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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC AND ULTRAVIOLET SPECTROPHOTOMETRIC METHOD FOR THE QUANTITATIVE ESTIMATION OF CEFUROXIME SODIUM IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Cefuroxime sodium is a semi-synthetic, broad-spectrum, cephalosporin antibiotic for parenteral administration. In the present paper a high-performance liquid chromatographic and an UV spectrophotometric method were developed and validated for the quantitative determination. The different analytical parameters such as linearity, precision, accuracy, specificity, limit of detection (LOD) and limit of quantification (LOQ) were determined according to International Conference on Harmonization ICH Q2B guidelines. Chromatography was carried out by gradient technique on a reversed-phase C-6 column with mobile phase based and optimized depending on the polarity of the molecules. The UV spectrophotometric determinations were performed at 275nm for Cefuroxime sodium. The linearity of the calibration curves for each analyte in the desired concentration range was excellent (r^2> 0.999 & 0.998) by both the HPLC and UV methods. Both the methods were accurate and precise with recoveries in the range of 97 and 103% and relative standard deviation (R.S.D) <2%. Moreover, the accuracy and precision obtained with HPLC correlated well with the UV method which implied that UV spectroscopy can be a cheap, reliable and less time consuming alternative for chromatographic analysis. The proposed methods are highly sensitive, precise and accurate and hence were successfully applied for the reliable quantification of drugs in the commercial formulations of Cefuroxime sodium.

Keywords: Cefuroxime sodium, UV spectrophotometric, RP-HPLC.

INTRODUCTION

Cefuroxime sodium (CS) is a semi-synthetic, broad-spectrum, cephalosporin antibiotic for parenteral administration[1]. HPLC method for the quantitative analysis of CS in formulations based on mobile phases containing compounds such as sodium acetate, acetonitrile, etc.[2-3]

A simple, harmonized approach to HPLC method screening can reduce cycle time for method development. Reducing expenses and improving efficiency have been a focus for many pharmaceutical companies. Several examples in the literature discuss the use of streamlined method development or screening processes. A

recent publication by Xiao et al. used the ChromSword® method development software in conjunction with automated column switching for challenging separations (e.g., alpha and beta methylepoxide) while utilizing columns from major vendors[4]. Other examples illustrate automation for peak tracking as well as column and mobile phasae screening in addition to the use of software tools for optimization (ChromSword®)[5-6].

The RP-HPLC method is widely employed in quality control assessment of drugs because of their sensitivity, repeatability and specificity[7-11]. On the other hand, the use of spectroscopic techniques can be considered a promising simple, faster, direct and relatively less expensive alternative for the determination of active drug content in pharmaceutical formulations with sufficient reliability[12-13]. The aim of present investigation was to developand validate simple, rapid UV Spectroscopic method as well as an alternative RP-HPLC method forroutine analysis.

EXPERIMENTAL

Chemicals and reagents

Cefuroxime sodium,orcinol and drug sample (Wockhardt Ltd. India), were used for the study. Water, acetonitrile, chloroform and methanol used were of HPLC grade (Merck, India). All the other chemicals used were of analytical grade (Merck, India).

Instrumentation

UV spectral measurements were recorded in (Shimadzu1601) UV-Visible spectrophotometer. RP-HPLC was performed by using RP-HPLC (Waters Alliance 2695). Chromatographic conditions (mobile phase composition and flow rate) were evaluated using the reverse phase, UV- 1575 UV- visible detector, column C18 (5µm, 250mm X 4.6mm ODS-A).

RP-HPLC method

The buffer solution was prepared by diluting 5.8ml orthophosphericacid in 1000ml of distilled water adjust the pH 3.0 ±0.05 Triethyl amine.The mobile phase was prepared by buffer solution and acetonitrile in the ratio 10:1 (V/V) and filtered through 0.45 µm whatmanfilter paper and sonicated before use. A stock solution was prepared by dissolving 100mg of CS in 100 ml mobile phase and was further diluted to obtain different concentration ranging16 µg /mlto 330 µg /mlof standard concentration.Fig. 3.Calibration curve was plotted between concentration against area were determine by duplicate analysis of six concentrations. These solutions were used to calculate the linear dynamic range and good correlation coefficient was found. The regression line almost passes through the origin. (Intercept). The quantification data and system suitability data are presented in Tables 1 and 2, respectively.

Table 1: System Suitability Parameters from linearity (RP-HPLC)

Parameters	CS for Injection
Mean	103054
%RSD	0.21
Resolution	5.12
Theoretical (plates/column)	10123.56
Asymmetry factor	1.35

UV spectroscopic method

The Stock solutions were prepared by dissolving100mg in 100mlultra pure water and further diluted with water to obtain working standards in different concentration ranges. From Stock solution 10µg/ml was prepared for wavelength selection and the maximum lambda max was selected Fig.1. Six standardsolutions were prepared from the stock solutions withdifferent concentration ranging from 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0respectively Fig.3. Calibration curve was plotted between concentration verses absorbance. All the standard solutions were scanned over the range of 200–400 nm. The analytical data arepresented in Table 2. All the solutions were prepared in triplicates.

Method Validation

In the present work, HPLC conditions listed in Table 1 have been developed and validated for detection and quantitation. In order to ensure the compound remained stable, HPLC analysis was conducted with a column temperature of 25 °C. The method showed excellent linearity, accuracy, precision, LOD, LOQ and Robustness when evaluated at the 20 ul (Table 2). Linearity & Range was determined using a six point calibration curve from 0.01% to 150% of the nominal concentration 20uL. Recovery of the sample matrix was assessed at three different concentrations using six solutions per concentration (n = 6). Results of the spike and recovery studies performed.

The UV spectroscopy Recovery studies were carried out by adding known quantities of standards at different levels (50 to150 %) to the pre-analyzed sample to study the linearity,

accuracy, precision, LOQ, LOD and Robustness of the proposed methods. The recovery studies also reveals whether there is a positive or negative influence on the quantification parameters by the additives usually present in the dosage forms. The linearity & Range study data are presented in Table 3.

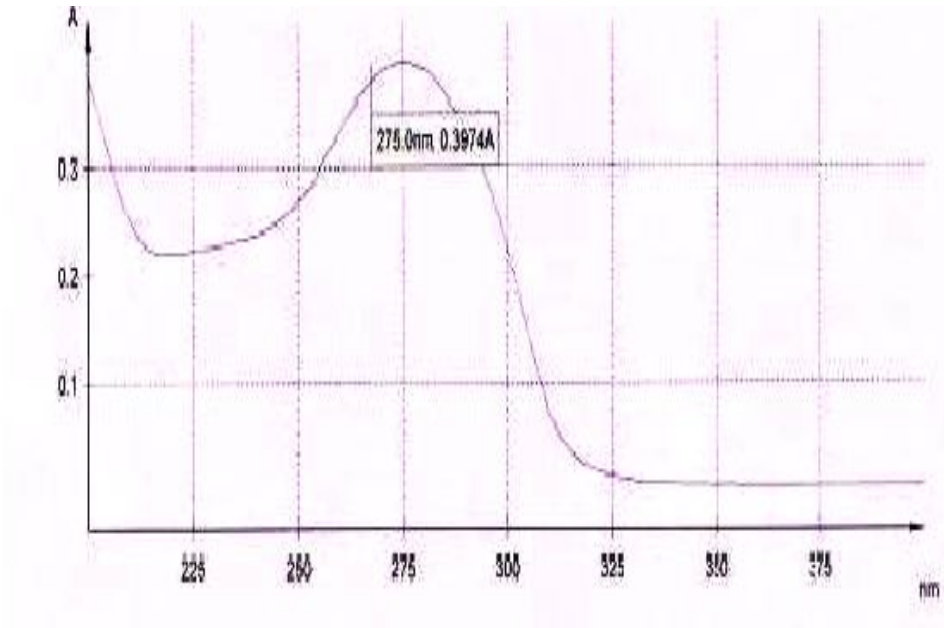

Fig. 1: Wavelength Selection on UV Spectrophotometer

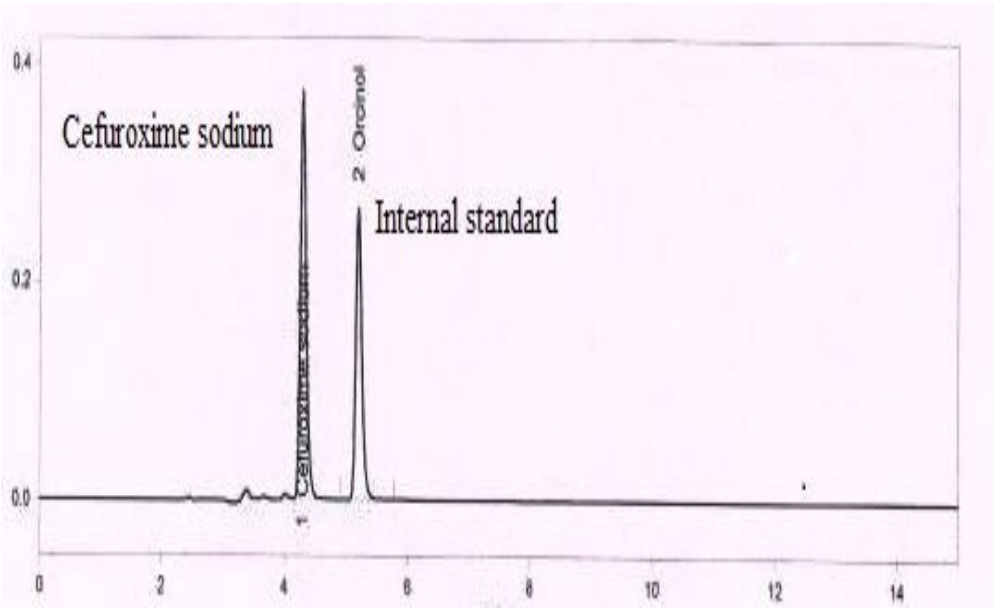

Fig.2:Representative Chromatogram of standard solution Cefuroxime sodium in pharmaceutical dosage form

Table 2: Validation parameters for UV spectroscopy and RP-HPLC

Validation Parameters	UV	RP-HPLC
Wavelength Selection	275nm	275nm
System Suitability	0.8%	0.2%
Linearity	0.9986	0.9986
Limit of Detection	22.48%	
Limit of Quantification	1.44%	0.4%
Range (µg/ml)	2.5 – 15	16 – 330

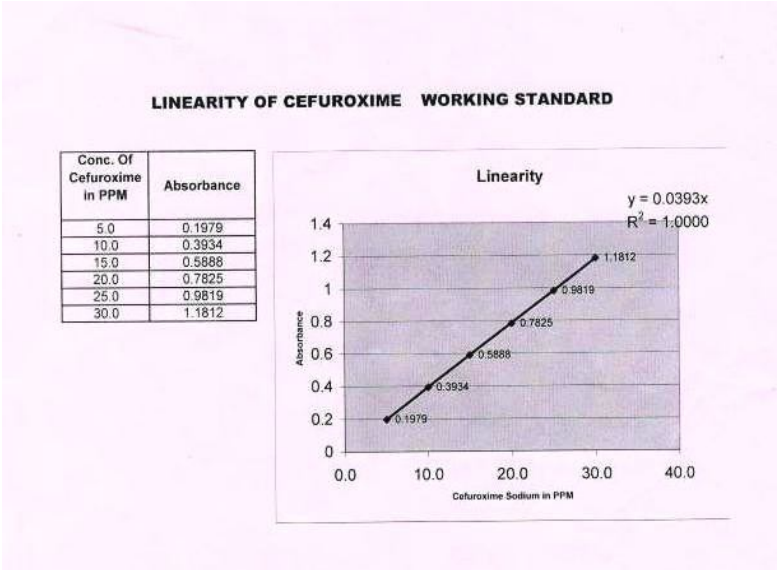

Fig.3: Linearity of Cefuroxime sodium

Table3:Study of Precision Parameter for RP-HPLC and UV

Method	Parameters	System Precision	Method Precision	Intermediate Precision	
				Inter-day	Intraday
RP-HPLC	Mean ± SD	2399860 11856.32	2153911 5319.240	2352246 1043.69	2331541 3401.184
	% RSD	0.49	0.71	0.04	0.14
UV	Mean ± SD	0.3986 0.031	0.4250 0.004	0.4041 0.003	0.4066 0.003
	% RSD	0.8	1.0	0.88	0.73

Table 4: Percent recoveries in commercial formulations by RP-HPLC &UV methods of analysis

Concentration	UV Method		% Recovery	RP-HPLC		% Recovery
	Mean	% RSD		Mean	% RSD	
50%	0.0202	0.28	97.016	10305	0.31	98.39
100%	0.4056	0.05	99.71	25675	0.06	99.04
150%	0.6044	0.32	99.81	498487	0.14	99.48

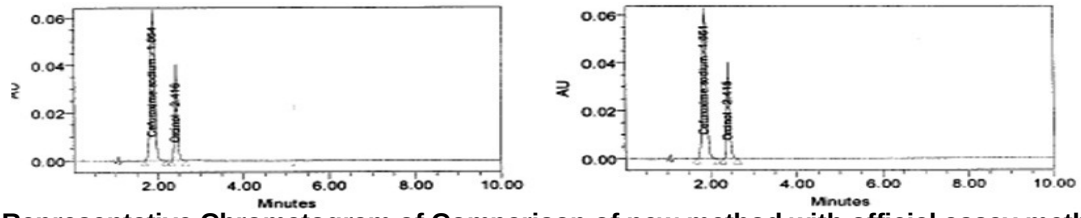

Fig.4:Representative Chromatogram of Comparison of new method with official assay method

RESULT AND DISCUSSION

The RP-HPLC method, system suitability (Table 1)was applied to a representative chromatograph to checkvarious parameters such as mean, RSD, asymmetric factor, resolution andtheoreticalplates. The methods were validated according to the International Conference on Harmonization[14-18].

Table 5: Comparison of new method with official assay method

Method	% Assay
HPLC as per USP	100.65%
RP- HPLC	100.35%
UV	100.72%

CONCLUSION

UV-Spectrophotometer and RP- HPLC method have been developed for determination of Cefuroxime sodium for injection dosage form. This intended study can be concluded that both the methods UV-spectrophotometric and RP-HPLC is simple, economical, rapid method and were found to be more precise, accurate, rugged and robust. Therefore the rapidity of the proposed method makes them useful in routine analysis. The validation results with the statistical treatment of the data and continuous study of the result during manufacturing process demonstrate the reliability of method. This method actually saves lot of time, chemicals and cost effective.

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