fe (III)] 0.25 M

Prepared by dissolving 12.0548 g of ammonium iron (III) sulfate dodecahydrate in 0.5 M sulfuric acid and diluting to 100 ml with 0.5 M sulfuric acid (for method A).

2.2.5. p-Phenylenediamine dihydrochloride (PPDD) solution 0.1 M

Prepared by dissolving 4.5268 g of PPDD in water and then diluting to 250 ml with the same solvent.

2.2.6. Iron (III) chloride hexahydrate [Fe (III)] solution 0.1 M

Prepared by dissolving 6.7587 g in 250 ml of distilled water and adding a few milliliters of 0.1 M sulfuric acid for stabilization (for method B).

2.3. Apparatus

A Gilson Minipuls 3MP4 (four channels) peristaltic pump was used to deliver the reagents. Samples were introduced via a 200 μl Teflon rotary valve (a Rheodyne RH-5020 injection valve) with interchangeable loops of different volumes, and propelled by the Gilson pump. The reaction coil was of Teflon (PTFE) tubing (0.8 mm i.d.), used for the remainder of the flow lines (manifold tubing).

The absorbances of the colored products formed were measured at 670 and 597 nm for ethylene blue and the violet dye, respectively, using an LKB Ultrospec II 4050 spectrometer equipped with a Helma 80 μl flow-cell and attached to a Chessel type BD 40 chart recorder. The peak heights obtained were measured manually.

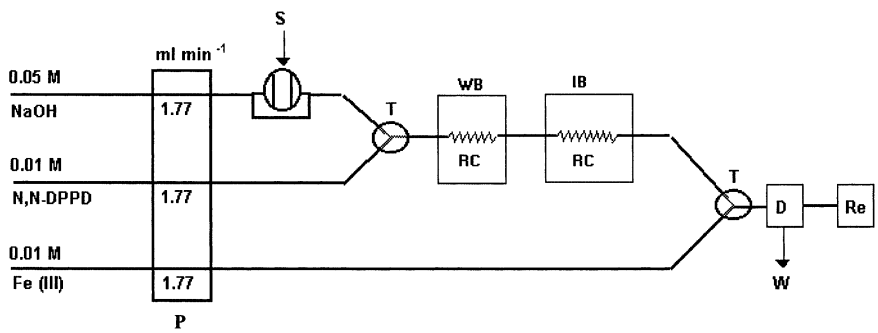

Fig. 1. Schematic diagram of the FI manifold used for procedure A. P, peristaltic pump; S, injection valve (200 ml); T, T-junction; WB, water bath (50 cm); IB, ice bath; (RC) reaction coil (100 cm); Re, recorder (speed 1 cm min^{-1}); W, waste; D, detector.

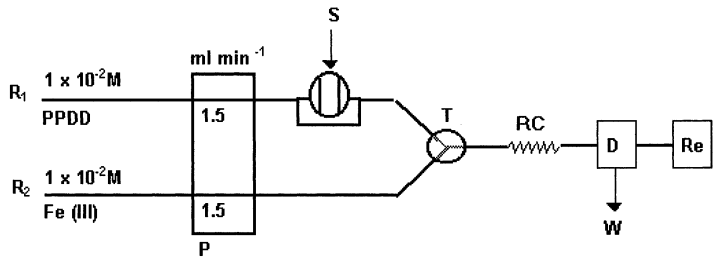

Fig. 2. A schematic diagram of the manifold used for procedure B. P, pump; S, injection valve (200 μl); (RC), reaction coil; D, detector; Re, recorder; W, waste.

2.4. FI Manifold

2.4.1. For the method based on the formation of ethylene blue

The FI manifold used is outlined in Fig. 1. It consists of three channels. The sample solution (200 μl) of the cephalosporin is introduced via the injection valve S into the NaOH stream and is pumped at a flow-rate of 1.77 ml min^{-1}, while *N*,*N*-DPPD is pumped through the other channel, then mixed. The mixture stream passes through a reaction coil 50 cm long for extra mixing and is submerged in a heated water bath at 70 and 80°C for cefadroxil and cefotaxime, respectively. Then the mixture is cooled in an ice bath using a 100 cm long reaction coil. The cold mixture is mixed with a solution of 0.01 and 0.1 M Fe (III) for cefadroxil and cefotaxime, respectively. All the three reagents are pumped at the same flow-rate (1.77 ml min^{-1} in each channel). The resulting dye is then monitored by the spectrophotometer at 670 nm, and the peaks are recorded by the chart recorder (Re).

2.4.2. For the method based on the formation of violet color

A schematic diagram of the manifold is shown in Fig. 2. An aqueous solution of PPDD (1×10^{-2} M) acting as a carrier stream for the cephalosporin is supplied through R_1 and the aqueous iron (III) solution (1×10^{-2} M) through R_2. The hydrolyzed drug sample is injected into the PPDD stream from a 50 μl Teflon rotary valve injector (Rheodyne type RH5020). Both the streams are propelled by a four-channel peristaltic pump (Gilson Minipuls 3MP4) and mixed at a PTFE T-piece. Then the mixture stream passes through a reaction coil of length 50 cm. Both reagents are pumped at the same flow-rate (1.5 ml min^{-1} in each channel).

The absorbance of the colored solution formed is measured in the flow-cells of the spectrophotometer at 597.3 nm and the peaks are recorded at a recorder speed of 1 cm min^{-1}.

2.5. Procedures

2.5.1. Analysis of authentic samples

By using the FI manifolds described in Fig. 1 (for method A) and Fig. 2 (for method B), sample solutions (200 and 50 μl) containing definite amounts of the cephalosporin were injected, for methods A and B, respectively. The maximum absorbance obtained was read and/or the signals recorded on the chart recorder.

2.5.2. Analysis of dosage forms

For injection: An accurately known weight of the drug sample, equivalent to 239 mg of the active constituent was transferred into a 50 ml standard flask, dissolved in 0.1 M NaOH and then made up to volume with 0.1 M NaOH. An aliquot of this solution giving an analyte concentration of 191 μg ml^{-1} was diluted to 25 ml using calibrated flask and analyzed as for authentic samples. The concentrations of the samples were calculated from the calibration curve.

For capsules: The contents of 10 capsules of the drug were weighed and powdered. An amount of powder equivalent to 181.7 mg of the active constituent was weighed accurately and transferred into a 50 ml calibrated flask. It was shaken with 25 ml of 0.5 M NaOH, and then the solution was filtered through a Whatman No. 1 filter paper. The residue was washed with 0.5 M NaOH and the volume was completed to 50 ml with 0.5 M NaOH. An aliquot of this solution giving an analyte concentration of 145 μg ml^{-1} was transferred into a 25 ml calibrated flask and analyzed as for authentic samples. The concentrations of the samples were calculated from the calibration curve.

3. Results and discussion

Different cephalosporins were shown to give different yields of the sulfide ion during their hydrolysis in sodium hydroxide solutions. This method characterizes a selective procedure for cephalosporins, since other β-lactam compounds such as pencillins do not give the sulfide ion under alkaline hydrolysis.

Method A: The proposed method is based on the hydrolysis of cefadroxil and cefotaxime with NaOH to give hydrogen sulfide, which is then reacted with *N*,*N*-DPPD and Fe (III) as an oxidizing agent to form the blue species (ethylene blue).

Method B: This also depends on generating hydrogen sulfide from cefadroxil and cefotaxime, and reacting the hydrogen sulfide produced with PPDD and Fe (III) to form violet species (violet-like dye).

The reactions variables and the FI variables were optimized in terms of sensitivity, precision, sampling rate and reagents consumption.

3.1. Influence of reaction variables for method A

3.1.1. Effect of *N*,*N*-DPPD concentration

The influence of the concentration of *N*,*N*-DPPD was studied by injecting 200 μl of a 50 μg ml^{-1} solution of the cephalosporin into a NaOH solution pumped at 1.77 ml min^{-1}. Different concentrations of *N*,*N*-DPPD were prepared, in the same manner as described in Section 2.2. The concentrations of Fe (III) were 0.01 and 0.1 M for cefadroxil and cefotaxime, respectively.

A maximum peak-height absorbance was obtained with 0.01 M *N*,*N*-DPPD. This concentration gave reproducible results with RSD values for the peak height equal to $\pm$ 0.21% and was, therefore, used in all further experiments.

3.1.2. Effect of Fe (III) concentration

From the result obtained with different concentrations of iron (III) in fixed concentrations of the other reagents, the peak-height absorbance of a 200 μl (50 μg ml^{-1}) sample solution was maximum at 0.01 M Fe (III) for cefadroxil and at 0.1 M for cefotaxime.

This concentration was chosen in optimizing the FI parameters for method A.

3.1.3. Effect of sulfuric acid concentration

Sulfuric acid was used as a solvent for both the *N*,*N*-DPPD and Fe (III) species, and the effect of its concentration was found to be critical. Therefore, it had to be carefully controlled, as changes in its concentration could easily have led to appreciable changes in the peak-height absorbance for the same cephalosporin solution. The results obtained with different concentrations of sulfuric acid as a solvent for fixed concentrations of *N*,*N*-DPPD and Fe (III) show that the peak-height absorbance of 200 μl (50 μg ml^{-1}) of sample solution increased for cefadroxil with 0.1 M acid as a solvent for *N*,*N*-DPPD and with 0.3 M acid for Fe (III). For cefotaxime it gave maximum peak height with 0.5 M acid as solvent for *N*,*N*-DPPD and with 0.1 M acid as solvent for Fe (III).

3.1.4. Effect of temperature

The peak-height absorbance of the ethylene blue formed was found to increase with increasing temperature. There the effect of changing the temperature in the range 40–80°C was studied using a reaction coil in a water bath. The results obtained indicate that the optimum temperature that must be chosen for further studies of both the cephalosporins was 70°C.

3.2. Influence of FI parameters for method A

3.2.1. Sample volume

Increasing the sample volume injected will lead to an increase in the sensitivity obtained until a steady-state signal is attained when the equilibrium reaches its maximum. For the manifold shown in Fig. 2, the influence of sample volume was evaluated for 50 μg ml^{-1} solutions of the cephalosporins. An increase in the peak-height absorbance was obtained by increasing the injected volume up to 200 μl, above which the increase in absorbance was not significant. This sample volume was, therefore, considered to be the optimum.

3.2.2. Reaction coil length

By using the FI parameters shown in Fig. 2, the effect of reaction coil length on the peak-height absorbance of a 50 μg ml^{-1} sample solution was evaluated. Various coils with increasing lengths were investigated (50–400 cm). An increase in *L* decreases the peak-height absorbance, due to the increasing sample dispersion, and consequently reduces the sample throughput. The maximum peak-height absorbance was obtained when the coil length was 50 cm for both the drugs. Therefore, a 50 cm reaction coil was chosen as the optimum to ensure high reproducibility of mixing of the sample with the reagents, high sensitivity (detection limits were 0.036 and 0.048 μg ml^{-1} by method A and 0.4 and 0.2 μg ml^{-1} by method B for cefadroxil and cefotaxime, respectively) and a high measurement rate (1.5 and 1 min per sample for method A and method B, respectively, which is the minimum time per sample).

3.2.3. Effect of flow-rate

The flow-rate is conveniently controlled by the peristaltic pump. The influence of total flow-rate was evaluated, at a flow-rate ratio (sample to reagent) of 1:1 by increasing the pump speed over the range 3.2–8.4 ml min^{-1}, keeping all other conditions constant and injecting a 50 μg ml^{-1} sample solution. The results obtained are summarized in Table 1 and indicate that the maximum absorbance was obtained at a total flow-rate of 5.3 ml min^{-1}; hence, this was selected as the optimum. It was known that when the flow-rates in all the channels are similar, reproducible and fast mixing is achieved in the T-junction [15].

Table 1
Summary of the parameters used for the determination of cefadroxil and cefotaxime by the proposed methods

Parameter	Method A		Method B	
	Cefadroxil	Cefatoxime	Cefadroxil	Cefatoxime
Reagents used	*N*,*N*-DPPD and Fe (III)		PPDD and Fe (III)	
Medium	sulfuric acid		aqueous medium	
Color produced	ethylene blue-like dye		violet color	
λ_{max} (nm)	670		597	
Reaction variables				
Conc. of reagents	0.01 M *N*,*N*-DPPD	0.01 M *N*,*N*-DPPD	0.01 M DPPD	0.01 M DPPD
Conc. of Iron (III) (M)	0.01	0.1	0.01	0.01
Sulfuric acid as solvent for				
N,*N*-DPPD (M)	0.1	0.5	aqueous	aqueous
Fe (III) (M)	0.3	0.1	aqueous	aqueous
Temperature (°C)	70	70	r.t.	
FI parameters				
Sample volume (μl)	200	200	50	50
Reaction coil length (cm)	50	50	50	50
Flow-rate (ml min^{-1})	5.3	5.3	3	3
Sampling rate (h^{-1})	40	40	60	60
Dispersion	1.46	1.46	1.76	1.76

3.3. Influence of reaction variables for method B

3.3.1. Effect of PPDD concentration

The dependence of the absorbance upon the PPDD concentration was examined in the range of 1×10^{-5}–1×10^{-1} M. The results show that the peak-height absorbance exhibits a maximum at 1×10^{-2} M for both the cephalosporins, and so it is selected for the analysis.

3.3.2. Effect of Fe (III) concentration

The optimization results for Fe (III) concentration indicate that the optimum Fe (III) concentrations for cefadroxil and cefotaxime are 1×10^{-2} M.

3.4. Influence of FI parameters for method B

3.4.1. Sample volume

The results obtained showed a decrease in the absorbance intensity with an increase in sample volume. The results obtained are summarized in Table 1. It was found that the 50 μl sample volume gave better sensitivity. Therefore, it was chosen in optimizing the FI parameters.

3.4.2. Effect of flow-rate

The flow system was operated using different flow-rates, from 2.5 to 6.0 ml min^{-1}. The best absorbance was obtained at a lower flow-rates, i.e. 3 ml min^{-1}, for both the drugs. Therefore, it was adopted for cefadroxil and cefotaxime determination.

3.5. Determination of cefadroxil and cefotaxime

Calibration graphs for the two methods were established under the optimized experimental conditions shown in Figs. 2 and 3. The calibration graphs were found to be linear. The calculated linear equations are presented in Table 2.

3.5.1. For method A

Beer's law was obeyed over the range 36.34–109.2 μg ml^{-1}, with slope 2.3×10^{-3} and correlation coefficient 0.999, with percentage recovery 100.4% for cefadroxil. It was also obeyed in the range 95.48–477.4 μg ml^{-1}, with slope 0.0015 and correlation coefficient 0.997, with recovery percentage 100.3% for cefotaxime Fig. 3.

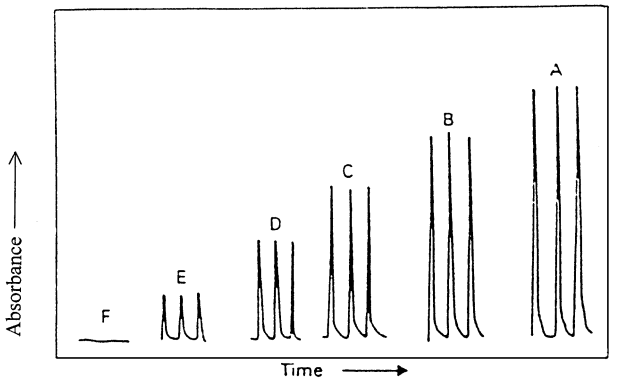

Fig. 3. Typical calibration peaks for the determination of cefotaxime by method A. The concentrations are: A, 477.4 μg ml^{-1}; B, 381.92 μg ml^{-1}; C, 286.44 μg ml^{-1}; D, 190.96 μg ml^{-1}; E, 95.48 μg ml^{-1}; F, 0.004 μg ml^{-1}.

Table 2
Performance of the results for the determination of cefadroxil and cefotaxime by the proposed methods

Result	Method A		Method B	
	Cefadroxil	Cefatoxime	Cefadroxil	Cefatoxime
Linearity range (μg ml^{-1})	36.34–109.20	95.48–477.40	0.5–400	0.5–450
Detection limits (μg ml^{-1})	0.036	0.048	0.4	0.2
Regression equation	$A = 0.0023C + 0.012$	$A = 0.0015C + 0.0147$	$A = 0.009C + 0.023$	$A = 0.007C + 0.016$
$S_{y/x}$ [a]	0.02	0.0202	0.01815	0.0201
S_a [b]	3.62×10^{-3}	2.80×10^{-3}	2.645×10^{-4}	1.698×10^{-4}
S_b [c]	1.433×10^{-4}	6.690×10^{-5}	5.27×10^{-5}	5.158×10^{-5}
Correlation coefficient	0.999	0.997	0.994	0.998
RSD (%)	0.83	0.77	0.90	0.80

[a] Standard deviation of the residuals.
[b] Standard deviation of the intercept.
[c] Standard deviation of the slope.

Table 3
Determination of cefadroxil in a commercial formulation as a capsule by the proposed (P_B) and Badway et al. (B) methods

Formulation	Amount of cefadroxil (μg ml^{-1})			% Recovery ± SD	
	Claimed	Found [a]			
		P_B	B	P_B	B
Cefadroxil Ultracef-capsules (500 mg) (Bristol)					
Sample 1	50.0	50.0	50.0	100.0 ± 0.7	100.0 ± 0.5
Sample 2	100.0	98.0	100.5	98.0 ± 0.9	100.5 ± 1.0
Sample 3	150.0	151.0	152.0	100.7 ± 0.2	101.3 ± 0.5

[a] Average of four determinations per sample.

Table 4
Determination of cefotaxime in a commercial formulation as an injection by the proposed automated methods (P_B) and Abdel-Khalek et al. (A) methods

Formulation	Amount of cefotaxime (μg ml^{-1})			% Recovery ± SD	
	Claimed	Found [a]			
		P_B	A	P_B	A
Cefotaxime Claforan i.v. injection (1 g)					
Sample 1	60.0	60.5	59.0	100.8 ± 0.9	98.3 ± 1.2
Sample 2	120.0	118.5	120.5	988 ± 0.7	100.4 ± 05
Sample 3	180.0	179.0	180.0	99.4 ± 0.3	100.0 ± 0.4

[a] Average of four determinations per sample.

It was possible to detect the drugs at as low as 0.036 (cefadroxil) and 0.0048 μg ml^{-1} (cefotaxime). The RSD values are 0.83% (50 μg ml^{-1}) and 0.77% (100 μg ml^{-1}) for cefadroxil and cefotaxime, respectively.

3.5.2. For method B

Under the mentioned optimized experimental conditions, the calibration graph was found to be linear over the range 0.5–400 μg ml^{-1}, with slope 0.009 and correlation coefficient 0.994, with percentage recovery 100.04% for cefadroxil. It was found to be linear over the range 0.5–450 μg ml^{-1}, with slope 0.007 and correlation coefficient 0.998, with percentage recovery 99.98% for cefotaxime. The detection limits obtained were 0.4 and 0.2 μg ml^{-1} for cefadroxil and cefotaxine, respectively. The RSD values are 0.9% (50 μg ml^{-1}) and 0.8% (100 μg ml^{-1}) for 1×10^{-4} M cefadroxil and cefotaxime, respectively (10 replicate injections) (Table 2).

The sampling rate was 40 h^{-1} with a dispersion of 1.76 and 60 h^{-1} with a dispersion of 1.46 for methods A and B, respectively, Table 1.

Table 5
Determination of cefadroxil in Ultracef-capsules by the proposed automated methods (P_A, P_B) and the method of Badawy et al. (B) compared with the B.P. method

	P_A	P_B	B	B.P.
Mean [a] ± SD	99.89 ± 0.68	99.60 ± 0.62	100.6 ± 0.66	100.23 ± 0.92
RSD	0.43	0.39	0.41	0.87
t [b]	0.713	1.337	1.060	(2.306) tabulated
F [b]	4.12	5.01	4.32	(9.01) tabulated

[a] Average of four determinations per sample for all methods and six determinations for the B.P. method.
[b] Theoretical values of t and F at $P = 0.05$ are 2.306 and 9.01, respectively.

Table 6
Determination of cefotaxime in Claforan i.v. injection by the proposed automated methods (P_A/P_B) and the Abdel-Khalek et al. (A) method, compared with the B.P. method

	P_A	P_B	A	B.P.
Mean [a] ± SD	99.28 ± 0.80	99.67 ± 0.68	99.57 ± 0.77	99.72 ± 0.955
RSD	0.517	0.431	0.489	0.910
t [b]	0.861	0.121	0.373	(2.306) tabulated
F [b]	3.146	4.479	3.506	(9.01) tabulated

[a] Average of four determinations per sample for all methods and six determinations for the B.P. method.
[b] Theoretical values of t and F at $P = 0.05$ are 2.306 and 9.01, respectively.

The proposed methods were also evaluated by analyzing some commercial formulations of cefadroxil and cefotaxime and comparing the results with those obtained using other spectrophotometric methods of the assay of cefadroxil and cefotaxime reported by Badawy et al. [11] and Abdel-Khalek and Mahrous [14], respectively. The results are accurate and precise, as indicated by the percentage recovery and RSD (Tables 3 and 4).

Application of the t- and F-tests at $P = 0.05$ showed no significant difference in accuracy and precision between the automated method, the Badawy method and the Abdel-Khalek method when compared with the official B.P. method [16], (Tables 5 and 6).

4. Conclusions

The FI methods described above for the determination of cefadroxil and cefotaxime in aqueous solutions, based on the formation of ethylene blue color or a violet color, provide precise, sensitive, simple and rapid techniques that satisfy most of the requirements in routine analysis.

References

[1] Reynolds J.E.F., Prasad A.B., Martindale the Extra Pharmacopoeia, 28th ed., Pharmaceutical Press, London, 1992.
[2] C. Shabadi, B.A. Shelar, A.R. Shelar, Simultaneous determination of cefadroxil and cephalexin in pharmaceutical preparation by quantitative TLC, Indian Drugs 35 (1998) 766–770.
[3] J.E. Aguero, F. San-Marino, Validation of a high-performance chromatographic method for determination cefotaxime in biological samples, J. Anal. Chem. 363 (1999) 289–293.
[4] V. Shinde, C. Shabadi, Simultaneous determination of cefadroxil and cephalexin from capsules by reverse phase HPLC, Indian Drugs 34 (1997) 399–402.
[5] L. Nuevas, R. Gonzalez, J. Rodrigue, J. Hoogmartens, Derivative spectrophotometric determination of the triethylammonium salt of cefotaxime in presence of related compounds, J. Pharm. Biomed. Anal. 18 (1998) 579–583.
[6] G. Saleh, Two selective spectrophotometric methods for the determination of amoxicillin and cefadroxil, Analyst 12 (1996) 641–645.
[7] J. Yang, G. Zhou, X. Cao, Q. Ma, J. Dong, Study on the fluorescence characteristics of alkaline degradation of cefadroxil, cefotaxime sodium and amoxicillin, Anal. Lett. 31 (1998) 1047–1060.
[8] F. Aly, N. Alaraffaj, A. Alwarthan, Permanganate based chemiluminscence analysis of cefadroxil monhydrate in pharmaceutical samples and biological fluids using flow-injection, Talanta 47 (1998) 471–478.
[9] N. Yilmaz, I. Biryal, Anodic voltammetry of cefotaxime, J. Pharm. Biomed. Anal. 17 (1998) 1335–1344.
[10] P.B. Issopoulos, Spectrophotometric determination of certain cephalosporins using molybdophosphoric acid, Analyst 114 (1989) 237–239.
[11] S.S. Badawy, F.M. Abdel-Gawad, M.M. Ibrahim, Spectrophotometric studies on determination of cefadroxil with copper (II) and vandium (V) in sulfuric acid medium, Anal. Lett. 26 (1993) 487.
[12] A. Alwarthan, F.H. Metwally, S.A. Al-Tamimi, Spectrophotmetric assay of certain cephalosporins based on formation ethylene blue, Anal. Lett. 26 (1993) 2619.
[13] A.A. Alwarthan, F.H. Metwally, S.A. Al-Tamimi, Spectrophotmetric determination of cefotaxime and cefadroxil by alkaline degradation to hydrogen sulfide, Arab Gulf J. Sci. Res. 13 (1995) 213.
[14] M.M. Abdel-Khalek, M.S. Mahrous, Use of ammonium molybdate in colorimetric assay of cephalosporins, Talanta 31 (1984) 635.
[15] A. Alwehaid, Flow injection spectrophotometric determination of 4-aminophenazone based on diazotisation and coupling reactions, Analyst 115 (1990) 1419–1422.
[16] British Pharmacopeia, HMSO, London, 1980, pp. 86, 87.