

SPECTROPHOTOMETRIC AND TITRIMETRIC ESTIMATION OF CEFTRIAXONE.

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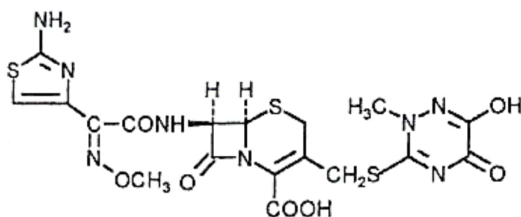
ABSTRACT:

Four simple rapid sensitive and accurate methods (A, B, C and D) for micro- and semimicro-determination of ceftriaxone in pure and dosage forms are developed. Method A is based on drug reaction with ammonium molybdate in acidified solution under heat treatment, the blue colour developed is measured at 697 nm. Beer's law is obeyed for 40-240 $\mu\text{g ml}^{-1}$ ceftriaxone. Method B is based on the oxidation with potassium permanganate in presence of sulphuric acid at room temperature. The decrease in permanganate colour (ΔA) is measured at 524.8 nm. Beer's law is obeyed for 5-40 $\mu\text{g.ml}^{-1}$. Method C is based on the direct titration against potassium bromate in hydrochloric acid medium using methyl red indicator (irreversible ind.). While method D is based on the oxidation of ceftriaxone with iron(III) in acidic medium. The liberated iron (II) reacts with 1,10-phenanthroline. The ferriox complex is colorimetrically measured at λ_{max} 510 nm against reagent blank. Beer's law is obeyed for 1-9 $\mu\text{g.ml}^{-1}$. The developed methods are successfully applied for determination of the cited drug in pharmaceutical product.

INTRODUCTION

Ceftriaxone is a long acting broad spectrum cephalosporine antibacterial drug⁽¹⁾. A number of methods for estimation of ceftriaxone are reported in literature, involving spectrophotometry⁽²⁻⁶⁾, chromatography⁽⁷⁻⁹⁾, potentiometry⁽¹⁰⁾ and voltammetry⁽¹¹⁻¹⁵⁾.

The aim of this work was the development of simple, easy, accurate and economical spectrophotometric and titrimetric methods for the analysis of ceftriaxone.



EXPERIMENTAL

Apparatus:

UV-Visible recording spectrophotometer shimadzu-260 corporation, Kyoto-Japan

Reagents and Materials

All reagents were of analytical grade and all solvents were of spectroscopic grade.

- Ceftriaxone, (EPICO 10th of Ramadan, Egypt) aqueous solutions, 0.5 mg.ml^{-1} , 1 mg.ml^{-1} and 0.001 M,

- Ammonium molybdate (2% aqueous solution).

- Sulphuric acid (3% & 20% w/v).

- Potassium permanganate (0.5 mg.ml^{-1}) stock solution and 0.001 M, prepared in double distilled water. The solutions should be freshly prepared and stored in a dark coloured container.

- Potassium bromate 0.01 N and 0.001M solutions, prepared in distilled water

- Methyl red indicator, Fluka AG.

- Hydrochloric acid 1.5 N.

- preparation of iron (III) -o- phenanthroline reagent⁽¹⁶⁾: mix. 0.198 g of 1,10-phenanthroline monohydrate (Fluka.Swiss) with 2 ml of 1.0 N HCl

and 0.16 g of ferric ammonium sulphate dodecahydrate (Fluka.Swiss) and dilute with water to 100 ml

- Pharmaceutical preparation

- Rocephine vial(1gm I.M.),Batch N°000468/B2064 (E.P.I.Co, 10th Of Ramadan, Egypt under license of Roche) containing dry substance equivalent to 1gm ceftriaxone .

General procedures

I - for authentic drug

(A) Ammonium molybdate method

Aliquots (0.2 – 2ml) of the ceftriaxone working solution (1 mg.ml^{-1}) were transferred into, separate test tubes followed by 2ml of 3% sulphuric acid and 3ml 2% ammonium molybdate solution. The mixture was shaken and heated in a thermally controlled water bath at $90 \pm 0.5^\circ\text{C}$ for 50 minutes, then cooled immediately to room temperature using cold water bath. The resulting blue solution was transferred quantitatively into a 25 ml calibrated flask and diluted to the mark with distilled water and homogeneously mixed . The absorbance was measured at 697 nm against a reagent blank treated similarly.

(B) Potassium permanganate method

Into a 10-ml calibrated flask 0.1 – 0.8 ml aliquots of the sample solution (0.5 mg.ml^{-1}) were transferred followed by 1ml of 20% sulphuric acid and 1 ml potassium permanganate solution.

The flasks were shaken and allowed to stand for 10 minutes at room temperature and diluted to the mark with distilled water. The decrease in absorbance (ΔA) at 524.8 nm was measured against a reagent blank.

(C) Potassium bromate method

Into separate 100- ml conical flasks, aliquots of the sample solution (2 – 10 mg ceftriaxone) were transferred and followed by 20 ml 1.5 N HCl solution and 3 drops of methyl red or methyl orange indicator. Titrate, with 0.01N -potassium bromate till colourless or even pale yellow colour. Blank determination must be carried out simultaneously.

(D) phenanthroline method

Aliquots of standard drug solution of ceftriaxone ranging from (10 to 90 μg) were transferred into a series of 10-ml volumetric flasks. The solutions of 2ml Fe(III) -*o*-phenanthroline reagent is added. The flasks were then heated in a thermally controlled water bath at 90 ± 0.5 °C for 20 minutes, then cooled immediately to room temperature using cold water bath and the volume was made up to the mark with distilled water. The developed coloured complex formed was measured at 510 nm against a reagent blank treated similarly.

II - Analysis of ceftriaxone in dosage formulation

The powder content of 3 vials was mixed, 25 mg was transferred into a 25-ml measuring flask, dissolved and completed to volume using distilled water. Different volumes were used equivalent to (0.5 - 2.0 mg) for molybdate method, (0.12 - 0.36 mg) for permanganate method, (3 - 11 mg) for bromate method and (15-75 μg) for phenanthroline method, then carry out as under general procedure 1 (A, B, C & D)

III - Stoichiometry of the proposed methods

(A) Potassium permanganate method: Job's method⁽¹⁷⁾ of continuous variation was adopted, using equimolar solution (1×10^{-3} M) of permanganate and ceftriaxone. A series of 5ml volumes of mixtures containing master solutions in different complementary preparations (0.5 - 5 ml) inclusive were prepared in 10 ml calibrated flasks. Sulphuric acid (1ml, 20%) was added and the flasks were completed to the mark using distilled water. The reaction had been allowed to stand at (25 ± 0.5 °C) for 10 minutes. The absorbance difference (ΔA) was measured at 524.8 nm against a blank of potassium permanganate.

(B) Potassium bromate method

A different series of 1×10^{-3} M ceftriaxone solution was transferred in separate flasks, and 20 ml 1.5 N HCl was added. Three drops of methyl red indicator, was added and titrate with 1×10^{-3} M potassium bromate solution till end point as under procedure (1-C).

RESULTS AND DISCUSSION

Ceftriaxone could be determined by four different methods. The first method depends on the measurement of the emerald - blue reaction product of the drug with ammonium molybdate against a reagent blank at 697 nm. The substance responsible for this blue colour is obtained by reduction⁽¹⁸⁾ of an acidified solution of MO(VI).

The absorbance (A) at 697 nm is a linear function of concentration (C) over the range (8 - 80 $\mu\text{g ml}^{-1}$) of ceftriaxone (Table 1).

Several parameters were studied for the reaction of ceftriaxone with ammonium molybdate, and the proportions of sulphuric acid and ammonium molybdate as stability of the reaction product and the

heating time. It is indicated that 2 ml of 3% H_2SO_4 and 3ml of 2% ammonium molybdate give maximum absorption with 1mg ceftriaxone. The heating time in boiling water bath, is about 50 minute to give complete reaction.

In the second method, potassium permanganate is used as strong oxidizing agent in sulphuric acid medium. The method depends on the measurements of the decrease in the absorbance of potassium permanganate colour upon the addition of different concentration of the drug.

The characteristic brilliant colour of permanganate solution has always attracted attention, in a dilute aqueous solution a well distinct band at 520 nm is detected.

The solution, in the presence of dilute sulphuric acid, reacts quantitatively with the investigated compound and it is used to estimate solution of the substance spectrophotometrically at 520 nm. The reaction was governed by the electronegativity difference, which provides a measure of the electronaffinity.

In solutions having hydrogen ion concentration of 0.5 M or greater, permanganate reduced all the way to manganous ion⁽¹⁹⁾. From a thermodynamic point of view, the increased acidity probably enhances the ease of protonation of such organic compound and therefore, the rate of its oxidation, ten minutes were sufficient to give complete reaction, and the reaction is stable at least for 30 minute.

Under the studied conditions, the absorbance decrease, ΔA against concentration of ceftriaxone was found to be linear over a wide range of drug concentrations (5-40 $\mu\text{g.ml}^{-1}$) with good correlation coefficients (-0.9999) (Table 1).

Job's method of continuous variation⁽¹⁷⁾ was employed to study the reaction stoichiometry under the working conditions at room temperature. It is evident from these obtained results and the typical plot that each molecule of ceftriaxone react with four molecules of potassium permanganate. This would account for a ratio of 20 electrons for one molecule of the cited drug.

In the third method, direct bromometry was used for the determination of ceftriaxone using irreversible redox indicator, such as methyl orange in acid medium

Free Br_2 liberated by self oxidation by the excess titrant can be detected by its yellow colour, but it is better to use an irreversible redox indicator⁽²⁰⁾. The concentration of hydrochloric acid used should be at least 1.5 N.

With all irreversible oxidation indicators the destruction of the indicator is often premature to a slight extent, so a little additional indicator is usually required near the end point and the "blank" must be carried out. Higher concentration of hydrochloric acid was tried but it gives lower results.

The ratio of stoichiometric reaction of ceftriaxone to potassium bromate under the studied condition was 1:2. The suggested procedure was used for the determination of ceftriaxone from 2 – 10 mg (Table 1)

The performance of the recommended procedure was assessed by calculation of the t-values, showing the absence of systemic error. No significant difference between the proposed methods and the used official method was observed Table (2).

In the fourth method 1,10-phenanthroline was used for determination of ceftriaxone in pure and dosage form. 1,10-phenanthroline is organic base containing the iron (II) specific group⁽²¹⁾. The method is based on the formation of tris(o-phenanthroline) iron(II) chelate upon the reaction of ceftriaxone with the reagent. The reaction proceeds through reduction of iron (III) ions to iron (II) and subsequent formation of the intensive orange – red colouration of the complex, the absorption spectra of the coloured complex in the proposed method show characteristic λ_{max} value. The experimental conditions were established by varying each parameter individually⁽²²⁾ and observing its effect on the absorbance of coloured complex. The addition of more than 2ml of iron(III)-phenanthroline reagent was required to obtain a maximum and reproducible absorbance, smaller amounts give incomplete complex formation. Therefore 2ml of the reagent was used throughout the experiments. A larger volume of reagent had no effect on the complex formation, although absorbance increased slightly due to background of the reagent used.

The effect of temperature on the formation of the coloured product was studied. The absorbance of the prepared solution as described under general procedure was measured at 510 nm, at different temperatures as function of time, the reaction proceeds very slowly at room temperatures. To accelerate it, higher temperatures were used. Maximum absorbance was obtained after heating for about 20min at $90 \pm 0.5^\circ\text{C}$ in thermally water bath. At lower temperature, the rate of colour development becomes progressively slower. There is no appreciable formation of the coloured compound at room temperature. For these reason, development time of 20 min at $90 \pm 0.5^\circ\text{C}$ was adopted in subsequent investigations. The sensitivity of the reaction increased by increasing the temperature and also time of heating till 20min which is the optimum condition for the reaction

In order to study the precision and the accuracy of the suggested methods, standard solution containing three different concentrations of the examined substance was prepared and analysed in seven replicates by suggested methods, Table (3). The percentage standard deviation and the percentage range of error at 95% confidence level can be considered to be satisfactory. The Results obtained by the suggested techniques correlated well with those obtained by the official pharmacopoeia method⁽⁹⁾

The proposed procedures were successfully applied to the determination of the investigated compound in the dosage form with good recovery (Table2).

Table 1: Ceftriaxone analysis by adopting the four suggested methods.

molybdate method		permanganate method		bromate method		Phenanthroline method	
Amount taken (mg)	Recovery %*	Amount taken (mg)	Recovery %*	Amount taken (mg)	Recovery %*	Amount taken (μg)	Recovery %*
0.20	106.16**			2	99.50	10	100.60
0.40	101.67	0.05	102.04	4	98.75	40	99.58
0.80	101.73	0.10	100.53	6	99.00	60	100.37
1.20	100.21	0.20	99.78	8	98.75	80	99.68
1.60	99.97	0.30	100.18	10	98.50	90	100.17
2.00	99.51	0.40	99.89				
Mean $p=0.05$	100.62	100.48		98.90		100.08	
N	5	5		5		5	
Variance	1.039	0.841		0.144		0.193	
SD	1.019	0.917		0.379		0.439	
SE	0.456	0.410		0.169		0.196	

*average of three experiments

**excluded

Table 2: Determination of ceftriaxone raw material and in Rocephine vial by the four suggested methods.

Parameter	U.S.P. official (9) Method	Molybdate method		Permanganate method		bromate method**		Phenanthroline method	
		Raw material	Vial	Raw material	Vial	Raw material	vial	Raw material	Vial
Mean _{p=0.05}	100.20	100.62	98.656	100.48	100.00	98.90	98.18	100.08	99.66
N	5	5	0.205	5	5	5	5	5	5
V	0.390	1.039	0.423	0.841	0.249	0.144	0.088	0.193	0.076
SD	0.628	1.019	0.185	0.917	0.499	0.379	0.297	0.439	0.265
SE	0.280	0.456	--	0.410	0.233	0.169	0.133	0.196	0.119
t-test(2,306)	--	0.785	--	1.136	--	3.980	--	0.352	--
F-test(5,05)	--	2.664	--	2.160	--	2.710	--	2.021	--
Regression equation*									
B		-0.0250	--	0.0011	--	--	--	4.3423	--
K		2.0459	--	0.6515	--	--	--	86.773	--
Correlation coefficient (r)		0.9996	--	-0.9999	--	--	--	0.9999	--
Relative standard deviation (rsd%)		1.013	0.188	0.913	0.499	0.383	0.303	0.299	0.266

*Conc. = K.Abs. + B

**0.5545 mg ceftriaxone = 0.334 mg potassium bromate

Table 3: Assessments of the precision, accuracy and reproducibility of the proposed methods.

Methods	Taken (mg)	Recovery (%)	SD	RSD (%)	SE
Permanganate	0.1	98.84	0.490	0.496	0.185
	0.3	99.67	0.795	0.798	0.300
	0.4	99.31	0.655	0.660	0.248
Mean	--	99.27	0.647	0.652	0.245
Molybdate	0.6	100.6	0.602	0.598	0.228
	0.8	99.53	0.791	0.795	0.299
	1.0	99.63	0.658	0.660	0.249
Mean	--	99.92	0.691	0.691	0.264
Bromate	3.0	98.69	0.346	0.351	0.134
	6.0	98.94	0.322	0.325	0.122
	9.0	98.69	0.309	0.313	0.117
Mean	--	98.77	0.326	0.330	0.123
Phenanthroline	10.0	98.68	0.352	0.357	0.133
	30.0	99.74	0.479	0.480	0.181
	50.0	99.85	0.286	0.286	0.108
Mean	--	99.42	0.372	0.374	0.141

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استخدام طرق طيفية وعيارية في تعيين السفتريكسون

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قسم الكيمياء التحليلية - كلية الصيدلة - جامعة الزقازيق - مصر

في هذا البحث تم استخدام أربع طرق جديدة لتعيين السفتريكسون. تعتمد الطريقة الأولى على تكوين لون أزرق نتيجة تفاعل السفتريكسون مع موليبدات الأمونيوم في وجود حامض الكبريتيك تحت تأثير الحرارة يتم قياسه طيفيا عند طول موجى يساوى ٦٩٧ نانومتر. أما الطريقة الثانية فتعتمد على تفاعل برمنجنات البوتاسيوم مع السفتريكسون في وجود حامض الكبريتيك حيث يتم نقص في لون البرمنجنات نتيجة اختزالها بالعقار وبالتالي يقل الامتصاص الطيفي ويتناسب النقص مع تركيز العقار. وفي الطريقة الثالثة تم معايرة السفتريكسون باستخدام برومات البوتاسيوم في وجود حامض الهيدروكلوريك وكاشف الميثيل البرتقالي أو الميثيل الأحمر. أما الطريقة الرابعة فتعين السفتريكسون في أقل كمية ممكنة حيث أنها ذات حساسية فائقة ويستخدم فيها كاشف الفينانثرولين حيث يختزل الحد يدك إلى حديدوز بواسطة العقار ثم يتكون معقد ملون من الحديدوز و الفينانثرولين يتم قياسه طيفيا عند طول موجى يساوى ٥١٠ نانومتر ويتناسب اللون مع تركيز العقار. كما تم تطبيق الطرق المقترحة على تعيين نسبة العقار الموجودة في حقن الروسيفين بنجاح .

وقد وجد أن الطرق المقترحة سهلة التنفيذ في أى معمل حيث أنها لا تحتاج إلى وجود أجهزة عالية الثمن أو ذات تقنية خاصة مثل HPLC كما أنها لا تستهلك وقت في التنفيذ.

وقد قورنت الطرق المقترحة بالطريقة الدستورية ووجد أنها لا تقل عنها في الدقة والحساسية.