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Reconstitution Stability of Ceftriaxone Sodium for Injection in Intravenous Diluents

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ABSTRACT

The objective of this study to evaluate reconstitution stability of Ceftriaxone in different diluents like sterile water for injection, 0.9% Sodium chloride solution, 10% Dextrose, 5% Dextrose + 0.9% NaCl solution, Sodium lactate over 3 days at room temperature and 10 days at refrigerated condition (2-8°C), and 10% invert sugar, 5% Sodium bicarbonate, Freamine III, 10% Mannitol over 24 hrs at room temperature, and Ceftriaxone admixture solution with Metronidazole HCl injection along with the diluents in 0.9% NaCl and 5% Dextrose over 24 hrs at room temperature. No data are available, however, about the Ceftriaxone stability at various temperatures in Freamine III, Metronidazole HCl injection, sodium lactate, 5% Sodium bi carbonate, 10% invert sugar and 10% Mannitol. Ceftriaxone was reconstituted with 19.2 mL of sterile water for injection and then injected to the bags of above mentioned diluents and stored the solutions at room temperature and refrigerator as specified above. The samples of Ceftriaxone solutions in sterile water for injection, 0.9% Sodium chloride solution, 10% Dextrose, 5% Dextrose + 0.9% NaCl solution, Sodium lactate were collected after 3 days at room temperature and 10 days at refrigerated stored, and the solutions in 10% invert sugar, 5% Sodium bicarbonate, Freamine III, 10% Mannitol were collected after 24 hrs at room temperature. The Ceftriaxone solutions in 0.9% NaCl and 5%Dextrose solutions admixed further with Metronidazole HCl injection (7.5 mg/mL). All these samples were tested with a specific stability indicating methods for the estimation of content of Ceftriaxone and Ceftriaxone impurities. A clinically significant loss of potency was defined as a greater than ten percent decrease from its initial concentration. The concentration of Ceftriaxone was 90% or more for 3 days at room temperature, 10 days at 2-8º C and 24 hrs at room temperature. Thus, Ceftriaxone can be stored in the solution having the concentration of 40mg/mL with Sterile water for Injection, 0.9% NaCl solution, 10% Dextrose Solution, 5% Dextrose + 0.9% NaCl solution are stable up to 3 days period at room temperature and 10 days if stored at refrigerated conditions, and concentration of 40mg/mL with Sodium Lactate, 10% Invert Sugar, 5% Sodium bi

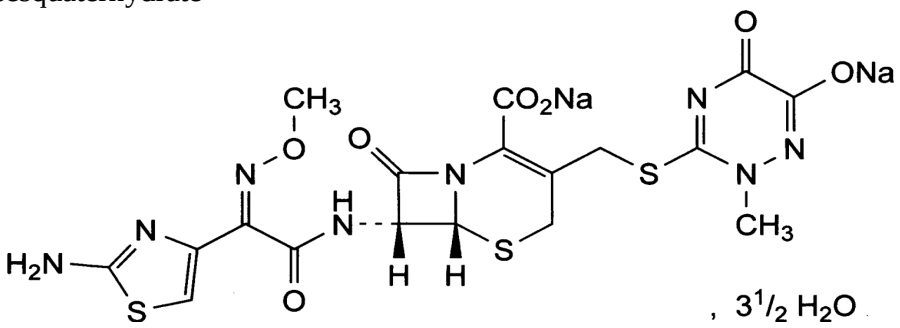
Carbonate, Freamine III, 10% Mannitol are stable up to 24 hrs at room temperature, and concentration of 10mg/mL with an admixture of Metronidazole HCl (concentration of 7.5mg/mL) + 0.9% NaCl solution and Metronidazole HCL (concentration of 7.5mg/mL) + 5% Dextrose in water are stable up to 24 hrs at room temperature.

Keywords: Ceftriaxone Sodium; Reconstitution study; Intramuscular and Intravenous.

INTRODUCTION

Ceftriaxone belongs to a group of antibiotics known as the Cephalosporin, which are closely related to Penicillin [1] Cephalosporin are classified as first , second or third generation on the basis of their spectrum of activity against gram-negative rods[2]. In 1981, the first third-generation drug, Ceftriaxone was introduced. Ceftriaxone is considered as a third-generation [3] molecule.

Ceftriaxone for injection is a sterile, semi synthetic, broad spectrum Cephalosporin antibiotic for intravenous or intramuscular administration. Ceftriaxone sodium is (6R, 7R-7-[2-(-Amino-4-thiazolyl) glyoxylamido]-8-oxo-3-[[(1, 2, 5, 6-tetrahydro-2-methyl-5, 6-dioxo-methyloxime) di sodium salt sesquaterhydrate [4]



Ceftriaxone sodium is a sterile white to yellowish crystalline powder which is really soluble in water, sparingly soluble in methanol and very slightly soluble in ethanol. The pH of 1% aqueous solution is approximately 6.7, the colour of Ceftriaxone sodium solutions ranges from light yellow to amber depending on the length of storage, concentration and diluent used.

The pKa value of Ceftriaxone is 3 (COOH), 3.2 (NH3+), 4.1 (enolic OH)[5]. Ceftriaxone for injection is indicated for the treatment of various infections when caused by susceptible organisms like lower respiratory tract infections. Acute bacterial otitis media, skin and skin structure infections, Meningitis and surgical Prophylaxis [6].

Literature survey reveals that the study conducted only in peritoneal dialysis fluids like dextrose 1.5% and 4.5%[7]. No reconstitution stability data available in the diluents Freamine III, Metronidazole HCl injection, sodium lactate, 5% Sodium bi carbonate, 10% invert sugar, 10% Mannitol. The objective of this study was to determine the reconstitution stability of Ceftriaxone in different diluents like sterile water for injection, 0.9% Sodium chloride solution, sodium

lactate, 10% invert sugar, 5% sodium bicarbonate, Freamine III, 10% Mannitol and Metronidazole HCl injection

Ceftriaxone sodium for injection 2g was reconstituted with above mentioned diluents, stored and withdrawn the samples at different time intervals. Ceftriaxone was measured by a stability indicating liquid chromatography method.

MATERIALS AND METHODS

Instrumentation:
The high pressure liquid chromatographic (HPLC) system was used bearing the configuration by equipped with pump (Waters 2695 separators module), auto sampler, thermostated column compartment and detector (Waters 2487 dual wavelength absorbance detector) controlled by Empower II software. A Hypersil C18, HPLC column (150 x 4.6 mm, 5µm) was used as a stationery phase.

Materials:
Working standards of Ceftriaxone sodium with potency 91.8% on as is basis as Ceftriaxone, Ceftriaxone E-isomer USP reference standard (Lot no.: H, Potency: 100%), di sodium hydrogen orthophosphate anhydrous (AR grade), tetra heptyl ammonium bromide (AR grade), sodium hydroxide (AR grade), ortho phosphoric acid and acetonitrile (HPLC grade, manufactured by Merck) were procured form commercial source. HPLC grade water was obtained using Millipore water purification system. The diluents sterile water for injection (mfg: Core healthcare limited, batch no.: 501533), 0.9% sodium chloride solution (mfg: Claris Life sciences limited, batch no. 3.11.6565), 10% dextrose solution (mfg: Albert David limited , batch no.: P4B971), 5% Dextrose solution (Mfg: Albert David limited, batch no.: 5AC97), sodium lactate solution (mfg: Baxter (India) Pvt. ltd, batch no: 957654), 10% Invert sugar (Mfg: Raptakos, Brett and co. Ltd, batch no: TW 4389 B), 5% Sodium bi carbonate (Mfg: Superb Drugs Pvt. Ltd., batch no: S93), Free amine III (Mfg: Claris Life science Limited, batch no.: 0-09-6174), Metronidazole HCl (Mfg: JB Chemicals and Pharmaceuticals Ltd, batch no: D8876) were procured from commercial source.

Methodology [4,8,9]
0.02M Phosphate buffer was prepared by dissolving 2.84g of anhydrous di sodium hydrogen ortho phosphate in 1000 mL of purified water. 0.4% w/v tetra heptyl ammonium bromide solution was prepared in the diluent acetonitrile. Mobile phase was prepared in the ratio of 300:250:2.2 of 0.02 M phosphate buffer and 0.4% w/v solution of tetra heptyl ammonium bromide solution and 20%w/v solution of ortho phosphoric acid. pH of the mixture was maintained between 6.6 and 6.8. This mobile phase was filtered and degassed. A resolution solution prepared at a concentration of 0.16mg/mL each of Ceftriaxone sodium and Ceftriaxone E-isomer and a Ceftriaxone sodium standard solution prepared at a concentration of 0.2mg/mL and further diluted to get the concentration of 0.002mg/mL. (Ceftriaxone sodium standard solution having a concentration of 0.2mg/mL is used for the estimation of Ceftiraxone and 0.002mg/mL is used for the estimation of impurities).

Procedure

Ceftriaxone sodium for injection 2g samples were reconstituted with 19.2mL sterile water for injection (100mg/mL) and further diluted with the best available intravenous diluents and subjected these solutions for chemical stability as detailed below.

A) Reconstitution solutions (100mg/mL) were diluted with the diluents as listed in table-1 to get the concentration of about 40mg/mL and the diluted solutions were then kept under said conditions in table-1.

Table – 1

S. No	Diluents	Storage Conditions	
		RT (NMT 25°C)	Refg. (2°-8°C)
1	Sterile Water for Injection	3D	10 D
2	0.9% NaCl Solution	3D	10 D
3	10% Dextrose Solution	3D	10 D
4	5% Dextrose + 0.9% NaCl solution	3D	10 D
-	-	RT (NMT 25°C)	
5	Sodium Lactate	24 Hrs	
6	10% Invert Sugar	24 Hrs	
7	5% Sodium bi Carbonate	24 Hrs	
8	Free amine III	24 Hrs	
9	10% Mannitol	24 Hrs	

RT-Room Temperature, NMT–Not more than, Refg.–Refrigerated, D–days

B) Reconstituted solutions (100mg/mL) were diluted with the diluents as listed in table-2 to get the concentrations of about 10mg/ml and the diluted solutions were then kept under said conditions in table-2.

Table – 2

S.No	Diluents	Storage Conditions
		RT (NMT 25°C)
10	Metronidazole HCl and 0.9% NaCl Solution	24 Hrs
11	Metronidazole HCl and 5% Dextrose in water	24 Hrs

RT - Room Temperature, NMT – Not more than

These diluted samples were analysed for the tests appearance, pH, assay and impurities at the time points mentioned in table-1 and analysed.

Sample preparation

The above solutions headed in “Procedure” were withdrawn at the specified time interval and made one more dilution to get final concentration 0.2 mg/mL and these sample solutions were injected into the chromatography and the responses were recorded.

A highly sophisticated instrument Waters high pressure liquid chromatography is equipped with UV at 254nm detector and ‘Hypersil BDS C18, 150 x 4.6 mm, 5µm’ were used for the estimation of Ceftriaxone and its impurities. The flow rate is 1.0mL/min and injection volume is 20 µL and run the chromatogram for 40 minutes. The retention time for Ceftriaxone is 8.0

minutes and Ceftriaxone E-isomer is 14 minutes. Injected separately a 20µL of blank, resolution solution, standard solution (duplicate each of 0.2mg/mL and 0.002mg/mL) and sample solutions into the chromatographic system. Recorded the chromatogram and measure the peak area counts.

The resolution found between Ceftriaxone and Ceftriaxone E-isomer was found as 7.0. Reported the individual known impurities with the relative retention times with respect to the retention time of Ceftriaxone for Imp-B, 7-ACA, Imp-C, E, D and A as 0.26, 0.30, 0.49, 0.76, 1.13 and 1.74 respectively. A response factor with respect to Ceftriaxone sodium applied into the calculations for Imp-B, 7-ACA, Imp-C, E, D and A as 0.99, 2.13, 1.65, 1.24, 1.49 and 1.86 respectively.

RESULT AND DISCUSSION

A clinically significant loss of potency is defined as a greater than 10% decrease from initial concentration [7]. The potency of Ceftriaxone sodium of the reconstituted solutions after the storage period as depicted in table-3 are found within the limit of 10% from the initial assay value.

Table-3

S.No	Diluent	Storage conditions		
		Initial	RT for 3D	Refg. for 10D
1	Sterile water for injection	103.46%	99.12%	100.73%
2	0.9% NaCl solution	101.34%	96.54%	100.09%
3	10% Dextrose solution	102.25%	95.47%	98.53%
4	5% Dextrose Solution + 0.9% NaCl Solution	100.62%	98.55%	100.61%
-	-		Initial	RT for 24 hrs
5	Sodium Lactate solution		100.35%	96.98%
6	10% Invert Sugar		99.82%	96.13%
7	5% Sodium bi Carbonate		100.09%	95.51%
8	Free amine III		104.28%	95.45%
9	10% Mannitol		101.22%	91.11%
10	Metronidazole HCl and 0.9% NaCl Solution		103.31%	98.11%
11	Metronidazole HCl and 5% Dextrose in water		103.38%	98.30%

D–Day, RT- Room temperature, Refg.-Refrigerated

The impurities (Imp-B, 7-ACA, Imp-C, E, D, A, single highest unknown impurity and total impurities) in the reconstitution solutions after the storage period as recorded in table-4 and found satisfactory.

Table-4

Storage	Imp-B	7-ACA	Imp-C	Imp-E	Imp-D	Imp-A	SHUNI	Total
Diluent: Sterile water for injection								
Initial	ND	0.03%	0.36%	ND	ND	ND	0.04%	0.45%
RT for 3D	ND	0.21%	1.40%	ND	0.04%	0.11%	0.20%	2.20%
Refg. For 10D	ND	0.14%	0.79%	ND	ND	0.03%	0.07%	1.06%
Diluent: 0.9% NaCl solution								
Initial	ND	0.08%	0.27%	ND	ND	ND	0.01%	0.36%
RT for 3D	ND	0.24%	1.33%	ND	0.04%	0.12%	0.20%	2.14%
Refg. For 10D	0.09%	0.17%	0.68%	ND	ND	0.03%	0.06%	1.06%
Diluent: 10% Dextrose solution								
Initial	ND	0.03%	0.26%	ND	ND	ND	0.01%	0.30%
RT for 3D	ND	0.22%	1.48%	ND	0.06%	0.11%	0.23%	2.40%
Refg. For 10D	0.16%	0.13%	0.70%	ND	ND	0.02%	0.09%	1.15%
Diluent: 5% Dextrose Solution + 0.9% NaCl Solution								
Initial	ND	0.03%	0.25%	ND	ND	ND	0.01%	0.29%
RT for 3D	ND	0.22%	1.37%	ND	0.04%	0.11%	0.19%	2.20%
Refg. For 10D	0.11%	0.17%	0.72%	ND	ND	0.02%	0.06%	1.11%
Diluent: Sodium Lactate solution								
Initial	ND	0.03%	0.25%	ND	ND	ND	0.01%	0.29%
RT for 24 hrs	ND	0.14%	0.76%	ND	ND	0.03%	0.15%	1.15%
Diluent: 10% Invert Sugar								
Initial	ND	0.02%	0.26%	ND	ND	ND	0.01%	0.29%
RT for 24 hrs	ND	0.21%	0.99%	ND	0.02%	ND	0.23%	1.53%
Diluent: 5% Sodium bi Carbonate								
Initial	ND	0.04%	0.25%	ND	ND	ND	0.03%	0.32%
RT for 24 hrs	ND	0.05%	1.53%	ND	0.23%	ND	0.61%	2.68%
Diluent: Free amine III								
Initial	ND	0.03%	0.27%	ND	ND	ND	0.02%	0.32%
RT for 24 hrs	0.69%	0.16%	0.71%	ND	0.06%	0.06%	1.07%	3.62%
Diluent: 10% Mannitol								
Initial	ND	0.02%	0.27%	ND	ND	ND	0.02%	0.31%
RT for 24 hrs	ND	0.09%	0.66%	ND	0.01%	ND	0.09%	0.90%
Diluent: Metronidazole HCl and 0.9% NaCl Solution								
Initial	ND	0.07%	0.26%	ND	ND	ND	0.01%	0.34%
RT for 24 hrs	ND	0.51%	0.94%	ND	0.01%	ND	0.15%	1.74%
Diluent: Metronidazole HCl and 5% Dextrose in water								
Initial	ND	0.04%	0.26%	ND	ND	ND	0.01%	0.31%
RT for 24 hrs	ND	0.45%	0.93%	ND	0.01%	ND	0.13%	1.67%

ND-Not detected, D–Days, RT-Room temperature, Refg–Refrigerated, SHUNI-Single highest unknown impurity

No abnormal colour developed in the reconstituted solutions for the solutions after storage periods mentioned in table-1. The reconstituted solution of Ceftriaxone Sodium for injection 2g

having concentration of 40mg/mL with Sterile water for Injection, 0.9% NaCl solution, 10% Dextrose Solution, 5% Dextrose + 0.9% NaCl Solution are stable up to 3 days period at room temperature and 10 days if stored at refrigerated conditions. The reconstituted solution of Ceftriaxone Sodium for injection 2g having concentration of 40mg/mL with Sodium Lactate, 10% Invert Sugar, 5% Sodium bi Carbonate, Free amine III, 10% Mannitol are stable up to 24 hrs at room temperature. The reconstituted solutions having concentration of 10mg/mL with an admixture of Metronidazole HCl (concentration of 7.5mg/mL) + 0.9% NaCl solution and Metronidazole HCl (concentration of 7.5mg/mL) + 5% Dextrose in water are stable up to 24 hrs at room temperature.

CONCLUSION

Ceftriaxone can be stored in the solution having the concentration of 40mg/mL with Sterile water for Injection, 0.9% NaCl solution, 10% Dextrose Solution, 5% Dextrose + 0.9% NaCl Solution are stable up to 3 days period at room temperature and 10 days if stored at refrigerated conditions, and concentration of 40mg/mL with Sodium Lactate, 10% Invert Sugar, 5% Sodium bi Carbonate, Freamine III, 10% Mannitol are stable up to 24 hrs at room temperature, and concentration of 10mg/mL with an admixture of Metronidazole HCl (concentration of 7.5mg/mL) + 0.9% NaCl solution and Metronidazole HCL (concentration of 7.5mg/mL) + 5% Dextrose in water are stable up to 24 hrs at room temperature.

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