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ROLE OF DIFFERENT HYDROTROPIC AGENTS IN SPECTROPHOTOMETRIC AND CHROMATOGRAPHIC ESTIMATION OF CEFIXIME

V. PAREEK[1], S. R. TAMBE* AND S. B. BHALERAO[1].

[1]Department of Pharmaceutical Chemistry, Mahatma Gandhi Vidya Mandir's Pharmacy College, Panchavati, Mumbai - Agra Road, Nashik- 422003, Maharashtra, India.

***Corresponding Author** santoshtambe@indiatimes.com

ABSTRACT

Present study deals with two Spectrophotometric methods, Conventional Spectrophotometric Estimation and Area under Curve Method and a chromatographic method for estimation of Cefixime by using five different hydrotropic agents. These include Ammonium acetate (6M), Potassium acetate (5M), Potassium citrate (0.5 M), Sodium citrate (1.25 M) and Urea (8M). Area under curve method was based on measurement of area under curve (AUC) in the wavelength range 279nm to 298nm. In both spectrophotometric methods, linearity of Cefixime was found in the concentration range 5 to 30µg/ml by using all above hydrotropic agents. HPTLC method was also developed .For HPTLC method, linearity of Cefixime was found to be in concentration range 100ng to 500ng. Mixture of methanol: ethyl acetate: triethylamine (7:5:0.05v/v) was used as a developing solvent. Recovery study was performed for both spectrophotometric and chromatographic methods. The results of analysis obtained by these methods were compared with those of USP standard limit. Limit of detection and quantitation in all methods were found laser in potassium acetate as compared to other hydrotropic agents used in studies.

KEYWORDS

Cefixime, Hydrotropic agents, Area under Curve method, HPTLC

INTRODUCTION

Cefixime trihydrate is chemically (6*R*,7*R*)-7-[[(*Z*)-2-(2-aminothiazol-4-yl)-2-[(carboxymethoxy)imino]acetyl]amino]-3ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid trihydrate. Cefixime is a β-lactam third-generation antibiotic used in treatment of various infections caused by gram negative bacteria [1] like Haemophilius *influenzae, Moraxella catarrhalis, Escherichia coli, Klebsiella* spp. Literature survey revealed HPTLC determination of Cefixime [2], Reversed phase HPLC determination of Cefixime [3] are the few methods available for it's estimation. Cefixime is poorly soluble in water. Special techniques are required to solubilize poorly water-soluble drugs; Hydrotropy is one of such technique. The proposed methods utilize solutions of non-toxic, non-volatile hydrotropic agents which are the substitutes and minimizes the use of organic solvents which are costlier, toxic and source of pollutant. Here we have presented two different

spectrophotometric methods, conventional spectrophotometric estimation (Method I), area under curve method (Method II) and chromatographic method by using different hydrotropic agents for estimation of Cefixime. The term "Hydrotropy" has been used to designate the increase in aqueous solubility of various poorly water soluble compounds due to presence of a large amount of additives [4]. Still the mechanism of hydrotropy is not understood very clearly. The concept of hydrotropy was first introduced in 1916 by Neuberg. According to his definition hydrotropes are metal salts of organic acids which at fairly high concentration increase the solubility of poorly water soluble compounds. Thoma and Arning (1976), have been used this term in the literature to designate non-micelle forming substances either liquids or solids, organic or inorganic, capable of solubilizing insoluble compounds. On the other hand Poochikian, Gradock (1979) studied that planarity of the hydrophobic part has been emphasized as an important factor in the mechanism of hydrotropic solubilization [5]. Hence, it seems rational to propose that molecules with a planar hydrophobic part and a polar group, which is not necessarily anionic, can act as hydrotropic agents. Saleh et al., in1985 extended the definition of a hydrotrope and said that it can be cationic, anionic or a neutral molecule provided it has a hydrophobic as well as a hydrophilic group [6.]

Coffman and Kildsig studied the mechanism of hydrotropic solubilization using the riboflavin–nicotinamide system. They concluded that the complexation of nicotinamide and riboflavin did not occur because nicotinamide is not able to quench riboflavin fluorescence and does not produce significant UV- spectral changes [7]. B.K. Roy, studied the hydro tropes have been reported to self-aggregate in aqueous medium like surfactants forming a term critical hydrotrope concentration (CHC) has been used in consonant with the critical micelle concentration [8-12]. Hydrotropic solutions can also be used as co-solvents, in solid dispersion technology [13], nanotechnology, parentral preparations [14], extraction purpose for solubilize [15] poorly water soluble drugs. When hydrotropes are added to aqueous surfactants or polymer solutions they produce strong synergistic effects [16].

MATERIALS AND METHODS

Pharmaceutical grade Cefixime was kindly supplied as a gift sample by Macleod Pharma Ltd. Mumbai. Tablets of Cefixime were procured from local market. Triethylamine and all hydrotropic agents used like ammonium acetate, potassium acetate, potassium citrate, sodium acetate, sodium citrate and urea are of analytical grade and methanol, ethyl acetate were of HPLC grade.

INSTRUMENTATION

UV-spectrophotometer

Shimadzu UV-2450 double beam spectrophotometer supported by Shimadzu UV-Probe software, version 2.21 was used for all spectrophotometric estimations.

Preparation of stock solution for spectrophotometric method:

Standard stock solution

Accurately weighed 25 mg of Cefixime and transferred to 25 mL volumetric flask, to it , sufficient volume of each hydrotropic agent was added separately and sonicated to dissolve the content of the flask completely. Final volume was made up to 25 mL by distilled water to obtain concentration of 1mg/mL.

Sample preparation

Twenty tablets of Cefixime were weighed and powdered. Powder equivalent to 25 mg of Cefixime was transferred to 25 ml volumetric

flask containing sufficient volume of different hydrotropic agents separately and sonicated for 5 minutes to solubilize the drug. These solutions were filtered through Whatman filter paper separately and then volumes were made up to the mark with distilled water to obtain sample concentration of 1mg/ml.

Preparation of stock solution in chromatographic method

Standard stock solutions

Preparation of Cefixime stock solution

Weighed accurately 10 mg of Cefixime and transferred to 10 mL volumetric flask, added to this sufficient volume of each hydrotropic agent separately and sonicated to dissolve the content of drug completely. Volumes were made up to 10 ml by distilled water (1000 µg/ml). From these stock solutions 1 ml was transferred to each 10 ml volumetric flask. The working solution of Cefixime (100 µg/ml) in each hydrotropic agent was prepared separately by diluting above solutions up to 10ml with methanol**.** Here methanol was used for final dilution as it is volatile in nature and gets easily evaporated from TLC plates which avoid interference of solubilizing solvent with developing solvent. It has also reduced the time of analysis and makes the selection of developing solvent easier.

Preparation of Linezolid (I.S.) stock solution

Linezolid was used as an internal standard for estimation of Cefixime. Weighed accurately 10 mg Linezolid and transferred to 10ml volumetric flask, volumes was made up to 10 ml by methanol (1mg/ml). Transferred 4 ml stock solution to 10 ml volumetric flask, made up the volume with methanol to obtain 400 µg/ml.

Instrumentation and optimization of chromatographic conditions

Precoated silica gel 60F-254 aluminium plates (20 cm x 10 cm) with 250 µm thickness; E. Merck, Darmstadt, Germany, supplied by Anchrom Technologist, (Mumbai) were used as stationary phase. Plates were pre washed with methanol and activated at 120 ^{o}C for 20 minutes in a hot air oven prior to chromatographic development. Sample application was done by using Camag 100µl syringe with Camag Linomat V sample applicator. Samples were sprayed on HPTLC plate at a constant rate of 2 µl/sec, in the form of narrow bands of 6 mm length. Linear ascending development was carried out in 20 cm x 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland).

Mixture of methanol: ethyl acetate: triethylamine in the ratio 7:5:0.05 v/v/v was used as a developing solvent for Cefixime. Time of chamber saturation with the developing solvent was optimized to 15 min. The length of chromatographic development was 70 mm and it took about 8 min to complete the development. Plates were then dried with hot air. Densitometric scanning was performed on Camag TLC scanner III at 269 nm which was supported by win CATS software (V 1.4.2.8121). The slit dimension was kept at 6.00 mm x 0.45 mm with 20 mm/s scanning speed. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 400 to 200 nm. R_f value of Cefixime and Linezolid were found 0.20 ±0.03 and 0.70 ± 0.03 respectively. Fig. 3 indicates integrated peak and R_f value of 200ng Cefixime in hydrotropic agents and 400ng for Linezolid used as an internal standard.

Selection of scanning wavelength

After chromatographic development bands were scanned over the range of 200-400 nm and the spectra were obtained. It was observed that Cefixime showed maximum absorption at 288 nm and Linezolid at 254 nm hence the

densitometric scanning was performed at isobestic point 269 nm for all the measurements. Fig. 3 shows overlay spectra of 200ng Cefixime and 400 ng Linezolid.

METHOD DEVELOPMENT

Linearity

In conventional spectrophotometric method, absorbance was noted in the concentration range of 5 µg/ml to 30 µg/ml. In area under curve method, area of spectra was noted between 278 nm to 298 nm (288 nm ± 10 nm). In chromatographic method three bands of each of 1 µl, 2 µl, 3 µl, 4 µl and 5 µl of 100 µg/ml working standard solution of Cefixime were applied to TLC plate. 4 µl working standard solution of Linezolid was over spotted as an internal standard. The calibration curve was plotted by peak area ratio of respective drug and Linezolid versus the corresponding drug concentration.

Limit of detection (LOD) and limit of quantitation (LOQ)

The detection limit and quantitation limit were computed to assess quantity of analyte which can be detected and minimum quantity of analyte which can be determined quantitatively by proposed UV- spectrophotometric and chromatographic methods

Accuracy

To study the accuracy of the proposed methods in both Spectrophotometric and chromatographic methods, recovery study were carried out by addition of known amount of standard drug in the pre analyzed tablet formulation, in 50%, 100% and 150 % of label claim. At each level of concentration, five determinations were performed. The required statistical parameters were evaluated to determine the accuracy of method.

RESULT AND DISCUSSION

Main criteria for the selection of hydrotropic agents in spectrophotometric methods include, 'sufficient concentration and volume of hydrotropic agents which completely solubilize content of drug' and these hydrotropic agents should not interfere in analyses. We have used five different hydrotropic solutions, which included ammonium acetate (6M), potassium acetate (5.0 M), potassium citrate (0.5 M), sodium citrate (1.25 M) and urea (10.0 M) in distilled water. Sufficient volumes of these hydrotropic solutions were used to solubilize the content of Cefixime completely. Hydrotropic solutions selected for this work in spectrophotometric methods have not shown any interference above 245 nm, depicted in fig. 1 and fig. 2; therefore Cefixime can be estimated by using these hydrotropic agents. In case of chromatographic method, selected hydrotropic agents not showed any interference in determining peak area of Cefixime, as depicted in fig 4. Spectrophotometric and HPTLC methods were developed and compared for estimation of Cefixime.

The linearity was found in concentration range of 5 to 30 µg/ml for Cefixime in all hydrotropic agents for both Spectrophotometric methods and summarized in Table 1.

The limit of detection and quantitation was computed for Cefixime in all hydrotropic agents and reported in Table 1 for spectrometric methods and table 2 for chromatographic method. In chromatographic method linearity was found to be 100 ng to 500 ng, which is depicted in table 2. Percentage recovery was found in the range of 97.4 % to 107.8 % for Cefixime by conventional Spectrophotometric estimation and 99.50 % to 104.7 % by AUC method .Results are summarized in Table 3, and chromatographic method recovery was found to be 98.2 % to 109.90 % showed in Table 4.

The LOQ and LOD values found were compared for each method and which clearly indicates that the planar chromatographic method is more

sensitive than spectrophotometric methods for the estimation of Cefixime. Both Spectrophotometric methods and chromatographic method showed more sensitivity for Cefixime estimation by using potassium acetate as a hydrotropic agent and minimum sensitivity observed for sodium citrate.

CONCLUSION

Developed spectrophotometric and chromatographic methods for estimation of Cefixime by using different hydrotropic agents was found to be the best alternative for estimations of poorly water soluble drugs and minimize the use organic solvents. The proposed method utilizes solution of non-toxic, non-volatile hydrotropic agents which give a novel, economical and environment friendly method for the estimation of Cefixime in tablet dosage forms.

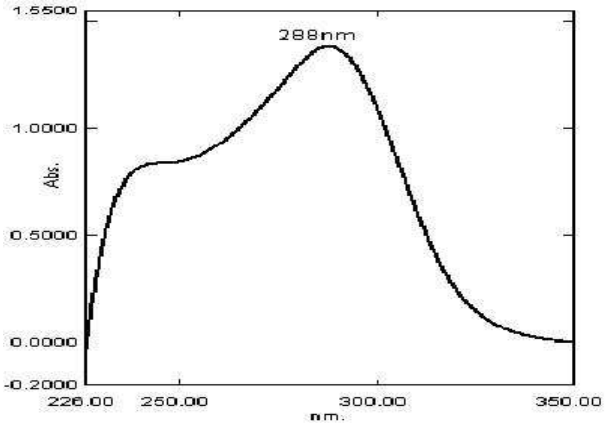

Figure1.
***Spectrum of Cefixime by using Ammonium acetate*.**

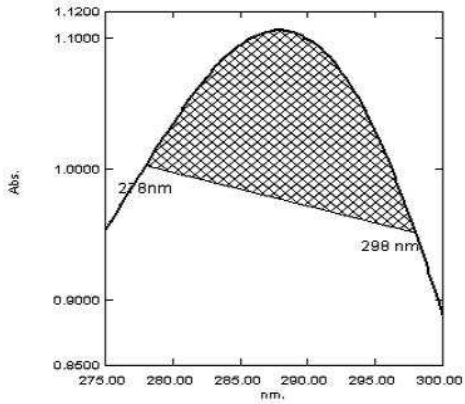

Figure 2.
Area under curve spectrum between 278 nm to 298 nm.

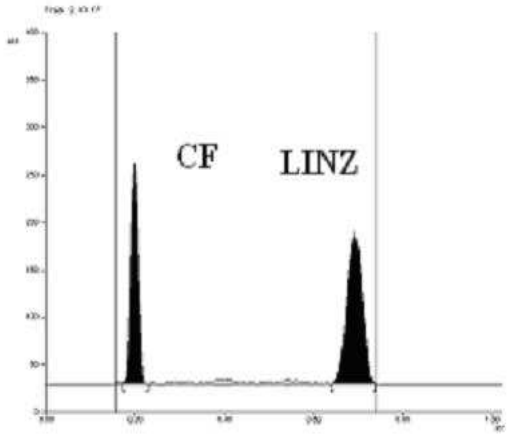

Figure 3.
Chromatogram cefixime in potassium acetate with Linezolid.

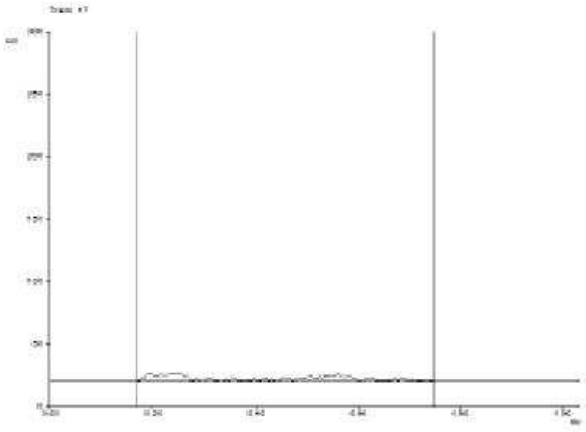

Figure 4.
Chromatogram of blank (Potassium acetate).

Table 1.
Results : Spectrophotometric methods

Hydrotropic Agents	Volume of hydrotropic solution (%v/v	Method –I			Method –II		
		Linearity* 4-30µg/ml	LOD µg/ml	LOQ µg/ml	Linearity* 4-30µg/ml	LOD µg/ml	LOQ µg/ml
6M Ammonium Acetate	10	y = 0.0407x–0.0045 r^2 = 0.9992	0.7	2.3	y = 0.0618x+0.056 r^2 = 0.9994	0.7	2.1
5M Potassium acetate	4	y = 0.0468x+0.0344 r^2 = 0.9991	0.5	1.2	y = 0.0713x+0.0042 r^2 = 0.9993	0.6	1.9
0.5M Potassium Citrate	4	y = 0.0459x+0.0476 r^2 = 0.9956	0.8	2.4	y = 0.0687x+0.051 r^2 = 0.9994	0.7	1.9
1.25M Sodium Citrate	10	y = 0.0456x+0.038 r^2 = 0.9997	0.9	2.8	y = 0.0694x+0.0011 r^2 = 0.9993	1.1	3.2
8M Urea	10	y = 0.0418x+0.0047 r^2= 0.9995	0.9	2.8	y = 0.0638x+0.030 r^2 = 0.9995	0.7	2.2

* Mean n = 3

Table 2.

Results: Chromatographic method

Hydrotropic Agents	Volume of hydrotropic solution (%v/v)	Linearity* 100-500ng	LOD µg/ml	LOQ µg/ml
6M Ammonium Acetate	4	y = 0.0015x –0.1463 r^2 = 0.9930	0.03	0.1
5M Potassium acetate	4	y = 0.0016x +0.1497 r^2= 0.9920	0.02	0.05
0.5M Potassium citrate	4	Y = 0.0015x +0.1535 r^2 = 0.9954	0.03	0.1
1.25M Sodium Citrate	4	Y = 0.0016x +0.1512 r^2 = 0.9929	0.03	0.1
8M Urea	10	y = 0.0015x -0.1544 r^2 = 0.9902	0.03	0.1

* Mean n = 3

Table 3.
Results of recovery study for Spectrophotometric method

Hydrotropic agent	Method	Amount of Standard drug added %	% Label Claim estimated* (Mean ± S.D.)	%RSD
Ammonium acetate	Method I	50	100.7±1.6	1.6
		100	101.3 ± 0.7	0.7
		150	107.8 ± 0.5	0.5
	Method II	50	103.9 ± 3.2	3.2
		100	102.7± 0.4	0.4
		150	101.5 ± 0.4	0.4
Potassium Acetate	Method I	50	104.8 ±0.5	0.4
		100	102.9± 0.7	0.6
		150	102.2 ± 0.8	0.8
	Method II	50	103.6 ± 1.8	1.1
		100	104.7± 0.3	0.3
		150	102.8± 0.9	0.8
Potassium citrate	Method I	50	97.4 ±3	3.1
		100	98.9 ± 0.4	0.4
		150	98.5 ± 0.8	0.8
	Method II	50	99.5 ± 2	2.0
		100	100.7± 0.3	0.3
		150	99.8 ± 0.9	0.9
Sodium Citrate	Method I	50	104.8 ±1.2	1.2
		100	100.0 ± 2	2.0
		150	99.9 ± 1.7	1.7
	Method II	50	99.5 ± 2.4	2.4
		100	100.0± 1	1.0
		150	101.9 ± 1.7	1.7
Urea	Method I	50	100.5 ±2.2	2.2
		100	98.6 ± 1.3	1.3
		150	98.5 ± 1.3	0.9
	Method II	50	101.4 ± 0.9	0.9
		100	100.8± 1.1	1.0
		150	99.0 ± 2.	2.0

- Mean n = 5

Table 4.
Results of recovery study for Chromatographic method

Hydrotropic agent	Amount of Standard drug added %	% Label Claim estimated* (Mean ± S.D.)	% RSD
Ammonium acetate	50	106.0±0.8	0.7
	100	104.8± 2.4	2.3
	150	106.4± 2.8	2.7
Potassium acetate	50	98.7 ±3.4	3.4
	100	102.5± 2.5	2.5
	150	103.7 ± 2.0	2.0
Potassium Citrate	50	100.1 ±6	6.0
	100	102.2± 2.5	2.4
	150	104.4 ± 1.4	1.3
Sodium Citrate	50	109.9±1.6	1.4
	100	108.7± 1.3	1.2
	150	102.3 ± 0.4	0.4
Urea	50	105.2 ±0.5	0.5
	100	98.2 ±2.0	2.1
	150	101.9 ± 0.9	0.8

* Mean n = 3

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