Research Article
ISSN: 0974-6943

Madhura Vishal Dhoka et al. / Journal of Pharmacy Research 2009, 2(11),1700-1704

Available online through
http://jprsolutions.info

Dissolution method development and validation for combination of Cefixime Trihydrate and Erdosteine capsules

Madhura Vishal Dhoka[*1], Vandana Tukaram Gawande[1], Pranav Pramod Joshi[1]
*[*1] Department of Quality Assurance, A.I.S.S.M.S. College of Pharmacy, Pune University, Pune-411014, India.*

Received on: 20-05-2009; Accepted on:15-07-2009

ABSTRACT

The aim of this work was to develop and validate a dissolution test for Cefixime and Erdosteine capsules using spectrophotometric method. The dissolution test conditions established were: 900 ml of 0.05 M Potassium phosphate buffer pH 7.2 adjusted with1 N NaOH as dissolution medium for 45 minutes, using a basket apparatus at a stirring rate of 100 rpm at temperature 37^0C. UV spectrophotometric method was developed for evaluating drug release namely absorption correction method (295 nm is λ_{max} of CEF ,where ERDO has practically nil absorbance,detection of ERDO was carried out at its λ_{max} 237 nm) The methods was validated to meet requirements for ICH guidelines.

Keywords: Dissolution method; Validation ; Cefixime Trihydrate; Erdosteine

INTRODUCTION

The dissolution performance test is a required test for all solid oral dosage forms for product release. It also is used commonly as a predictor of an in-vivo performance of a drug product. To help satisfy dissolution requirements, the USP provides information in the way of a general chapter on dissolution, as well as related chapters on disintegration, drug release and development and validation of dissolution procedure [1]. Parameters to set up the dissolution test should be researched and defined for drugs that do not possess official Monographs. FDA also provide guidelines on development and validation of dissolution procedures [2-4]. There are two common techniques of analyzing dissolution test samples, spectrophotometric (UV) and HPLC. Spectrophotometric determinations are the most common method of analysis because they are faster, simpler, and require less solvent than HPLC. ICH gives guidelines for validation of analytical procedures [5].

Cefixime trihydrate, is the third generation cephalosporin antibiotic. Cefixime is given orally in the treatment of susceptible infections including gonorrhoea, otitis media, pharyngitis, lower respiratory-tract infections such as bronchitis, and urinary-tract infections[1]. It is official in USP. Chemically it is 5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-corboxylic acid,7-[[2-amino-4-thiazolyl)[(carboxymethoxy)imino]acetyl]amino]-3-ethenyl-8-oxo-,trihydrate,[6R-[6∝,7β(Z)]]-(6R,7R)-7-[2-(-amino-4-thiazolyl)glyoxylamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4,2,0]oct-2-ene-2-carboxylic acid, 7^2-(Z)-[O-carboxymethyl)oxime] [1].

There are several investigations concerning the determination of cefixime alone and in combination with other drugs in pharmaceutical preparations and plasma by UV, HPLC, LC-MS, HPTLC methods [6-10].

Erdosteine [[2-Oxo-2-[(tetrahydro-2-oxo-3-thienyl) amino] ethyl] thio] acetic acid is a mucolytic and is official in Martindale [11]. It Modulates mucus production, viscosity and increases mucociliary transport, thereby improving expectoration and thus it shows mucolytic and antitussive activity [12-13]. One stability indicating HPTLC method is reported for erdosteine [14]. Erdosteine and its optical active metabolite have been analyzed by high-performance liquid chromatography using a fluorescent chiral tagging reagent [15]. Sensitive determination of erdosteine in human plasma has been achieved by automated 96-well solid-phase extraction and LC–MS–MS [16].

Investigations are done to study effect of mucolytic on antibiotic penetration in sputum and it reveals that mucolytics improve the same [17-19]. Hence combination of an antibiotic with a mucolytic is a treatment of choice for acute exacerbations of chronic bronchitis. Also comparative evaluation of Cefixime plus Erdosteine and Amoxicillin plus Bromhexine shows that former gives faster and better symptomatic relief and was also better tolerated than later [20]. As Combination is not available in the market it was also developed.

Capsules having composition of Cefixime Trihydrate equivalent to Cefixime 200 mg and Erdosteine 300mg were prepared by dry granulation and powder blend was filled in hard gelatin capsules (size 00). The average fill weight of one capsule is 600 mg. Dissolution test was conducted in USP XXV dissolution test apparatus type I with basket rotation at 100 rpm. The dissolution medium was 900 ml of 0.05 M

***Corresponding author.**
Madhura Vishal Dhoka
Department of Quality Assurance,
A.I.S.S.M.S. College of Pharmacy,
Pune University, Pune-411014, India.
Tel.: + 91-9422079037
Telefax: +91-
E-mail: madhura1777@yahoo.com

Potassium phosphate buffer pH 7.2 adjusted with1 N NaOH as dissolution medium for 45 minutes and temperature was maintained at 37 ±5°C. One capsule was taken in each basket. 5 ml of samples were withdrawn at 15, 30, and 45minutes. 5 ml of dissolution medium was replaced in each sampling. Two UV spectrophotometric methods namely absorption correction method and dual wavelength were developed for its analysis.

In this paper we report simple, accurate, precise and sensitive dissolution method for quality control of Cefixime trihydrate and Erdosteine in combined capsule dosage form. The proposed method is optimized and validated according to ICH guidelines.

MATERIALS AND METHODS

Materials

Samples of Cefixime trihydrate and Erdosteine were obtained as gift samples from Maxim pharmaceuticals, Pune and Glenmark pharmaceuticals respectively. Empty hard gelatin capsules of size 00 were received from Mycon pharmaceuticals, pune. All reagents and solvents used were analytical grade. Stock solutions (1mg/ml) of both drugs were prepared in Methanol AR grade. For further dilutions dissolution medium that is 0.05 M Potassium phosphate buffer pH 7.2 was used.

Instrumentation

Double-beam UV-Visible spectrophotometer used in the experiment was from Jasco, Japan model V-550. The dissolution apparatus from Dissolution apparatus from Electrolab, TDT-08L, USP were used in the development and validation.

The media were deaerated by vacuum filtration and were maintained at 37.0 ± 0.5°C by using a thermostatic bath. The Delux 101 potentiometer was used to determine the pH of all solutions.

Dissolution test conditions

Various dissolution Media were tested for the development of a suitable dissolution method for the dissolution study of Cefixime Triydrate and Erdosteine capsules. The following parameters were finalized:

Medium: 0.05 M Potassium phosphate buffer ph 7.2
Volume: 900 ml
Apparatus: USP type-I (Basket)
RPM: 100
Temperature: 37^0C
Time interval: every 15 min till 45 min.

Preparation of 0.05 M Potassium phosphate buffer pH 7.2

Dissolve 6.8g of monobasic potassium phosphate in 1000ml water; adjust pH 7.2 with 1N NaOH

Preparation of test solution:

A capsule was dropped into each of the six dissolution vessels containing preheated dissolution media, 0.05 M Potassium phosphate buffer pH 7.2. 10 ml aliquot of the sample was withdrawn at 15, 30 and 45 mints intervals replacing 10ml of dissolution medium each time. 2 ml of aliquot was diluted to 10ml with dissolution media and filtered through 0.45µm nylon membrane filter.

Preparation of standard stock solution

25 mg of each Cefixime Triydrate and Erdosteine were weighed separately and dissolved in Methanol AR grade and then volume was made up to 25 ml, so as to get the concentration of 1mg/ml for both.

Selection of Analytical Wavelength

Using appropriate dilution of standard stock solution, two solutions were scanned separately. The two wavelengths selected should be such that one of the drugs wavelengths shows practically nil absorbance at λ_{max} of the other drug. Wavelengths (λ_1) 295 nm, which is λ_{max} of CEF where ERDO has practically nil absorbance. The other wavelength (λ_2) selected is 237 nm, which is λ_{max} of ERDO and CEF also absorbs at that wavelength. Overlain spectra of both drugs is shown in Fig.1

Selection of analytical concentration range

For each drug, from Stock solution (1mg/ml) 1ml was diluted to 10 ml with 0.05 M Potassium phosphate buffer pH 7.2 to get concentration of 100 µg/ml.Of this appropriate dilutions were done using 0.05 M Potassium phosphate buffer pH 7.2. The absorbance of each aliquot were measured at selected wavelength and plotted against concentration. The concentration range over which the drugs obeyed Beer's law was chosen. The range was found to be 2-50 µg/ml for CEF and 7.5-75 µg/ml for ERDO.

Determination of absorptivity at analytical wavelength

For each drug appropriate aliquots were pipetteted out from standard stock solution and a series of dilutions of different concentration were made. For CEF, the concentration range taken 2-50 µg/ml and that of ERDO was 7.5-75 µg/ml. The absorbances of said concentrations for both the drugs were noted at selected analytical wavelength. These absorbances were then divided by concentration in gm/lit to get absorptivities as given in table 1.

Equations for calculation:

$C_{CEF} = A1/ay1$ $\quad C_{CEF} = A1/42.8455$

$C_{ERDO} = A2\text{-}ay2\ C_{CEF}/ax2$ $\quad C_{ERDO} = A2\text{-}33.1515\ C_{CEF}/21.444$

Method validation

The UV spectrophotometric method used to analyze the Cefixime trihydrate and Erdosteine samples in 0.05M phosphate buffer pH 7.2 was validated for specificity, linearity, precision and accuracy, according to USP Pharmacopoeia and ICH guidelines.

RESULT AND DISCUSSION

Method development

USP discusses important parameters to be considered during dissolution method development as follows.

Medium

When selecting the dissolution medium, physical and chemical data for the drug substance and drug product must be considered like the solubility and solution state stability of the drug as a function of the pH value. Other critical drug product properties include the release mechanism (immediate, delayed, or modified) and disintegration rate as affected by formulation hardness, friability, presence of solubility enhancers, and presence of other excipients. The most common dissolution medium is probably dilute hydrochloric acid; however, other media used include buffers in the physiologic pH range of 1.2 to 7.5, simulated gastric or intestinal fluid (with or without enzymes), water,

and surfactants (with or without acids or buffers) such as polysorbate 80, sodium lauryl sulfate, and bile salts. Solubility and solution stability of both drugs was determined in common dissolution media and it was found that both drugs were completely soluble and stable for two days in 0.05M phosphate buffer pH 7.2. As formulation is of conventional type release of both drugs is of immediate type and hence single dissolution medium is sufficient for study. Literature survey revels that both drugs are absorbed from upper part of small intestine and hence this medium was finalized. Media volume is typically in the range of 500–1000 ml, with 900 ml the most common volume. In order to maintain sink conditions volume of medium that is at least three times that required to form a saturated solution of drug substance is used. Hence volume selected was 900ml. Media deaeration is usually required and can be accomplished by heating the medium or (more commonly) filtering the medium or placing it under vacuum for a short period of time. In this study deaeration is achieved by filtration of medium through membrane filter with aid of vacuum.

The Dissolution Apparatus

USP chapter 711 lists seven types of dissolution apparatuses. The choice of apparatus is based upon the dosage form performance in the in-vitro test system. For solid oral dosage forms, the most frequently used apparatus are Apparatus 1 (basket) and Apparatus 2 (paddle). Basket type is used for testing of capsules and hence it was used. Agitation is also an important part of the dissolution procedure. Apparatus 1 (baskets) at 100 rpm or Apparatus 2 (paddles) at 50 or 75 rpm are used most commonly. Higher or lower rates are usually inappropriate because of the inconsistency of hydrodynamics below 25 rpm and increased turbulence above 150 rpm. Hence 100 rpm speed is selected.

Dissolution Study Design

Dissolution is evaluated by measuring release rate profiles, or the amount dissolved over time. Single or multiple points in time can be measured, depending upon the dosage type or data desired. For immediate-release dosage forms, the procedure duration is usually 30 – 60 min; and in most cases, a single time point specification is adequate. So sampling is done every 15 min till 45 minutes because both drugs are released completely within 45 mins. Sampling is another important experimental design consideration. For immediate release formulation tests using one time point over a short (less than 1 h) period, sampling can be done manually. Hence aliquots are removed manually, replacing fresh dissolution medium each time. Ideally sampling should be done from middle position in the jar. Filtration is necessary to prevent undissolved drug particles from entering the analytical sample and further dissolving, skewing the test results. Also, filtration removes insoluble excipients that might otherwise cause high background or turbidity in the assay technique. Aliquots are filtered through membrane filter.

With all these parameters, dissolution profile of capsules was observed and it was found satisfactory as both drugs show more than 90% drug release in 45 minutes.

Analytical Method validation

There are two common techniques of analyzing dissolution test samples, spectrophotometric (UV) and HPLC. Spectrophotometric determinations are the most common method of analysis because they are faster, simpler, and require less solvent than HPLC. Absorption correction method and dual wavelength method were validated as per ICH guidelines.

Specificity (placebo interference)

Scanning and absorbance measurement carried out for the blank (diluents used in the method), placebo and test solution. Placebo consisted of all the excipients and shell capsules without the drug. There was no interference from the blank and placebo at analytical wavelengths as shown in fig.3

Linearity

The concentrations of Cefixime Trihydrate and Erdosteine from 2-50 µg mL-1 and 7.5-75 µg mL-1 respectively were prepared from stock solution (1 mg mL-1) and absorbance of measured at 237 and 295nm. The graph was plotted between concentration and absorbance for linearity and it showed good correlation coefficient 0.999 for both drugs at analytical wavelengths. Calibration curves are shown in Fig.4

Precision

The precision of the method was determined by measuring the repeatability (intra-day precision) and the intermediate precision (inter-day precision), both expressed as RSD (%).All the data (Table 2) are within the acceptance criteria of 5%.

Accuracy

The placebo samples were spiked with 80, 100 and 120% of the standard Cefixime Trihydrate and Erdosteine in triplicate. And recovery of both drugs at these 3 levels is determined in formulation. Compiled recovery data shown in Table-3 for accuracy study. These expressed overall % RSD of 2.97 (for Cefixime Trihydrate) and 1.72 (for Erdosteine) in three levels which was the less than the acceptance level of 10%.

Robustness

The robustness of an analytical procedure is the measure of its capacity to remain unaffected by small but deliberate variations in parameters internal to the procedure. For dissolution testing, parameters to be varied include medium composition (for example, buffer or surfactant concentration), pH, volume, agitation rate, sampling interval and temperature. These parameters are varied and % RSD of absorbance of both drugs was calculated and it was found within limit that is less than 5%.

Solution stability

In the solution stability studies the percent drug present in the sample for 24 hrs was determined and it showed maximum cumulative %RSD of 2.71 which was less than 5% of acceptance criteria. The standard and sample solution was stored at room temperature for 24 hr and drug content was compared using freshly prepared solutions.

Table.1. Absorptivity Measurement at analytical wavelengths

Components	Absorptivity at 295nm	Absorptivity at 237nm
Erdosteine(x)	NIL (ax_1)	21.444(ax_2)
Cefixime Trihydrate (y)	42.8455 (ay_1)	33.1515 (ay_2)

Table.2. Result for precision Studies

Sr.No.	% RSD (n=3)	Concentration (µg/ml) Cefixime Trihydrate			Erdosteine		
		20	30	40	30	45	60
1	Intra day precision	0.16	0.86	1.29	0.96	1.01	1.31
2	Inter day precision	1.04	1.66	1.71	1.39	1.13	1.72

Table..3. Result for Recovery Studies

Drug		80	100	120
Cefixime Trihydrate	Mean % Recovery	103.11	100.26	101.2
	% RSD (n=3)	2.79	3.17	2.95
Erdosteine	Mean % Recovery	102.7	97.26	98.2
	% RSD (n=3)	2.12	1.25	1.79

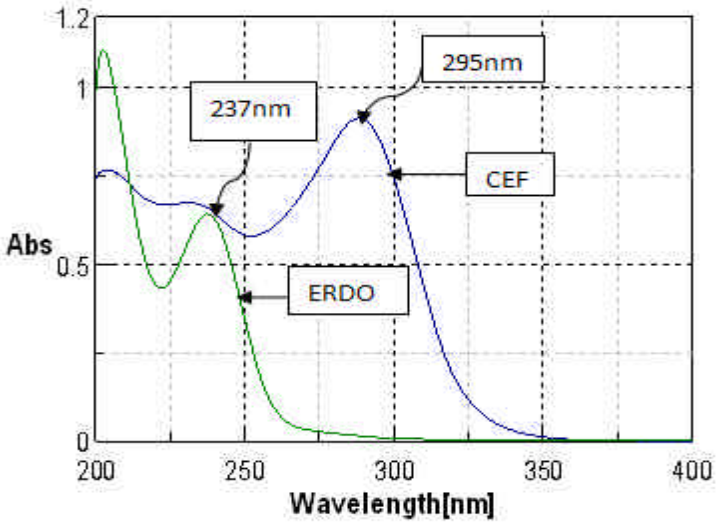

Fig.1. Overlain Spectra of Cefixime Trihydrate (CEF) and Erdosteine (ERDO)

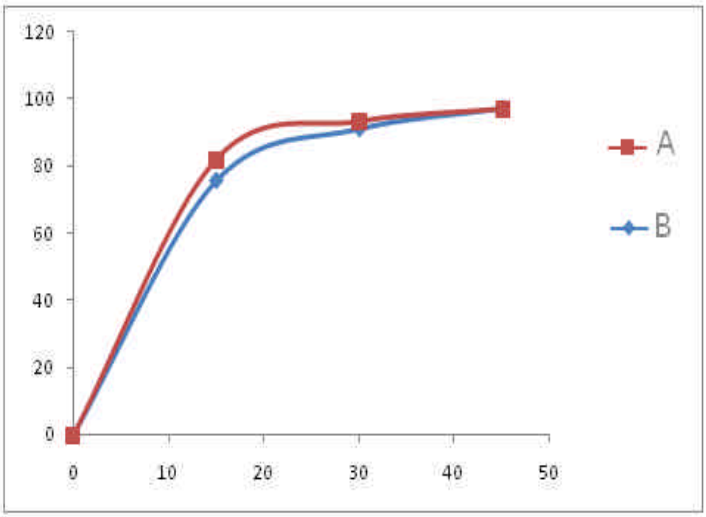

Fig.2.Dissolution Profile 1-Cefixime Trihydrate 2- Erdosteine

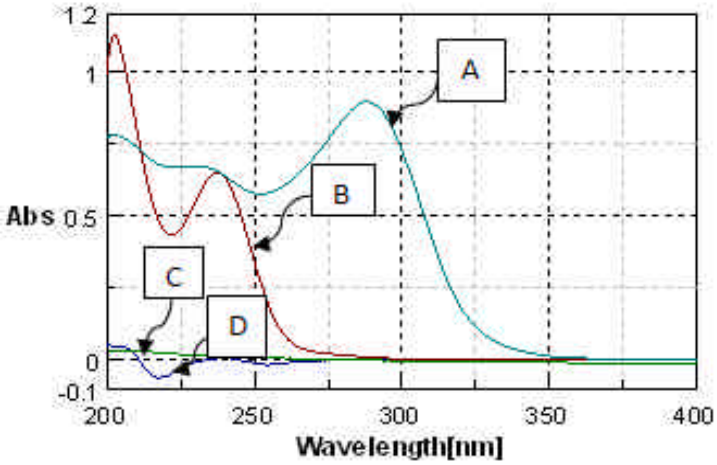

Fig. 3. Absorption Spectrum A-Cefixime Trihydrate B-Erdosteine C-Placebo D-Blank

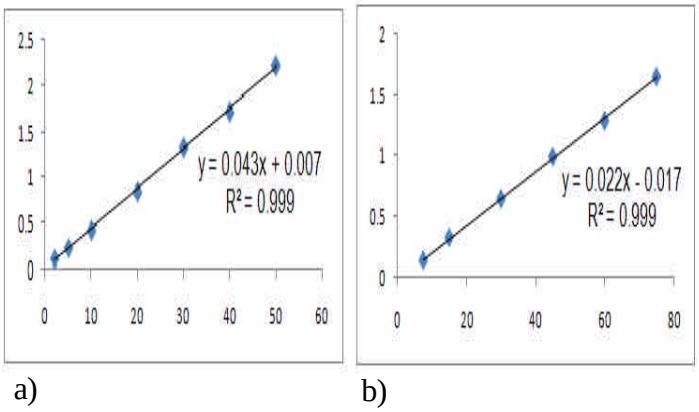

Fig.4. Calibration Curve A- Cefixime Trihydrate B-Erdosteine

CONCLUSION

The developed dissolution method is precise, accurate and simple. The results from the statistical analysis prove that the method is repeatable for the dissolution studies of Cefixime Trihydrate and Erdosteine capsules. The % drug release was higher than 90% in 45 minutes for both drugs. The use of 900 ml of 0.05 M Potassium phosphate buffer pH 7.2. at 37 °C, basket at the stirring speed of 100 rpm and 45 min of test provided satisfactory results.

ACKNOWLEDGEMENT

The authors are grateful to Dr. A. R. Madgulkar, Principal, AISSMS College of pharmacy, India, for providing all the facilities during this study.

REFERENCES

1. The United States Pharmacopoeia 30th Ed.,United States Pharmacopoeial Convention, Rokville, 2007,Chapter 701,711,724, 1092,1225,1654.
2. FDA Guidance for Industry: Dissolution Testing of Immediate Release Solid Oral Dosage Forms, Food and Drug Administration, Rockville MD,1997.

3. FDA Guidance for Industry: SUPAC-MR: Modified Release Solid Oral Dosage Forms, Food and Drug Administration, Rockville MD,1997.
4. FDA Guidance for Industry: Waiver of In-Vivo Bioavailability and Bioequivalence Studies for Immediate Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System, Food and Drug Administration, Rockville MD,2000.
5. ICH – Q2 (R1) Guideline on Validation of Analytical Procedure. Text and Methodology,2005.
6. Falkowski AJ, Look M,Determination of cefixime in biological samples by Reversed-phase high-performance liquid chromatography,J chromatogr biomed appli,422,1987,145-152.
7. Meng F, Chen XY, Zeng YL, Zong DF,Sensitive liquid chromatography–tandem mass spectrometry method for the determination of cefixime in human plasma: Application to a pharmacokinetic study,J Chromatogr B,819, 2005, 277.
8. Al-Momani IF, Spectrophotometric Determination of selected cephalosporins in drug formulation using flow injection analysis,J Pharm Biomed Anal,25, 2001,751-757.
9. Eric-Jovanovic S, Agbaba D, Zivanov-stakik D, Vladimirov S HPTLC determination of ceftriaxone, cefixime and cefotaxime in dosage forms,J Pharm Biomed Anal,184,1998,893.
10. Shah PB, Pundarikakshudu K.Spectrophotometric, difference spectroscopic and high performance liquid chromatographic methods for the determination of cefixime in pharmaceutical formulations, J AOAC Int,89,2006, 987-994.
11. Martindale; the complete drug reference,31st edi, pp 1068.2
12. Dechant KL, Noble S, Erdosteine, Drugs,52,1996,875-81.
13. Negro RW. Erdosteine: Antitussive and Anti-inflammatory Effects, lung.2008; 186:70-73.
14. Mhaske DV, Dhaneshwar SR, High-performance Thin-layer chromatographic method For determination of erdosteine In pharmaceutical dosage forms acta chromatographica,19,2007,170-184.
15. Muramatsu M, Toyo'oka T, Yamaguchi K, Kobayashi S, High-performance liquid chromatographic determination of erdosteine and its optical active metabolite utilizing a fluorescent chiral tagging reagent, *R*-(-)-4-(*N*,*N*-dimethylaminosulfonyl)-7-(3-aminopyrrolidin-1-yl)-2,1,3-benzoxadiazole, J Chromatogr B,719,1998,177–189.
16. Kim H, Chang KY, Lee HJ, Han SB, Lee KR, Sensitive determination of erdosteine in human plasma by use of automated 96-well solid phase extraction and LC–MS/MS, J Pharm Biomed Ana,34,2004, 661–669.
17. Marchioni CF, Polu JM, Taytard A, Hanard T, Noseda G, Mancini C. Evaluation of efficacy and safety of erdosteine in patients affected by chronic bronchitis during an infective exacerbation phase and receiving amoxycillin as basic treatment. ECOBES, European Chronic Obstructive Bronchitis Erdosteine Study.
18. *Ricevuti G, Mazzone A, Uccelli E, Gazzani G, Fregnan GB. Influence of erdosteine, a mucolytic agent, on amoxycillin penetration into sputum in patients with an infective exacerbation of chronic bronchitis ,Thorax,43,1988,585-590.*
19. Combinations of erdosteine and beta-2 agonists for treating COPD. European Patent EP1857106. http://www.freepatentsonline.com/EP1857106.html
20.Sharma A, Bagchi A ,Gupta H ,Kinagi SB, Sharma Y.B, Baliga VP.Comparative evaluation of the efficacy, safety and tolerability of the fixed dose combinations of cefixime plus erdosteine and amoxicillin plus bromhexine in patients with acute exacerbations of chronic bronchitis.COPD Treatment II, Wednesday, October 24, 2007, 12:30 PM - 2:00 PM.

Source of support: Nil, Conflict of interest: None Declared