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Thin Layer Chromatodraphy Method for the Determination of Ternary Mixture Containing Salbutamol Sulphate, Bromhexine Hydrochloride and Etofylline

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Abstract:
Simple, rapid, accurate, precise, reliable and economical thin layer chromatographic and spectrophotometric methods have been proposed for the resolution and determination of Salbutamol sulphate (SS), Brombexine hydrochloride (BH) and Etofylline (ET) in pure and pharmaceutical formulations respectively. The developed methods show best results in terms of resolution, linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ) for standard laboratory mixtures of pure drugs and marketed formulations. The R_f value for SS was found to be 0.25, for BH R_f value was found to be 0.91 and for ET R_f value was found to be 0.71. The range for SS, BH and ET were found to be 1-35 µg mL^{-1}, 4-40 µg mL^{-1} and 5-80 µg mL^{-1} respectively. The values of LOD were 0.245 µg mL^{-1}, 0.521 µg mL^{-1} and 0.930 µg mL^{-1} and the values LOQ were 0.816 µg mL^{-1}, 1.733 µg mL^{-1} and 3.071 µg mL^{-1} for SS, BH and ET respectively. The precision values were less then 2 % in terms of % relative standard deviation for the developed method. The common excipients and additives did not interfere in their determinations.

Keywords: Bromhexine hydrochloride, Etofylline, Salbutamol sulphate, Spectrophotometry, Thin layer chromatography,

Introduction:

Salbutamol sulphate (SS), chemically known as bis [(1RS)-2-[(1, 1-dimethylethyl) amino]-1-[4-hydroxy-3-(hydroxymethyl) phenyl] ethanol] sulphate, is beta-adenocepter agonist used as an anti-asthmatic drug, Bromhexine hydrochloride (BH**)**, N-(2-amino-3, 5-dibromobenzyl)-N-methyl cychlohexanamine hydrochloride, is an expectorant use in the treatment of various respiratory disorders. Etofylline (ET), 7-(2-hydroxyethyl)-1, 3-dimethyl-3, 7-dihydro-1, 4-purine-2, 6-dione, is a xanthine bronchodilator used for the treatment of respiratory diseases and asthma in combination with SS. SS [1], BH [2] and ET [3] are official in BP. The official methods involve determination of SS [1], BH [2] and ET [3] using Potentiometry. SS, in the combination with BH and ET is widely used in the treatment of various respiratory disorders.

Some procedures have been described for the assay of either SS or BH or ET in single dosage forms [4–7]. A spectrophotometric method has been reported for determination of SS and BH in combine dosage forms [8]. Determination of SS and ET has been reported spectrophotometrically in their combined dosage forms [9]. Some spectrophotometric methods were developed for the simultaneous determination of SS, BH and ET in combined dosage forms [10]. The ternary combination, SS, BH and ET, is not yet official in any pharmacopoeia. As par literature, no chromatographic methods could be traced for the analysis of SS, BH and ET in their combined dosage forms. Therefore simple, rapid, economical and reliable chromatographic method for estimation of these drugs in mixture seemed to be necessary.

Thin layer chromatography (TLC) is widely used that it has become the essential technique for analyst and research workers. TLC is the most rapid, simplest and economical chromatographic technique for separation and identification of the compounds [11]. The compounds, which are separated, can be recovered easily using this chromatographic technique. TLC is equally easy to describe and more straightforward to explain, is a simple, quick, and inexpensive procedure that gives the chemist a quick

answer as to how many components are in a mixture as well as the profile of impurity [12].

This research deals with the use of TLC and spectrophotometric method for the resolution and estimation of the ternary mixture respectively as to become most rapid and economical as well as reliable technique for multicomponant analysis. An attempt was made to develop chromatographic method for resolution of SS, BH and ET in the combined dosage forms and then individual components of the resolved mixture were estimated spectrophotometrically and the developed method was validated successfully.

Materials and methods:

Instruments

Spectrophotometric measurements were made on a Shimadzu 1700 double beam UV Visible spectrophotometer with a fix slit width of 1 nm coupled HP7540 computer loaded with Shimadzu UV PC software of version 2.0 and EPSON-300 printer.

Reagents

All chemicals used were of analytical grade and double distilled water was used throughout. Pure SS and BH were obtained from Dial Pharmaceuticals Pvt. Ltd., India and ET was obtained from Cadila Healthcare Pvt. Ltd., India. Various pharmaceutical formulations of SS, BH and ET in their combined dosage forms were obtained commercially.

Mobile phase system used for the separation

Mobile phase used for the separation of ternary mixture was methanol: n-hexane in the proportion of 2:1.

Preparation of TLC plates and solvent system

TLC plates (20 × 10cm × 0.5mm) were prepared by spreading slurry of silica gel GF_{254} (50gm) in double distilled water. The plates were dried in air and activated in oven at 110ºc for 30minutes. For the solvent system development, after various trials, finally methanol: n-hexane (2: 1) ratio was used.

Chromatographic method and visualization of spot

Stock solutions, 1 mg mL^{-1} of pure samples of SS, BH and ET were freshly prepared individually in methanol. Solutions for standard laboratory mixture and commercial formulation of SS: BH: ET was prepared in 1: 4: 50 ratio using methanol as solvent. Solution of pure drugs, standard laboratory mixture and commercial formulation were applied to the plate at 10 µl level. The chromatogram was developed at room temperature for 30 minutes. Different solvent systems in different ratio were used. Glass chambers were pre-equilibrated with solvent system for 30 minutes. The developed plates were dried at room temperature. The detection of the samples was carried out by UV-chamber at short wavelength (254 nm) and by iodine chamber.

Procedure for spectrophotometric quantification of resolved compound by TLC

For spectrophotometric quantification of the developed method, sufficient quantity of powder samples were scrap out for the three drugs individually and simultaneously each drugs of standard laboratory mixture and commercial formulation were also scrap out separately after resolution. Each powder samples were than transferred to separate centrifuge tubes which were diluted up to 10ml with methanol. The samples were centrifuge at 3000 rpm for 5 minutes and clear solutions were collected. The absorbance of the resultant solutions was measured at 227 nm, 248.8 nm and 272.6 nm for SS, BH and ET respectively. The whole experiment was repeated five times.

Validation parameters

Accuracy

For studying the accuracy of the proposed methods, and for checking the interference

Tables 1: Trials of mobile phases for the resolution of ternary mixture

Mobile phase	***Observation***
Methanol: strong ammonia solution	Only SS can be detected.
Methanol: chloroform	All three can be detected but R_f values for BH (0.88) and ET (0.9) are vary close.
Methanol: n-hexane: iso propyl alcohol	All the three can be detected but R_f value for BH was found to be 0.97(vary close to solvent front).
N- hexane: iso propyl alcohol	SS can't be detected.
Methanol: chloroform: n- hexane	All three can be detected but no reproducibility was found.
Methanol: cyclohexane: chloroform	All three can be detected but R_f value for BH was found to be 0.99 (almost on the solvent front).
Methanol: n-hexane: toluene	All three can be detected but R_f value for BH was found to be 0.99 (almost on the solvent front).
Methanol: toluene	ET can't be detected.
Toluene: ethyl acetate: diethyl amine	SS and ET can't be detected.
Methanol: cyclohexane: toluene: diethyl amine	SS can't be detected.
Toluene: ACN	SS can't be detected.
Methanol: toluene: ethyl acetate	SS can't be detected.
Methanol: chloroform: iso propyl alcohol	ET can't be detected.
Methanol: cyclohexane: benzene	SS can't be detected.
Methanol: n-hexane (2:1)	**All three are detected with better resolution, R_f values and reproducibility.**

[a] – Mean and % relative standard deviation for 10 determinations

Table 2: Results of recovery study

Amt added (µg mL^{-1})			**% Recovery[a]**		
SS	BH	ET	SS	BH	ET
1.04	4.16	52	101.98	98.67	98.79
1.3	5.2	65	103.24	98.27	99.10
1.56	6.24	78	102.14	97.39	98.28
Mean Recovery			102.45	98.11	98.75
%RSD			0.54	0.54	0.35

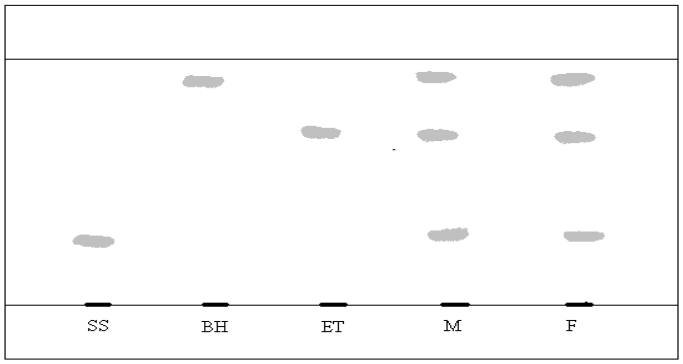
Table 3: Validation parameters

Parameters			
	SS	BH	ET
Range(µg mL^{-1})	1-35	4-40	5-80
Slope	0.0387	0.0241	0.0333
Intercept	0.0081	0.332	-0.0186
Correlation-coefficient(R^2)	0.9991	0.9993	0.9994
Accuracy	102.45 ± 0.54	98.11 ± 0.54	98.75 ± 0.35
Precision	%RSD – 0.55	%RSD – 0.61	%RSD – 0.33
LOD (µg mL^{-1})	0.245	0.521	0.930
LOQ(µg mL^{-1})	0.816	1.733	3.071
Reproducibility	%RSD – 0.52	%RSD – 0.57	%RSD – 0.39

Table 4: Results of commercial formulation analysis

Formulation	**% Labeled Claim obtained for SS [d]**	**% Labeled Claim obtained for BH [d]**	**% Labeled Claim obtained for ET [d]**
AIR VENT[a]	102.34 ± 0.55	98.12 ± 0.51	98.62 ± 0.37
BUTABROM[b]	101.48 ± 0.64	100.98 ± 0.71	99.47 ± 0.74
SAANS[c]	100.37 ± 0.68	99.72 ± 0.67	101.11 ± 0.57

[a] – Brand A tablets; [b] – Brand B tablets; [c] – Brand C tablets; [d] – Mean and standard deviation for 10 determinations. Here ± sign indicates the upper and lower limits of standard deviation of 10 determinations.



SS-Salbutamol sulphate
BH-Bromhexine hydrochloride
ET-Etofylline
M-Standard laboratory mixture
F-Market formulation

Figure 1: Pure SS, BH, ET, Standard laboratory mixture and commercial formulation on TLC plate.

from excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. This study was performed by addition of known amounts of salbutamol sulphate, bromhexine hydrochloride and etofylline to a known concentration of the commercial tablets. The amounts of standard recovered were calculated in the terms of mean recovery with the upper and lower limits of percent relative standard deviation.

Precision

Intra day precision and inter day precision for the developed methods were measured in terms of % RSD. The experiments were repeated five times a day for intra day precision and on five different days for inter day precision. The concentration values for both intraday precision and interday precision were calculated five times separately and percent relative standard deviation were calculated. Finally the mean of % RSD (% RSD = [S/X] 100, where S is standard deviation and X is mean of the sample analyzed) were taken for conclusion.

Limit of Detection (LOD) and Limit of Quantitation (LOQ).

Limit of Detection (LOD) and Limit of Quantitation (LOQ) were calculated according to the 3 s/m and 10 s/m criterions, respectively, where *s*, is the standard deviation of the absorbance (n = 10) of the sample and m is the slope of the corresponding calibration curve.

Reproducibility

The reproducibility of the method was determined by the use of different instruments: Shimadzu UV 1700 and Shimadzu UV 1601. The average value of % RSD (% RSD = [S/X] 100, where S is standard deviation and X is mean of the sample analyzed) of the responses for the determination of SS, BH and ET were found as mentioned below which reveals the reproducibility of the method.

Result and Discussion:

Various mobile phases at different proportion were tried and were listed in following table 1. The R_f value for SS was found to be 0.25, for BH R_f value was found to be 0.91 and for ET R_f value was found to be 0.71 using methanol : n-hexane (2:1) as a mobile phase. BH spot and ET spot was detected by UV-chamber, SS spot was detected by iodine chamber Fig. 1. After total separation and identification, the separated compounds were estimated spectrophotometrically using methanol as a solvent. The developed method was validated accurately and results of accuracy were shown in Table 2, summary of various validation parameters were listed in Table 3, results of marketed formulation analysis were listed in Table 4.

Conclusion:

It is concluded that TLC method for the resolution of ternary mixture and spectrophotometric quantification of the resolved components were developed and validated successfully. The method was found to be simple, rapid, economical, selective and reliable. It is helpful without the use of much sophisticated instruments and therefore useful for routine analysis of ternary mixture of salbutamol sulphate, bromhexine hydrochloride and etofylline.

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