www.sphinxsai.com

International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.2, No.1, pp 337-340, Jan-Mar 2010

DETERMINATION OF CEFIXIME TRIHYDRATE AND CEFUROXIME AXETIL IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORMS BY ELECTROPHORETIC METHOD

***K. Azhagesh Raj**

Department of Pharmacy, Annamalai University, Annamalai Nagar, Chidambaram, Post Code No.608002, Tamil Nadu, India.

***E-mail: azhageshraj@yahoo.com**

ABSTRACT: The simple, reliable and reproducible Electrophoretic method were developed for analysis of cefixime trihydrate and cefuroxime axetil. The anionic detergent sodium dodecyl sulphate (SDS) is used in combination of a reducing agent (Mercaptoethanol) so as to dissociate the proteins. The sample and stacking gel contains Tris HCl (pH 8.8).
All the components contains 0.1% SDS. The chloride ions in the sample and the stacking gel from the leading edge of the moving boundary is a zone of lower conductivity ans steeper voltage gradient which sweeps the polypeptides from sample and deposite them onto the ionization of glycine and the resulting glycine ions frees from the moving boundary. Western blotting is rapid and sensitive assay method for the detection and characterization of proteins. On staining SDS – Polyacrylamide gel, different proteins appeared as dark bands against light blue background.
KEY WORDS: Cefixime trihydrate, Cefuroxime axetil, HPLC analytical method.

INTRODUCTION

The chemical name of cefixime trihydrate is 5-Thia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylic acid, 7-[[(2-amino-4-thiazolyl) [(caroxymethoxy)imino] acetyl] amino]-3-ethenyl-8-oxo-trihydrate[3][5].
The chemical name of cefuroxime axetil is 5-Thia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylic acid, 3-[[(amino carbonyl)oxy]methyl]-7-[[2-furanyl(methoxy imino)acetyl] amino-8-oxo-1-(acetoxy)ethyl ester[3][5].
Cefixime is effective against a wide spectrum of sensitive Gram –Ve, Gram +Ve and anaerobic bacterial pathogens including β- lactamase producing strains[9].
Cefuroxime is effective against β- lactamase, Haemophilus influenzae, Neisseria gonorrhea and lyme disease[9].

EXPERIMENTAL WORK

Reagents used for preparation of gel:

30% Acrylamide

30gm of acrylamide and 0.8% N, N- methylene bis acrylamide is dissolved in 70ml of distilled water, it is filtered and make upto 100ml with distilled water.

Separating gel buffer[1][7]

18.17g of tris is dissolved in 70ml of distilled water. The pH is adjusted to 8.8 with conc.HCl and made upto 100ml with distilled water.

Stacking gel buffer

12.11g of tris is dissolved in 70ml of distilled water. The pH is adjusted to 6.8 and make up to 100ml with distilled water.

10% Sodium dodecyl sulphate

10g of SDS is dissolved in distilled water and made up to 100ml.

0.5% (V/V)NNN TEMED

Running buffer

3.03g of tris and 14.41g of glycerin are dissolved in a small quantity of distilled water. The pH is adjusted to 8.3 with concentratedhydrochloric acid. To this 5g of SDS is added and made up to 1000ml with distilled water.

Sample buffer

4g of sucrose and 800mg of SDS is dissolved in 5ml of stacking buffer and made up to 10ml with stacking buffer.

0.45% Ammonium persulphate

Water saturated isobutanol

Staining solution

50ml of methanol, 10ml of acetic acid and 0.25 mg comassie brilliant blue are dissolved in distilled water. The mixture is filtered and stored.

De-staining solution

100ml methanol and 100ml acetic acid are dissolved and made up to 1000ml with distilled water.

Sample

To 0.05ml of sample, 0.05ml of distilled water and 0.1ml sample buffer is added[4].

Sample 1 : Cefuroxime axetil
Sample 2 : Cefixime trihydrate
Sample 3 : Mixed drugs (1) and (2)
Sample 4 : Cefakind
Sample 5 : Ceftum
Sample 6 : Biotax
Sample 7 : Mahacef

Supporting gel

1. Distilled water - 0.5ml
2. Acrylamide - 0.5ml
3. TEMED - 0.002ml
4. APS - 0.009ml

Separating gel(15%)

1. Distilled water - 3.265ml
2. 30% Acrylamide - 4.5ml
3. 3M Tris buffer(pH 8.8) - 1.125ml
4. 10% SDS - 0.09ml
5. 10% APS - 0.015ml
6. TEMED - 0.005ml

Stacking gel (10 %)

1. Distilled water - 3.132ml
2. 30% Acrylamide - 0.75ml
3. 0.5m Tris buffer (pH 6.8)- 0.568ml
4. 10% SDS - 0.045ml
5. 10% APS - 0.0075ml
6. TEMED - 0.005ml

Procedure for casting the gel[8]

1. Assemble the plates for casting gel.
2. Clamp the assembly of plates to fix it in a gel casting apparatus. Ensure the assembly is leak proof by filling water between the plates. Silicon grease can be applied to spacer to make a water tight seal.
3. Add 50µl of APS solution to 5ml of SDS separating gel mix and pour the gel solution between the plates till the level is 2cm beloe the top edge of notched plate.
4. Add 200 to 250µl of water to make the surface even.
5. After the gel is set (approximately 20 – 30 min),wash the top of the separating gel with distilled water and drain off the water completely.
6. Add 20µl of APS solution to 2ml of stacking gel mix and pour directly onto the polymerized separating gel.
7. Insert the comb into the gel solution carefully without trapping any bubbles about 1cm above the separating gel. The stacking gel will set in approximately 10 min.
8. Add 25µl of sample loading buffer to protein sample.
9. Add 25µl of sample loading buffer of 25µl of protein marker.
10. Place it in a boiling water bath for 5 minutes.
11. After the stacking gel has set, carefully remove the comb and the bottom spacer. Wash the wells immediately with distilled water to remove non-polymerized acrylamide. Fill the bottom reservoir with 1 x reservoir buffer are carefully fix the plate to the apparatus without trapping any air bubbles between the buffer and the bottom of the gel. Fix the plates to page apparatus. Fill the top reservoir with 1x reservoir buffer.
12. Load 30µl protein marker in well 1,40µl of protein sample in well 2 and5µl of protein sample in well 4. Connect the cords to the power supply according to the convention red: anode, black: cathode.
13. Set voltage at 100 V and switch on the power supply.
14. When the dye front comes to 0.5cm above the bottom of the gel, turn off the power. This will take approximately 1 to1 ½ hours.
15. Remove the gel plates and gently dry the plates apart using a spatula or similar tool, not at the notch.
16. Transfer the gel to a tray containing water, wash the gel for 1-2 minutes at room temperature.
17. Decant water, cut the gel among lane 3.
18. Transfer lane 4 i.e., protein sample in 10ml of blotting taken in a petridish, keep at room temperature for 10 minutes. Following incubation, proceed for electro blotting as described in step 22.
19. To the gel piece (lanes 1 & 2) add minimum of 20ml water
20. Decant the water, add minimum 20ml of Ezee blue stain.Stain at room temperature for 1-2 hours.
21. Decant the staining solution, add minimum quantity of water to cover the gel.

Electroblotting:[2][6]

22. Assemble the blotting sandwich within the blotting cassette. Take care to avoid air bubbles between the gel and NC membrane.
23. Insert the cassette into the apparatus filled with blotting buffer and connect blotting unit to power supply as per the convention, red: anode, black:cathode.
24. Electrophoreses the sample at 50 V for 2 hrs for blotting to occur.
25. Remove the NC membrane gently from the cassette and place the membrane in 10ml of freshly prepared blocking buffer taken in a petri dish. Leave it over night at 4°C.
26. Discard blocking buffer.
27. Immerse blot in 10ml of primary antibody solution and mix gently for 30 minutes. Discard the primary antibody solution.
28. Wash the blot by immersing in 10ml wash buffer for 3-5 minutes. Repeat the wash process two times. Discard the buffer each time.
29. Immerse the blot in 10ml of 1 x HRP labeled antibody. Mix gently for 30 minutes. Discard the HRP labeled antibody.
30. Wash the blot by immersing in 10ml wash buffer for 3-5 minutes.
31. Immerse the washed blot in 10ml substrate solution, mix gently for 5-10 minutes, within this time coloured band will appear.
32. Remove the blot, wash with distilled water, discard and dry.
33. Compare the SDS-Polyacrylamide gel with the developed Nitrocellulose membrane.

RESULTS AND DISCUSSION

On standing SDS-Polyacrylamide gel, different proteins appeared as dark bands against a light blue background.

On Electrophoresis of drugs Cefixime and Cefuroxime with the marketed brands showed a clear single blue coloured band observed on nitrocellulose membrane as shown in fig 1 ..

Compared to all the drugs,

Sample No: 4 –CEFAKIND

Sample No: 5-CEFTUM

Sample No: 7-MAHACEF showed clear dark blue bands against the light blue background, compared to other drugs and marketed brands.

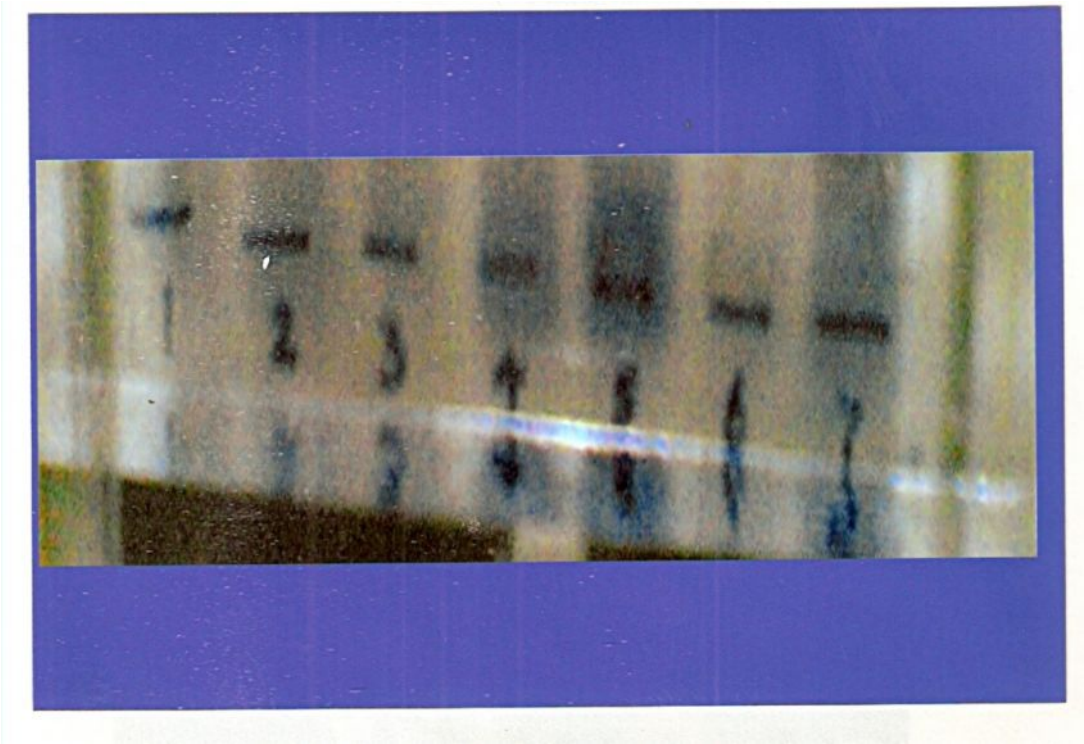

FIG -1: DEVELOPMENT OF BANDS FOR DRUGS

REFERENCES

1. Bentley's text book of Pharmaceutics, E.A. Eawlins, 8th edition.
2. Fundamentals of Analytical Chemistry, Skoog, West Holler, Crouch, 8th edition.
3. Aadvanced Chemistry, Philip Mathews, 133,353.
4. Fundamentals of Analytical Chemistry, Douglas A. Skoog.
5. Foye's Principles of Medicinal Chemistry, David A. WillIams, Thomas L. Lemke, 5th edition,846.
6. Pharmaceutical Analysis Vol. II ,Dr .A.V. Kasture, Dr. K.R. Mahadik, Dr.S.G. Wadodkar, Dr. H.N. More, 48-57.
7. Instrumental Methods Of Analysis, Willard, Merrit, Dean, Settle, 7th edition, 580-613.
8. Fundamentals of Analytical Chemistry, Skoog, West, Holler, Crouch, 8th edition.
9. Indian Drugs, Vol. 40.No. 12,Dec-2003, 707-710.

*****