EJAC
Eurasian Journal of Analytical Chemistry

Eurasian J. Anal. Chem. 5(3): 227-238, 2010

Application of HPLC and HPTLC for the Simultaneous Determination of Cefixime Trihydrate and Ambroxol Hydrochloride in Pharmaceutical Dosage Form

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Received: *13 August 2009****; Accepted:*** *17 Februray 2010*

Abstract

Two sensitive and reproducible methods are described for the quantitative determination for the simultaneous estimation of cefixime trihydrate and ambroxol hydrochloride. The first method was based on HPTLC followed by densitometric measurements of their spots at 254 nm. The separation was on HPTLC aluminium sheets of silica gel 60 F254 using acetonitrile: methanol: triethylamine (8.2:1:0.8, v/v/v) as mobile phase. The linear regression analysis was used for the regression line in the range of 200 - 1000 ng spot^{-1} for cefixime and ambroxol, respectively. This system was found to give compact spots for cefixime and ambroxol, after development. The second method was based on HPLC separation of the two drugs on the column [C18 (5 µ, 25 cm×4.6 mm, i.d.)] at ambient temperature using a mobile phase consisting of acetonitrile: methanol (50:50, v/v). Quantitation was achieved with UV detection at 254 nm based on peak area with linear calibration curves at concentration ranges 4 - 18 and 4 - 28 µg mL^{-1} for cefixime and ambroxol, respectively. Both methods have been successively applied to pharmaceutical formulation. No chromatographic interference from the tablet excipients was found. Both methods were validated in terms of precision, robustness, recovery and limits of detection and quantitation.

Keywords:

Cefixime; ambroxol; HPTLC; HPLC; method validation

1. Introduction

Cefixime (CFX) ((6*R*, 7*R*)-7-[(*Z*)-2-(2-amino-4-thiazolyl)-2-(carboxy-methoxyimino) acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo-[4,2,0]-oct-2-ene-2-carboxylic acid), is an orally absorbed third generation cephalosporin antibiotic. It has a broad antibacterial spectrum against various gram-positive bacteria and gram-negative bacteria, including Haemophilus influenzae, Neisseria gonorrhoeae, Escherichia coli, and Klebsiella pneumoniae resistant to ampicillin, cephalexin, cefaclor, and trimethoprim- sulfamethoxazole. Ambroxol, (AMB) *trans*-4-(2-amino-3, 5-dibromobenzylamino) cyclohexanol hydrochloride is a compound with potent mucolytic activity, for which it is used as an expectorant and bronchosecretolytic in therapeutics [1-5]. The structures of drugs are shown in (Fig.1). Literature survey revealed many chromatographic methods for determination of Cefixime alone or in combinations with other drugs from pharmaceutical formulations and biological fluids [6-10]. Several chromatographic methods have also been reported for determination of Ambroxol from

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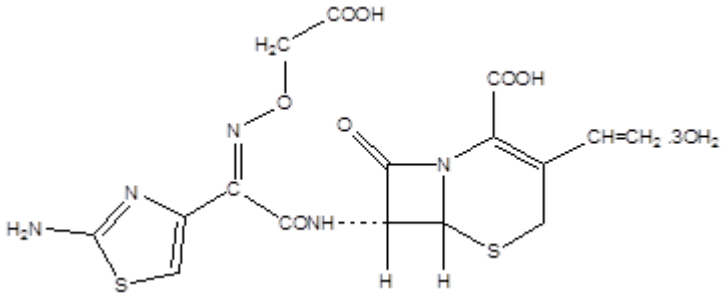
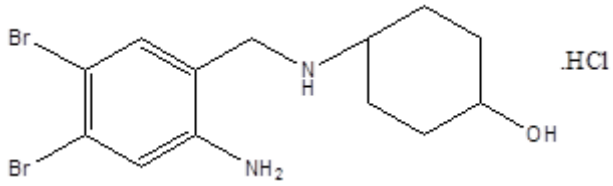
ISSN: 1306-3057,

pharmaceutical formulations and biological fluids [11-17]. But none of these methods demonstrate the simultaneous determination of cefixime trihydrate and ambroxol hydrochloride in tablet dosage form. Aim of present work was to develop simple, economical, rapid, precise and accurate method for simultaneous determination of binary drug formulation using HPLC and HPTLC.



Cefixime trihydrate



Ambroxol hydrochloride

Fig.1 Structures of analytes to be analyzed

2. Experimental

2.1. Materials

Pharmaceutical grade of cefixime trihydrate was kindly supplied by Macleod's pharmaceuticals (Daman, India) and ambroxol hydrochloride was kindly supplied as a gift sample by Glenmark pharmaceuticals Ltd. (Nashik, India) used without further purification and certified to contain. All chemicals and reagents used were of HPLC grade and were purchased from Merck Chemicals, India.

2.2. Instrumentation and chromatographic conditions

2.2.1. For TLC densitometry

The samples were spotted in the form of bands of width 8mm with a Camag 100 µl sample (Hamilton, Bonaduz, Switzerland) syringe on precoated silica gel aluminium Plate 60 F-254 (20 cm×10 cm) with 250 µm thickness; E. Merck, Darmstadt, Germany, supplied by Anchrom Technologists, (Mumbai) using a Camag Linomat V (Switzerland). The plates were prewashed by methanol and activated at 110 ^{o}C for 5 min prior to chromatography. A constant application rate of 0.1 µL s^{-1} was employed and space between two bands was 10 mm. The slit dimension was kept at 6 mm×0.45 mm and 10 mm s^{-1} scanning speed was employed. The monochromator bandwidth was set at 20 nm with K 320 cut off filter, each track was scanned thrice and baseline correction was used. The mobile phase consisted of acetonitrile–methanol–triethylamine (8.2:1:0.8, v/v/v) and 20 mL of mobile phase was used per chromatography. Linear ascending development was carried out in 20 cm×10 cm twin trough glass chamber (Camag, Muttenz, Switzerland). Dimensions: length×width×height = 12 cm×4.7 cm×12.5 cm. It was saturated (lined on the two bigger sides with filter paper that had been soaked thoroughly with the mobile phase) and the chromatoplate development was

carried out in dark with the mobile phase. The optimized chamber saturation time for mobile phase was 20 min at room temperature (25 ^{o}C±2) at relative humidity of 60%±5. The length of chromatogram run was 7 cm and approximately 10 min. Subsequent to the development, TLC plates were dried in a current of air with the help of an air dryer in wooden chamber with adequate ventilation. The flow of air in the laboratory was maintained unidirectional (laminar flow, towards exhaust). Densitometric scanning was performed on Camag TLC scanner III in the reflectance-absorbance mode at 254 nm for all measurements and operated by WIN CATS software (V 1.4.2, Camag). The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. Concentrations of the compound chromatographed were determined from the intensity of diffusely reflected light. Evaluation was via peak areas with linear regression.

2.2.2. For HPLC method

The HPLC system consisted of a pump (model KNAUER HPLC, smartline HPLC pump) with injecting facility programmed at 20 µl capacity per injection was used. The detector consisted of a UV–vis (UV 2600) model operated at a wavelength of 254 nm. The software used was chromgate version 1.5. The columns used were C-18 (250mm×4.6 mm, 5.0 µ). Different mobile phases were tested in order to find the best conditions for separating both the drugs simultaneously. The optimal composition of the mobile phase was determined to be acetonitrile: methanol: triethylamine (50:50:0.1, v/v/v) pH 3.0. The flow rate was set to 1.0 mL min^{-1} and UV detection was carried out at 254 nm (Fig.2).

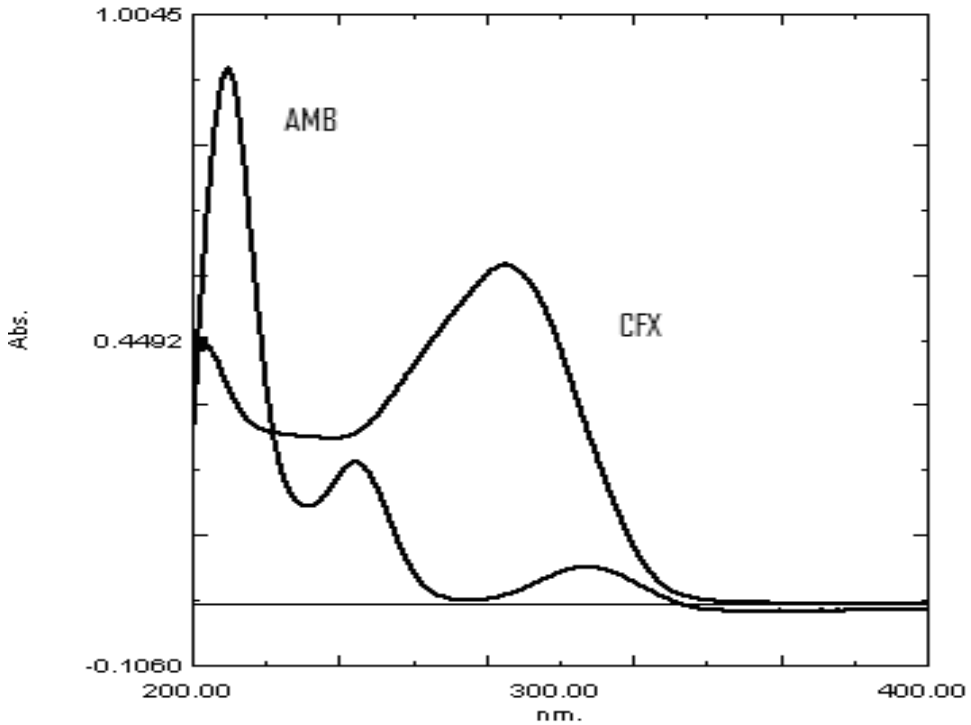


Fig.2 Overlain spectra of CFX and AMB (10 µg mL^{-1} for CFX and 6 µg mL^{-1} for AMB)

2.3. Standard solutions and calibration graphs

Stock standard solution was prepared by dissolving 100 mg of cefixime and ambroxol in 10 mL methanol. The stock solution was protected from direct light by using amber colored volumetric flasks and keeping the solution at room temperature.

2.3.1. For HPTLC–densitometric method

The standard solutions were prepared by dilution of the stock solution with methanol to reach a concentration range 100 μg mL^{-1} for cefixime and ambroxol respectively. These standard solutions were spotted on the TLC plate to obtain final concentration 200 - 1000 ng $spot^{-1}$ for cefixime and ambroxol respectively. Each concentration was spotted three times on the TLC plate. The plate was developed on previously described mobile phase. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

2.3.2. For HPLC method

The standard solutions were prepared by dilution of the stock solution with mobile phase to reach a concentration range 4-18 μg mL^{-1} and 4-28 μg mL^{-1} for cefixime and ambroxol, respectively. Triplicate 20 μL injections were made six times for each concentration for cefixime and ambroxol, respectively and chromatographed under the conditions described above. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

2.4. Sample preparation

To determine the content of cefixime and ambroxol simultaneously in conventional tablets (label claim: 100 mg cefixime and 30 mg ambroxol per tablet, combination tablet containing both analytes), the twenty tablets were weighed, their mean weight determined and they were finely powdered and powder equivalent to 100mg cefixime and 30 mg ambroxol was weighed. Then equivalent weight of the drug was transferred into a 100 mL volumetric flask containing 50 mL methanol, sonicated for 30 min and diluted to 100 mL with methanol. For HPTLC same dilutions of tablets were made using methanol.

2.4.1. For HPTLC–densitometric method

Different microlitres (2, 3 and 4 μL) of sample solution were applied three times to the HPTLC plate to give concentration 200, 300, 400 ng $spot^{-1}$ for cefixime and ambroxol, respectively. The plate was developed in the previously described chromatographic conditions. The peak area of the spot was measured at 254 nm for cefixime and ambroxol, respectively and their concentration in the samples were determined using multilevel calibration developed on the same plate under the same conditions using linear regression equation.

2.4.2. For HPLC method

A 20μl volume of sample solution (10 and 3 μg mL^{-1} of cefixime and ambroxol, respectively) was injected into HPLC, six times, under the conditions described above. The peak area were measured at 254 nm for cefixime and ambroxol, respectively and their concentrations in the samples were determined using multilevel calibration developed on the same HPLC system under the same conditions using linear regression equation.

2.5. Method validation [18-31]

Both methods were validated in compliance with ICH guidelines. The following parameters were validated.

2.5.1. Precision

Precision of the method was determined with the product. An amount of the product powder equivalent to 100% of the label claim of cefixime and ambroxol was accurately

weighed and assayed. System repeatability was determined by six replicate applications and six times measurement of a sample solution at the analytical concentration. The repeatability of sample application and measurement of peak area for active compound was expressed in terms of relative standard deviation (%R.S.D.) Method repeatability was obtained from R.S.D. value by repeating the assay six times in same day for intra-day precision. Intermediate precision was assessed by the assay of two, six sample sets on different days (inter-day precision). The intraday and inter-day variation for determination of cefixime and ambroxol was carried out at three different concentration levels 200, 400, 600 ng $spot^{-1}$ for HPTLC. For HPLC 8, 10, 12 µg mL^{-1} of cefixime and 12, 16, 20 µg mL^{-1} of ambroxol.

2.5.2. Limit of detection and limit of quantitation

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy.

2.5.2.1. For HPTLC–densitometry and HPLC method

The detection limit (LOD) for the proposed methods were calculated using the following equation

$$LOD = 3.3s/k$$

Where s is the standard deviation of replicate determination values under the same conditions as for sample analysis in the absence of the analyte and k is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, the detection limits obtained for the absorbance's were calculated and listed in Table 1. The limits of quantitation, LOQ, defined as

$$LOQ = 10s/k$$

Table 1. Linearity regression data for calibration curves

Parameter	TLC densitometry		HPLC	
	Cefixime	Ambroxol	cefixime	Ambroxol
Linearity range	200-1000 ng $spot^{-1}$	200-1000 ng $spot^{-1}$	4-18 µg mL^{-1}	4-24 µg mL^{-1}
Correlation coefficient	0.9991	0.9999	0.997	0.998
Slope	4.549	2.978	53883	41954
Intercept	237.9	217.2	41372	15289

2.5.3. Specificity

2.5.3.1. For HPTLC–densitometric method

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for cefixime and ambroxol, in sample was confirmed by comparing the *R*f and spectra of the spot with that of standard. The peak purity of cefixime and ambroxol, was assessed by comparing the spectra at three different levels, i.e., peak start (*S*), peak apex (*M*) and peak end (*E*) positions of the spot (Fig.3).

2.5.3.2. For HPLC method

The specificity of the HPLC method was determined by the complete separation of cefixime and ambroxol, along with other parameters like retention time (*tr*), capacity factor (*k*), tailing or asymmetrical factor (*T*), etc (Fig.4).

2.5.4. Recovery studies

For both methods recovery studies was carried out by applying the method to drug sample to which known amount of cefixime and ambroxol corresponding to 50, 100 and 150% of label claim had been added (standard addition method). At each level of the amount six determinations were performed and the results obtained were compared with expected results.

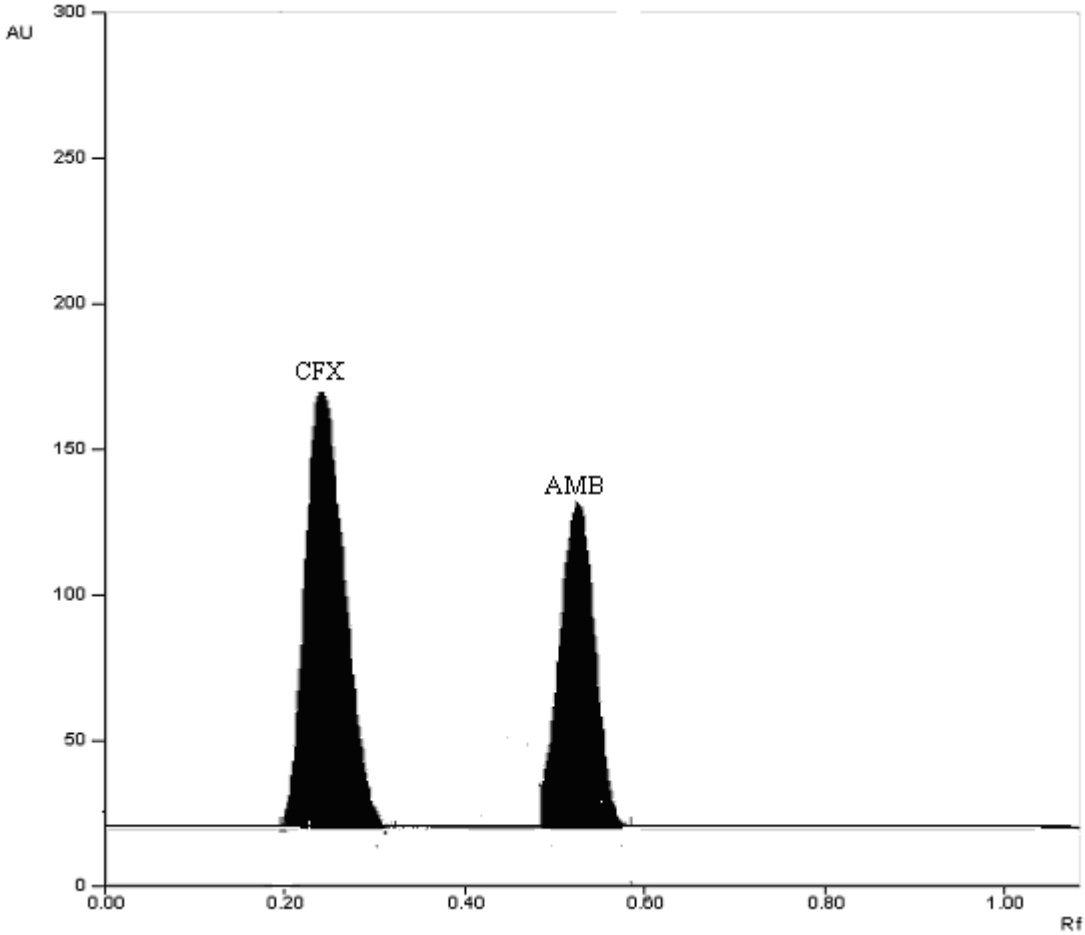


Fig.3 Typical HPTLC Chromatogram of CFX and AMB Standard Drug Solution measured at 254 nm, mobile phase acetonitrile: methanol: triethylamine (8.2: 1: 0.8 v/v/v)

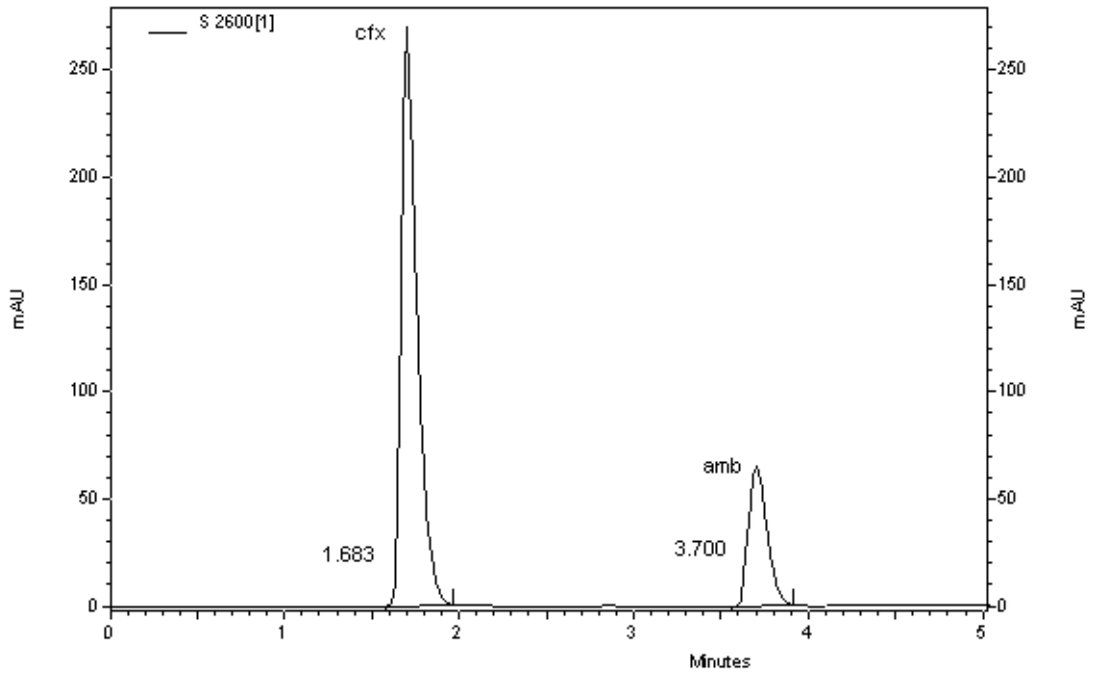


Fig.4 Chromatogram of standard cefixime (10 μg mL^{-1}): Rt: 1.6, ambroxol (6 μg mL^{-1}): Rt: 3.7, measured at 254 nm, Mobile phase: Acetonitrile: methanol: triethylamine (50:50:0.1v/v/v), pH 3.0

3. Result and discussion

3.1. Optimization of procedures

3.1.1. Optimization of HPTLC–densitometry method

Initially toluene and methanol in the ratio of 5:5 (v/v) was tried for both drugs simultaneously. The spots were not developed properly and dragging was observed. Then toluene and methanol in the ratio of 3:7 (v/v) was tried. The developed Spots were diffused and *R*f was near to solvent front. Then the reverse ratio of same mobile phase was tried. The distance travelled by developed spots was less and dragging was observed. To the above mobile phase carbon tetrachloride and acetonitrile in different ratios were added but the developed spots lack compactness and were less persistent. Also the *R*f values of cefixime and ambroxol were not satisfactory because of less resolution between them. Ultimately mobile phase consisting of acetonitrile: methanol: triethylamine (8.2:1:0.8, v/v/v) gave good resolution. Both the peaks were symmetrical in nature and no tailing was observed when plates were scanned at 254 nm. Well-defined spots were obtained when plate was activated at 110^0C for 5 min. and the chamber was saturated with the mobile phase for 20 min at room temperature

3.1.2. Optimization of HPLC method

Both the drugs are freely soluble in Methanol and acetonitrile so the mixture of methanol and acetonitrile was selected as mobile phase. Initially, acetonitrile and methanol (ratio of 55:45 v/v) was tried for both the drugs, simultaneously. The peaks were not separated properly. Then, the proportion of methanol in the mobile phase was decreased so as to give a mobile phase consisting of methanol 50 % v/v, and was attempted. In this mobile phase, the compounds were separated properly, but the peak for CFX exhibited splitting, and peak for AMB showed large tailing, which were successfully overcome by an addition of

small amount of triethylamine (0.1 %) and pH of the mobile phase is 3.0 This mobile phase (Acetonitrile: methanol: triethylamine (50:50:0.1v/v/v), pH 3.0) was finalized and selected for the analysis and this ratio was selected for validation purpose.

3.2. Linearity

Cefixime and ambroxol showed good correlation coefficient in concentration range of 200 - 1000 ng spot^{-1} (r = 0.9991 and r = 0.9999) for HPTLC where as cefixime in the concentration range of 4 - 14 µg mL^{-1} and ambroxol 4-24 µg mL^{-1} (r =0.997 and r= 0.998) for HPLC, respectively. Linearity was evaluated by determining five standard working solutions containing 200 - 1000 ng spot^{-1}, of Cefixime and ambroxol in triplicate for HPTLC and 4-14 µg mL^{-1} and 4-24 µg mL^{-1} for cefixime and ambroxol, respectively for HPLC. For both methods the linearity of calibration graphs and adherence of the system to Beer's law was validated by high value of correlation coefficient and the S.D. for intercept value was less than 2%. No significant difference was observed in the slopes of standard curves. The results of the linearity studies are as shown in Table 1.

3.3. Precision

3.3.1. For HPTLC–densitometric method

The repeatability of sample application and measurement of peak area were expressed in terms of %R.S.D. and were found to be 0.36, 0.9, 1.8, and 1.7, 1.3, 1.3 for cefixime and ambroxol for intra-day variation, respectively and 0.65, 1.9, 1.3 and 1.7, 2.0, 1.0 for cefixime and ambroxol for inter-day variation. The % R.S.D. values depicted in Table 2 shows that proposed method provides acceptable intra-day and inter-day variation of cefixime and ambroxol.

3.3.2. For HPLC method

The within-run precision and between-run precision of the proposed HPLC method were determined by assaying the tablets in six times per day for consecutive six days and expressed as %R.S.D. were found to be 0.60 and 1.4 for cefixime and ambroxol, respectively. The intra-day and inter-day precision has been depicted in Table 2.

Table 2. Intra and inter- day precision of cefixime and ambroxol

Drug	TLC densitometry		HPLC	
	Intra-day precision (n=3) *% R.S.D.*	Inter-day precision (n=3) *% R.S.D.*	Intra-day precision (n=3) *% R.S.D.*	Inter-day precision (n=3) *% R.S.D.*
Cefixime	0.36	0.65	1.6	0.60
	0.9	1.9	1.1	1.2
	1.8	1.3	0.9	1.1
Ambroxol	1.7	1.7	1.0	1.4
	1.3	2.0	1.7	1.5
	1.3	1.0	1.2	1.4

3.4. LOD and LOQ

3.4.1. For HPTLC–densitometric method

The LOD and LOQ were found to be 19, 58 ng spot^{-1} and 42, 128 ng spot^{-1}, respectively for cefixime and ambroxol.

3.4.2. For HPLC method

The LOD and LOQ were found to be 0.615, 1.86 µg mL^{-1} and 0.78, 2.365 µg mL^{-1}, respectively for cefixime and ambroxol.

3.5. Recovery studies

Both the proposed methods when used for extraction and subsequent estimation of cefixime and ambroxol from pharmaceutical dosage form after spiking with additional drug afforded recovery of 98 - 102% and mean recovery for cefixime and ambroxol from the marketed formulation are listed in Table 3.

Table 3. Standard addition technique for determination of cefixime and ambroxol by TLC densitometry and HPLC

Drug	TLC densitometry				HPLC			
	Excess drug added to the analyte (%)	Initial Conc.	Recovery (%)	% R.S.D.	Excess drug added to the analyte (%)	Initial conc.	Recovery (%)	% R.S.D
Cefixime	50	400	99.36	0.58	50	10	100.26	0.32
	100	400	99.50	0.32	100	10	100.5	1.99
	150	400	103.15	1.9	150	10	101.1	1.97
Ambroxol	50	400	99.43	0.16	50	3	100.1	1.3
	100	400	100.29	0.11	100	3	100.5	1.8
	150	400	99.62	0.94	150	3	98.69	0.86

3.6. Specificity

3.6.1. For HPTLC–densitometric method

The peak purity of cefixime and ambroxol was assessed by comparing their respective spectra at peak start, peak apex and peak end positions of the spot, i.e., r (S, M) = 0.9995, 0.9997 and r (M, E) = 0.9992, 0.9996. Good correlation (r = 0.9998 and r = 0.9997) was also obtained between standard and sample spectra of cefixime and ambroxol respectively.

3.6.2. For HPLC method

The specificity of the HPLC method is illustrated in Fig. 4 where complete separation of cefixime and ambroxol was noticed in presence of tablet excipients. The average retention time ±standard deviation for cefixime and ambroxol were found to be 1.6±0.05 and 3.7±0.07 min, respectively, for six replicates. The peaks obtained were sharp and have clear baseline separation.

3.7. Analysis of the marketed formulation

3.7.1. For HPTLC–densitometric method

The spots at *R*f 0.25 (for cefixime) and 0.54 (for ambroxol) were observed in the densitogram of the drug samples extracted from tablets. There was no interference from the excipients commonly present in the tablets. The drug content was found to be 100.94%±0.078 (%R.S.D. of 0.78) and 98.59%±0.140 (%R.S.D. of 0.99) for cefixime and ambroxol, respectively. It may therefore be inferred that degradation of cefixime and ambroxol had not occurred in the marketed formulations that were analyzed by this method as shown in Table 4. The low %R.S.D. value indicated the suitability of this method for routine analysis.

Table 4. Applicability of the proposed methods for the determination of cefixime and ambroxol in commercial tablets (n=6)

Parameters	HPTLC densitometry		HPLC	
	Cefixime (mg)	Ambroxol (mg)	Cefixime (mg)	Ambroxol (mg)
Label claim (mg)	100	30	100	30
Drug content (%) ± S.D.	100.94± 0.078	98.59± 0.140	101.3±0.03	100.6 ±0.017
% R.S.D.	0.78	0.99	0.33	0.57

3.7.2. For HPLC method

The peaks at *t*r 1.683 (for cefixime) and 3.700 min (for ambroxol) were observed in the chromatogram of the drug samples extracted from tablets. Experimental results of the amount of cefixime and ambroxol in tablets, expressed as percentage of label claim were in good agreement with the label claims, thereby suggesting that there is no interference from any excipients, which are normally present in tablets. The drug content was found to be 101.30%±0.03 (%R.S.D. of 0.33) and 100.60%±0.017 (%R.S.D. of 0.57) for cefixime and ambroxol, respectively as shown in Table 4.

4. Conclusion

The proposed HPTLC and HPLC methods provide simple, accurate and reproducible quantitative analysis for simultaneous determination of cefixime and ambroxol in tablets. Both the methods were validated as per ICH guidelines. Six real samples of tablets were determined simultaneously by HPTLC and HPLC methods and the results were correlated. Statistical tests indicate that the proposed HPTLC and HPLC methods reduce the duration of analysis and appear to be equally suitable for routine determination of cefixime and ambroxol simultaneously in pharmaceutical formulation.

Acknowledgement

The authors express their gratitude to Macleod's Pharmaceuticals Ltd. (Daman, India) and Glenmark Pharmaceuticals Ltd. (Nashik, India) for the gift sample of pure cefixime trihydrate and ambroxol hydrochloride. Thanks are also extended to Mr.V.M. Aurangabadkar, Principal, and Dr. S.B. Kasture, HOD Pharmacology Dept., MGV'S college of pharmacy, Nashik, India for providing necessary facilities and constant support.

References

1. British Pharmacopoeia (2003) Her Majesty's Stationery Office, London, vol. I p 104, 371.
2. The Merck Index (2006) An Encyclopedia of Chemicals, Drugs and Biologicals 14th Edition, Merck, USA, p 386, 1974.
3. Sweetman S (2002) Martindale: The Complete Drug Reference 33rd Edition, Pharmaceutical Press, p 166, 1703.
4. United States Pharmacopeia, The National Formulary, (USP/NF) (2004) United States Pharmacopeial Convention, Rockville, MD, p 359.
5. Brunton L, Lazo J, Parker K (2005) Goodman & Gilman's The Pharmacological Basis of Therapeutics, Eleventh Edition McGraw Hill New York p.1807.
6. Xiaoyan F M, Dafang Y Z (2005) Sensitive liquid chromatography–tandem mass spectrometry method for the determination of cefixime in human plasma: Application to a pharmacokinetic study. Journal of Chromatography B, 819:277.
7. Jovanovic S E, Agbaba D, Stakic D Z, Vladimirov S (1998) HPTLC determination of ceftriaxone, cefixime and cefotaxime in dosage forms. Journal of Pharmaceutical and Biomedical Analysis 18:893.
8. Al-Momani I F (2001) Spectrophotometric determination of selected cephalosporins in drug formulations using flow injection analysis. J Pharma Biomed Anal 25:751-757.
9. Quintiliani R (1996) Cefixime: A Pharmacoeconomic Perspective. Current Therapeutic Research 57:12.
10. Fattah A, El-Walily M, Gazy A A, Belal S F, Khamis E F (2000) Quantitative determination of some thiazole cephalosporins through complexation with palladium (II) chloride. J Pharm Biomed Anal. 22:385.
11. Hang T, Zhang M, Song M, Shen J, Zhang Y (2007) Simultaneous determination and pharmacokinetic study of roxithromycin and ambroxol hydrochloride in human plasma by LC-MS/MS. Clinica Chimica Acta 382:20.
12. Heinanen M, Barbas C (2001) Validation of an HPLC method for the quantification of ambroxol hydrochloride and benzoic acid in a syrup as Pharmaceutical form stress test for stability evaluation. Journal of Pharmaceutical and Biomedical Analysis 24:1005.
13. Hadad G M, El-Gindy A, Mahmoud M M (2008) HPLC and chemometrics-assisted UV-spectroscopy methods for the simultaneous determination of ambroxol and doxycycline in capsule. Spectrochimica Acta Part A Molecular and biomolecular spectroscopy; 70:655.
14. Kim H, Yoo J Y, Han S B, Lee H J (2003) Determination of ambroxol in human plasma using LC-MS/ MS. Journal of Pharmaceutical and Biomedical Analysis 32:209.
15. Meiling Q, Wang P, Cong R, Yang J (2004) Simultaneous determination of roxithromycin and ambroxol hydrochloride in a new tablet formulation by liquid chromatography. Journal of Pharmaceutical and Biomedical Analysis 35:1287.
16. Dincer Z, Basan H, Goger N G (2003) Quantitative determination of ambroxol in tablets by derivative UV spectrophotometric method and HPLC. Journal of Pharmaceutical and Biomedical Analysis 31: 867.

17. Satınsky D, Lucia M L, Santos D, Sklenarov H, Solich P, Conceic M¸ Montenegro B S M, Araujo A N (2005) Sequential injection chromatographic determination of ambroxol hydrochloride and doxycycline in pharmaceutical preparations. Talanta 68:214.
18. ICH/CPMP guidelines Q2A (1994) Text on Validation of Analytical Procedures.
19. ICH/CPMP Guidelines Q2B (1996) Validation of Analytical Procedures-Methodology.
20. The United State Pharmacopoeia (USP NF) (2004) The Official Compendia of Standards, Asian Edition, p. 2622.
21. Chan C C, Lee Y C, Lam H, Zhang X M (2004) Analytical Method Validation and Instrument Performance Verification, John Wiley and Sons, INC., p 11.
22. Sethi P D (1996) HPTLC: Quantitative Analysis of Pharmaceutical Formulation, CBS Publications, New Delhi, p 162-65.
23. Sethi P D (1996) HPLC: Quantitative Analysis of Pharmaceutical Formulation, CBS Publications, New Delhi, p 1-28.
24. Heftman E (2004) Chromatography, Journal of Chromatography Library 69A, 6th (ed.), Elsevier, Amsterdam, p 254-60.
25. Christian G D (2004) Analytical Chemistry, 6th Edn, Wiley International; p. 604-620.
26. Mendham J, Denney R C, Barnes J D, Thomas M J K (2004) Vogel's Textbook of Qualitative Chemical Analysis, 6th Edn, Pearson Publications; p. 268.
27. Skoog D A, Holler F J, Timothy A, Nieman N W (1985) Principle of Instrumental Analysis, 3rd Edn., Stanford University, Saunders College Publications, London, p. 1-4.
28. Sethi P D (1997) Quantitative Analysis of Drugs in Pharmaceutical Formulations, 3rd Edn., CBS Publishers and Distributors, New Delhi, p. 50-64.
29. Svelha G. (1986) Wilson and Wislson's Comprehensive Analytical Chemistry, Analytical Visible and UV Spectroscopy, p.339-358.
30. Mellon M G (1952) Analytical Absorption Spectroscopy, Absorptiometry and Colorimetry, John Wiley and sons, New York; p. 20.
31. Skoog D A, Holler F J, Nieman T A (2005) Principles of Instrumental Analysis, 5th (ed.), Thomson Brook/ cole, p 761-65.