Physicochemical and Microbiological Study of Different Brands of Ceftriaxone Sodium Available In Libyan Market

Abdulrhman. A. Akasha, Maha Mahmoud Nashwan, Raihan Jamal Ashour, and Nehal Abdulrhman Hegazi

***Abstract*—**Cephalosporin is considered to be equivalent to cefotaxime in terms of safety and efficacy. It has broad spectrum activity against Gram-positive and Gram-negative bacteria, Ceftriaxone sodium is marketed under the trade nme. The five brands of Ceftriaxone sodium, Triaxon(U.A.E), Ceftriaxone (INDIA), Zetraxon(SWITZERLAND) ,Cefaxon(TUNISIE) and Nevakson (TURKEY) were physically and chemically characterized. The basic function groups was identified by Infra Red (IR) spectrophotometer. The particle size and moisture content of ceftriaxone sodium brands was determined by light obscuration particle count test and Karl-Fischer titration ,respectively. The validate a stability-indicating LC method for quantitative determination of ceftriaxone was studied using different storage conditions.

The bactericidal activity of ceftriaxon sodium and their brands were investigated by using Escherichia coli) (E. coli), Pseudomonas aeruginosa (P. aeruginosa), Staphylococcus aureus (S. aureus), methicillin-resistant staphylococcus aureus (MRSA). The resultant bactericidal activity of Ceftriaxone sodium brands against this bacteria were exhibited similarity of bacterial activity.

***Keywords*—**Bacteria, Bactericidal activity Ceftriaxone, HPLC, IR and Karl-fischer.

I. Introduction

ANTIBACTERIAL compounds are chemically semisynthetic modified natural compounds. Cephalosporins are semisynthetic β-lactam antibiotic, which produce their effect on the bacterial cell membrane.

Ceftriaxone sodium is a third-generation Cephalosporin which is broad spectrum antibiotic [1],[2]. It is considered to be equivalent to cefotaxime in terms of safety and efficacy. It has activity against Gram-positive and Gram-negative bacteria Ceftriaxone sodium is marketed under the trade name ROCEPHIN®. ceftriaxone can be stressed under various conditions of hydrolysis, high temperature and photolysis according to the ICH (international conference on harmonization) stability testing guidance. [3]

Light obscuration particle count test is used on the basis of light blockage which allows an automatic determination of the size number of particles. The test is carried out under conditions limiting particulate contamination, preferably in a laminar-flow cabinet [4].

The determination of water content (karl-fischer) is the coulometric titration of water is based upon the quantitative reaction of water with sulphur dioxide and iodine in an anhydrous medium in the

presence of a base with sufficient buffering capacity [5]. Infrared spectrophotometry is used for recording spectra in the region of 4000-650 cm^{-1} (2.5-15.4 µm) or in some cases down to 200 cm^{-1} (50 µm)[6]. Ceftriaxone potency can be tested on the several bacterial strains for the minimum inhibitory concentration MIC of the antibiotics. The MIC of each antimicrobial agent was determined by broth two fold serial dilution procedure against the following microbes: Escherichia coli (E.coli) Pseudomonas aeruginosa (P.aeruginosa), Staphylococcus aureus (S.aureus) & Methicillin-resistant staphylococcus aureus (MRSA) [7].

II. Procedure for Paper Submission

A. Determination Of Water Content (Karl-Fischer)

The compartments of the reaction cell were filledwith electrolyte reagent for the micro determination of water and performed the coulometric titration to a stable end-point. The prescribed amount of the substance to be examined was introduced into the reaction cell, then stirred for 30s. The value from the instrument's output was estimated and calculated the amount of water that is present in the substance. When appropriate to the type of sample and the sample preparation, perform a blank titration as shown in Fig: 1.

Fig.1 Water Content (Karl-Fischer) Test

Abdulrhman. A. Akasha, Maha Mahmoud Nashwan, Raihan Jamal Ashour, and Nehal Abdulrhman Hegazi are with Department of Pharmaceutics, Faculty of Pharmacy, Tripoli University, Libya, P O Box 13645. Tel: +218-924378509, Fax: +21821-4625121. Email Id: Akashaabdu@yahoo.co.uk.

B. Particulate Contamination: Sub-Visibe Particle

To determine the particulate contamination of five samples and count the number of particles equal to or greater than 10 µm and 25 µm by using: Light Obscuration Particle Count Test

The sealing closure was removed and the outer surfaces of the vials was cleaned. Each contents of a of vial were diluted to 10ml with particle-free water R (sterile water for injection). Gas bubbles were eliminated by allowing to stand for 2 min, then the number of particles were counted. The mean number of particles for the preparation to be examined was calculated.

C. Infrared Spectrophotometry

are used for recording spectra in the region of 4000-650 cm^{-1} (2.5-15.4 µm) or in some cases down to 200 cm^{-1} (50 µm).

1-2 mg of the substance to be examined was titrated with 300-400 mg of finely powdered and dried potassium bromide R. These quantities are usually sufficient to give a disc of 10-15mm diameter and a spectrum of suitable intensity . Carefully grind the mixture, spread it uniformly in a suitable die, and submit it to a pressure of about 800 MPa (8 t•cm^{-2}) as shown in Fig. 2.

Fig. 2 Infrared Spectrophotometry instrumentation.

D. Chemical Stability Of Ceftriaxne By A Validated Stability-Indicating Liquid Chromatographic Method

The objectives of this study to develop and validate a stability-indicating LC method for quantitative determination of ceftriaxone and determine the chemical stability of ceftriaxone for injection with sterile water, at different storage conditions.

Ceftriaxone was stressed under various conditions of hydrolysis, high temperature and photolysis according to the ICH (international conferenceon harmonization) stability testing guidance. Ceftriaxone was considered to be stable if the concentration was ≤ 90% of the initial concentration.

Stock solution of Ceftriaxone sodium and cephradine,standard solution 250 mL acetonitrile(MP); 50 mL phosphate buffer (pH 7.4; 0.1M), 3.2 g tetrabutylammonium bromide made up to a volume of 1 L with water. Flow rate: 1.0 mL min^{-1} and UV detection at: 260 nm as shown in Fig. 3.

Fig. 3 Chromatographic Method to evaluate Chemical Stability Of Ceftriaxne

E. Antimicrobial Susceptibility Test

The sensitivity of test microorganisms to ceftriaxone preparations was evaluated by determining the minimum inhibitory concentration (MIC) of the antibiotics. The MIC of each antimicrobial agent was determined by broth two fold serial dilution procedure against the following microbes:

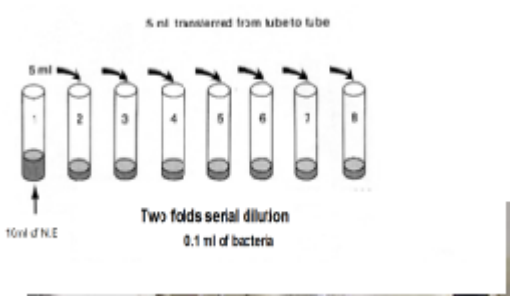

Fig. 4 Showed, Antimicrobial Susceptibility Test of Ceftriaxone on Bacterial strains

Escherichia coli (E.coli), Pseudomonas aeruginosa (P.aeruginosa), Staphylococcus aureus (S.aureus) Methicillin-resistant staphylococcus aureus (MRSA). As shown in Fig. 4.

III. Results And Discussion

A. Antimicrobial Susceptibility Test

Ceftriaxone potency has tested on the several bacterial strains for the minimum inhibitory concentration MIC of the antibiotics. The MIC of each antimicrobial effect exhibited the similarity of very effective against E. Coli as shown in Table 1. In marked contrast, all the brands showed intermediate antimicrobial activity against P. aeruginosa which required a concentration of 32 µg/ml to reach the visible inhibitory results as shown in Table 2. The minimal inhibitory concentrations of Zetaxon, Nevakson, Cetriaon and Rocephine for complete inhibition at low concentration. On the other hand Triaxon and Cefaxone had low activity at higher concentration of 16 µg/ml as shown in Table 3.

Further more, We have tested all selected brands on Methicillin-resistant staphylococcus aureus (MRSA) only Cefaxone and Rocephine have completed inhibition at 8 µg/ml. But Zetaxon, Nevakson, Triaxon and Ceftriaxonehave showed intermediate effect at high concentration of 8 µg/ml as

shown Table 4.

-Antimicrobial Susceptibility Test-

TABLE I
COMPARATIVE ACTIVITY OF CERTIAXONE PREPARATIONS AGAINST E.COLI

	MIC (ug/ml) Interpretive standard			MIC (µg/ml)							
	S	I	R	125	62.5	32	16	8	4	2	1
Zetaxon	≤8	16-32	≥64	-	-	-	-	-	-	-	+
Nevakson				-	-	-	-	-	-	-	+
Triaxon				-	-	-	-	-	-	+	+
Cefaxone				-	-	-	-	-	+	+	+
CetriaXone				-	-	-	-	-	-	-	+
Roephine				-	-	-	-	-	-	-	+

TABLE II
COMPARATIVE ACTIVITY OF CERTIAXONE PREPARATIONS AGAINST P. AERUGINOSA

	MIC (ug/ml) Interpretive standard			MIC (µg/ml)							
	S	I	R	125	62.5	32	16	8	4	2	1
Zetaxon	≤8	16-32	≥64	-	-	+	+	+	+	+	+
Nevakson				-	-	+	+	+	+	+	+
Triaxon				-	-	+	+	+	+	+	+
Cefaxone				-	-	+	+	+	+	+	+
CetriaXone				-	-	+	+	+	+	+	+
Roephine				-	-	+	+	+	+	+	+

TABLE III
COMPARATIVE ACTIVITY OF CERTIAXONE PREPARATIONS AGAINST S.AUREUS

	MIC (ug/ml) Interpretive standard			MIC (µg/ml)							
	S	I	R	125	62.5	32	16	8	4	2	1
Zetaxon	≤8	16-32	≥64	-	-	-	-	-	-	-	+
Nevakson				-	-	-	-	-	-	-	+
Triaxon				-	-	-	+	+	+	+	+
Cefaxone				-	-	-	+	+	+	+	+
CetriXaone				-	-	-	-	-	-	-	+
Roephine				-	-	-	-	-	-	-	+

TABLE IV
COMPARATIVE ACTIVITY OF CERTIAXONE PREPARATIONS AGAINST MRSA

	MIC (ug/ml) Interpretive standard			MIC (µg/ml)							
	S	I	R	125	62.5	32	16	8	4	2	1
Zetaxo	≤8	16-32	≥64	-	-	-	+	+	+	+	+
Nevakson				-	-	-	+	+	+	+	+
Triaxon				-	-	-	+	+	+	+	+
Cefaxone				-	-	-	-	+	+	+	+
CetriaXone				-	-	-	+	+	+	+	+
Rocephine				-	-	-	-	+	=	+	+

B. Particulate Contamination: Sub-Visibe Particle

From our results the investigated particulate contamination of Ceftriaxone sodium, Triaxon(U.A.E), Ceftriaxone (INDIA), Zetraxon (SWITZERLAND), Cefaxon(TUNISIE) and Nevakson (TURKEY) by obscuration method showed there were any negative results towards these brands as shown in Table 5.

TABLE V
SHOWED THE PARTICULATE CONTAMINATION OF CEFTRIAXONE SODIUM

Size p (µm)	No. p.s	Size (µm)	No. P.s	Size (µm)	No. P.s
2	34242	2	23325	2	46099
5	1536	5	1672	5	6603
7.5	1536	7.5	492	7.5	1956
10	227	10	271	10	555
15	63	15	86	15	70
20	8	20	20	20	12
25	2	25	7	25	5
30	0	30	6	30	3

Cefaxone ceftriaxone Nevakson
Normal limit of :
10 µm not more than 6000P ,25 µm not more than 600P

C. Determination Of Water Content(Karl-Fischer)

In the present study the investigated Ceftriaxone sodium, Triaxon (U.A.E), Ceftriaxone (INDIA), Zetraxon (SWITZERLAND), Cefaxon (TUNISIE) and Nevakson (TURKEY) showed the percentage of water content of each brand as determined by Karl Fischer method in Figure 5. Our results exhibited the normal limilt of moisture as compared to standard range of 0.4 to 9.90 %.

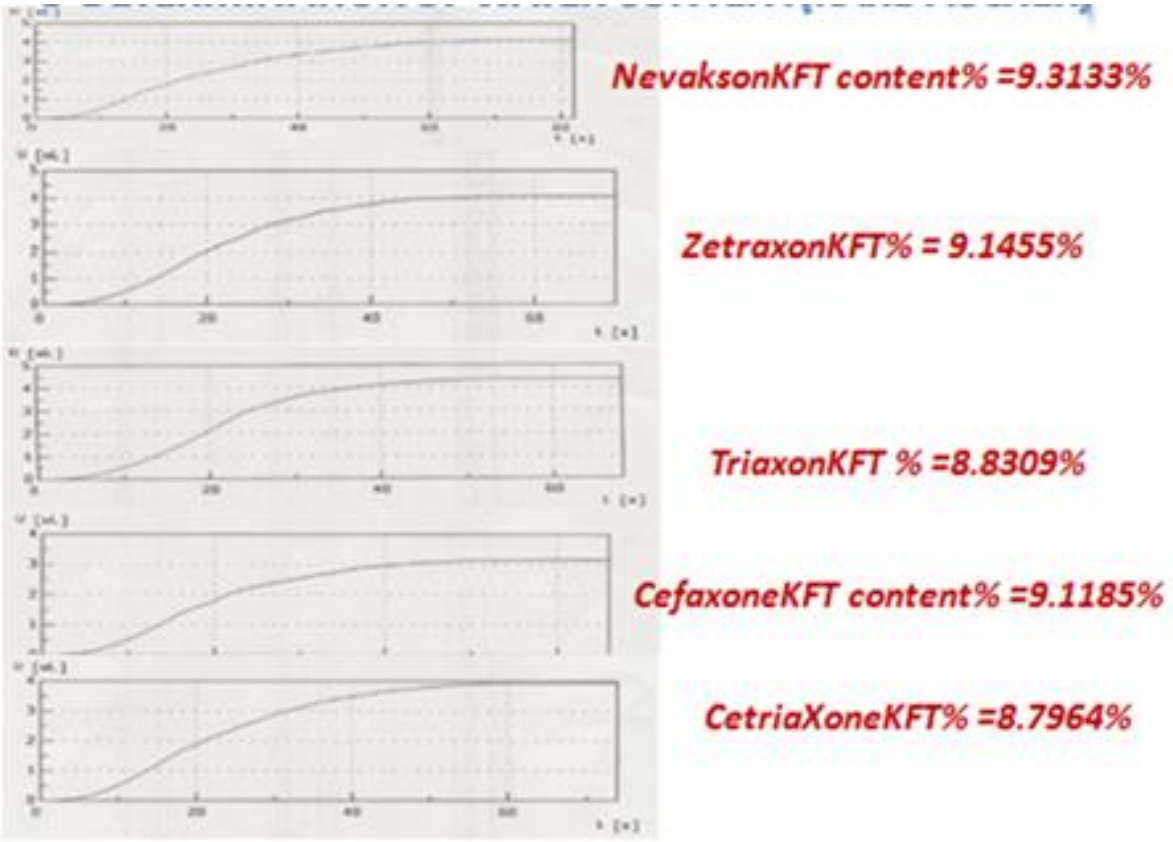

Fig. 5 The % of water content in different five Ceftriaxone sodium brands

D.Infrared Spectrophotometry

The spectroscopic investigation of Ceftriaxone sodium, Triaxon (U.A.E), Ceftriaxone (INDIA), Zetraxon (SWITZERLAND), Cefaxon (TUNISIE) and Nevakson (TURKEY) revealed the band 1690-1640 cm, which represents the amono group, the band of 1680-1630cm, represents carbonyl amide group, the band 1730-700cm represents carboxylic group and the presence of band 3500-3180cm represents amide group. IR chart revealed the identical function groups of all Ceftriaxone brands and the standard drug, as showed in Fig. 6.

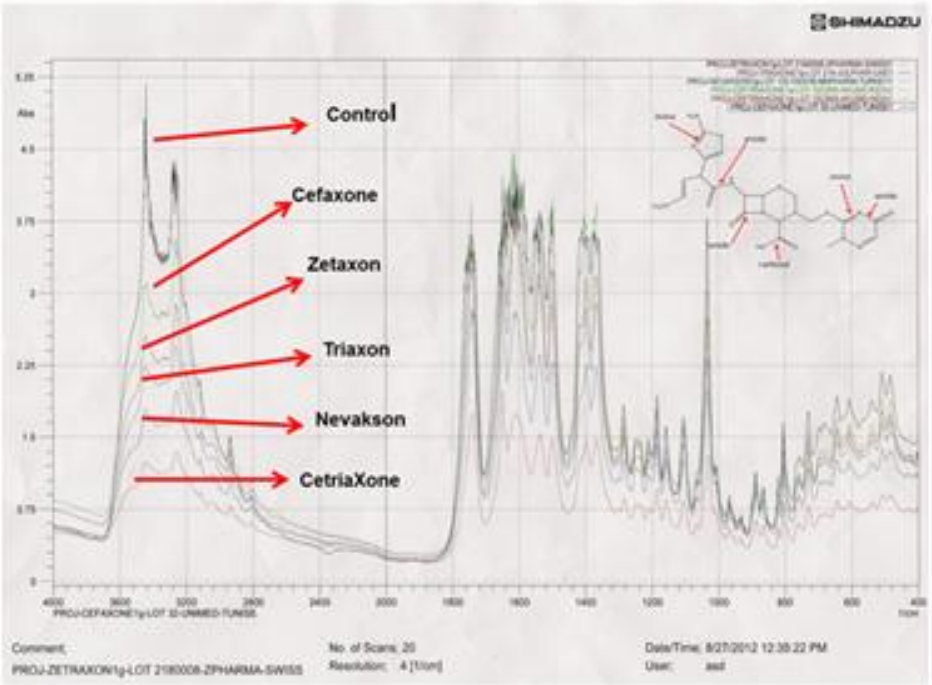

Fig. 6 The IR Spectra of different five Ceftriaxone sodium brands.

E. Chemical Stability of Ceftriaxne

Following the different conditions of Ceftriaxone exposure, the drug was subjected to the chromatographic determination to estimate the drug degradation. Ceftriaxone was considered to be stable if the concentration ≤ 90% of the initial concentration. At room temperature and at + 8 °C the degradation of ceftriaxone followed first-order kinetics and the samples were chemically stable for at least 4 and 41 days respectively. At - 20 °C the concentration of all samples remained higher than 90% of the original concentration. As showed in Table 6.

TABLE VI
DEGRADATION KINETIC CEFTRIAXONE SODIUM FOR INJECTION

Drug Sample	Storage Condition	K a Days -1	T90 b Days	C R2
A	23 ºC ± 2C light protection	0.0214	4.92	0.9843
	23 ºC ± 2C light exposure	0.0200	4.92	0.9850
	8 ºC ± 1C	0.0023	4.92	0.9834
B	23 ºC ± 2C light protection	0.0198	4.92	0.9661
	23 ºC ± 2C light exposure	0.0200	4.92	0.9705
	8 ºC ± 1C	0.0025	4.92	0.9647

a: Degradation rate constant
b: Time at which concentration fell to 90%of original concentration
c: Determination Coefficients

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