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Visible Spectrophotometric method for the estimation of Cefepime

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Abstract

A simple, accurate and reproducible visible spectrophotometric method was developed for the estimation of cefepime in pure and vial formulation. Cefepime with 1-Chloro-2,4-dinitrobenzene in presence of dimethyl sulfoxide at 45^{o}C have formed a yellow colored complex having maximum absorbance at 420nm.The calibration graph was linear in the range 20µg/ml to50µg/ml.

Keywords: *Visible spectrophotometry, Cefepime, 1-Chloro-2, 4-dinitrobenzene,Dinitrobenzene.*

1. Introduction

Cefepime is 7-(Z)-2-(2-aminothiazol 4-yl)-2-methoxyiminoacetamido)-3-(1-methylpyrrolidino) methyl-3-cephem-4-carboxylate[1] is a fourth generation cephalosporin for parental administration. This is mainly used in the treatment of various microbial infections caused by gram+ve and gram-ve microorganisms[2,4]. Cefepime is official in USP. The USP[3] describe a HPLC method for the estimation of cefepime formulations. A review of literature revealed that there are HPLC[6-12], U.V Spectrophotometric[5] and Colorimetric method[13] using Folins-Ciocaltue reagent. Thus the present method aims at developing newer colorimetric method which is rapid, accurate, precise, sensitive and reliable.

2. Experimental

2.1. Instruments used:

All colorimetric measurements were done on a Shimadzu Pharmaspeck 1800, Double beam spectrophotometer with 2 quartz cell 1cm path length. The point of maximum absorbance was at 420nm.

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2.2. **Chemicals and reagents:**

Cefepime was received as a gift sample from Sans Pharmaceutical Ltd, Kerala, India. 1-Chlor-2,4-dinitro benzene and Dimethyl Sulfoxide were of AR grade. This was procured from SD Fine chemicals Mumbai, India.

2.3. **Preparation of standard drug solution:**

The stock solution of cefepime was prepared by dissolving 50mg of the drug in 50ml dimethylsulfoxide.From that 5ml was taken and made up to 50ml. The new concentration was 100µg/ml.To 1ml of the above solution was added 3.6ml of 0.8M 1-Chloro-2,4- dinitro benzene. This was heated at 45^{o}C for ten minutes. The solution was cooled and made up to 5ml with dimethyl sulfoxide.

2.3.1. **Optimization of concentration of 1-chloro-2,4-dinitrobenzene:**

To 1ml drug solution 3.6ml of various concentrations ranging from 0.5 to 1M of 1-chloro-2,4-dinitrobenzene were added and heated at 45^{o}C for 10min. The yellow coloured solution was measured at 420nm. The absorbance was maximum at a concentration 0.8M.Table.1.

2.3.2. **Optimization of volume of 0.8M 1-chloro-2,4-dinitrobenzene:**

Different volumes ranging from 3 to 4ml of 1-chloro-2,4-dinitrobenzene were added into 1ml drug solutions and the same procedure as above was carried out. The maximum absorbance was found to be at 3.6ml; 0.8M.Table.2.

2.3.3. **Optimization of temperature:**

The drug solution along with 3.6ml; 0.8M 1-chloro-2,4-dinitrobenzene were heated for 10min at different temperatures ranging from 30^{o}C to 63^{o}C.The maximum absorbance was found to be at 45^{o}C.Table.3.

2.3.4**. Optimization of heating time:**

After fixing the above parameters, the contents were heated at 45^{o}C at regular intervals of time from 5min to 25min. The absorbance intensity was maximum at 10min.Table.4.

2.3.5. **Preparation of calibration graph:**

From the working standard, concentrations ranging from 10µg/ml to60µg/ml of cefepime were taken. To this 3.6ml; 0.8M 1-chloro-2,4-dinitrobenzene was added. Then the test tubes were heated at 45^{o}C for 10 min.Cooled and made up to 5ml with dimethyl sulfoxide. A blank was also prepared in the same manner without the drug. The absorbance was measured at 420nm.The measured absorbance was plotted against the concentration. From the graph it was found that the Beer's law concentration range was between 20µg/ml to 50µg/ml.Figure.1.

2.4. **Analysis of pharmaceutical formulation:**
An aliquot quantity equivalent to 50mg of cefepime was weighed and transferred to a 50ml standard flask. The contents were dissolved and made up to 50ml with dimethylsulfoxide.From this six individual concentrations were taken. To this was added 3.6ml; 0.8M 1-chloro-2,4-dinitrobenzene and heated at 45$_o$C for 10min.It was cooled and the volumes were made up to 5ml with dimethyl sulfoxide. The absorbance was measured at 420nm. The concentration of the drug was determined by single point standardization. The results are given in Table.5,6 & 7.

2.5. **Recovery studies:**
A known quantity of the standard was added to the pre-analyzed sample formulation and the contents were analyzed by the proposed method. The result of the recovery studies are shown in Table.7.

3. **Result and discussion:**
The proposed colorimetric method for estimation of cefepime in vials by single point standardization was simple, accurate and rapid and can be employed for routine analysis.

The cefepime concentration at a range of 20µg/ml to 50µg/ml has followed the Beer Lambert's law range at 420nm. The percentage deviation from the recovery was at 100% indicating the reproducibility and accuracy of the method. The cefepime content was found to be 95.8553% in vial formulation by the newly developed method. The method was found to be reproducible and can be used for routine estimation of cefepime in bulk and its vial dosage form.

4. **Conclusion:**

A simple, precise, rapid and accurate method was developed for the estimation of cefepime by visible spectrophotometry in vial formulations. The drug sample was heated with 3.6ml;0.8M 1-chloro-2,4-dinitrobenzene for 10minutes at 45^{o}C.A yellow color complex was obtained which showed a λmax at 420nm.The Beer's-Lamberts range was found to be from 20µg/ml to 50µg/ml.The same method was done in the case of vial formulation and concentration was calculated by single point standardization. The percentage recovery was also calculated. The method gave a satisfactory label claim and recovery value. So this method can be used for the routine analysis of cefepime in bulk and vial formulation.

(M)	Absorbance
0.5	0.2903
0.6	0.3412
0.7	0.3904
0.8	0.5008
0.9	0.4813
1	0.4901

Table-1: Optimization of concentration of 1-Chloro-2,4-Dinitrobenzene

Table-2: Optimization of volume of 1-Chloro-2,4-Dinitrobenzene

Volume(ml)	Absorbance
3	0.5421
3.2	0.5813
3.4	0.6004
3.6	0.6278
3.8	0.6130
4	0.6007

Table-3: Optimization of Temperature

Temperature(^{0}C)	Absorbance
30	0.6401
33	0.6401
36	0.6401
39	0.6401
42	0.6401
45	0.6649
48	0.6401
51	0.6401
54	0.6401
57	0.6401
60	0.6521
63	0.6312

Table-4: Optimization of heating time

Time(min)	Absorbance
5	0.6531
10	0.7049
15	0.6840
20	0.6622
25	0.6130

Table-5: Absorbance of Standard solutions at 420nm:

Absorbance of Standard solutions:						
Replicate	20µg	25µg	30µg	35µg	40µg	45µg
1	0.0206	0.0250	0.0302	0.0350	0.0403	0.0452
2	0.0204	0.0255	0.0304	0.0354	0.0401	0.0452
3	0.0206	0.0248	0.0298	0.0349	0.0403	0.0456
Mean	0.0205	0.0251	0.0301	0.0351	0.0402	0.0453
SD	±0 .005	±0.007	±0.005	±0.005	±0.005	±0.005

Table-6: Absorbance of Test solutions at 420nm:

Absorbance of Test solutions:						
Replicate	20µg	25µg	30µg	35µg	40µg	45µg
1	0.0202	0.0249	0.0302	0.0405	0.0450	0.0348
2	0.0202	0.0248	0.0301	0.0399	0.0449	0.0348
3	0.0205	0.0254	0.0307	0.0400	0.0455	0.0355
Mean	0.0203	0.0250	0.0303	0.0401	0.0451	0.0350
SD	±0.005	±0.005	±0.005	±0.005	±0.005	±0.005

Table-7 Analysis of vial formulation of cefepime at 420nm

S.I.No	Astd	Atest	Cstd(µg/ml)	C test(µg/ml)	Labelled Claim(mg)	Amount present(mg)	%labelclaim
1	0.0205	0.0203	20	19.8048	1000	954.9	95.49
2	0.0251	0.0250	25	24.90039	1000	952.838	95.2838
3	0.0301	0.0303	30	30.1993	1000	969.755	96.9755
4	0.0351	0.0350	35	34.90028	1000	956.103	95.6103
5	0.0402	0.0401	40	39.9004	1000	961.91899	96.191899
6	0.0453	0.0451	45	44.801	1000	955.8036	95.58036

Mean = 95.8553% S.D = ± 0.393058

Table-8: Recovery studies of cefepime:

% Recovery Values at:		
Replicate	30µg	40µg
1	102.95%	97.010%
2	102.96%	97.018%
3	102.92%	97.006%
Mean	102.94%	97.011%
SD	±0.005	±0.005

Table-9: Recovery studies of cefepime by the new method

S.I.No	Concentration of sample (mg)	Amount of standard added (mg)	Total amount of drug estimated	Amount of standard drug recovered	%recovery
1	0.0198048	0.010	0.03009967	0.01029487	102.9487
2	0.0301993	0.010	0.0399004	0.097011	97.011

Mean = 99.97985 % S.D = ±0.002078

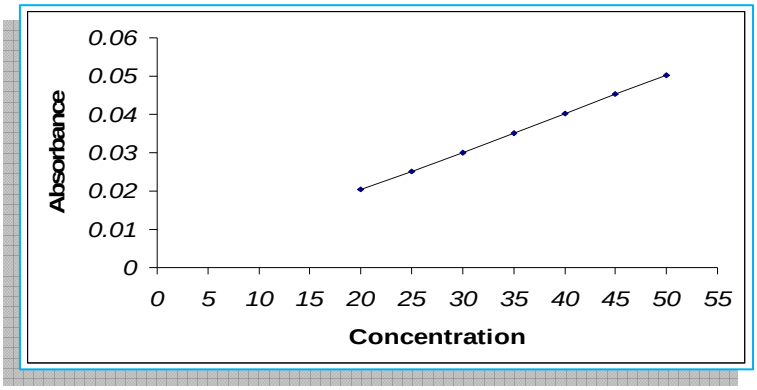

Fig.1. Beer Lambert's concentration range at 420nm.

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