RESEARCH ARTICLE

Stability Indicating Development and Validation of Ultraviolet Spectrophotometric Methods for the Simultaneous Estimation of Bromhexine and Cephalexin in their Combined Dosage Form

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Abstract

Aim: The aim of this study is to develop two simple, rapid, accurate, economical validated spectrophotometric for simultaneous estimation of bromhexine and cephalexin in bulk and dosage form. **Materials and Methods:** The Method-I is first derivative spectrophotometric and Method-II is the simultaneous equation method. Both the methods were employed for the analysis of drugs bromhexine and cephalexin using methanol and ammonium hydrogen phosphate (pH 7.7) in the ratio of 60:40 as a diluent. **Results and Discussions:** Both the methods are used for the simultaneous determination of bromhexine and cephalexin at 2155 nm and 266.5 nm, respectively, over the concentration range of 0.2–1.2 µg/ml and 12.5–75 µg/ml with the correlation coefficient (r^2) 0.9996 and 0.9994. **Conclusions:** The recovery studies confirmed the accuracy of proposed method and low-value standard deviation confirmed precession of method. The method is validated as per the International Council for Harmonization guidelines.

Key words: Bromhexine and cephalexin, development, first derivative, spectrophotometer, validation

INTRODUCTION

Bromhexine [Figure 1], 2, 4-dibromo-6-{[cyclohexyl (methyl) amino] methyl} aniline is widely used in medicine as a mucolytic drug. It works through decreasing the amount of respiratory tract fluid and reduces its viscosity by activating enzymes that hydrolyze mucopolysaccharides.[1,2] The drug is official in the Indian Pharmacopoeia (IP)[3] and British Pharmacopoeia (BP).[4] Several methods have been reported spectrophotometry,[5-7] high-performance liquid chromatography (HPLC).[8-14] Cephalexin [Figure 1] is chemically {(6R, 7R)-7-[(R)-2-amino-2-phenylacetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid monohydrate}[15] which is a first-generation cephalosporins for oral administration which is bactericidal and mainly used in the treatment of various bacterial infections caused by Gram-positive and Gram-negative microorganisms.[16] Cephalexin is official in IP,[17] United States Pharmacopoeia,[18] and BP.[19] Literature survey revealed that spectrophotometric,[20-22] HPLC,[23-27] and one HPLC method[28] have been reported for this combination drug.

MATERIALS AND METHODS

Chemicals and materials

Pure standard drugs such as bromhexine and cephalexin were obtained as gift samples from reputed pharmaceutical company. Methanol and ammonium hydrogen phosphate (Merck, Mumbai, India) were of analytical grade. Formulations of Cep-Bro tablet pharmaceutical dosage form of cephalexin and bromhexine containing labeled amount

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of cephalexin-250 mg and bromhexine-4mg were procured from local market.

Instrumentation

Teccomp ultraviolet (UV)-2301 double-beam UV-visible spectrophotometer was used to carry out spectral analysis, and the data were recorded by Hitachi software. Standard and sample drugs were weighed using Denver electronic analytical balance (SI-234).

Preparation of standard and sample solution

10mg of each standard drug was accurately weighed and dissolved in 5 ml diluent, methanol, and ammonium hydrogen phosphate (pH 7.7) in the ratio of 60:40 v/v, then transferred to a 10ml volumetric flask and sonicated for 5 min, and finally, volume was made up to the mark with the same solvent to make 1000 µg/ml stock solution. 100 µg/ml solution of each drug was prepared by diluting the above stock solution to 10ml and was used for preparing solutions of required concentrations. Combined solution of 1 ml of each drug solution was used for the simultaneous estimation of bromhexine and cephalexin.

Preparation of sample solution

For the analysis of tablet formulation, 10 tablets (Cep-Bro; cephalexin-250mg and bromhexine-4 mg) were weighed and powdered finely. From the tablet powder, an amount powder equivalent to 10mg of cephalexin standard was weighed accurately, dissolved in 10ml solvent, and the content was sonicated for 10–15 min to dissolve the drug completely in the solvent. Then, it was filtered and made up to 10 ml with same diluents methanol and ammonium hydrogen phosphate (pH 7.7) in the ratio of 60:40 v/v to prepare 1000 µg/ml cephalexin stock solution. From this stock solution, 50 µg/ml cephalexin sample solution was prepared. As per the label claim of the two drugs, a bromhexine concentration of 0.8 µg/ml was obtained. The resultant solution was used for the simultaneous estimation of cephalexin and bromhexine in combined dosage form.

Method-I: First-order derivative spectrophotometry

In this method, standard stock solutions of bromhexine and cephalexin were prepared and scanned in the spectrum mode from 200 nm to 400 nm. The absorption spectrum obtained was derivatized from first to fourth order. A first-order derivative spectrum was selected for the analysis of drugs. From the spectra of drugs, the maximum absorbance was observed at 266.5 nm for bromhexine and 215.5 nm for cephalexin and zero cross = 279 nm, and these two wavelengths can be applied for the determination of bromhexine and cephalexin without any interference from the other drug in their pure and combined dosage forms [Figure 2]. The proposed method was validated as per the International Council for Harmonization (ICH) guideline.[29]

Method-II: Simultaneous equation method

Working standard solutions of bromhexine and cephalexin were prepared separately from standard stock solution. These solutions were scanned in the spectrum mode from 400.0 nm to 200.0 nm. The maximum absorbance of bromhexine and cephalexin was observed at 266.5 nm and 215.5 nm, respectively [Figure 3]. The linearity of bromhexine and cephalexin was found to be in the concentration ranges of 0.2–1.2 µg/ml and 12.5–75 µg/ml, respectively, at their respective maxima. Two simultaneous equations were formed using these absorptivity coefficient values.

$$C_x = A_2a_{y1}\text{-}A_1a_{y2}/a_{x2}a_{y1}–a_{x1}a_{y2}$$

$$C_y = A_1a_{x2}\text{-}A_2a_{x1}/a_{x2}a_{y1}–a_{x1}a_{y2}$$

a_{x1} = Absorptivity of bromhexine at 266.5 nm

a_{x2} = Absorptivity of bromhexine at 215.5 nm

a_{y1} = Absorptivity of cephalexin at 266.5 nm

a_{y2} = Absorptivity of cephalexin at 215.5 nm

RESULTS AND DISCUSSIONS

First-order derivative spectrophotometric method (Method-I)

The proposed method was based on first-order derivative spectrophotometry for the simultaneous estimation of bromhexine and cephalexin in UV region using methanol and ammonium hydrogen phosphate (pH 7.7) in the ratio of 60:40 v/v. Beer's law obeyed in the concentration range of 0.2–1.2 µg/ml and 12.5–75 µg/ml for bromhexine and cephalexin, respectively. The correlation coefficient (r^2) values were found to be 0.999 for both drugs which show that absorbance of both drugs was linear within the mentioned

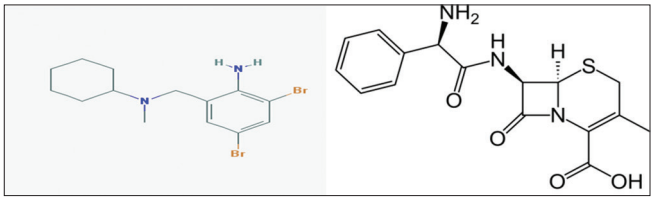

Figure 1: Chemical structures of bromhexine and cephalexin

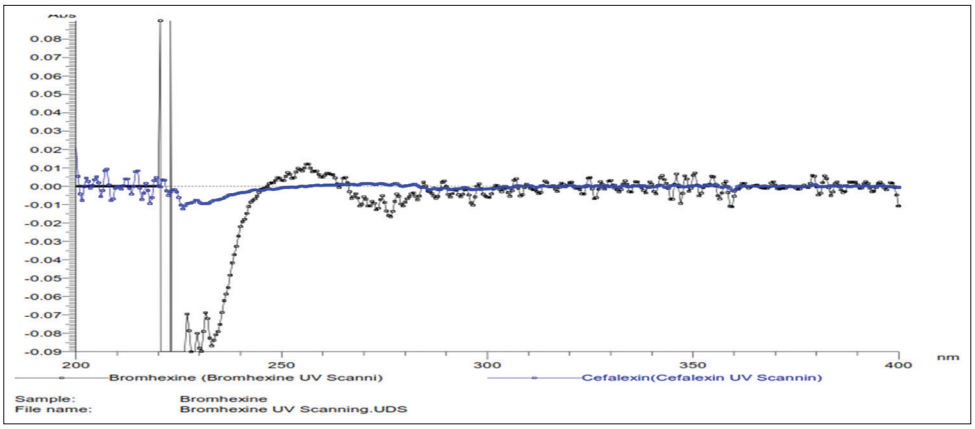

Figure 2: Overlay spectra of bromhexine and cephalexin for first-order spectrophotometric method

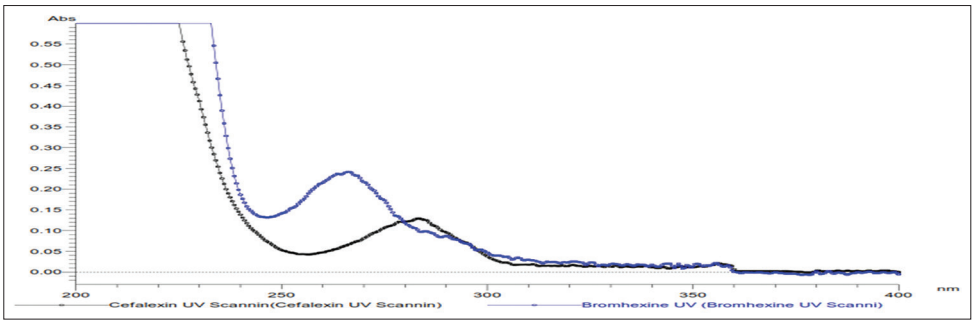

Figure 3: Overlay spectra of bromhexine and cephalexin for simultaneous equation method

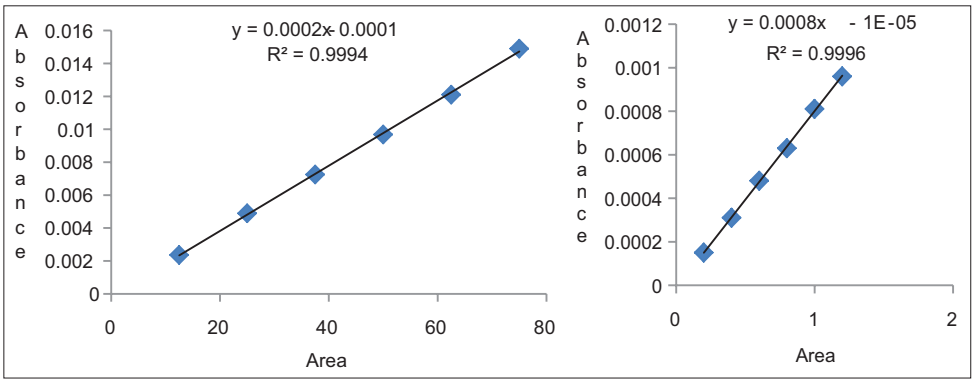

Figure 4: Linearity graphs of bromhexine and cephalexin for first-order derivative spectrophotometric method

Table 1: Results of linearity for first-order derivative spectrophotometric method

Bromhexine		Cephalexin	
Concentration µg/ml	**Absorbance**	**Concentration µg/mL**	**Absorbance**
0.2	0.00015	12.5	0.00236
0.4	0.00031	25	0.00489
0.6	0.00048	37.5	0.00725
0.8	0.00063	50	0.00968
1	0.00081	62.5	0.0121
1.2	0.00096	75	0.0149

concentration range [Table 1 and Figure 4]. All the validation parameters were found to be within the permitted level and were given in Table 2. Marketed brand of the tablet was analyzed and the amount of drugs determined by proposed method was found to be 99.125 and 99.142 for bromhexine and cephalexin, respectively [Table 3].

Simultaneous equation method (Method-II)

A six-point calibration curves were obtained in a concentration range of 0.2–1.2 µg/ml and 12.5–75µg/ml for bromhexine and cephalexin, respectively. The response of the drugs was found to be linear in the concentration range, and the linear regression equations were y = 0.2553x + 0.0415 with the correlation coefficient r^2 = 0.9993 for bromhexine and y = 0.0192x + 0.0524 with the correlation coefficient r^2 = 0.9996 for cephalexin [Table 4 and Figure 5]. All the validation parameters were found to be with in the permitted level and were given in Table 5. Marketed brand of the tablet was analyzed and the amount of drug determined by proposed methods was found to be 98.064 and 98.263 for bromhexine and cephalexin, respectively [Table 5].

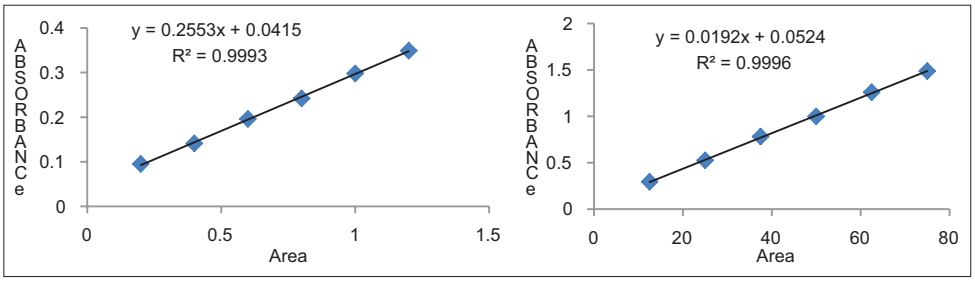

Figure 5: Linearity graphs of bromhexine and cephalexin for simultaneous equation method

Table 2: Summary of validation for bromhexine and cephalexin for first-order derivative spectrophotometric method

Validation parameter	Values for bromhexine	Values for cephalexin
Intraday precession (%RSD)	1.13	0.338
Interday precession (%RSD)	1.442	0.2422
Ruggedness (%RSD)	1.749	0.2176
Recovery (%)	99.39–99.63	99.65–100.04
LOD µg/ml	0.005	0.20
LOQ µg/ml	0.02	0.70
Formulation assay (µg/ml)	99.125	99.142

RSD: Relative standard deviation, LOD: Limit of detection, LOQ: Limit of quantification

Table 3: Results of stability studies of bromhexine and cephalexin

Time in hours	Bromhexine		Cephalexin	
	Average absorbance	% assay	Average absorbance	% assay
0	0.243	100.413	0.997	99.8998
2	0.241	99.5868	0.996	99.7996
4	0.241	99.5868	0.991	99.2986
8	0.239	98.7603	0.989	99.0982
12	0.238	98.3471	0.981	98.2966
24	0.237	97.9339	0.979	98.0962

Table 4: Results of linearity for simultaneous equation method

Bromhexine		Cephalexin	
Concentration µg/mL	Absorbance	Concentration µg/mL	Absorbance
0.2	0.095	12.5	0.294
0.4	0.141	25	0.525
0.6	0.196	37.5	0.781
0.8	0.242	50	0.998
1	0.298	62.5	1.258
1.2	0.349	75	1.487

Table 5: Summary of validation for bromhexine and cephalexin for simultaneous equation method

Validation parameter	Values for bromhexine	Values for cephalexin
Intraday precession (%RSD)	0.568	0.233
Interday precession (%RSD)	0.733	0.161
Ruggedness (%RSD)	1.159	0.542
% Recovery	99.00–99.08	99.36–99.74
LOD µg/ml	0.005	0.20
LOQ µg/ml	0.02	0.70
Formulation assay µg/mL	98.064	98.263

RSD: Relative standard deviation, LOD: Limit of detection, LOQ: Limit of quantification

The stability studies indicated that appreciable changes were observed by treating the drug with UV light, thermal stress, oxidation, acidic hydrolysis, and basic hydrolysis; however, no appreciable change was observed with sunlight. The results of stability studies were given in Table 5. Thus, the methods are useful for the simultaneous determination of bromhexine and cephalexin in bulk and pharmaceutical formulations.

CONCLUSIONS

The proposed methods (I and II), i.e., first-order derivative spectrophotometric and simultaneous equation are found to be very simple and rapid, can be performed using spectrophotometer, and does not require much costly instruments. It also shows good linearity and sensitivity. Results of the analysis were validated as per the ICH guidelines. Stability testing study including the effect of temperature, oxidation, photolysis, and susceptibility to hydrolysis across a wide range of pH is performed. Thus, the methods are applicable for simple economical estimation of bromhexine and cephalexin in their pharmaceutical dosage forms.

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