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Spectrophotometric and Atomic Absorption Spectrometric Determination of Cephalexin and Cephradine in Dosage Forms

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ABSTRACT

This paper describes two simple, sensitive and selective spectrophotometric and atomic absorption spectrometric (AAS) procedures for the determination of two compounds of cephalosporins (Cephalexin monohydrate and Cephradine). These procedures are based on the formation of ion-pair complexes between the drugs and ammonium reineckate. The formed precipitates are quantitatively determined either spectrophotometrically or by AAS procedures. The methods consist of reacting drugs with Reinecke's salt in an acidic medium at a temperature of 25 ± 2°C. The spectrophotometric procedure (procedure I) is based on dissolving the formed precipitate with acetone. The volume was completed quantitatively and absorbance of the solution was measured at 525 nm against blank. Also, the AAS procedures (procedure II) are quantitatively determined directly or indirectly through the chromium precipitate formed or the residual unreacted chromium in the filtrate at 358.6 nm. The optimum conditions for precipitation have been carefully studied. Beer's law is observed for the studied drugs in the ranges 0.1-1.5 mg mL^{-1} or 5-70 μg mL^{-1} using spectrophotometric or AAS methods, with correlation coefficients ≥ 0.9965, respectively. Both procedures I and II were accurate and precise when applied to the analysis of the cited cephalosporins in different dosage forms with good recovery percent ranged from 98.90 ± 0.94 to 100.15 ± 0.97 without interference from additives.

Key words: cephalosporins, reinecke's salt, spectrophotometry, atomic absorption, dosage forms

INTRODUCTION

Cephalosporins are a major group of semi-synthetic β-lactam antibiotics used in clinical medicine for treatment of bacteria-related infections. They are closely related in fundamental structure and antibactericidal action mechanism to penicillins. They are used for the treatment of infections caused by both gram-negative and gram-positive bacteria. Cephalosporins are among the oldest and most frequently prescribed naturally occurring antimicrobial agents[1]. Cephalosporins were determined by titrimetric[2-4], spectrophotometric[5-17], fluorimetric[18,19], chemiluminescence[20,21], chromatographic[22-26], potentiometric[27], and voltammetric[28,29] methods.

Reineck's salt is ammonium tetrathiocyanotodiamminochromate (III) monohydrate which can be used for quantitative determination of many pharmaceutical compounds applying titrimetric[30] and spectrophotometric[31,32] procedures.

This paper describes the development of two simple and accurate spectrophotometric procedures. Furthermore, a selective and sensitive atomic absorption spectrometric (AAS) procedure was used for the analysis of cephalexin and cephradine in the pure form as well as in pharmaceutical preparations.

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MATERIALS AND METHODS

I. *Apparatus*

– A Shimadzu UV1601, UV-visible spectrophotometer (Tokyo, Japan).

– A Shimadzu atomic absorption flame spectrophotometer model AA.640-13 with a chromium hollow cathode lamp under the following observations: height above burner 1 cm; single slot type burner; air flow-rate 21.51/min; acetylene flow-rate 3.41/min operation conditions: lamp current 29 mA; slit width 0.7 nm; wavelength current 29 mA; slit width 0.7 nm; wavelength 358.6 nm.

II. *Materials and Reagents*

All solvents and reagents were of analytical grade. Double distilled water was used to prepare all solutions. Pharmaceutically pure Cephalexin monohydrate (Arab

Drug Co., Cairo, Egypt), and Cephradine (Sigma Chemical Co., St. Louis, USA) were used as working standards.

(I) *Pharmaceutical Preparations*

The following commercial dosage forms were subjected to the analytical procedure.

– Ceporex® tablets (Glaxo Wellcome, S.A.E., Cairo, Egypt), labeled to contain 250 mg cephalexin monohydrate per tablet.

– Cephalexin® capsules (Army Factory of Drugs, Cairo, Egypt), labeled to contain 250 mg cephalexin monohydrate per capsule.

– Velosef® capsules (Bristol Myers-Squibb Pharmaceutical Co., Cairo, Egypt) containing Cephradine equivalent to 250 mg per capsule.

– Velosef® vials (Bristol Myers-Squibb Pharmaceutical Co., Cairo, Egypt) containing Cephradine equivalent to 250 mg per vial.

– Stock solutions 5.0 mg mL^{-1} were prepared by accurately weighing (0.5 g) of the examined pure drug into a 100-mL calibrated flask, dissolved in double distilled water and kept in the dark to avoid degradation of the drugs.

(II) *Reagent*

Stock solution, 5×10^{-3} M ammonium reineckate (Aldrich product) solution was also prepared by dissolving the appropriate weight in 100 mL of double distilled water.

III. *Procedures*

(I) *Procedure I (Spectrophotometric Procedure)*

An aliquot containing 1.00–15 mg of the investigated drugs was transferred into a 10-mL calibrated flask; 3.0 mL of 5×10^{-3} M of ammonium reineckate and 1.0 mL of 0.01 M HCl were added successively. The mixture was left to stand for 10 min and completed the volume with water. The precipitate was filtrated through a sintered glass funnel (G4) after 1 hr, and washed three times with 3 mL of ice water. Afterwards, the precipitate was dried in a vacuum desiccator. The formed precipitate in the crucible was dissolved with acetone into a 10-mL volumetric flask together with the successive washings of the funnel and filtration device. The volume was completed quantitatively with acetone to the appropriate volume. Absorbance of the solutions was measured at 525 nm, against a reagent blank solution prepared in the same way without the drug. The calibration graph was obtained by applying the procedure and using standard drug solutions.

(II) *Procedure II (Atomic Absorption Procedure)*

1. *Direct Procedure*

The above (drug-reineckate) precipitates were:

(1) Collected on a G4 sintered glass crucible and washed with five 2 mL portions of ice water.

(2) Dissolved in 25 mL of acetone.

The solution was then nebulized in an air–acetylene flame of AAS measurement of chromium ion concentration at 358.6 nm. Concentration of the tested drug was calculated from the relevant calibration graph.

2. *Indirect Procedure*

The filtrate and washings from the direct procedure were:

(1) Collected in a 100-mL volumetric flask; and

(2) Completed to volume with acetone.

The resulting solution (2 mL) was diluted to 25 mL with acetone. A blank (omitting addition of drugs) was prepared and absorbance was measured at the above flaming conditions. Chromium concentration was calculated from a calibration curve.

IV. *Preparation of Samples*

(I) *Tablets*

Twenty tablets of the drug were thoroughly ground. A quantity equivalent to 50 mg of drug was accurately weighed into a 100-mL volumetric flask, completed to volume with water and filtered per method I or II.

(II) *Capsules*

The contents of 20 capsules were weighed and finely ground to powder. A portion of the powder, equivalent to 50 mg of drug, was dissolved in water (by shaking for 5 min) and filtered when necessary. The solution was completed to 100 mL with distilled water and the procedure was completed twice per method I or II.

(III) *Vials*

An accurately measured weight of vials equivalent to 50 mg of drug was dissolved in water (by shaking for 5 min) and filtered when necessary. The solution was completed to 100 mL with water and the procedure was completed per procedure I or II.

RESULTS AND DISCUSSION

Mixing each aqueous solution of cephalexin monohydrate or cephradine with ammonium reineckate in acidic medium at 25 ± 2°C resulted in the formation of purple precipitate. The precipitate is based on the formation of ion-pair complexes between the drugs and ammonium reineckate. Formation of drug-reineckate ion-pair complexes allows indirect determination of these drugs by atomic absorption spectrometric measurement of the chromium content of the reineckate counter anion.

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For the spectrophotometric procedure (procedure I), the absorption spectrum of the reaction products was measured at 525 nm (Figure 1). For the atomic absorption spectrometric procedure (procedure II), acidic solutions of the drugs yielded purple coagulated precipitates with ammonium reineckate. These precipitates form the basis of the micro-quantitative determinations of the cited cephalosporins. The chromium ion content could be determined either directly in the precipitate or indirectly in the filtrate by atomic absorption spectrometry.

The different variables that affect the spectrophotometric (procedure I) and atomic absorption spectrometric (procedure II) determinations of all cephalosporins with ammonium reineckate were optimized.

I. *Optimization of Experimental Parameters*

(I) *Type and Concentration of the Acid*

The cited cephalosporins reineckate salts were prepared using the same concentration of the drug and the ammonium reineckate, while varying the type and amounts of the acid. The absorbencies of the final salt solutions in the appropriate solvent were taken as a measure of better precipitation.

Different acid media was used to increase the intensity of the formed precipitated ion-pair. Sulfuric, phosphoric, hydrochloric and acetic acids were tested. The optimum concentration was hydrochloric acid of 0.01M concentration, since the results are highly concordant at this media. Moreover, 1.0 mL of 0.01 M HCl added to 10 mL gave the best results.

(II) *Ammonium Reineckate Concentration*

The general procedure was applied using different concentrations of the reagent, while the cited cephalosporins and acid concentrations were kept constant. The absorbencies of the final salt solutions in the appropriate solvent were taken as a measure of better precipitation.

Experiments were carried out in which the volume was kept constant at 10 mL while the concentration of reagent was increased; revealed that 3.0 mL of 5×10^{-3} M is the optimum concentration (Figure 2). The excess reagent used is probably a result of dissociation in aqueous medium as fraction of the ion-pair formed.

(III) *Temperature*

The effect of temperature upon the formation and solubility of ion associates was investigated at different temperature levels (25, 35, 45, and 60°C). The results show that the studied drugs are best determined at room temperature (25 ± 2°C). This is because increasing temperature increases the solubility where the process of dissolution of the precipitates is endothermic. Furthermore, the lattice energy is usually greater than the solvation energy and hence the stability of ion associates decreases.

(IV) *Reaction Time*

A series of flasks containing equal concentrations of the cephalosporin was analyzed using the corresponding standard procedure, but filtering the precipitate after various time intervals. Absorbance of the final cephalosporins-reineckate solution in the appropriate solvent was taken as a measure for the best precipitation time. One hour was

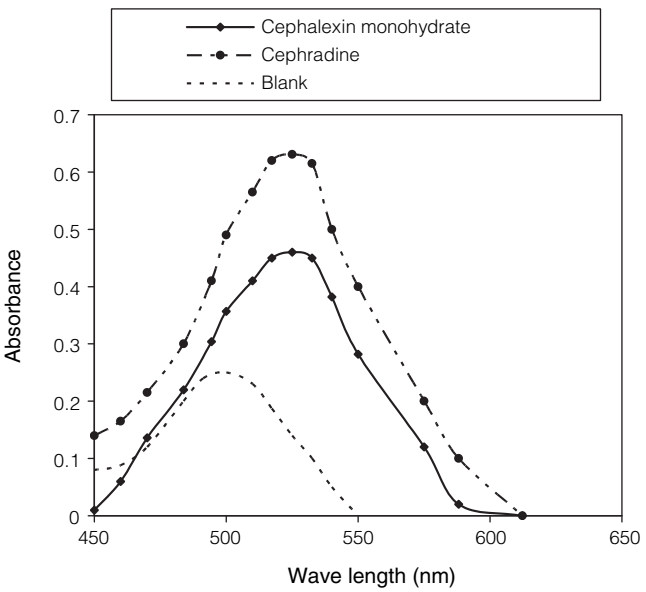


Figure 1. Absorption spectra of the complex formed through reaction of 1.0 mg mL^{-1} of the studied drugs with ammonium reineckate blank.

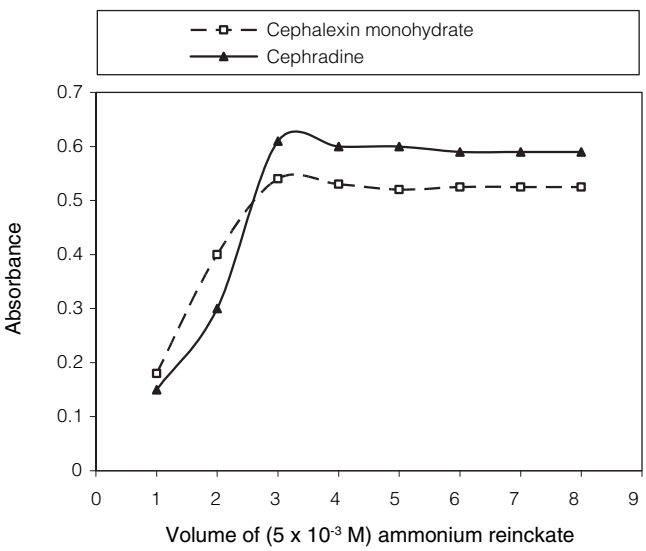


Figure 2. Effect of (5×10^{-3} M) ammonium reineckate on the absorbance of the complex formed with 1.0 mg mL-1of the studied drugs.

found to be sufficient for complete precipitation of cephalosporins-reineckate standing at a temperature of 25°C.

(V) *Solubility, Washing Liquid and Stability of the Precipitated Reineckates*

Trials to find out the best solvent to dissolve cephalosporins-reineckate precipitate were performed using distilled water, acetone, dioxane, methanol, ethanol, and propanol. The washing liquid of choice was ice water. Acetone was found to be the best solvent for dissolving the precipitated ion-pair formed in aqueous acidic media. Then the stability of the colour of cephalosporins-reineckate acetone solution was found to be stable for at least 24 hr and was examined periodically at different time intervals.

II. *Analytical Performance*

(I) *Beer's Law*

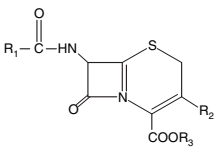
Standard curves were constructed by plotting the observed absorbency readings versus concentrations of cephalosporins in mg mL^{-1} of the final solution. Conformance to Beer's law was evident. Plots show good linearity with high correlation coefficients (Table 2). Beer's law is valid within the concentration range 0.1-1.2 and 0.2-1.5 mg mL^{-1} for cephalexin monohydrate and cephradine, respectively. For more accurate analysis, Ringbom optimum concentration range was calculated to be 0.25-1.1 and 0.3-1.3 mg mL^{-1} for cephalexin monohydrate and cephradine, respectively. The molar absorptivity, Sandell sensitivity, detection, and quantification limits were calculated (Table 2).

The reproducibility of the proposed methods was assessed by running six replicate samples, each containing 600 μg mL^{-1} using the spectrophotometric method of the studied drugs in the final assay solution. The relative standard deviations were calculated (Table 2).

For AAS method, calibration graphs with good linearity were obtained (Table 3). The linear regression equations were also calculated. A correlation coefficient, y-intercept, and slope for the calibration data were calculated using the least squares method. Detection and quantification limits were also evaluated (Table 3).

Statistical analysis of the results obtained by the proposed procedures (I and II) compared with those of the official method (based on Liquid chromatography)[33] are given in (Table 4) showed comparable accuracy (*t*-

Table 1. Structure of the studied cephalosporins



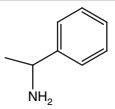
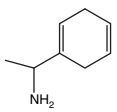
Name	R_1	R_2	R_3
Cephalexin monohydrate		$-CH_3$	H
Cephradine		$-CH_3$	H

Table 2. Spectral characteristics and precision data

Parameter	Cephalexin monohydrate	Cephradine
Beer's law limits (mg mL^{-1})	0.1-1.2	0.2-1.5
Ringbom range (mg mL^{-1})	0.25-1.10	0.3-1.3
Stability of ion-pair (hr)	36	48
Molar absorptivity × 10^2 (L mol^{-1} cm^{-1})	2.733	1.5713
Sandell sensitivity (μg cm^{-2})	1.337	2.224
Detection limits (μg mL^{-1})	12.18	6.96
Quantification limit (μg mL^{-1})	40.60	23.20
Repeatability (RSD %)	0.440	0.4656
Regression equation[a]		
Slope (a)	0.4816	0.5076
Intercept (b)	0.0852	-0.0282
Correlation coefficient (r)	0.9965	0.9991

[a] $A = a + bC$, where C is the concentration in μg mL^{-1}.

Table 3. Analytical characteristic of the AAS procedure

Drug	Procedure	Conc. Range (μg mL^{-1})	*a*	*b*	R	C.V. (%)	LOD (μg mL^{-1})	LOQ (μg mL^{-1})
Cephalexin monohydrate	Direct AAS	5-50	0.1151	0.9852	0.9996	0.544	1.66	5.53
	Indirect AAS	10-60	- 0.783	1.0151	0.9992	0.352	1.04	3.47
Cephradine	Direct AAS	5-50	0.6502	0.9721	0.9997	0.61	1.88	6.28
	Indirect AAS	10-70	0.2467	0.9984	0.9993	0.52	1.56	5.21

a, intercept; b, slope; R, correlation coefficient; C.V.%, coefficient of variance; LOD, detection limits; LOQ, quantification limit.

Table 4. Statistical analysis of the results obtained using the proposed procedures and reference method for analysis of authentic samples

Drug		Spectrophotometric procedure	AAS procedure		Official method[33]
			Direct	Indirect	
Cephalexin monohydrate	X ± SD	100.17 ± 0.403	99.39 ± 0.541	100.04 ± 0.352	99.80 ± 0.40
	V	0.162	0.293	0.124	0.16
	t-test	1.45	1.36	1.01	
	F-test	1.015	1.83	1.29	
Cephradine	X ± SD	99.785 ± 0.465	99.77 ± 0.609	99.96 ± 0.521	99.20 ± 0.60
	V	0.216	0.371	0.271	0.36
	t-test	1.74	1.49	2.14	
	F-test	1.67	1.03	1.33	

Number of experiments (n) = 6; X, mean of recovery; *V*, variance; tabulated (*t*) = 3.85; tabulated (*F*)= 4.28 for five degree of freedom and 95% confidence limit.

Table 5. Determination of cephalosporins in their pharmaceutical preparations by the proposed and official methods

Dosage form		Spectrophotometric procedure	AAS procedure		Official method[33]
			Direct	Indirect	
Cephalexin® capsules (250 mg/capsule)	*X ± SD*	99.45 ± 0.81	99.25 ± 0.69	99.55 ± 0.84	98.52 ± 0.73
	V	0.656	0.476	0.706	0.533
	t-test	1.91	1.63	2.07	
	F-test	1.23	1.12	1.32	
Ceporex® tablets (250 mg/tablet)	*X ± SD*	99.25 ± 0.64	100.10 ± 0.59	99.94 ± 0.66	99.70 ± 0.71
	V	0.41	0.348	0.436	0.504
	t-test	1.05	0.969	0.554	
	F-test	1.23	1.45	1.15	
Velosef® capsules (250 mg/capsule)	*X ± SD*	98.90 ± 0.94	99.45 ± 0.87	100.15 ± 0.97	99.82 ± 1.02
	V	0.88	0.757	0.941	1.04
	t-test	1.484	0.534	0.524	
	F-test	1.182	1.374	1.11	
Velosef® vials (250 mg/vial)	*X ± SD*	99.50 ± 0.69	99.74 ± 0.75	99.90 ± 0.82	100.21 ± 0.86
	V	0.48	0.563	0.672	0.74
	t-test	1.44	0.981	0.583	
	F-test	1.54	1.31	1.10	

Number of experiments (n) = 6; X, mean of recovery; *V*, variance; tabulated (*t* = 3.85); tabulated (*F* = 4.28) for five degree of freedom and 95% confidence limit.

test) and precision (*F*-test), since the calculated values of *t*- and *F*-tests at 95% confidence limits for five degrees of freedom were less than the theoretical data[34].

(II) *Stoichiometric Relationships*

For the atomic absorption spectrometric method, Job's method of continuous variation[35] indicated a molar ratio of 1:2 drugs to reineckate.

(III) *Reaction Equation*

Cephalosporin hydrochloride + ammonium reineckate $\overset{HCl}{\rightleftharpoons}$ ammonium chloride + cephalosporin reineckate

Cephradine + $2NH_4$ [Cr $(NH_3)_2(SCN)_4$] $\overset{0.01\ M\ HCl}{\rightleftharpoons}$ [Cephradine] $^{+2}$ [Cr $(NH_3)_2(SCN)_4]_2$ $^{-2}$ + $2NH_4Cl$

III. *Analytical Applications*

The proposed procedures were applied to determine the cephalosporins in their pharmaceutical formulations. The results indicate high accuracy and precision (Table 5). As can be seen from Table 5, the proposed methods have the advantages of being virtually free from interferences by excipients such as glucose, lactose, and starch or from common degradation products. The results obtained were compared statistically by the student's *t*-test (for accuracy) and the variance ratio *F*-test (for precision) with those obtained by the pharmacopoeial method[33] on samples of the same batch (Table 5). The values of *t*- and *F*-tests obtained at 95% confidence level and five degrees of freedom did not exceed the theoretical tabulated value, indicating lack of significant difference between the methods compared.

CONCLUSIONS

The developed procedures are simple, sensitive and accurate for the determination of two cephalosporins (Cephalexin monohydrate and Cephradine). The methods consist of reacting drugs with Reinecke's salt in an acidic medium at 25 ± 2°C. The spectrophotometric procedure (procedure I) is based on dissolving the formed precipitate with acetone, where the volume was completed quantitatively and the absorbance of the solution was measured at 525 nm against blank.

On the other hand, the formed precipitates on the atomic absorption spectrometric procedure (procedure II) are quantitatively determined directly or indirectly through the chromium precipitate formed or the residual unreacted chromium in the filtrate at 358.6 nm.

Beer's law is obeyed by the studied drugs in the ranges 0.1-1.5 mg mL^{-1} or 5-70 μg mL^{-1} using spectrophotometric or AAS methods, with correlation coefficients ≥ 0.9965, respectively. In addition, both procedures I and II are selective and suitable for routine quality control.

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