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Method Development & Comparative Statistical Evaluation of HPLC & HPTLC Method for Simultaneous Estimation of Cefodrixil Monohydrate & Ambroxol Hydrochloride

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ABSTRACT: Rapid, precise, accurate, specific, and sensitive high performance liquid chromatographic method and high performance thin layer chromatographic methods have been developed for simultaneous determination of cefadroxil monohydrate and ambroxol hydrochloride in their tablet formulation. The HPLC method was standardised using Purospher BDS C18 column (25cm X 4.6mm, 5µm) with mobile phase constituted of 0.5M ammonium acetate buffer- acetonitirile (50:50v/v), pH adjusted to 7 using orthophosphoric acid delivered at the flow rate of 1.0 $mlmin^{-1}$ and detection was performed at 247nm. For HPTLC analysis separation was carried out on precoated TLC plates, coated with silica gel 60F-254 and using mobile phase methanol- potassium dihydrogen phosphate (0.067M) (35:65v/v) scanned at 254nm with CAMAG TLC scanner controlled by Cats Software. Different analytical performance parameters such as linearity, accuracy, precision, repeatability, robustness LOD and LOQ were determined according to International conference of Harmonization ICH Q2B guidelines. Results of developed methods were compared with reported method using ANOVA.

KEYWORDS: Cefadroxil Monohydrate; Ambroxol Hydrochloride; HPLC; HPTLC, ANOVA.

INTRODUCTION

Cefadroxil monohydrate, 7-[(CR)-2-amino- (4-hydroxyphenyl) acetamido]-3-methyl-3-cephem-4-carboxyl acid monohydrate is a first generation, oral cephalosporin antimicrobial agent active against Gram-positive organisms. It is official in IP [1], USP [2] and BP [3].

Ambroxol hydrochloride, [2-amino-3, 5 dibromo-N (trans-4-hydroxy cyclohexyl)] benzyl amine hydrochloride is a metabolite of bromhexin and act to reduce viscosity of tanaceous mucus secretions via fragmentation of long mucopolysaccharide chains. It is official in IP [1], BP [3].

Cefadroxil Monohydrate alone and in combination with other drug was determined by several methods including UV spectroscopy [4, 5], HPLC [6], HPTLC [7]. Ambroxol hydrochloride alone was determined by UV spectrophotometry [8, 14], HPLC

[14], colorimetry [16]. Ambroxol hydrochloride was also determined simultaneously in combined dosage form by HPLC [9-13], HPTLC [15].

A spectrophotometric method is found reported for simultaneous quantitative determination of cefadroxil monohydrate and ambroxol hydrochloride in pharmaceutical preparations. In Present work attempt is made to develop HPLC and HPTLC method and compare them statistically with reported UV methods [5].

MATERIALS & METHODS

Ammonium acetate buffer and potassium dihydrogen phosphate (Analytical reagent grade) was procured from Loba Chem Pvt. Ltd. Acetonitrile was obtained from Merk Laboratories Pvt Ltd, Mumbai. Methanol (analytical reagent grade) was procured from Sisco laboratories Pvt. Ltd. Cefadroxil monohydrate was supplied by Maxim Pharmaceuticals, Pune and Ambroxol hydrochloride was supplied by Centaur Pharmaceuticals Goa as gift sample. The purity of cefadroxil monohydrate and ambroxol hydrochloride was 98% and 99.7% respectively.

1. HPLC Method (Method-1)[18]

HPLC Model- Jasco equipped with UV/ Visible detector (Jasco UV-2075 plus), Column Dimensions, Purospher C-18 (4.6×250mm, 5µm particle size) with isocratic mode was used at flow rate of 1.0 ml/min at room temperature with injection volume of 20µl and wavelength detection at 247nm.

1.1 Preparation of Mobile phase

35.54gm of Ammonium acetate AR grade was dissolved in 1000ml of double distilled water to prepare 0.5M Ammonium acetate buffer solution adjusted to pH-7 with orthophosphoric acid. Mobile phase comprises of ammonium acetate buffer (pH-7; 0.5M)-Acetonitrile (50:50 v/v).

1.2 Preparation of Standard stock solution

Standard stock solutions were prepared in mobile phase. 10mg each of cefadroxil monohydrate and Ambroxol hydrochloride were dissolved in sufficient mobile phase with the aid of sonication. Volume was made up to 10ml with mobile phase to prepare standard stock solution of 1000µg/ml each of cefadroxil monohydrate and Ambroxol hydrochloride.

1.3 Linearity Study

In to a series of 10 ml volumetric, suitable dilutions were made with mobile phase to get final concentration of 5-25 µg/ml for cefadroxil monohydrate and 3-15 µg/ml for Ambroxol hydrochloride. A 20 µl of sample solution was injected into injection port of chromatographic system having fixed volume loop injector. Chromatograms were noted and area was plotted against concentration to get calibration curve.

1.4 Sample preparation

Twenty tablets were weighed and finely powdered. Tablet powder equivalent to 25 mg of cefadroxil monohydrate and 3mg of ambroxol hydrochloride were taken in 10mLvolumetric flask, and dissolved in sufficient mobile phase with aid of sonication and volume was made up to 10mLwith mobile phase. Resultant solution was filtered through 0.45µ filter paper and further dilutions were made with mobile phase to get 3 µg/ml of Ambroxol hydrochloride and 25 µg/ml of cefadroxil monohydrate.

2. HPTLC Method (Method-2) [19, 20]

2.1 Instrumentation and chromatographic condition

1. Sample syringe: Camag 100 µl syringe (Hamilton, Bonaduz, Switzerland)
2. Sample applicator: Camag linomat 5 (Switzerland)
3. Stationary phase: Precoated silica gel aluminium plate 60 F254 (10 × 10cm)
4. Sample application: Band size of 6 mm
5. Slit dimension: 5 mm × 0.45 mm
6. Wavelength selected: 254nm
7. Saturation time: 15 min
8. Development time: 30 min
9. Scanning: Densitometric scanning on Camag TLC scanner 3 at 254 nm with scanning speed of 10 mm/spot.

2.2 Preparation of Mobile Phase

About 9.118 gm of potassium dihydrogen phosphate was dissolved in water and made up to the volume of about 1000ml. 350ml of methanol and 650 ml of buffer were mixed to get the mobile phase. All the chemicals and reagents used are of AR grade.

2.3 Standard Stock Solution

10mg each of cefadroxil monohydrate and Ambroxol hydrochloride dissolved in methanol with the aid of sonication in two 10 ml volumetric flask. A standard stock solution of cefadroxil monohydrate and Ambroxol hydrochloride was prepared in methanol to have the concentration of 1 mg/ml respectively.

2.4 Linearity and Calibration

For linearity, 0.3 to 1.8 µl of cefadroxil monohydrate stock solution was spotted on TLC plate to get concentration of 300 to 1800 ng/spot, similarly 0.2 to 1.2 µl of Ambroxol hydrochloride Stock solution was spotted on TLC plate to get concentration of 200 to 1200 ng/spot. Twin through chamber was saturated with mobile phase. The chromatoplates were then developed in twin through chamber. After development, plates were dried with dryer and scanned at 254nm and peak areas were measured.

2.5 Sample Preparation

Twenty tablets were weighed accurately and ground to fine powder. Powder equivalent to 0.88mg of cefadroxil monohydrate and 0.11 mg and Ambroxol hydrochloride was weighed and dissolved in 10ml of methanol. The solution was sonicated for 10 minute and

filtered through whatman filter paper no-41. 1 µl was then spotted on chromatoplate to get concentration of 0.88 µl/ml of cefadroxil monohydrate and 0.11µl/ml of Ambroxol hydrochloride.

3. Method validation

Both Methods were validated according to ICH guidelines.

3.1 Precision:

Sample analysis is repeated 6 times (n=6) for repeatability study. The reproducibility of the proposed method was determined by repeating sample analysis at different time intervals on same day (n=3) (Intra-day precision) and on three different days (n=3) (Inter-day precision) and were expressed in % RSD.

3.2 Accuracy:

To ascertain the accuracy of proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% & 120%).Percent recovery for cefadroxil monohydrate and Ambroxol hydrochloride for both methods were found to be in the range of 98.10 % to 101.00%

3.3. LOD and LOQ:

Limit of detection was calculated by using formula of 3.3 σ/S and for limit of quantitation formula of 10 σ/S was used where σ is standard deviation of response and S is slope of calibration curve [Table 1].

3.4 Specificity

Specificity is checked by carrying out analysis of sample in presence of excipients which are expected to be present in tablet sample

3.5 Robustness

Robustness study was carried out by changes in process parameters such as flow rate, pH, ratio of mobile phase, time from spotting to development to scanning

4. Statistical Evaluation [21]

The analytical characteristics of the tested methods in HPLC and HPTLC were validated to ensure the suitability of the analytical requirements and reliability of the results. The statistical ANOVA treatments were performed with the Graphpad Instat statistical software.

RESULTS & DISCUSSION

HPLC Method (Method-1)

Mobile phase consisting of Ammonium Acetate Buffer (pH-7; 0.5M) - Acetonitirile (50:50v/v), pH 7 adjusted with orthophosphoric acid offered a good separation at a flow rate of 1.0 ml/min and a runtime of 10 min. Cefadroxil monohydrate elutes first and then

Ambroxol hydrochloride gets eluted as shown in the chromatogram, **Fig.1** which illustrate the separation of both active ingredients in this system. The detection wavelength of 247 nm was chosen in order to achieve a good sensitivity for quantitative determination of cefadroxil monohydrate and Ambroxol hydrochloride in tablet dosage form. The isocratic program throughout HPLC method was adopted to analyze both components in a single run. The proposed method is simple and do not involve laborious time-consuming sample preparation. System Suitability Testing is used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed [17].

Table-1 Results of parameters used for validation of cefadroxil monohydrate and Ambroxol hydrochloride

Parameters	HPLC Method (Method-1)		HPTLC Method (Method-2)	
	Cefadroxil	Ambroxol	Cefadroxil	Ambroxol
Linearity (R^2)	0.9987	0.997	0.9992	0.9991
%RSD (n=6) (Indicates precision)	< 2 %	< 2 %	< 2 %	< 2 %
Mean % Recovery	99.95	99.5	100.3	99.4
Limit of Detection	0.73 µg/ml	0.071 µg/ml	3.10 ng/ml	0.30 ng/ml
Limit of Quantitation	2.20 µg/ml	0.22 µg/ml	9.40 ng/ml	0.93 ng/ml
Range	5-25 µg/ml	3-15 µg/ml	0.3-1.8 µg/ml	0.2-1.2 µg/ml
Assay(n=5)	100.2	100.6	99.56	100.8
Robustness study	Robust	Robust	Robust	Robust
Specificity Study	Specific	Specific	Specific	Specific

[a] n: No of times analysis repeated

Table-2 Interday and Intraday Precision Data

Method used	Drug added µg /ml		Intraday precision RSD (%)(n=3)		Interday precision RSD (%)(n=5)	
	Cefadroxil	Ambroxol	Cefadroxil	Ambroxol	Cefadroxil	Ambroxol
Method-1 HPLC Method	10	6	0.72	0.80	1.55	0.59
	15	9	0.97	0.79	0.96	0.56
	20	12	1.03	0.78	0.89	1.11
Method-2 HPTLC Method	0.9	0.6	1.6	0.68	1.643	1.15
	1.2	0.8	1.10	1.77	1.264	1.77
	1.5	1.0	1.472	1.56	1.677	1.43

[a] n: No of times analysis repeated

The plot of peak area response against concentration is linear over the concentration range of 5-25 µg/ml and 3-15 µg/ml for cefadroxil monohydrate and Ambroxol hydrochloriderespectively. The precision of the method was established by carrying out the analysis of the analyte (n=6) using the proposed method. The low value of standard deviation showed that the method was precise (**Table 1**). Intraday (n=3) and interday (n=5) precision was carried out to and % RSD was found <2 ensuring repeatability of procedure (**Table 2**).

Table-3 Results for recovery study

% drug added	Amount of drug added		Mean Area (n=3)		Amount Found		% Recovery	
	Cefadroxil	Ambroxol	Cefadroxil	Ambroxol	Cefadroxil	Ambroxol	Cefadroxil	Ambroxol
Method-1 HPLC Method								
80	8	8	268607	252260	8.07	7.97	100.9	99.73
100	10	10	314526	309155	9.95	9.81	99.5	98.17
120	12	12	358901	378963	11.7	12.07	98.16	100.6
Method-2 HPTLC Method								
80	0.8	0.8	3008.07	3455.39	0.81	0.79	101	99.75
100	1.0	1.0	3707.07	4265.46	0.999	0.989	99.93	98.9
120	1.2	1.2	4462.22	5108.04	1.2	1.198	100	99.83

[a] n No of times analysis repeated

To ensure the reliability and accuracy of the method recovery studies were carried out at three different levels (80%, 100%, and 120%). The results of recovery studies were presented in **Table 3**. Robustness of the method was determined by small deliberate changes in flow rate, mobile phase ratio and pH. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was robust. The results of robustness were presented in **Table 4**. During assay study, there was no change in the content of drug due to presence excipient, which reveals that the method is specific [17].

Table-4 Results for Robustness Study

Drug Used	%RSD (n=3) for Method-1 HPLC Method						%RSD (n=3) for Method-2 HPTLC Method	
	Flow Rate (1 ml/min)		pH (7)		Ratio Of Mobile Phase (50:50v/v)		Time (Spotting to development)	Time (Development to Scanning)
	+0.5%	-0.5%	+0.1	-0.1	+1%	-1%	+30min	+30min
Cefadroxil	1.08	0.43	0.75	0.49	1.0	0.9	1.30	0.66
Ambroxol	1.06	0.63	0.70	0.71	1.09	0.8	1.14	1.78

[a] n No of times analysis repeated

HPTLC Method (Method-2)

It is well-established fact that chromatographic techniques are more specific than other analytical methods. The Rf value of cefadroxil monohydrate was found to be in the range of 0.40-0.55 and that of Ambroxol hydrochloride 0.85-0.9. Spectrum of all tracks was recorded between 200-400nm wavelengths using deuterium lamp. A typical spectrum of cefadroxil monohydrate and Ambroxol hydrochloride are depicted in **Fig.2**.Caliberation curve was constructed by plotting area of respective drug against concentration in µg/ml. A linear relationship was observed for cefadroxil monohydrate and Ambroxol hydrochloride in Concentration range of 0.3-1.8 µg/ml and 0.2-1.2 µg/ml respectively (**Table 1**).

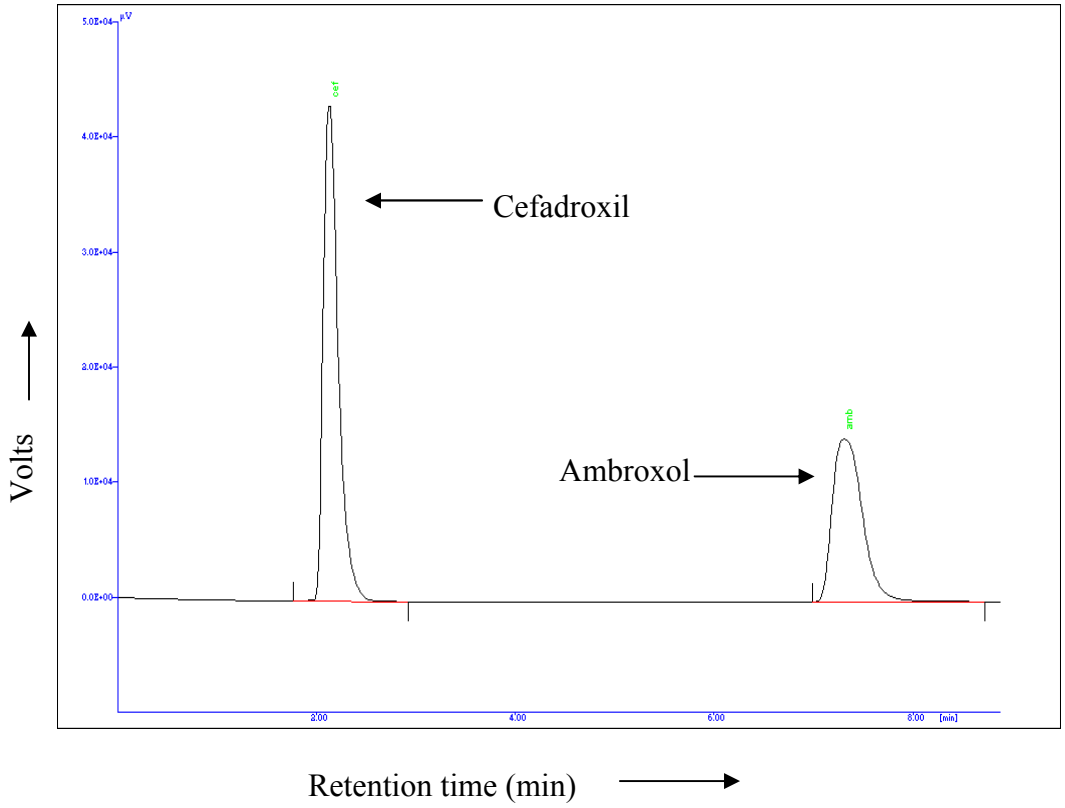
Table-5 Results of ANOVA Analysis

Source of Variation	Degree of freedom	Sum of square	Mean square	F Value
Cefadroxil				
Treatments (between columns)	3	3.42	1.14	
Residual (within columns)	16	7.67	0.4794	2.378
Total	19	11.09	1.6194	
Ambroxol				
Treatments (between columns)	3	6.263	2.088	
Residual (within columns)	16	13.489	0.8431	2.476
Total	19	19.742	2.9311	

The low value of standard deviation showed that the method was precise (**Table 1**). Intraday (n=3) and interday (n=3) precision was carried out and % RSD was found <2, ensuring repeatability of procedure (**Table 2**).

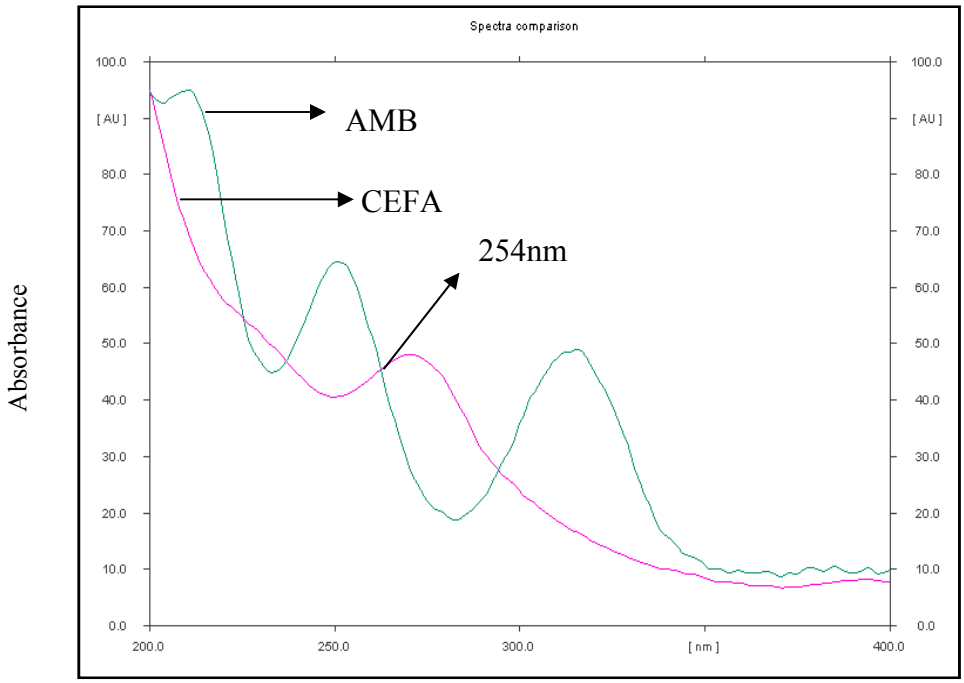
Fig.1. Resolution Study for Cefadroxil monohydrate and Ambroxol hydrochloride

[Rt value of cefadroxil monohydrate : 2.0-2.5 min. and that of Ambroxol hydrochloride: 7.5-8.0 min.]



The result of recovery analysis for cefadroxil monohydrate and Ambroxol hydrochloride are tabulated in **Table 3**. From the result it is revealed that there is good correlation between amount of standard added and amount of drug found at all concentration level.

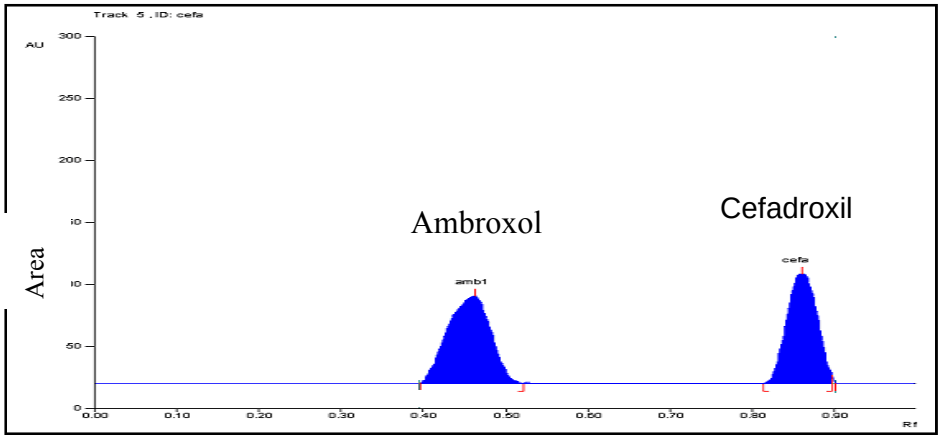
Fig.2. UV Spectra for Cefadroxil monohydrate and Ambroxol Hydrochloride after densitometric Analysis



Robustness of the method was determined by small but deliberate changes in time from spotting to development and development to scanning. No change in the content of drug was observed which showed that the method was robust (**Table 4**). There was no change in the content of drug due to presence excipient, which reveals that the method is specific.

Fig.3. Peaks for Cefadroxil monohydrate and Ambroxol Hydrochloride

[Rf value of cefadroxil monohydrate : 0.40-0.55 and that of Ambroxol hydrochloride: 0.85-0.9]



Rf Value

Statistical Evaluation[21]:

The results of analysis of methods developed were compared with the reported UV spectrophotometric method by performing one-way ANOVA studies. Using software Graphpad Prism performed ANOVA studies. F Test value at confidence interval 95% was found to be less than table F value. Results of analysis of ANOVA studies are given in **Table 5**.

CONCLUSION

Cefadroxil monohydrate and Ambroxol hydrochloride was simultaneously determined in tablet matrix using two different analytical methods. The methods developed are simple, accurate, rapid, sensitive and specific. RP-HPLC and HPTLC may be recommended for routine and Quality control analysis of investigated drugs in two component pharmaceutical preparation. Comparing two methods, HPLC method found more precise, robust whereas HPTLC method found more sensitive. Comparative statistical evaluation reveals that observed F value is less than table value, the difference in results of analysis between the developed methods and reported method were not significant.

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REFERENCES

[1] Indian Pharmacopoeia 2007, Vol-1, p 868, 701.
[2] United States Pharmacopoeia 2007, p 1646.
[3] British pharmacopoeia 2002, p 99, 100, 341.
[4] J. Makchit, S. Upalee, C. Thongpoon et al, Determination of cefadroxil by sequential injection with spectrophotometric detector. Analytical sciences. 2006; 22: 591-597.
[5] M. V. Dhoka, S. J. Sandage, S. C. Dumbre, Simultaneous estimation of cefadroxil and ambroxol in tablet dosage form. Indian drugs. 2010; 47(5): 66-68.
[6] V. M. Shinde, C. V. Shabadi, Simultaneous determination of cefadroxil and cephalexin from capsules by reverse phase HPLC. Indian drugs, 1997: 399-402.
[7] C. V. Shabadi, B. A. Shelar, A. R. Shelar, Simultaneous determination of cefadroxil and cephalexin in pharmaceutical preparation by quantitative TLC. Indian drugs, 1998: 766-770.
[8] B. S. Kuchekar, G. S. Shinde, I. T. Naikawadi et al, Spectrophotometric estimation of ambroxol hydrochloride in tablets, Indian J Pharm Sci., 2003: 193-195.
[9] K. M. Kothekar, B. Jaykar, A. P. Khandhar, R. K. Mishra, Quantitative determination of levofloxacin and ambroxol hydrochloride in pharmaceutical dosage form by reverse phase HPLC. Eura J Anal Chem. 2007; 2(1): 21-26.
[10] J. E. Koundourellis, E. T. Malliou, T. A. Broussali, HPLC determination of ambroxol on the presence of different preservatives in the pharmaceutical formulations. J Pharma. Biomed. Anal. 2000; 23: 469-475.
[11] M. Nobilis, J. Pastera, D. Svoboda, J. Kvtina, K. Macek, High-performance liquid chromatographic determination of ambroxol in human plasma. J Chromatogr. 1992; 581(2): 251.
[12] N.M. Bhatia, S.K. Ganbavale, M.S. Bhatia et al. RP-HPLC and spectrophotometric estimation of ambroxol hydrochloride and cetirizine hydrochloride in combined dosage form. Indian J Pharma. Sci. 2008; 70(5): 603-608.
[13] T. P. Ruiz, C. M. Lzano, A. Sanz, E. Bravo, Determination of bromhexin and ambroxol in pharmaceutical dosage forms, urine and blood serum. J Chromatogr B. 1997; 692: 199-205.
[14] Z. Dincer, H. Basan, N. G. Goger, Quantitative determination of ambroxol in tablets by derivative UV spectrophotometric method and HPLC. J Parma. Biomed. Anal. 2003; 31(5): 867-872.

[15] K. Laxmi, V Niraimathi, HPTLC determination of ceterizine and ambroxol in tablet. Indian drugs. 2003, Vol. 40(4): 227-230.
[16] P. N. S. Pai, N. Lalitha, G. K. Rao, B. Balakrishna, Determination of ambroxol hydrochloride using dithiocarbamic acid colorimetric method. Indian J Pharma. Sci. 2006; 68(4): 501-502.
[17] ICH Harmonised- Tripartite Guideline (2005) Validation of analytical procedures text and methodology Q2 (R1).
[18] P. R. Brown, Advances in Chromatography Vol.41, Marcel Dekker, New York, 2001, p. 1-3.
[19] P. D. Sethi. Quantitative analysis of pharmaceutical formulations. In: HPTLC – High performance thin layer chromatography, 1st ed. Mumbai: CBS publishers and distributors, 1996, p 3-5, 10, 18-20, 24, 27-28.
[20] E. Stahl. Thin Layer chromatography- Apparatus and general techniques in TLC. In: Thin Layer Chromatography- A laboratory handbook, 2nd ed. Heidelberg: Springer International edition, 2005. p. 52, 59, 63, 64.
[21] S. C. Gupta, V. K. Kapoor. Fundamentals of mathematical statistics. 9th ed. New Delhi: Sultan Chand and sons, 1994. p. 12.4-12.6.

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