INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

PHYSICAL AND CHEMICAL STABILITY STUDIES ON CEFOTAXIME AND ITS DOSAGE FORMS BY STABILITY INDICATING HPTLC METHOD

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ABSTRACT

The influence of temperature and relative humidity on the stability of cefotaxime sodium in the solid state, continuous infusion and in different doses was investigated. Changes in the concentration of cefotaxime sodium were followed by a HPTLC method. The physical stability parameters of colour change and pH were calculated. The physical and chemical stability of cefotaxime sodium was determined at three different temperatures (5°C, 25°C, and 45°C) and quantified by using a stability indicating HPTLC method. The stability of a drug may also be concentration dependent and hence the stability of cefotaxime IV available in three different doses (1gm, 500mg, and 250 mg) was determined when stored at 45°C. The drug solutions were clear and pale yellow initially with the intensity increasing by time, eventually becoming reddish yellow. HPTLC analysis indicated that 100mg/ml concentration of cefotaxime sodium maintained adequate stability for 2 hours at 45°C and up to 24 hours at 25°C and up to 5 days at 5°C. Decrease in drug concentration by more than 10% from initial concentration was considered unstable (chemical instability). Change in pH by more than 1 was considered unstable. The drug exhibited physical and chemical stability consistent with previously reported stability studies.

Keywords: Stability studies, Cefotaxime sodium, Cephalosporin, HPTLC.

INTRODUCTION

Stability testing of an active substance or finished product gives evidence about the quality of the active substance or finished product which varies over time because of the influence of environmental factors like temperature, humidity and light. Stability testing also provides vital informations about the interaction of drug with its ingredients, possible degradations and their mechanisms, and degraded products. The results of stability studies are widely used by the pharmaceutical industry in deciding the storage conditions, suitable packaging material, shelf life and expiration date of the product (ICH guidelines, 2000, Grimm et al., 2000, Dean et al., 2000).

Stability is defined as the capacity of a drug substance or drug product to remain within established specifications to maintain its identity, strength, quality, and purity throughout the retest or expiration dating periods (FDA guidelines, 1998). Drug stability is aimed at ensuring that the drug product remains within specifications established to ensure its identity, strength, quality and

purity. Drug stability and compatibility are critical issues controlling accurate and appropriate delivery of drug therapy to patients. Impurities and degradation can lead to change in the pharmacological, chemical and toxicological properties of the drug which will affect its safety and efficacy (Ahuja, 1998, Ahuja, 2003, FDA guidelines, 1998). Stability is very important for antibacterial agents especially those given by Intra venous (I .V route) as they reach systemic circulation directly, and the clinical outcome and safety are directly correlated to drug levels in blood.
The increased use of parenteral drugs is revealed in surveys that show in the average hospital, 40% of the total dosage forms dispensed to patients are in the form of injections (Salvotore, 1994). Newer generation of parenteral antibiotics have lead to increased role of parenteral therapy. Continuous infusion is an efficient means of administering beta-lactams to maintain drug concentrations higher than the Minimum Inhibitory concentration (MIC) throughout the dosing interval. Continuous infusion has a pharmacoeconomic advantage over intermittent dosing by achieving the same effect with a lower daily dose of drug.
Cefotaxime sodium is a semisynthetic, broad spectrum cephalosporin antibiotic for parenteral administration. Cefotaxime is effective in treating patients with complicated urinary tract and lower respiratory tract infections (AHFS drug information, 2004, Data sheet, TAXIM). Most monograph literatures on cefotaxime indicate stability of the drug at room temperature (25ºC) and refrigeration (5ºC) (USP, 2004, AHFS drug information, 2004, Trissel, 1998). Hence we were interested in studying if cefotaxime which is extensively used intravenously maintains its stability when used clinically as continuous infusion, in a temperate country like India, where temperature may reach up to 45ºC in summer.

MATERIALS AND METHODS

Materials

Cefotaxime sodium was gifted by Orchid pharma, Chennai. Cefotaxime sodium vials were procured from Alkem Chemicals, Mumbai. Water for injection was purchased from Core health care, Mumbai. All other chemicals and reagents used were of laboratory or analytical grade.

Chromatographic Conditions

Chromatography was performed on HPTLC plates (10 x 10 cm) (Camag) chromatographic system by the Ascending chromatography method. Compounds of interest were separated, employing ethylacetate: acetone: water: acetic acid (10:5:3:2 v/v) as mobile phase and Silicagel $60F_{254}$ as the stationary phase. During the investigation, the mobile phase composition remained unaltered. The samples were injected at a quantity of 10µl using Linomat injector. The peak areas were measured at 254 nm using UV detector and scanned (Camag scanner) (Thangadurai et al., 2002).

Standard Stock solution

1gm of cefotaxime sodium was dissolved in 10 ml of sterile water to produce 100mg/ml and serially diluted to produce 200µg/ml and final dilutions were made to the concentrations ranging 0.2 – 1 µg/spot respectively.

Standardization of Cefotaxime sodium

Cefotaxime sodium corresponding to graded concentrations of 0.2 - 1µg/spot were spotted in HPTLC plates of size 10 × 10 cm. The spots were dried and developed using ethylacetate: acetone: water: acetic acid (10:5:3:2) as solvent system (Thangadurai.et.al 2002) and peak areas were measured at 254nm.

Stability testing of cefotaxime sodium at three different temperatures (Fabre and Eddine, 1982, Fabre et al., 1984):

Three 1gm vials of Cefotaxime sodium I .V were reconstituted with 10ml of sterile water for injection and were stored at 5ºC, 25ºC and 45ºC. From the above reconstituted solutions 0.2 ml solution was withdrawn and made up to 10ml and further 1ml was withdrawn and made up to 10ml and serially diluted to produce 200µg/ml. From the above solution

0.5ml of drug solution was withdrawn and made up to 1ml with sterile water, 10µl of drug solution was spotted in HPTLC plates of size 10 × 10 cm and developed in a solvent system [11] comprising ethyl acetate: acetone: water: acetic acid (10:5:3:2), dried and peak areas were measured at 254nm. The above procedure was repeated 5 days with samples withdrawn from vials stored at 5ºC, 25ºC, 45ºC at various time intervals of 0min, 1hr, 2hrs, 4hrs, 7hrs, 24 hrs, 72 hrs, 120 hrs. pH and clarity were noted. The results obtained were observed and recorded.

Stability testing of cefotaxime sodium at 45ºC and in different dosage forms

Three vials (1gm, 500mg & 250 mg) of cefotaxime sodium I.V were reconstituted with 10ml of sterile water for injection and stored at 45ºC. From the above reconstituted solutions 0.2 ml, 0.4ml, and 0.8ml solutions were withdrawn and serially diluted to 200µg/ml. From the above solution 0.5ml of drug solution was withdrawn and made upto 1ml with sterile water, 10µl of drug solution was spotted in HPTLC plates of size 10 × 10 cm, developed in a solvent system ethyl acetate: acetone: water: acetic acid (10:5:3:2) dried and peak areas were measured at 254nm. The above procedure was performed upto 24 hours, with samples withdrawn from vials kept in 45ºC at various time intervals of 0min, 0.5hr, 1hr, 2hrs, 6hrs & 24 hrs. pH, colour change and clarity were noted.

RESULTS AND DISCUSSION

Stability study of Cefotaxime sodium infusion at three different temperatures

Stability study was conducted on pure drug of the investigated compound (Cefotaxime sodium). The mobile phase was selected with reference to Thangadurai et al., 2002 and preliminary experiments showed the elution of cefotaxime sodium in the solvent system. Cefotaxime sodium I.V. infusion (100mg/ml) was stored at different temperatures such as room temperature (25ºC), refrigeration (5ºC) and (45ºC). Samples were withdrawn at different time intervals for 5 days and quantitated by HPTLC method. The stability of cefotaxime sodium I.V. infusion stored at refrigeration temperature proved to be more stable even after 72 hours (% deviation = 5.05) than the other solutions stored at room temperature (% deviation = 11.11) and 45º (% deviation = 27.83) (Table 2).

At 45ºC, the % deviation was > 10% (considered unstable) after nearly 2 hours of storage (value indicates between 2-4 hours). The degradation was also confirmed by the physical color change from colorless to pale yellow at the 2 hours sample (Table 1). At 25ºC > 10% degradation was seen between 24 hours and 48 hours. The physical color change was seen at 24 hour sample from colorless to dark yellow and at the end of 5 days the sample was reddish yellow in color indicating complete degradation (Table 3). At 5ºC drug solution was stable up to 5 days. There was no color change in the solution at the end of 5 days, indicating the sample was stable (Table 1). The results obtained from pH, colour and clarity test showed physical stability at 5ºC even after 5 days (Table 1).

Stability study of cefotaxime sodium i.v. infusion at three different dosage forms

Cefotaxime sodium I.V. infusion (100mg/ml) was stored at 45º. Different dosage forms of (1gm, 500mg & 250mg) were used in the study. Samples were withdrawn at different time intervals for 24 hours and quantitated by HPTLC method. The observations at the end of 24 hours were noted. The stability study of cefotaxime sodium I.V. infusion stored at 45º, in different dosage forms showed very little difference and almost similar stability was observed in all the three dosage forms.

The drug solutions were stable up to 2 hours of study, % deviation was > 10% (considered unstable) (Table 6) after nearly 2 hours of storage (value indicates between 2-4 hours) (Table 5). The results obtained for pH and clarity test showed little change even after 24 hours, but the color intensified with time (Table 4). The color started appearing at the 2nd hour samples and intensified further in the 6th hour sample and became reddish yellow at the 24hour sampling time,

indicating degradation progressing over time (Table 4).

Results of our study involving cefotaxime sodium (by HPTLC method) were found similar to those reported by Fabre.H. et al., 1982; where cefotaxime sodium was stable up to 5 days at refrigeration temperature (5ºC) and 24 hours at room temperature (25ºC), our results indicate cefotaxime sodium was stable only for 2 hours at 45ºC.

Our work on the dose related stability of cefotaxime sodium (at doses 1gm, 500mg & 250mg and quantitated by stability indicating HPTLC assay) at 45ºC did not show any significant changes in the degradation pattern. Stability was seen only for 2 hrs at all three doses at 45ºC.

There was no visible change in the HPTLC chromatogram of cefotaxime during our entire study excepting in the case of 45°C exposure of cefotaxime after 72hrs where no recordable peak corresponding to cefotaxime was found indicating complete degradation of cefotaxime.

Table 1: Physical Stability of Cefotaxime Sodium I.V. at Three Different Temperatures

Temperature (°C)		0 hour	1 hour	2 hour	4 hour	7 hour	24 hour	72 hour	120 hour
5	pH	5.9	5.9	5.9	5.9	5.9	5.8	5.8	5.4
	Clarity	clear	clear	clear	clear	clear	clear	clear	Clear
	Color	colorless	colorless	colorless	colorless	colorless	colorless	colorless	colorless
25	pH	5.9	5.9	5.9	5.9	5.7	5.4	5.4	5.2
	Clarity	Clarity	clear	clear	clear	clear	clear	clear	clear
	Color	Color	colorless	colorless	colorless	colorless	Dark yellow	Dark yellow	Reddish yellow
45	pH	5.9	5.9	5.8	5.8	5.6	5.2	-	-
	Clarity	clear	clear	clear	clear	clear	clear	-	-
	Color	colorless	colorless	Pale yellow	Pale yellow	Dark yellow	Reddish yellow	-	-

Table 2: Chemical Stability of Cefotaxime Sodium I.V. at Three Different Temperatures

Temp	Expected concentration	0 hour	1 hour	2 hour	4 hour	7 hour	24 hour	72 hour	120 hour
5°	1µg/spot	5220.7 (0.99)	5156.3 (0.98)	5145.2 (0.97)	5086.4 (0.95)	5064.7 (0.95)	5011.8 (0.94)	5003.6 (0.94)	4936.8 (0.91)
25°	1µg/spot	5220.7 (0.99)	5053.6 (0.96)	5041.7 (0.96)	4982.3 (0.94)	4933.7 (0.93)	4812.6 (0.91)	4654.9 (0.88)	4423.3 (0.82)
45°	1µg/spot	5220.7 (0.99)	5032.2 (0.94)	4822.6 (0.91)	4573.9 (0.87)	4324.3 (0.82)	3756.2 (0.71)	-	-

Values recorded are the peak area values and the values given within brackets are the concentration of cefotaxime sodium I.V. in mcg/ml.

$$\text{Percentage deviation} = \frac{\text{Initial concentration} - \text{Final}}{\text{Initial Concentration}} \times 100$$

Table 3: Degradation Profile of Cefotaxime Sodium at Three Different Temperatures

Sampling Time	Amount of Drug Remaining (%)		
	5°	25°	45°
0 time	100	100	100
1 hour	98.99	96.97	94.95
2 hour	97.98	96.97	91.92
4 hour	95.96	94.95	87.88
7 hour	95.96	93.94	82.83
24 hour	94.95	91.92	71.72
72 hour	94.95	88.89	-
120 hour	91.92	82.83	-

Table 4: Physical Stability of Cefotaxime Sodium I.V. at 45°c and In Different Dosage Forms

(Temperature 45°C) Dosage		0 hour	0.5 hour	1 hour	2 hour	6 hour	24 hour
1 gm	pH	5.9	5.9	5.8	5.7	5.7	4.9
	Clarity	clear	clear	clear	clear	clear	clear
	Color	colorless	colorless	colorless	Pale yellow	Dark yellow	Reddish yellow
500 mg	pH	5.9	5.9	5.8	5.7	5.7	4.9
	Clarity	clear	clear	clear	clear	clear	clear
	Color	colorless	colorless	colorless	Pale yellow	Dark yellow	Reddish yellow
250 mg	pH	5.9	5.9	5.8	5.7	5.6	5.0
	Clarity	clear	clear	clear	clear	clear	clear
	Color	colorless	colorless	colorless	Pale yellow	Dark yellow	Reddish yellow

Table 5: Chemical Stability of Cefotaxime Sodium I.V at 45°C and in Different Dosage Forms

Dosage	Expected concentration	0 hour	0.5 hour	1 hour	2 hour	6 hour	24 hour
1gm	1µg/spot	5225.8 (0.99)	5183.2 (0.98)	5047.3 (0.96)	4832.4 (0.91)	4392.3 (0.84)	3762.2 (0.72)
500mg	1µg/spot	5123.6 (0.97)	5107.4 (0.96)	4996.8 (0.95)	4858.9 (0.92)	4363. (0.82)	3766.4 (0.72)
250mg	1µg/spot	5208.7 (0.99)	5185.4 (0.98)	5080.8 (0.97)	4865.8 (0.92)	4374.4 (0.83)	3771.4 (0.71)

Values recorded are the peak area values and the values given within brackets are the concentration of cefotaxime sodium I.V. in mcg/ml.

$$\text{Percentage deviation} = \frac{\text{Initial concentration} - \text{Final}}{\text{Initial Concentration}} \times 100$$

Table 6: Degradation Profile of Cefotaxime Sodium at 45°C and in Different Dosage Forms

Sampling Time	Amount of Drug Remaining (%)		
	1gm	500mg	250mg
0.5 hour	98.99	98.97	98.99
1 hour	96.97	97.94	97.98
2 hour	91.92	94.85	92.93
6 hour	84.85	84.54	83.84
24 hour	72.73	74.23	71.72

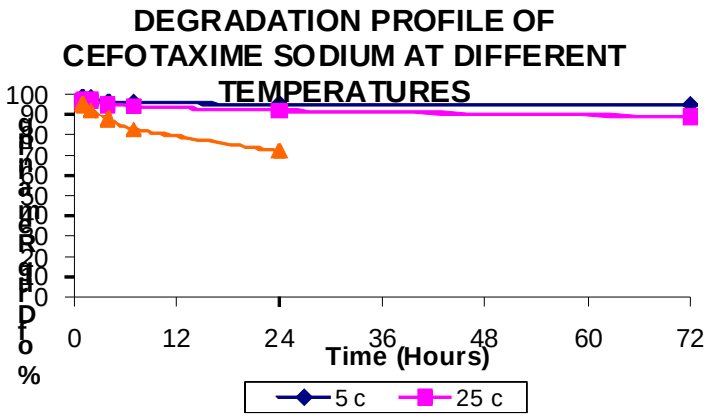

Fig. 1: Degradation of Cefotaxime Sodium at different temperatures (5°C, 25°C and 45°C). Samples at 45°C showing degradation at shorter period of time

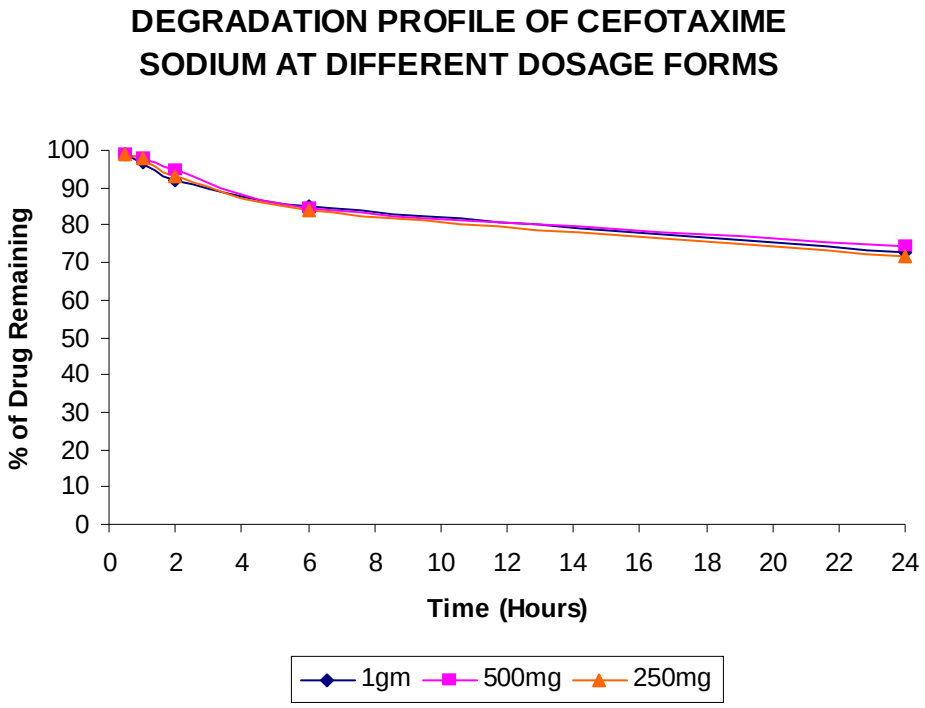

Fig. 2: Degradation profile of Cefotaxime sodium at different Dosage Forms (1g, 500mg and 250 mg). The stability patterns are almost the same

CONCLUSION

Cefotaxime Sodium infusion was stable at the end of 5 days when stored at 5°C, retaining its color and pH. Hence our datas agree with the datas of the marketed product (Data sheet, TAXIM). When stored at 25°C, the degradation started around 24 hours, indicating stability for a day. At the accelerated stability temperature of 45°C, the degradation started around 2 hours itself. There was little change in the different dosage forms when stored at accelerated temperature, with stability for all samples retained to the end of 2 hours. Identifying the degraded products can pave way for a more detailed study on stability.

ACKNOWLEDGEMENT

The authors are thankful to Principal, Management and Staff of Sri Ramakrishna Institute of Paramedical sciences, Coimbatore, Tamilnadu for providing the facilities.

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