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Quantitative determination of some thiazole cephalosporins through complexation with palladium (II) chloride

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Abstract

A simple and sensitive spectrophotometric method has been developed for the determination of five cephalosporins namely cefpodoxime (CFPD), ceftizoxime (CTIZ), ceftazidime (CZD), ceftriaxone (CTRX), and cefixime (CXIM). This method is based on the formation of yellow to yellowish brown complex between palladium (II) chloride and the investigated cephalosporins in the presence of sodium lauryl sulphate (SLS) as surfactant. The reaction conditions were studied and optimized. The procedure was validated. For each drug, the composition of this complex as well as its stability constant were also investigated. The proposed method was used for the determination of the above-mentioned drugs in their commercial preparations. The results were compared statistically with either official or published methods and showed no significant difference between the two methods.

Keywords: Cephalosporins; Palladium (II) chloride; Spectrophotometry; Chelate complex; Tablets; Vials

1. Introduction

Cefpodoxime (CFPD), ceftizoxime (CTIZ), ceftazidime (CZD), ceftriaxone (CTRX) and cefixime (CXIM) represent some of the semi-synthetic third generation cephalosporins. Being the drugs of choice in Gram-negative bacillary meningitis, CXIM and CFPD are administered orally and are used in the treatment of UTI, soft-tissue infections, gonorrhea, otitis media and upper and lesser respiratory tract infections. Parenterally, CZD, cefoperazone, CTIZ and CTRX have the same action as the oral drugs besides to their use in infections in the immunocompromised patients, serious and life-threatening infections such as brain abscess and gastroenteritis [1].

The USP 23 [2] gives four HPLC procedures for the determination of CTIZ, CZD CTRX and CXIM. While BP [3] describes two HPLC methods for the assay of CTRX and CXIM. CFPD is not official in both pharmacopoeias.

Several methods for the quantitative measurement of these antibiotics in pharmaceutical preparations and biological fluids have been described. They utilized such techniques as colorimetry [4], spectrophotometry [5], differential pulse polarography [6,7], HPTLC [8] and HPLC [9–12]. CTRX

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and CZD were determined in aqueous and biological media using cathodic-stripping voltammetry [13–15]. Some of them were also determined colorimetrically by complexation with metals [16].

The use of palladium (II) chloride as a complexing agent for the quantitation of drugs is fairly wide. The chelate complex of palladium (II) ions bears the advantage of being water-soluble, and hence does not necessitate any extraction procedure. Several drugs were determined spectrophotometrically by measuring the color intensity of the complex formed between their molecules and palladium (II) ions, e.g. captopril [17], metoclopramide [18], prazosin [19], norfloxacin and ciprofloxacin [20] and propylthiouracil [21].

Some of the published methods suffer interference from tablet base, while others are not simple for routine analysis as they need sophisticated instruments and expensive reagents, not yet available in many control laboratories. Therefore, it was considered worthwhile to develop a rapid, simple and accurate procedure suitable for application in quality control laboratories.

This paper reports a simple, sensitive and accurate spectrophotometric method for the determination of five thiazole-containing cephalosporins namely CFPD, CTIZ, CZD, CTRX and CXIM. The proposed method is based on the reaction between the investigated drugs and palladium (II) with the formation of a yellow to yellowish-brown chelate complex. The optimum conditions (volume of the reagent, pH, …, etc.) were established before the application of the method to the analysis of drugs as bulk or in different pharmaceutical preparations. The composition of this complex as well as its stability constant for each drug was also investigated. Other advantages of the method are cheapness and relative speed.

2. Experimental

2.1. Instrumentation

A Perkin-Elmer 550 double-beam spectrophotometer (Norwalk, CT, USA) with matched 10 mm quartz cells and attached to a Hitachi Model 561 recorder with a scan speed of 60 nm min^{-1} was employed for all absorbance measurements.

2.2. Materials and reagents

Pharmaceutical grade CFPD was obtained from Hoechst Orient SAE (Cairo, Egypt), UL of Roussel Uclaf (France) and was certified to contain 99.2%, CTIZ and CXIM were donated by Kahira Pharm. & Chem. Ind. Co. (Egypt), UL of Hikma Pharmaceuticals (Jordan) and were certified to contain 99.7 and 98.7%, respectively. CTRX was kindly supplied by Egyptian Int. Pharmaceutical Industries Co., EIPI Co. (ARE), in Co-operation with F. Hoffmann-La-Roche Ltd. (Basle, Switzerland) and was certified to contain 99.7%. CZD was obtained from Glaxo Operations UK Ltd. (Greenford, England) and was certified to contain 98.9%. They were used without further purification. Palladium (II) chloride solution was prepared as a 2×10^{-3} M solution by dissolving about 35.5 mg of palladium (II) chloride (Sigma, Milwaukee, WI, USA) in 1 ml of concentrated hydrochloric acid, with the aid of heat, followed by the addition of 50 ml of boiled water and diluting to 100 ml with water in a volumetric flask. A 0.2% w/v of sodium lauryl sulfate (SLS) (Cambrian Chemicals, Beddington, England) was prepared by dissolving 0.2 g of SLS in the minimum amount of water and diluted to 100 ml either with water or methanol (according to the procedure). Walpole's acetate buffer [22] was prepared by mixing different volumes of 0.2 M acetic acid with 0.2 M sodium acetate. The pH of the solutions should be adjusted by adding more of the 0.2 M acetic acid or the 0.2 M sodium acetate solution as required. The pharmaceutical preparations were obtained from the market. Drug for injection is essentially stable in dry state and can be stored at room temperature, but should be protected from light. When reconstituted with water for injection it is recommended that it should be used within 6 h if stored at room temperature and 24 h if kept in refrigerator [1].

2.3. Standard solutions

Solutions of 0.2 mg ml^{-1} were prepared by dissolving the equivalent of 20 mg of the studied cephalosporins in water (CZD, CTIZ and CTRX) or methanol (CFPD and CXIM) in five 100 ml volumetric flasks. Dilute to volume with the same solvent used.

2.4. Construction of the calibration graphs

To five sets of 10 ml volumetric flasks, appropriate volumes of 2×10^{-3} M palladium (II) chloride were transferred, then the specified volumes of Walpole's acetate buffer was added followed by the proper volumes of SLS (Table 1). Aliquots of the cephalosporins, within the concentration range cited in Table 1, were pipetted each into its corresponding set. Each set was allowed to stand in a thermostated water bath for the specified time and was diluted to volume with methanol for CFPD and water for the rest. The solution was then cooled to room temperature (about 25°C) and the intensity of the developed yellow color was measured at the corresponding λ_{max} against a blank solution similarly prepared but omitting the cephalosporins.

2.5. Assay of pharmaceutical preparations

2.5.1. For tablets (CFPD and CXIM)

Twenty tablets were powdered and a quantity of the powder equivalent to 20 mg of the free base was extracted by shaking with 60 ml methanol for 15 min with the aid of a magnetic stirrer. The mixtures were filtered through Whatman no. 41 filter-paper into a 100 ml volumetric flask and then diluted to volume with the same solvent. The procedure was completed as described in Section 2.4.

2.5.2. For vials (CTIZ, CZD and CTRX)

The power content of a vial was transferred quantitatively to a 100 ml volumetric flask with the help of about 3 ml water. The content was shaken to dissolve, then diluted to volume with methanol for CZD or with water for CTIX and CTRX to a final estimated concentration of 0.2 mg ml^{-1}. The procedure was completed as described in Section 2.4.

3. Results and discussion

Palladium (II) chloride reacts with five cephalosporins, namely CFPD, CTIZ, CZD, CTRX and CXIM with the formation of a yellow to yellowish-brown chelate complex.

The resulting chelate is possibly due to the complexation between palladium (II) ions and the sulfur atoms of the β-lactam and thiazole ring. The chelate was found to be soluble only in the presence of a surfactant. Several surfactants were tested and SLS was selected as it gave a good effect besides to its cheapness and availability. The absorption spectra of the complexes were recorded over the range of 300–500 nm and the absorption maxima are recorded in Table 1. Fig. 1 shows the absorption spectrum of CFPD–Pd complex as an example for these cephalosporins.

Table 1
Optimal conditions for the formation of the complex with five cephalosporins at the corresponding λ_{max}

Drug	Volume[a] (ml)	$PdCl_2$ volume (ml)	Buffer pH	Buffer volume (ml)	SLS volume[b] (ml)	Temperature (°C)	Time (min)	λ_{max} (nm)
CFPD	0.4–1.1	2	4.8	4	2	60	45	357
CTIZ	0.25–0.6	2	4.8	4	1	80	45	340
CZD	0.4–1.4	2	4.8	4	1	80	45	354
CTRX	0.2–0.7	2	4.8	4	1	100	45	344
CXIM	0.25–0.6	2	4.8	4	1	80	45	347

[a] Stock solutions were prepared as 0.02 mg ml^{-1}.
[b] SLS was prepared in water for all the cephalosporins except CFPD (methanol).

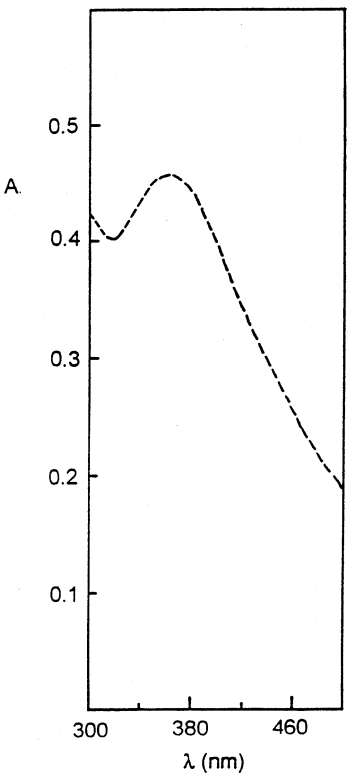

Fig. 1. Absorption spectrum of the complex of 14 μg ml^{-1} CFPD in its determination by complexation with Pd (II).

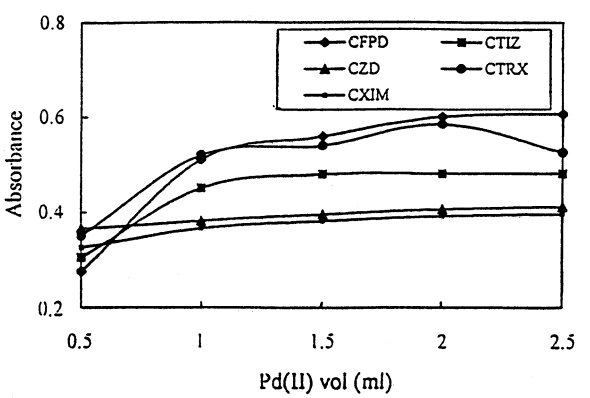

Fig. 2. Effect of palladium (II) chloride volume on the color intensity of the complexes of 40, 30, 20, 20 and 20 μg ml^{-1} CFPD, CTIZ, CZD, CTRX and CXIM, respectively.

3.1. Optimization of the conditions

An investigation of the complex formation showed that an increasing volume of palladium (II) chloride produced an increase in the absorbance of the complex till it reached a constant value or even decreased. A 2.0 ml volume was selected (Fig. 2). The effect of the pH of the acetate buffer on the color intensity of the complex was not critical, pH 4.8 was selected for all investigated drugs (Fig. 3a). Furthermore, the effect of optimal buffer volume was studied aiming to reach maximum absorbance (Fig. 3b). Fig. 4a, b show the effect of temperature and heating time on the color development. CFPD showed maximum absorbance at 60°C after 45 min where higher temperature showed either turbidity formation or blackening. Maximum color intensity for CTIZ was attained at 80°C after 45 min as boiling water-bath temperature showed the disappearance of the peak. A temperature of 80°C was selected for CZD and CXIM where heating was for 45 min. As for CTRX, heating in a boiling water-bath for 45 min gave the highest color intensity. Table 1 summarizes the optimal conditions for the complexation of each cephalosporin with palladium (II) chloride. The color of the complex was stable for at least 1 h.

3.2. Constitution of the complex

The composition of the complex was established by the continuous-variation method (Job's method) [23]. The plot reached a maximum at a fraction of 0.3 indicating the formation of 3:7 cephalosporin–palladium (II) complex (more likely 1:2) (Fig. 5). The stability constant (B) for each of the five drugs was calculated using the continuous variation method according to the following equation [24].

$$B = \frac{(A/A_{ex})C_x}{(C_M - A/A_{ex}C_x)(C_L - nA/A_{ex}C_x)^n}$$

where, B is stability constant; M, metal; L, ligand; x the molar fraction of the ligand; $n = x/1 - x$; A/A_{ex} the ratio of the observed absorbance to that indicated by the tangent for the same wavelength (extrapolated); C_x, C_M and C_L are the concentrations of the complex, the metal and the ligand, respectively.

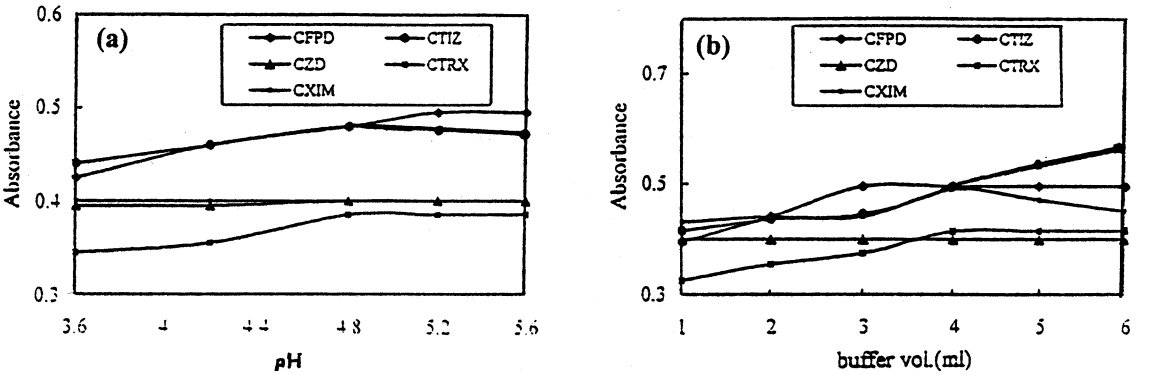

Fig. 3. Effect of (a) pH of acetate buffer and (b) buffer volume on 30, 30, 20, 14 and 20 μg ml^{-1} CFPD, CTIZ, CZD, CTRX and CXIM, respectively.

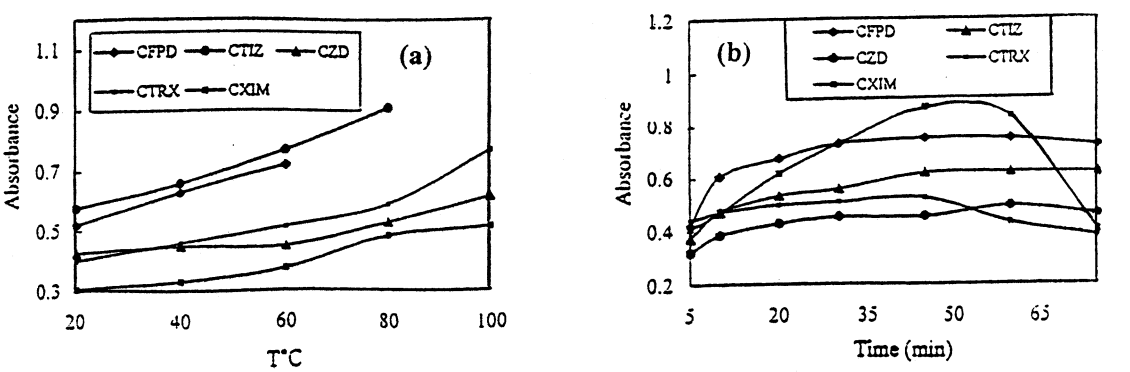

Fig. 4. Effect of (a) temperature on 30, 30, 20, 14 and 14 μg ml^{-1} CFPD, CTIZ, CZD, CTRX and CXIM, respectively (b) heating time on 20, 10, 15, 8 and 12 μg ml^{-1} CFPD, CTIZ, CZD, CTRX and CXIM, respectively.

The stability constant as well as its log complex are presented in Table 2 and indicate the high stability of the complexes.

3.3. Validation

A prospective validation protocol described by the USP 23 [2] for quantitation of active ingredients was applied for the proposed method.

3.3.1. Linearity of the method

Under the optimum conditions described above, the graphs obtained by plotting the ab-

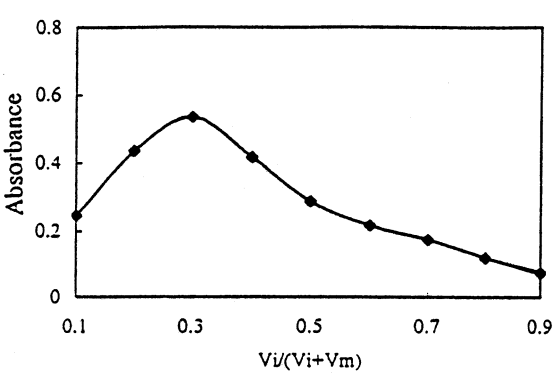

Fig. 5. Continuous variation graph of CFPD–palladium (II) chloride, (1×10^{-3} M).

Table 2
Optical characteristics and statistical data of the regression equations for the complexes of CFPD, CTIZ, CZD, CTRX and CXIM

Parameter	CFPD	CTIZ	CZD	CTRX	CXIM
Concentration range (μg ml^{-1})	8–22	5–12	8–28	4–14	5–12
Apparent absorptivity (l mol^{-1} cm^{-1})	1.27×10^{4}	2.53×10^{4}	1.64×10^{4}	3.39×10^{4}	2.63×10^{4}
Sandell's sensitivity (μg cm^{-2} 0.001 A^{-1})	3.25×10^{-2}	1.51×10^{-2}	3.33×10^{-2}	1.64×10^{-2}	1.72×10^{-2}
Regression equation					
Intercept (a)	7.03×10^{-3}	0.14	-3.28×10^{-3}	2.86×10^{-3}	0.11
Slope (b)	0.0307	0.0512	0.0300	0.0583	0.0470
Correlation coefficient (r)	0.9997	0.9992	0.9999	0.9997	0.9995
Variance (S_o^2)	1.60×10^{-5}	3.34×10^{-5}	1.40×10^{-5}	2.78×10^{-5}	1.74×10^{-5}
Detection limit	0.31	0.26	0.29	0.21	0.21
S.D. of intercept (S_a)	4.88×10^{-3}	7.93×10^{-3}	3.92×10^{-3}	6.01×10^{-3}	5.67×10^{-3}
S.D. of slope (S_b)	3.19×10^{-4}	8.97×10^{-4}	2.10×10^{-4}	6.30×10^{-4}	6.46×10^{-4}
S_b rel %	1.04	1.75	0.70	1.08	1.37
Stability constant (B)	3.25×10^{6}	3.31×10^{6}	5.48×10^{6}	1.16×10^{6}	3.41×10^{6}
Log B	6.51	6.52	6.74	6.06	6.53

Table 3
Evaluation of the precision of the proposed colorimetric method for the selected cephalosporins

Drug	Added[a]	Found ± S.D.[b]	RSD (%)[c]	SAE[d]	Confidence limits[e]
CFPD	10.0	10.04 ± 0.055	0.548	0.025	10.04 ± 0.0694
	15.0	15.02 ± 0.038	0.263	0.017	15.02 ± 0.0472
	20.0	20.01 ± 0.019	0.095	0.008	20.01 ± 0.0222
CTIZ	6.0	6.01 ± 0.027	0.449	0.012	6.01 ± 0.0333
	9.0	9.01 ± 0.019	0.211	0.008	9.01 ± 0.0222
	12.0	12.00 ± 0.017	0.142	0.008	12.00 ± 0.0222
CZD	10.0	10.00 ± 0.019	0.190	0.008	10.00 ± 0.0694
	15.0	15.00 ± 0.023	0.153	0.103	15.00 ± 0.2859
	20.0	20.00 ± 0.029	0.145	0.003	20.00 ± 0.0361
CTRX	6.0	6.00 ± 0.011	0.183	0.005	6.00 ± 0.0139
	9.0	9.00 ± 0.019	0.211	0.008	9.00 ± 0.0232
	12.0	11.99 ± 0.016	0.134	0.007	11.99 ± 0.0194
CXIM	6.0	6.01 ± 0.050	0.850	0.017	6.01 ± 0.0472
	9.0	9.00 ± 0.049	0.501	0.018	9.00 ± 0.0499
	12.0	11.99 ± 0.028	0.229	0.017	11.99 ± 0.0472

[a] Final concentration in μg ml^{-1}.
[b] Mean ± standard deviation for five determinations.
[c] RSD, relative standard deviation.
[d] SAE, standard analytical error.
[e] Confidence limits at $P = 0.95$ and four degrees of freedom.

sorbance at the specified λ_{max} against concentration were found to be linear over the Beer's law ranges given in Table 2. The optical characteristics and statistical data of the regression equations for the complexes of the five cephalosporins are also shown in Table 2 and indicate a good linearity of the calibration graph.

3.3.2. Accuracy and precision

The accuracy and repeatability of the proposed procedure was checked by standard addition method. The good percentage recoveries and the values of standard deviation (Table 4) indicate the good accuracy and repeatability of the proposed method.

Table 4
Determination of CFPD, CTIZ, CZD, CXIM and CTRX in their commercial formulations using the proposed methods compared statistically with the official method (not for CFPD)

Drug	Formulation	Recovery ± S.D.[a]	
		Proposed methods	Official method
CFPD	Orelox tablets[e]	100.09 ± 0.28	99.87 ± 0.17
		t[b] = 1.24	
		F[c] = 2.71	
Recovery[d]		100.23 ± 0.2	
CTIZ	Cefizox vials[f]	100.13 ± 0.26	99.98 ± 0.39
		t = 0.71	
		F = 1.45	
Recovery[d]		100.34 ± 0.39	
CZD	Fortum vials[g]	99.77 ± 0.47	100.03 ± 0.53
		t = 0.69	
		F = 1.33	
Recovery[d]		100.09 ± 0.21	
CTRX	Rocephin vials[h]	99.75 ± 0.86	100.06 ± 0.71
		t = 0.57	
		F = 1.47	
Recovery[d]		100.07 ± 0.20	
CXIM	Suprax tablets[i]	100.00 ± 0.10	99.92 ± 0.17
		t = 1.26	
		F = 2.89	
		100.1 ± 0.33	

[a] Mean ± standard deviation of five determinations.
[b] Tabulated t-value for $P = 0.05$ and 8 degrees of freedom is 2.306.
[c] Tabulated F-value for $P = 0.05$ and $F_1 = F_2 = 4$ is 6.39.
[d] Standard addition method (mean of five determination ± standard deviation).
[e] Orelox, labeled to contain 130.45 mg of CFPD proxetil equivalent to 100 mg of CFPD Hoechst Orient SAE, (Cairo, Egypt), UL of Roussel Uclaf (France)], UL of Hikma Pharmaceuticals (Jordan)].
[f] Cefizox vials, labeled to contain 1 g CTIZ sodium [Kahira Pharm. & Chem. Ind. Co. (Egypt), UL of Hikma Pharmaceuticals (Jordan)].
[g] Fortum vials, labeled to contain CZD pentahydrate equivalent to 1 g of CZD [Glaxo Operations UK Ltd. (Greenford, England)].
[h] Rocephin vials, labeled to contain 1.193 g of CTRX disodium equivalent to 1 g of CTRX [Egyptian Int. Pharmaceutical Industries Co., EIPI Co. (ARE), in cooperation with F. Hoffmann-La-Roche Ltd. (Basle, Switzerland)].
[i] Suprax tablets, labeled to contain 400 mg CXIM [Kahira Pharm. & Chem. Ind. Co. (Egypt), UL of Hikma Pharmaceuticals (Jordan)].

In order to determine the precision of the method, solutions containing three different concentrations of each cephalosporin were prepared and analyzed in five replicates. The analytical results obtained from this investigation are summarized in Table 3. The mean standard deviation (RSD %) and standard analytical error (SAE) are also shown in the same table and their low values indicate the high precision and accuracy of the method.

3.3.3. Specificity and interference studies

The influence of commonly used excipients and additives (lactose, microcrystalline cellulose, talc, magnesium stearate and starch) was investigated before the determination of these antibiotics in their dosage forms. No interference could be observed. Also the assay was applied for the determination of endogenous components of biological fluids and shows no interference. The five investigated drugs were dispensed and used as single components (vials or tablets), so the assay in presence of other drugs is of little important.

3.4. Analysis of commercial pharmaceutical dosage forms

The proposed method for the determination of the five cephalosporins was applied to their pharmaceutical formulations (tablets or vials). It was carried out on the same batch of samples together with the official USP 23 [2], (for CFPD a reported procedure was used [11]). These determinations were carried out on the same batch of samples. The results were compared statistically by the Student's t-test and Variance ratio F-test (Table 4). The experimental values did not exceed the theoretical ones indicating the absence of any significant difference between the methods compared.

4. Conclusion

The chelate complex formed under the above mentioned conditions and measured spectrophotometrically can offer a sensitive, simple cheap and rapid procedure for the determination of the

five cephalosporins in bulk, tablets and vials dosage forms. Also the complex formed was found to be soluble in aqueous media in presence of a surfactant, without the need for organic solvent to extract the reaction products. From the calculation of stability constant of the complex, the latter was found to be stable. The method had been validated for the determination of these antibacterials. The statistical analysis of the results confirmed that the developed method was accurate and precise and could be recommended for use in quality control labs.

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