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Research Article

SIMULTANEOUS QUANTIFICATION OF SALBUTAMOL SULPHATE AND AMBROXOL HYDROCHLORIDE BY RP-HPLC AND HPTLC IN BULK DRUG AND DOSAGE FORM

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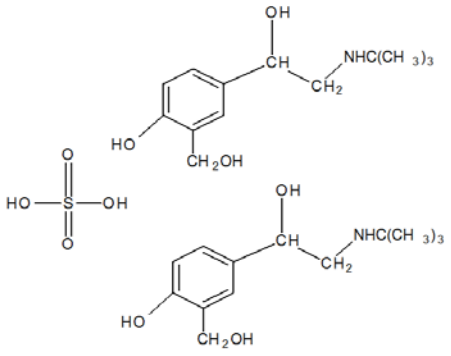
ABSTRACT

Present work describes a precise, accurate and reproducible Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) and High Performance Thin Layer Chromatographic (HPTLC) methods for simultaneous estimation of Salbutamol sulphate and Ambroxol hydrochloride. In the RP-HPLC method, the drugs were resolved using a mobile phase of acetonitrile: 50 mM disodium hydrogen phosphate buffer (containing 0.1% triethylamine, pH adjusted to 4.2 using orthophosphoric acid) (28:72 v/v) on an Inertsil, ODS-3V C18 (250 X 4.6 mm), 5µm column in isocratic mode. The retention time of salbutamol sulphate and ambroxol hydrochloride were 2.59 and 6.15 min, respectively. In the HPTLC method, the chromatograms were developed using a mobile phase of methanol: ethyl acetate: toluene: ammonia (4:1.5:5.6:1.0 v/v) on precoated plate of silica gel 60 F254 and quantified by densitometric absorbance mode at 231 nm. The R_f values of salbutamol sulphate and ambroxol hydrochloride were, 0.38 and 0.59 respectively. Recovery values of 98.09-102%, percentage relative standard deviation of <1.38 and correlation coefficient of 0.996–0.998 shows that the developed methods were accurate and precise. As per ICH guidelines the results of the analysis were validated in terms of specificity, limit of detection, limit of quantification, linearity, precision and accuracy and were found to be satisfactory. These methods can be employed for the routine analysis of tablets containing salbutamol sulphate and ambroxol hydrochloride.

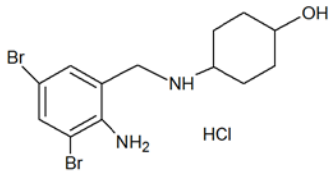
Keywords: Salbutamol sulphate, Ambroxol hydrochloride, RP-HPLC, HPTLC, Validation

INTRODUCTION

Salbutamol sulphate (SAL), bis [(1RS)-2-[(1, 1-dimethylethyl) amino]-1-[4-hydroxy-3-(hydroxymethyl) phenyl] ethanol] sulphate, is beta-adrenoceptor agonist used for the relief of broncho-spasm in conditions such as asthma and chronic obstructive pulmonary disease [1, 2]. Ambroxol hydrochloride (AMB), trans-4-((2-amino-3, 5-dibromobenzyl) amino) cyclohexanol hydrochloride. Ambroxol reduces bronchial hyper-reactivity and acts as a mucolytic and cough suppressant. Both SAL and AMB are official in Indian pharmacopoeia. Combination of SAL and AMB is used for the treatment of asthma and bronchitis [3, 4]. Literature survey reveals that salbutamol in combination with other drugs has been estimated by UV spectrophotometric methods [5-9], HPTLC [10], RP-HPLC [11], TLC method[12]. The methods reported for simultaneous determination of ambroxol in combination with other drugs are UV spectrophotometric methods [13-16], RP-HPLC [17-21], HPTLC [22] and LC-MS/MS [23]. No HPTLC method and only one HPLC method [24] have been found to be reported for the simultaneous estimation of salbutamol sulphate and ambroxol hydrochloride in combination. The aim of the work was to introduce a simple, accurate and reproducible isocratic RP-HPLC and HPTLC methods for simultaneous determination of SAL and AMB. The proposed methods were optimized and validated as per ICH guidelines [25].



Salbutamol sulphate



Ambroxol hydrochloride

Fig. 1: Chemical structures of Salbutamol sulphate and Ambroxol hydrochloride

MATERIALS AND METHODS

Chemicals and Reagents

SAL and AMB were obtained as gift samples from Gens Pharma International Pvt. Ltd., Pune and Elder Pharmaceuticals Ltd., Mumbai respectively. Disodium hydrogen phosphate, HPLC grade Water and Acetonitrile were procured from Merck Ltd, Mumbai, India. Orthophosphoric acid was purchased from Research Lab., Fine Industries, Mumbai. Methanol, ethyl acetate, toluene, ammonia all were of analytical grade (Merck Ltd., Mumbai). The commercial formulation of SAL and AMB is available in ratio of 1:15{Sal-Mucolite} tablet procured from local market.

Instrumentation

The HPLC system, Jasco PU-2080 Plus, with manual Rheodyne injector facility operates at 20 µl capacity per injection was used. The column used was Inertsil, C18 (250 X 4.6 mm), 5µm and the detector consisted of UV/VIS (Jasco UV 2075-Plus) operated at 231 nm. The data were acquired and processed using Borwin software version 1.5

HPTLC was performed in Camag HPTLC (Camag, Muttenz, Switzerland) system, equipped with linomat 5 sampler applicator, twin trough plate development chamber, and CAMAG TLC III scanner.

RP-HPLC method

Chromatographic Conditions

Optimizations of chromatographic condition were carried out using mobile phase, acetonitrile: 50 mM disodium hydrogen phosphate buffer, containing 0.1% triethylamine (28:72 v/v), pH 4.2 was adjusted using orthophosphoric acid. Prior to deliver into the system, mobile phase was filtered through 0.45 µ membrane filter and sonicate for 10 min. The samples were introduced by injector with a 20 µl sample loop. The analysis was carried out under isocratic conditions using flow rate 1 ml/min at 18^0C and chromatograms were recorded at 231 nm.

Preparation of standard stock solution

Weighed accurately 2 mg of SAL and 30 mg of AMB, transferred to a 10 ml volumetric flask, it was dissolved in 5 ml of mobile phase and sonicate for 10 min. Finally the volume was made up to mark with mobile phase.

Preparation of working standard solution

From the standard stock solution, 0.1ml solution was pipetted out in 10 ml volumetric flask and volume was made up to the mark with mobile phase.

Analysis of tablet formulation

Twenty tablets (sal-mucolite) were weighed, triturated, mixed thoroughly and average weight of tablet was calculated. Accurately weighed quantity of tablet powder equivalent to 2 mg of SAL was transferred to 10 ml volumetric flask, it was dissolved in 5 ml of mobile phase and sonicate for 10 min. The resultant solution was filtered through 0.45µ membrane filter, diluted to volume with mobile phase. 0.1 ml of resultant solution further diluted to 10 ml with mobile phase and injected to HPLC system (Table 1).

HPTLC method

Chromatographic conditions

The experiment was performed on a precoated plate of silica gel 60 F254, (10 cm × 10 cm with layer thickness of 0.2 mm) prewashed with methanol. Mobile phase comprising of methanol: ethyl acetate: toluene: ammonia (4:1.5:5.6:1.0 v/v). The developing solvent was run upto 80 mm in Camag chamber previously saturated with 10 ml of mobile phase for 20 min. 10 µl samples were applied as band at a distance of 8 mm from lower edge and the distance between two bands was 7 mm. The development was performed at 24°C and the average development time was 15 minutes. After development, the plate was air dried and scanned densitometrically at 231 nm with slit dimensions 6.00 x 0.30 mm, using CAMAG TLC scanner III.

Preparation of standard stock solution

Weighed accurately 2 mg of SAL and 30 mg of AMB, transferred to a 10 ml volumetric flask, add 5 ml of methanol and sonicate for 10 min. Finally the volume was made up to mark with methanol. Working standard solutions was prepared by serial dilution of stock solutions with methanol.

Analysis of tablet formulation

Accurately weighed quantity of tablet powder equivalent to 2 mg of SAL was transferred to 10 ml volumetric flask, add 5 ml of methanol and sonicate for 10 min. The resultant solution was filtered through 0.45µ membrane filter, diluted to volume with methanol (Table 1).

Table 1: Analysis data of Salbutamol Sulphate and Ambroxol Hydrochloride

Sample	Label claim	RP-HPLC		HPTLC	
		% estimated ± S.D. % RSD		% estimated ± S.D. % RSD	
SAL	2 mg	99.52 ± 1.7414	1.7497	98.97 ± 1.9086	1.9284
AMB	30 mg	98.64 ± 1.2545	1.2718	98.49 ± 0.9627	0.9774

SD -Standard deviation, RSD- Relative standard deviation

Validation

Limit of detection (LOD) and limit of quantification (LOQ)

The signal-to-noise ratio (*S/N*) method was adopted for the determination of limit of detection and limit of quantification. The limit of detection was estimated as three times the *S/N* ratio and the limit of quantification was estimated as ten times the *S/N* ratio.

Specificity

Specificity is the ability of a method to discriminate between the analyte of interest and other components that may present in the sample. The specificity of the method was evaluated to ensure separation of SAL and AMB and was demonstrated by assaying samples of SAL and AMB tablet.

Linearity

For RP-HPLC method, diffrent standard solutions were prepared by diluting standard stock solution with mobile phase in concentration 0.5, 1.0, 1.5, 2.0, 2.5, 3 µg/ml for SAL and 7.5, 15, 22.5, 30, 37.5, 45 µg/ml for AMB, injected and chromatogram were taken under standard chromatographic conditions.

For HPTLC the standard solutions were prepared by diluting standard stock solution with methanol in concentration range of 100, 120, 140, 160, 180, and 200 ng/spot for SAL and 1500, 1800, 2100, 2400, 2700 and 3000 ng/spot for AMB. The peak area was plotted against corresponding concentrations to obtain the calibration graph.

Precision

Precision of analytical methods were expressed in relative standard deviation (RSD) of a series of measurements. The intra-day and inter-day precisions of the proposed methods were determined by estimating the corresponding responses (i.e. three concentrations / three replicates each) of the sample solution on the same day and on three different days respectively.

Recovery

To check the accuracy of the proposed method, recovery studies were carried out by standard addition method. A known amount of standard SAL and AMB corresponding to 80, 100 and 120% of the label claim was added to preanalysed sample of tablet. The recovery studies were carried out in triplicate at each level.

RESULTS AND DISCUSSION

In RP-HPLC method, chromatographic conditions were optimized to obtain an adequate separation of eluted compounds. Mobile phase and flow rate selection was based on peak parameters such as tailing, theoretical plates, capacity factor etc. The system with acetonitrile: 50 mM disodium hydrogen phosphate buffer (containing 0.1% triethylamine, pH 4.2 adjusted by using orthophosphoric acid) (28:72 v/v) with 1 ml/min flow rate is quite robust. A typical chromatogram for SAL and AMB is shown in Fig 2.

The optimum wavelength for detection was 231 nm at which better detector response for drugs was obtained. The average retention times for SAL and AMB was found to be 2.59 and 6.15 min with

linear range of 0.5-3 µg/ml (r^2 = 0.998) and 7.5-45 µg/ml (r^2 = 0.998) respectively (Table 2). The method was found to be precise after quantification of six replicates of SAL and AMB with RSD less than 2.0% (Table 3). The recovery values were found to be 98.48-100.82 % (Table 4).

In HPTLC method, chromatographic separation of the drugs was performed on aluminium plates precoated with silica gel 60 F254, with methanol: ethyl acetate: toluene: ammonia (4:1.5:5.6:1.0 v/v) as mobile phase. Chromatographic evaluation of the separated bands was performed at 231 nm. SAL and AMB were resolved satisfactorily with R_f values 0.38 and 0.59 respectively (Fig.3).

The method showed good linear response in concentration range of 100-200 ng /spot (r^2 =0.998) and 1500 -3000 ng /spot (r^2 = 0.996) for SAL and AMB respectively (Table 2).

The method was found to be precise after quantification of six replicates of SAL and AMB and RSD was found to be less than 2.0% (Table 3). The recovery values were 98.09-102 % with S.D. of <1.28 (Table 4).

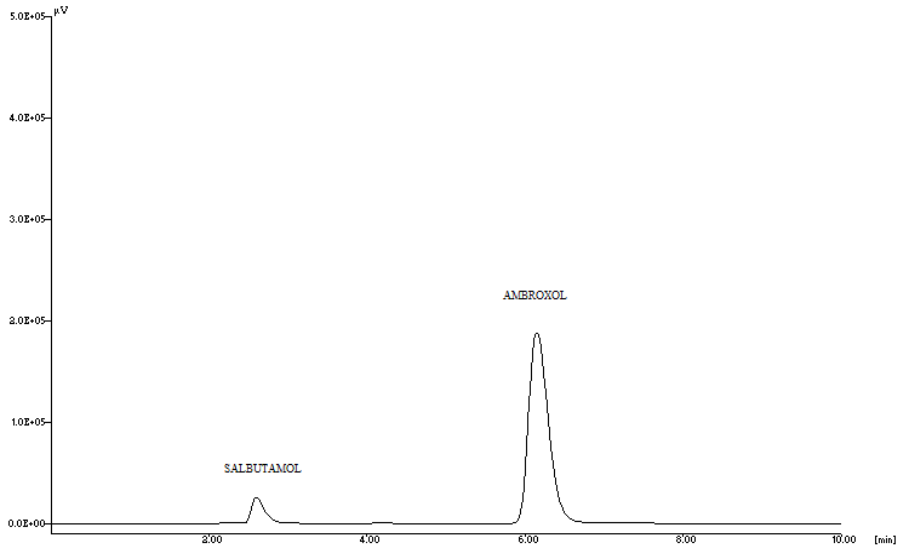


Fig. 2: RP-HPLC Chromatogram of Salbutamol Sulphate and Ambroxol Hydrochloride

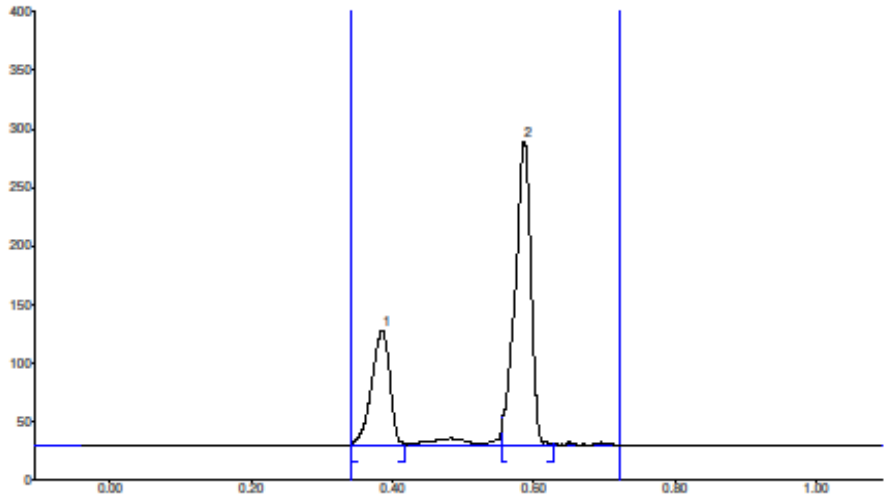


Fig. 3: HPTLC Chromatogram of Salbutamol Sulphate and Ambroxol Hydrochloride

Table 2: Linear Regression data of Salbutamol Sulphate and Ambroxol Hydrochloride

Parameter	RP-HPLC		HPTLC	
	SAL	AMB	SAL	AMB
Limit of detection	0.15 (µg/ml)	0.5 (µg/ml)	25 (ng/spot)	50 (ng/spot)
Limit of quantitation	0.45 (µg/ml)	1.5 (µg/ml)	90 (ng/spot)	160 (ng/spot)
Retention time (min) and Rf	2.59	6.15	0.38	0.59
Correlation coefficient (r^2)	0.998	0.998	0.998	0.996
Calibration range	0.5-3.0 (µg/ml)	7.5-45 (µg/ml)	100-200 (ng/spot)	1500-3000 (ng/spot)
Regression equation	y = 23445x + 3595	y = 17219x + 45935	y = 2119.x - 197.5	y = 1556.x + 956.9

Table 3: Precision data of Salbutamol Sulphate and Ambroxol Hydrochloride

Parameter	RP-HPLC		HPTLC	
Precision (n = 3)	SAL	AMB	SAL	AMB
	100.12 ± 1.7993	100.29 ± 0.3775	102.35 ± 0.5978	102.02 ± 1.8243
Intra-day*	100.18 ± 1.8458	100.13 ± 0.6959	99.57 ± 1.3984	98.19 ± 1.4623
(% estimated ± R.S.D.)	99.69 ± 1.7644	100.61 ± 1.1645	100.36 ± 1.0915	101.60 ± 1.2479
	100.12 ± 1.1319	100.32 ± 0.4031	102.82 ± 0.8645	101.07 ± 1.3613
Inter-day*	99.73 ± 1.0126	100.31 ± 0.6797	99.59 ± 1.8103	98.0 ± 0.6552
(% estimated ± R.S.D.)	98.87 ± 1.1870	99.62 ± 0.7307	100.66 ± 0.9714	102.31 ± 1.5230

* Mean of three replicates

Table 4: Recovery data of Salbutamol Sulphate and Ambroxol Hydrochloride

Level of standard	RP-HPLC		HPTLC	
Addition (%)	% Recovery ± S.D.	% RSD	% Recovery ± S.D.	% RSD
SAL				
80	98.70 ± 0.8293	0.8402	101.1 ± 1.1343	1.1521
100	100.82 ± 1.128	1.1189	98.54 ± 1.2668	1.2895
120	98.99 ± 0.8259	0.8343	98.09 ± 0.7375	0.7451
AMB				
80	98.48 ± 0.7924	0.8045	100.10 ± 0.5142	0.5133
100	99.17 ± 1.3777	1.3891	102 ± 0.6740	0.6647
120	98.75 ± 0.9383	0.9502	99.28 ± 0.5958	0.5875

CONCLUSION

The proposed RP-HPLC and HPTLC methods for the quantification of SAL and AMB in tablet were simple, precise, accurate, rapid and selective. The methods were found to be linear in wide range of concentration. The developed methods were free from interference due to the excipients present in tablet and can be used for routine simultaneous quantitative estimation of SAL and AMB in tablet.

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REFERENCES

1. Indian Pharmacopoeia, volume III Published by the controller of publication, New Delhi. 2007; P.1687.
2. Wilson and Gisvold's, Textbook Organic Medicinal and Pharmaceutical chemistry, London, UK: 2004. P. 96-99.
3. Indian Pharmacopoeia, volume III Published by the controller of publication, New Delhi. 2007; P. 143, 250, 701.
4. Maryadele J. O'Neil. The Merk Index, An Encyclopedia of Chemicals, Drugs and Biologicals, 14th ed.: published by Merk laboratories: 2006; P. 385.
5. Dave HN, Mashru RC, Thakkar AR. Simultaneous determination of salbutamol sulphate, bromhexine hydrochloride and etofylline in pharmaceutical formulations with the use of four rapid derivative spectrophotometric methods. Anal Chim Acta 2007; 597:113–120.
6. Mukherji G, Aggarwal N. Quantitative estimation of salbutamol sulphate by derivative UV spectroscopy in the presence of albumin, Int J Pharm 1992; 86: 153-158.
7. Mukherji G, Aggarwal N. Derivative UV-spectroscopic determination of salbutamol sulphate in the presence of gelatin. Int J Pharm 1991; 71:187-191.
8. Parimoo P, Umapathi P, Ilango K. Simultaneous quantitative determination of salbutamol sulfate and bromhexine hydrochloride in drug preparations by difference spectrophotometry. Int J Pharm 1993; 100: 227-231.
9. Patel PA, Dole MN, Sawant SD, Shedpure PS. Simultaneous determination of salbutamol and ambroxol in fixed dose combination by spectrophotometry. Int J Pharma Sci and Research 2011; 2(5): 1225-1230.
10. Colthup PV, Dallas FA, Saynor DA, Carey PF, Skidmore LF and Martin LE. Determination of Salbutamol in human plasma and urine by high-performance thin-layer chromatography, J Chromatogr. 1985; 345:111-118.
11. Jacobson GA and Peterson GM. High-performance liquid chromatographic assay for the simultaneous determination of ipratropium bromide, fenoterol, salbutamol and terbutaline in nebulizer solution. J Pharm & Biomed Anal 1994; 12: 825-832.
12. Dave HN, Mashru RC, Patel AK. Thin Layer Chromatography Method for the Determination of Ternary Mixture Containing Salbutamol Sulphate, Bromhexine Hydrochloride and Etofylline. J Pharm Sci & Res 2010; 2(2): 143-148
13. Gowekar NM, Pande VV, Kasture AV, Tekade AR, Chandorkar JG. Spectrophotometric estimation of ambroxol and cetirizine hydrochloride from tablet dosage form. Pak J Pharm Sci 2007; 20(3): 250-1.
14. Bhatia NM, Ganbavale SK, Bhatia MS, More HN, Kokil SU. RP-HPLC and Spectrophotometric estimation of ambroxol hydrochloride and cetirizine hydrochloride in combined dosage form. Ind J Pharm Sci 2008; 70: 603-608
15. Makarand A, Bonde CG. Development and validation of simultaneous UV spectrophotometric method for the determination of levofloxacin and ambroxol in tablets. Int J ChemTech Research 2009; 1: 873-888
16. Hadad GM, Gindy AE, Waleed MM. HPLC and chemometrics-assisted UV-spectroscopy methods for the simultaneous determination of ambroxol and doxycycline in capsule. Spectrochim Acta Part A 2008; 70: 655–663
17. Dinçer Z, Basan H, Göger NG. Quantitative determination of ambroxol in tablets by derivative UV spectrophotometric method and HPLC. J Pharm & Biomed Anal 2003: 1: 31(5): 867-872.
18. Nobilis M, Pastera J, Svoboda D and Kvstina J. High-performance liquid chromatographic determination of ambroxol in human plasma. J Chromatogr 1992; 581: 25l-255.
19. Meiling Qi, Wang P, Cong R, Yang J. Simultaneous determination of roxithromycin and ambroxol hydrochloride in a new tablet formulation by liquid chromatography. J Pharm & Biomed Anal 2004; 35:1287–1291.
20. Nagappan KV, Meyyanathan SN, Raja RB, Reddy S, Jeyaprakash MR, Birajdar AS. A RP-HPLC Method for Simultaneous Estimation of Ambroxol Hydrochloride and Loratidine in Pharmaceutical Formulation. Research J Pharm and Tech 2008; 4: 366-369.
21. Maithani M, Raturi R, Vertika Gautam. Simultaneous estimation of ambroxol hydrochloride and cetrizine hydrochloride in

tablet dosage form by RP-HPLC method. Int J Comprehensive Pharm 2010; 1: 1-3.

22. Jain PS. Stability-indicating HPTLC determination of ambroxol hydrochloride in bulk drug and pharmaceutical dosage form. J Chromatogr Sci 2010; 48(1): 45-48.
23. Hohyun Kim, Jeong-Yeon Yoo, Sang Beom Han, Hee Joo Lee, Kyung Ryul Lee. Determination of ambroxol in human plasma using LC-MS/MS. J Pharm & Biomed Anal 2003; 32: 209-216.
24. Sohan S. Chitlange, Ashish G. Dhole, Sagar V. Pandkar, Sagar B. Wankhede, Development and validation of RP-HPLC method for the simultaneous estimation of salbutamol sulphate and ambroxol hydrochloride in tablet dosage form. Inventi Rapid: Pharm Ana and Qual Assur 2011; 2:131.
25. Validation of analytical procedures: Text & Methodology, Q2 (R), ICH Harmonized Tripartite Guidelines Nov 2005.