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A Comparative Validation Study for the Estimation of Cefotaxime Sodium by Spectrophotometric and Fluorimetric Methods

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Abstract

In present research, a comparative validation study for the estimation of cefotaxime sodium in pharmaceutical formulations by spectrophotometric and fluorimetric methods were performed.

The developed methods depend on the determination of cefotaxime sodium by using 2, 4-Dinitro phenyl hydrazine. By using 2, 4-Dinitro phenyl hydrazine, these drug form ion-pair complex in acidic medium. This coloured complex shows maximum absorbance at 408nm by spectrophotometric method (Method A) and fluorescent intensity was measured by using fluorimetric method (Method B).Linearity calibration curve were obtained in a concentration range of 1.0-6.0 µg/ml for cefotaxime sodium by using both of these methods. The result of analysis of these methods shows that the amount of drugs present in the formulation has a very good correlation with the label claim of the formulation.

The developed methods were validated for various parameters as per ICH guidelines like Accuracy, Precision, Linearity, and range, LOD and LOQ, Ruggedness and Robustness. %RSD will be less than 2 for all the validation parameters. Recoveries studies revealed that results within the specified limits. Hence the proposed spectrophotometric and fluorimetric methods were found to be satisfactory and could be used for the routine analysis of cefotaxime sodium in their marketed formulations. Statistical comparison of the results with the reference methods showed excellent agreement and proved that no significant difference in the accuracy and precision.

Keywords: Cefotaxime sodium; 2, 4-Dinitrophenyl hydrazine; Fluorimeter and UV-Visible Spectrophotometer.

Introduction

Cefotaxime sodium (Fig. 1) is a third-generation cephalosporin antibiotic. Like other third generation cephalosporins, it has broad spectrum activity against Gram-positive and Gram-negative bacteria. Cefotaxime injection is used to treat certain infections caused by bacteria including pneumonia and other lower respiratory tract (lung) infections, gonorrhoea, meningitis and other brain spinal cord infections.[1-3]

In literature survey several analytical methods like HPLC[4-13] and spectrophotometric[14-19] immunoassay for screening, fluorimetry and Mass spectrometry[20-25] methods have been reported on the determination of cefotaxime sodium in combination with other drugs. The purpose of this study was to develop and validate a simple analytical method to quantify cefotaxime sodium in injectables, using spectrophotometry and fluorimetric methods. The methods have been successfully applied to the determination of the cited drugs in their commercial pharmaceutical formulations. The results obtained by these methods were statistically compared.

Materials and Methods

Drugs and Formulations

The reference samples of Cefotaxime Sodium (API) were provided from Lupin Pharmaceuticals, Mumbai. The commercial formulation was procured from the local market.

Chemicals and Solvents

2,4-Dinitro Phenyl Hydrazine (1% w/v), ethanol and dilute H_2SO_4 were used for these studies. Freshly prepared double distilled water was used throughout the experiment.

Instrumentation

Systronics-2203 UV Visible double beam spectrophotometer (10mm optical path length matched quartz cell,50 nm min-1 scan speed) and 5 nm fixed slit width) was used for recording spectra and absorbance measurements; Elico fluorimeter with a xenon discharge lamp, excitation and emission slit of 10 nm and quartz cuvettes of1x1 cm was used for emission studies. Other instruments like pH-Meter (MKV1, Systronics) and Digital Balance are used for this study.

Preparation of Standard Solution

100 mg of cefotaxime sodium was weighed and transferred in to 100 ml volumetric flask. The drug was dissolved and the volume was made up to the mark with water to obtain final concentration of 1000µg/ml (Stock -A solution).

Preparation of working standard solution

From the stock-A solution, 1 ml was pipette out and transferred in 100 ml volumetric flask and add 10 ml of 2, 4-DNP and 5ml dilute sulphuric acid then the volume was made up to the mark with water to obtain the final concentration of 10µg/ml (stock –B solution).

Results and Discussions

The main step in the development of an analytical method is to improve the conditions and parameters which should be followed in the development and validation. Different solvents were studied (methanol, ethanol, acetone and water), the criteria employed were the sensitivity of the method and availability of the solvent. From a solvent effect studies and spectral behaviours of Cefotaxime sodium, water was selected as solvents for the suggested spectrophotometric and fluorimetric methods. The method sensitivity such as LOD and LOQ and analytical parameters such as correlation coefficient, intercept and slope of the calibration equations was tested.

Method Development

In this method, based on spectrophotometric and fluorimetric determination of cefotaxime sodium in pure and dosage forms were developed by using 2, 4-Dinitro Phenyl Hydrazine in dilute sulfuric acid. The analytical conditions were selected, keeping mind that the chemical structure of Cefotaxime sodium.

Spectrophotometric method (Method-A)

Cefotaxime sodium react with 2, 4-DNP in dilute sulfuric acid (pH-3.2) to get a yellow-coloured ion-pair complex and measured the maximum absorbance at 408nm (Fig. 1). The ion-pair formation is due to Cefotaxime sodium contains carbonyl keto group react with 2, 4-DNP n acidic medium. So, 2,4-Dinitro Phenyl Hydrazine was converted to 2, 4-Dinitro Phenyl Hydrazones give yellow coloured ion pair complex, which is quantitatively determined by spectrophotometric methods.

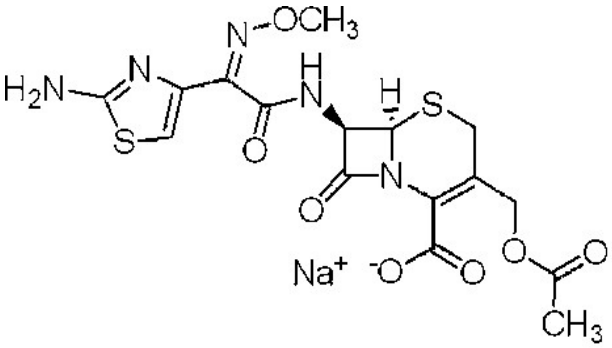

Fig. 1: Structure of Cefotaxime Sodium.

Fuorimetric method (Method-B)

Cefotaxime sodium react with 2, 4-DNP in dil. H_2SO_4 (pH-3.2). Yellow colored fluorescent compound is formed and measure the fluorescent intensity. This was formed due to Cefotaxime sodium contains carbonyl keto group react with 2,4-DNP in acidic medium. 2,4-Dinitro Phenyl Hydrazine was converted to 2,4-Dinitro Phenyl Hydrazones give yellow colored ion pair complex, which is quantitatively determined fluorimetrically.

Analysis of Cefotaxime sodium

Quantitative analysis of Cefotaxime sodium content (Claimed concentration 100 mg/1ml) in commercially available injection by UV and fluorimetric methods. The assay procedure was repeated for 6 times, mean weight of standard drugs, of sample were taken and calculated. Prepare 5 µg/ml of standard and sample solution were also prepared and assayed for content of cefotaxime sodium against the reference standard. The content of cefotaxime sodium in the marketed brands was determined, the percentages of individual drugs found in formulations, amount and relative standard deviation in formulations were calculated. The result of analysis shows that the amount of drugs present in the formulation has a very good correlation with the label claim of the formulation Table1.

Table 1: Estimation of cefotaxime Na.

Factors (n = 5)	UV-Visible method		Fluorimetric method	
	Brand A	Brand B	Brand A	Brand B
Mean (%w/v)	99.0%	99.7%	98.5%	98.4%
Labelled amount (mg)	0.01	0.01	0.01	0.01
Amount found (mg)	0.00985	0.0099	0.0099	0.00994
SD	0.00008	0.00004	0.00010	0.00008
R.S.D (%)	0.804	0.402	1.02	0.808

Method Validation

A) *Accuracy*

The recovery studies (Table 2 &3) were carried out 3

times at 50%, 100% and 150% levels and the percentage recovery and percentage relative standard deviation of the percentage recovery were calculated. Recoveries studies revealed that results within the specified limits and the method were found to be accurate.

Table 2: Recovery Studies for Cefotaxime Na.

Level of addition (% pure drug)	Con. of drug in formulations (µg/ml)		Conc. of pure drug (µg/ml)		Total conc. of drug found (µg/ml)	
	Method A	Method B	Method A	Method B	Method A	Method B
50%	5	5	2.	2.5	7.47 7.48 7.47	7.46 7.47 7.46
100%	5	5	5	5	10.07 10.05 9.98	10.01 10.09 9.95
150%	5	5	7.5	7.5	12.44 12.45 12.44	12.43 12.45 12.43

Table 3: Statistical validation data for accuracy determination of Cefotaxime Na.

Level of Recovery %	Mean		Standard Deviation		%RSD		% Analytical recovery	
	A	B	A	B	A	B	A	B
50%	7.4733	7.46	0.0057	0.0057	0.076	0.07	99.64	99.6
100%	10.17	10.01	0.0041	0.412	0.040	0.409	101.4	101.7
150%	12.44	12.43	0.0016	0.0019	0.012	0.014	99.94	99.52

B) *Precision*

The intra-day and inter-day precision studies of the developed method (Table 4 & 5) confirmed adequate sample stability and method reliability, where all the RSDs were less than 2%.

Table 4: Precision studies for cefotaxime Na-Method A.

Intraday (n=6)				Interday (n=6)			
Conc. (µg/ml)	Ab.(1)	Ab.(2)	Ab.(3)	Conc. (µg/ml)	Ab. (D-1)	Ab. (D-2)	Ab. (D-3)
5	0.695	0.695	0.695	5	0.695	0.695	0.695
5	0.696	0.696	0.694	5	0.696	0.694	0.694
5	0.695	0.694	0.695	5	0.695	0.695	0.695
5	0.696	0.695	0.694	5	0.696	0.696	0.696
5	0.695	0.696	0.696	5	0.695	0.695	0.695
5	0.695	0.695	0.695	5	0.696	0.696	0.694
MEAN	0.6953	0.6951	0.6948	MEAN	0.6955	0.6951	0.6948
S.D	0.000517	0.00075	0.000753	S.D	0.000547	0.00075	0.000753
%RSD	0.0743	0.1078	0.1084	%RSD	0.0786	0.1078	0.1079

Table 5: Precision studies for cefotaxime Na-Method B.

Intraday (n=6)				Interday (n=6)			
Conc. (µg/ml)	**Ab.(1)**	**Ab.(2)**	**Ab.(3)**	**Conc. (µg/ml)**	**Ab. (D-1)**	**Ab. (D-2)**	**Ab. (D-3)**
4	66	66	66	4	66	66	67
4	67	67	67	4	67	67	66
4	66	66	66	4	66	67	67
4	66	66	67	4	67	67	66
4	67	67	67	4	66	67	67
4	66	67	67	4	66	67	66
MEAN	66.33	66.5	66.66	MEAN	66.33	66.83	66.5
S.D	0.5164	0.5477	0.5164	S.D	0.5164	0.4082	0.5477
%RSD	0.7785	0.8236	0.7746	%RSD	0.7785	0.6108	0.8236

C) *Linearity and range*

For linearity six points calibration curve were obtained by both method A and B in a concentration range 1.0-6.0 µg/ml. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation for cefotaxime Na. are shown in (Fig. 3 & 4). Linearity results both Method A and B were depicted in Table 6.

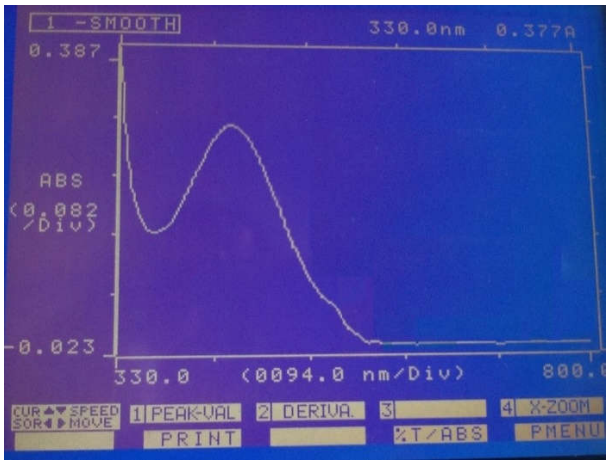

Fig. 2: Spectrum of Cefotaxime sodium using 2, 4-DNP.

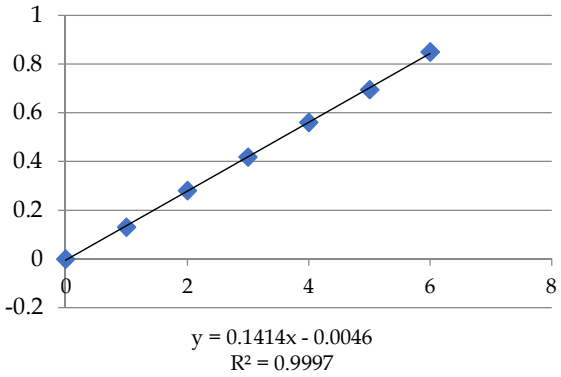

Fig. 3: Calibration curve of Cefotaxime sodium (Method A).

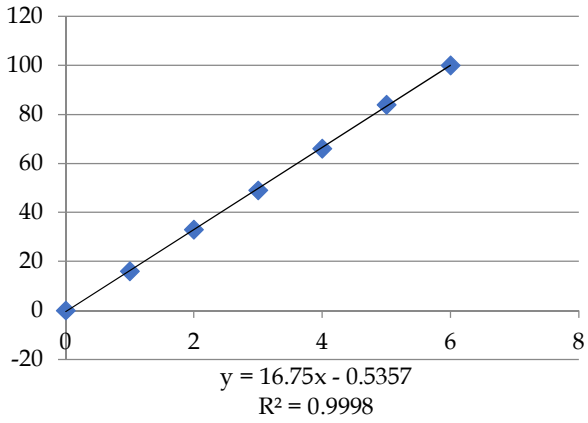

Fig. 4: Calibration curve of Cefotaxime sodium (Method B).

Table 6: Overview of the linearity data for cefotaxime sodium by the UV Visible spectrophotometric and Fluorimetric methods.

Parameters	UV-Visible method	Fluorimetric method
λmax	408nm	-
Regression Coefficient (r^2)	0.9997	0.9998
Slope	0.1414	16.75
Intercept	0.0046	0.5357
Standard Deviation	0.000517	0.6575
Concentration Range (µg mL^{-1})	1.0-6.0	1.0-6.0
Number of Points	6	6

D) *Limit of Detection (LOD) & Limit of Quantification (LOQ)*

The limit of detection and limit of quantification were calculated from calibration curve and LOD and LOQ of Cefotaxime sodium in is given in Table 7.

Table 7: Limit of Detection (LOD) & Limit of Quantification (LOQ) for Cefotaxime Na.

Method	LOD µg/mL	LOQ µg/mL
Method-A	0.141	0.036
Method-B	0.129	0.392

E) *Ruggedness*

The ruggedness of the methods was demonstrated by conducting the experiment on two different analyst and %RSD was calculated by Method A and B shown in Table 8. Variation in percentage content was found to be within the limit so the method is rugged in nature.

Table 8: Ruggedness of Cefotaxime Na.

SL NO:	Conc.(µg/ml)		Method-A		Method-B	
			Analyst-1	Analyst-2	Analyst-1	Analyst-2
	A	B	Absorbance	Absorbance	Absorbance	Absorbance
1	5	4	0.695	0.695	66	66
2			0.696	0.694	65	66
3			0.695	0.695	66	65
4			0.696	0.694	65	67
5			0.695	0.696	66	66
6			0.695	0.695	66	66
Ab. Mean			0.6953	0.6948	65.66	65.833
S.D			0.000517	0.000753	0.5164	0.6575
%RSD			0.0743	0.1084	0.7864	0.9987

F) *Robustness*

The result of robustness study of the developed assay method was established in Table 9. The result shown that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. Hence the analytical method would be concluded as robust.

Table 9: Robustness of Cefotaxime Na.

SL NO:	Conc.(µg/ml)		Method-A		Method-B	
			Room Temp.	AC. Temp.	Room Temp.	AC. Temp.
	Method A	Method B	Absorbance	Absorbance	Absorbance	Absorbance
1	5	4	0.678	0.671	0.684	0.683
2			0.671	0.670	0.684	0.683
3			0.673	0.669	0.681	0.683
4			0.675	0.672	0.686	0.684
5			0.674	0.673	0.684	0.684
6			0.676	0.674	0.684	0.684
Ab. Mean			0.674	0.671	0.6838	0.6835
S.D			0.0022	0.0035	0.0096	0.0045
%RSD			0.326	0.521	1.40	0.658

Comparative Study

The two methods were successfully applied for the determination of the studied drugs in injections. Statistical comparisons between the results obtained by the suggested fluorimetric and UV-Visible spectrophotometric methods of analysis of the cited drugs were carried out, and no significant difference between them.

A linear relationship was found between cefotaxime sodium concentration and response time of both UV and Fluorimetric methods. The precision data obtained for the two methods are tabulated in Table 4 and 5. Table 6 shows regression analysis data for both the methods. Both UV and flourimetric method shows the regression coefficient of 0.999. Both UV-visible spectrophotometric and fluorimetric methods showed R.S.D. values lower than 2% presenting good precision, however UV Visible spectrophotometric method was more precise than florimetric method. Accuracy was investigated by means of % recovery experiments (n=3) using developed methods. Both spectrophotometric and flourimetric methods exhibited recoveries close to 100%, however better recovery was achieved by UV Visible-method. The LOD and LOQ for UV Visible method were found to be

0.141 µg mL-1 and 0.036 µg mL-1, respectively. Forfluorimetric method, LOD and LOQ were found to be 0.129 µg mL-1 and 0.392 µg mL -1, respectively.

Conclusion

This study showed a possibility to use 2, 4-DNP as a reagent for the spectrophotometric and flourimetric methods determination of cefotaxime sodium. The developed method was validated for various parameters as per ICH guidelines like Accuracy, Precision, Linearity and range, LOD and LOQ, Ruggedness, Robustness. The results obtained were within the acceptance criteria for the parameter. Hence it can be concluded that, the proposed methods (both UV Visible Spectrophotometric and fluorimetric methods) were found to be satisfactory and used for the routine analysis of cefotaxime sodium in their marketed formulation. However, the UV- Visible spectroscopic method is showed to be more sensitive, accurate and precise than fluorimertic method.

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