Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2014, 6 (6):315-321
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Simultaneous estimation and validation of bromhexine and cephalexin in bulk and pharmaceutical dosage form by RP-HPLC method

B. Siddartha and Ch. Kiranya Bharathi

Department of Pharmaceutical Analysis, Malla Reddy College of Pharmacy, Secunderabad

---

ABSTRACT

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the simultaneous determination Bromhexine and Cephalexin in pharmaceutical dosage form. The column used was ODS C_{18} (250mm x 4.6 mm, 5µ) in isocratic mode, with mobile phase containing phosphate buffer and acetonitrile (45:55 v/v). The buffer is prepared by adding 1.42gm of sodium dihyrogen ortho phosphate in a 1000ml of volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then pH adjusted to 5.0 with dil. orthophosphoric acid solution. The flow rate was 1.0ml/ min and effluents were monitored at 254 nm. The retention times of Bromhexine and Cephalexin were found to be 2.661 min and 4.271 min, respectively. The linearity for Bromhexine and Cephalexin were in the range of 1-6 µg/ml and 62.5-375 µg/ml respectively. The recoveries of Bromhexine and Cephalexin were found to be 98.58% to 101.45% w/v and 98.13% to 101.51%w/v, respectively. The proposed method was validated and successfully applied to the estimation of Bromhexine and Cephalexin in combined tablet dosage forms.

Key words: Bromhexine, Cephalexin, Buffer, Methanol, Validation and ICH Guidelines.

INTRODUCTION

Bromhexine is an expectorant/mucolytic agent. Chemically it is 2, 4-dibromo-6-{[cyclohexyl(methyl) amino]methyl}aniline. The chemical formula is C14H20Br2N2. The molecular weight is 376.13g/mol. Bromhexine is an oral mucolytic agent with a low level of associated toxicity. Bromhexine acts on the mucus at the formative stages in the glands, within the mucus-secreting cells. Bromhexine disrupts the structure of acid mucopolysachharide fibres in mucoid sputum and produces less viscous mucus, which is easier to expectorate [1, 2].

Cephalexin is an antibiotic useful for the treatment of a number of bacterial infections. Chemically, it is (6R, 7R)-7-[(2R)-2-amino-2-phenylacetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid. The chemical formula and molecular weight is C16H17N3O4S and 347.389g/mol. Cephalexin, like the penicillins, is a beta-lactam antibiotic. By binding to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall, it inhibits the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that cephalexin interferes with an autolysin inhibitor [3].

Different analytical methods have been reported in the literature for the assay of Bromhexine and Cephalexin in pharmaceuticals and include spectrophotometry and HPLC [4-10]. The present study was to establish a simple,

sensitive and low cost RP-HPLC method for simultaneous estimation of Bromhexine and Cephalexin in bulk as well as in other dosage forms. The developed method was validated as per ICH guidelines [11-12].

MATERIALS AND METHODS

Reagents

Bromhexine and Cephalexin were kindly supplied by Zephyr Medicare Pvt Ltd. Acetonitrile, water (HPLC grade, Merck) and all the other reagents of AR grade were purchased from M R Enterprisers. A tablet BROCIF (Zephyr Medicare Pvt Ltd) containing 4mg of Bromhexine and 250mg of Cephalexin were used.

Instrumentation

The LC system consisted of a Waters model 515, PDA detector 2998 with 20 µL sample loop. The output signals were monitored and integrated using Empower 2 software.

Chromatographic conditions

The elution was isocratic and the mobile phase consisted of a mixture of buffer (Accurately weighed 1.42gm of Sodium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then pH adjusted to 5.0 with dil. Orthophosphoric acid solution) and acetonitrile (45:55 v/v). The mobile phase was filtered through a 0.45-µm (HVLP, Germany) membrane filter prior to use. A ODS C_{18} (100mm x 4.6 mm, 5µ) was used for determination. The flow rate was 1.0 ml/min and the column was operated at ambient temperature (~30^{o}C). The volume of sample injected was 10 µL. Prior to injection of the solutions, column was equilibrated for at least 30min with mobile phase flowing through the system. The UV detector was set at wavelength of 254nm. A typical RP-HPLC chromatogram of Bromhexine and Cephalexin is shown in Figure-1.

Diluent: Methanol

Standard Preparation

Accurately weighed and transferred 4mg of Bromhexine and 250mg of Cephalexin working Standards into a 100 ml clean dry volumetric flask, add 70ml of diluent, sonicated for 10 minutes and make up to the final volume with diluent. From the above solution 1ml was transferred into 10ml to get concentration of 4µg/ml and 250µg/ml for Bromhexine and Cephalexin.

Sample Preparation

About 20 tablets were taken and their average weight was calculated. The tablets were crushed to a fine powder and drug equivalent to 100mg were transferred to a 100ml volumetric flask, dissolved in diluent filtered through 0.45µ membrane filter. From the above solution 1ml was transferred into 10ml to get concentration of 4µg/ml and 250µg/ml for Bromhexine and Cephalexin.

METHOD VALIDATION

The developed method was validated as per ICH guidelines for its accuracy, linearity, precision, specificity, robustness, ruggedness, limit of detection and limit of quantification by using the following procedures. The parameters are validated as shown in table-9.

System suitability

System suitability and chromatographic parameters were validated such as asymmetry factor, tailing factor and number of theoretical plates were calculated.

Linearity

Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solutions of Bromhexine and Cephalexin at different concentration levels. Absorbance of resulting solutions was measured and the calibration curve was plotted between absorbance vs concentration of the drug (Figure: 2 & 3). The response was found to be linear in the range 1-6µg/ml & 62.5-375µg/ml for Bromhexine and Cephalexin. The data was given in table-1.

Precision

A) Method Repeatability

Six sample solutions of the same concentration (100% i.e., 4µg/ml of Bromhexine and 250µg/ml of Cephalexin) were prepared and injected into the HPLC system as per test procedure. The results were given in table-2.

Accuracy

Accuracy was performed in triplicate for various concentrations of Bromhexine and Cephalexin equivalent to 50%, 100% and 150% of the standard amount was spiked to 100% sample solution and they were injected into the HPLC system per the test procedure. The average % recovery was calculated. The data was given in table-3.

Limit of detection and Limit of Quantification

LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve as per ICH guidelines. The LOD and LOQ of Bromhexine were found to be 0.055µg/ml and 0.167µg/ml respectively. The LOD and LOQ of Cephalexin were found to be 4.370µg/ml and 13.242µg/ml respectively.

Robustness

Robustness was done by small deliberate changes in the chromatographic conditions and retention time of Bromhexine and Cephalexin were noted. The factors selected were change in flow rate and variation in the mobile phase composition. The results remained unaffected by small variations in these parameters as shown in table-4 and 5.

Ruggedness

Ruggedness of the method was checked by using different days and with different instruments. Six sample solutions of the same concentration (100% i.e., 4µg/ml of Bromhexine and 250µg/ml of Cephalexin) were prepared and injected into the HPLC system as per test procedure. The relative standard deviation of the results obtained from different days and with differnt instruments were found to be <2.0%. The results were given in table-6 and 7.

Assay

The assay and % purity were calculated for brand BROCIF with label claim 4mg and 250mg of Bromhexine and Cephalexin. The observed value was compared with that of standard value without interference from the excipients used in the tablet dosage form. The results were given in table-8.

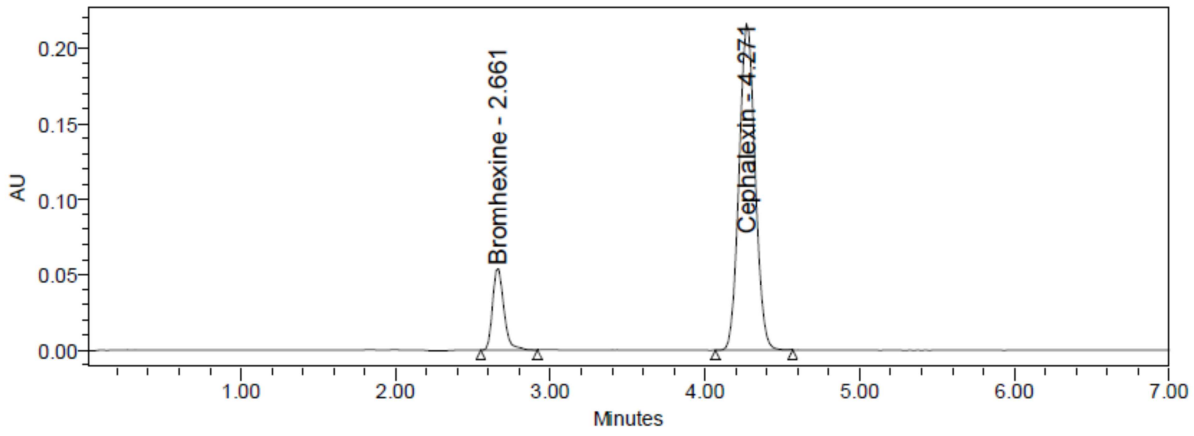

Figure-1: HPLC Chromatogram of Bromhexine and Cephalexin in optimized chromatographic conditions

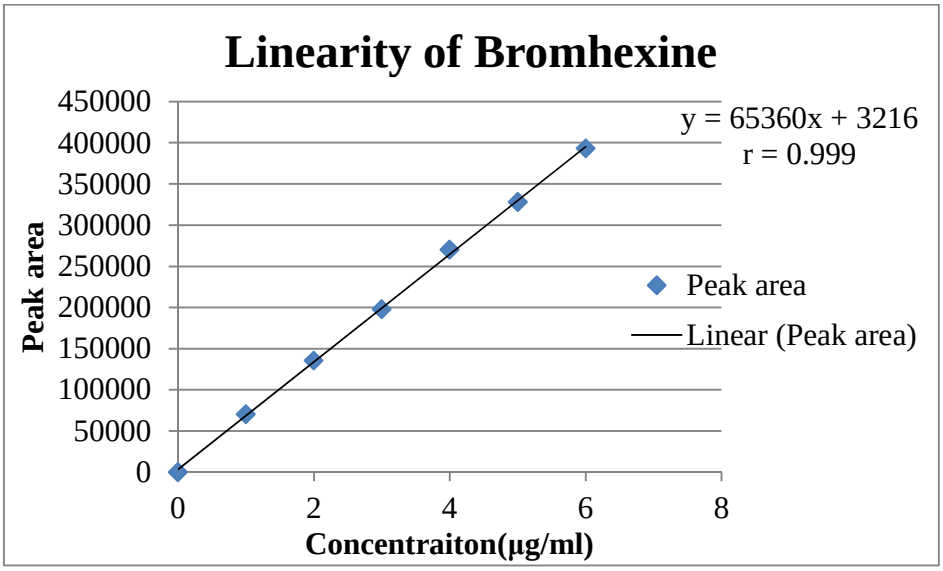

Figure-2: Linearity of Bromhexine in the range 1 to 6µg/ml

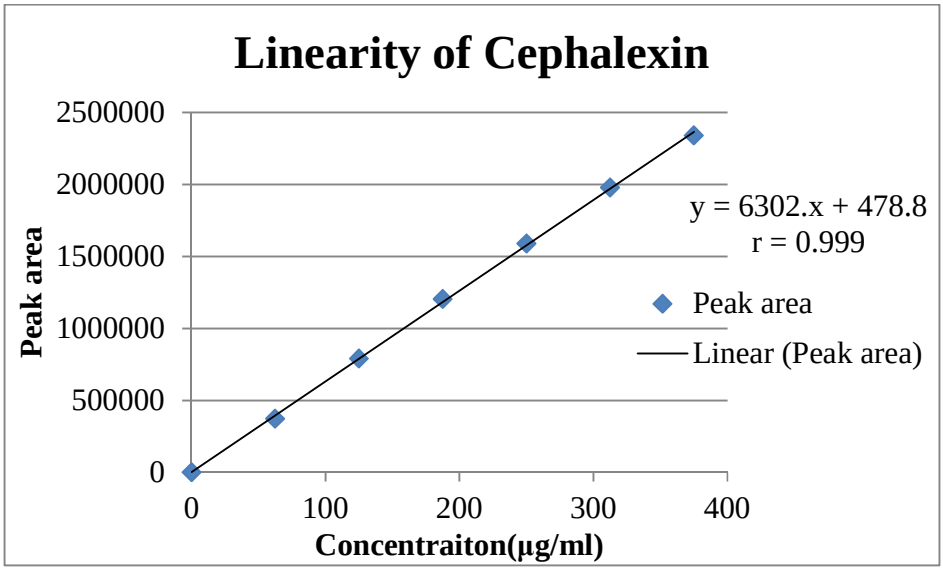

Figure-3: Linearity of Cephalexin in the range 62.5 to 375µg/ml

Table-1: Linearity data of Bromhexine and Cephalexin

S.No	Bromhexine			Cephalexin		
	Conc(µg/ml)	Rt(mins)	Area	Conc(µg/ml)	Rt(mins)	Area
1	1	2.594	70257	62.5	4.023	372918
2	2	2.591	135407	125	4.003	790194
3	3	2.586	197967	187.5	4.011	1204456
4	4	2.595	270064	250	4.007	1589609
5	5	2.595	328192	312.5	4.010	1979489
6	6	2.586	393184	375	4.034	2339117
	r = 0.9997 y = 65360x + 3216			r = 0.9996 y = 6302.x + 478.8		

Table-2: Precision data of Bromhexine and Cephalexin

S.No	Bromhexine			Cephalexin		
	Conc(µg/ml)	Rt(mins)	Area	Conc(µg/ml)	Rt(mins)	Area
1	4	2.600	267260	250	4.041	1558761
2	4	2.606	267358	250	4.068	1544958
3	4	2.616	269266	250	4.110	1550625
4	4	2.646	268107	250	4.205	1564732
5	4	2.661	266679	250	4.271	1553615
6	4	2.663	269291	250	4.284	1566462
Mean			267994			1556526
SD			1094			8345
%RSD			0.41			0.54

Table-3: Accuracy data of Bromhexine and Cephalexin

S.No	Bromhexine				Cephalexin		
	Spiked level	Amount added (µg/ml)	Amount present (µg/ml)	Average %Recovery* ± %RSD	Amount added (µg/ml)	Amount present (µg/ml)	Average %Recovery* ± %RSD
1	50%	1.00	1.01	100.59 ± 1.30	62.50	62.71	100.34 ± 0.53
2	100%	2.00	1.98	99.19 ± 0.54	125.00	126.57	101.26 ± 0.29
3	150%	3.00	3.00	100.11 ± 0.41	187.50	185.82	99.10 ± 1.02

**n=3 (Average of 3 determinations)*

Table-4: Robustness data relating to change in flow rate (1.2ml/min)

S.No	Bromhexine				Cephalexin		
	Flow rate (ml/min)	Average Peak Area*	SD	%RSD	Average Peak Area*	SD	%RSD
1	Flow rate-1-(0.9ml)	270412	1455	0.54	1584804	5915	0.37
2	Flow rate-1-(1.0ml)	269879	526	0.19	1585413	3766	0.24
3	Flow rate-1-(1.1ml)	269733	1868	0.69	1579770	6752	0.43

**n=3 (Average of 3 determinations)*

Table 5: Robustness data relating to change in mobile phase composition

S.No		Bromhexine			Cephalexin		
	Mobile phase variation (%)	Average peak area*	SD	%RSD	Average peak area*	SD	%RSD
1	M.P-1-(BUFFER:ACN::46:54)	272623	3355	1.23	1562883	13692	0.88
2	M.P-2-(BUFFER:ACN::45:55)	270818	1092	0.40	1591912	4607	0.29
3	M.P-3-(BUFFER:ACN::44:56)	272247	2366	0.87	1556291	14120	0.91

**n=3 (Average of 3 determinations)*

Table 6: Ruggedness data relating to change of day

S.No	Inter-day precision					
	Day-1			Day-2		
	Peak area			Peak area		
	Conc (µg/ml)	Bromhexine	Cephalexin	Conc (µg/ml)	Bromhexine	Cephalexin
1	4/250	278472	1599383	4/250	278651	1577913
2	4/250	274618	1582927	4/250	274906	1561785
3	4/250	275512	1592232	4/250	275717	1576451
4	4/250	275219	1582927	4/250	276998	1566159
5	4/250	278171	1593223	4/250	274218	1553418
6	4/250	271199	1582922	4/250	275173	1586291
Mean		275532	1588936		275944	1570336
SD		2658	7025		1621	12051
%RSD		0.96	0.44		0.59	0.77

Table 7: Ruggedness data relating to change of instrument

S.No	Instrument to Instrument					
	Inst-1			Inst-2		
	Peak area			Peak area		
	Conc (µg/ml)	Bromhexine	Cephalexin	Conc (µg/ml)	Bromhexine	Cephalexin
1	4/250	270272	1583654	4/250	268622	1593737
2	4/250	275897	1588464	4/250	269131	1586352
3	4/250	274987	1598437	4/250	274987	1586252
4	4/250	274677	1593645	4/250	273482	1599263
5	4/250	269158	1583532	4/250	269659	1583822
6	4/250	275754	1562521	4/250	270846	1599249
Mean		273458	1585042		271121	1591446
Std.dev		2956	12462		2567	6904
%RSD		1.08	0.79		0.95	0.43

Table-8: Results of analysis of laboratory samples (Assay)

S.No			Bromhexine		Cephalexin	
	Sample	Label	Amount found	%Purity ± RSD*	Amount found	%Purity ± RSD*
1	Brand-1 (BROCIF)	4mg/250mg	4.00	99.94 ± 0.15	248.58	99.04 ± 0.37

**n=3 (Average of 3 determinations)*

Table 9: System suitability parameters

Validation parameter	Results	
	Bromhexine	Cephalexin
Linearity range (µg/ml)	1-6	62.5-375
Regression equation	y = 65360x + 3216	y = 6302.x + 478.8
Correlation Coefficient(r)	0.9997	0.9996
Precision (%RSD)	0.41	0.54
Accuracy	98.58% to 101.45%	98.13% to 101.51%
Robustness (%RSD)		
Flow rate: (0.9ml/min & 1.1ml/min)	NMT 0.69	NMT 0.43
Mobile phase: Buffer : ACN(45:55)	NMT 1.23	NMT 0.91
Ruggedness (%RSD)		
Interday – (Day 1 & Day 2)	NMT 0.96	NMT 0.77
Instrument to Instrument (Inst-1 & Inst-2)	NMT 1.08	NMT 0.79

RESULTS

A reverse-phase column procedure was proposed as a suitable method for the simultaneous estimation of Bromhexine and Cephalexin dosage form. The chromatographic conditions were optimized by changing the mobile phase composition. Different ratios were experimented to optimize the mobile phase. Finally, phosphate buffer and acetonitrile in the ratio 45:55 v/v was used as mobile phase, which showed good resolution of Bromhexine and Cephalexin peak. The wavelength of detection selected was 254nm, as the drug showed optimized absorbance at this wavelength. By our proposed method the retention time of Bromhexine and Cephalexin were about 2.661mins and 4.271mins and none of the impurities were interfering in its assay.

DISCUSSION

The statistical analysis of data and the drug recovery data showed that the method was simple, rapid, economical, sensitive, precise and accurate. It can thereby easily adopt for routine quality control analysis. The results of this analysis confirmed that the proposed method was suitable for determination of drug in pharmaceutical formulation with virtually no interference of additives. Hence the proposed method can be successfully applied in simultaneous estimation of Bromhexine and Cephalexin in marketed formulation.

CONCLUSION

The proposed method is rapid, accurate and sensitive. It makes use of fewer amounts of solvents and change of set of conditions requires a short time. This method can be suitably analyzed for the routine analysis of Bromhexine and Cephalexin in bulk and its pharmaceutical dosage forms. It does not suffer from any interference due to common excipients present in pharmaceutical preparation and can be conveniently adopted for quality control analysis.

Acknowledgements
The authors are thankful to Malla Reddy College of Pharmacy, Secunderabad for providing all facilities to complete the work and Zephyr Medicare Pvt Ltd for providing the Bromhexine and Cephalexin as gift sample.

REFERENCES

[1] Indian Pharmacopoeia, Govt. of India, Ministry of health and Family Welfare, Publication by The Indian commission Ghaziabad; **1996**; (1): 111.
[2] British Pharmacopoeia, London, HMSO Publication **1996**; 2: 207.
[3] S.C.Sweetman (Ed), "Martindale – The complete Drug Reference, "Pharmaceutical Press, London (U.K) **2009**; 36th Edn: 218.
[4] K. Susmitha et al., ISRN *Analytical Chemistry* **2013**; Article ID 861851, 1-7.
[5] I. Nageswar Rao et al., *Indian Journal of Chemical Technology* **2005**; 12: 170-174.
[6] G.Vijaya Raja et al., *International Journal of Pharma Sciences and Research* **2010**; 1(2): 90-94.
[7] Sagar Suman Panda et al., *Scientia Pharmaceutica* **2013**; 81: 1029-1041.
[8] M. C. Damle et al., *International Journal of PharmTech Research* **2009**; 1(3): 527-536.
[9] Najib N. M. et. al., *Journal of Clinial pharmacy and therapeutics*, **2008**; 12: 419-426.
[10]Prayanka P. and Suresh P., *Asian Journal of Pharmaceutics*, **2008**; 2: 120–122.
[11]ICH validation of Analytical procedures: Text and Methodology Q2 (R1) in proceedings of International Conference on Harmonization; Geneva: Switzerland **2005**; 205-210.
[12]ICH guidelines, Validation of Analytical procedures technical requirements for registration of Pharmaceuticals for Human use Text and Methodology Q2 (R1), International Conference on Harmonization; Geneva: Switzerland **2005**; 325-340.