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Simultaneous RP-HPLC Estimation of Salbutamol, Ambroxol and Theophylline in Tablets

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A simple, fast, precise and accurate liquid chromatographic method was developed for the simultaneous estimation of salbutamol, ambroxol and theophylline in tablets. This combination is used for the treatment of bronchial asthma. Drugs are chromatographed on a reverse phase Hypersil C_{18} column using mobile phase, phosphate buffer (pH 7.0), methanol and acetonitrile in the ratio 40:30:30 (v/v). The retention time of salbutamol, ambroxol and theophylline was 3.07, 4.91 and 1.77 min, respectively. The validation of the proposed method was also carried out. The method was found to be linear (correlation coefficient r > 0.999), precise (relative standard deviation: 0.42 % for salbutamol, 0.61 % for ambroxol and 0.73 % for theophylline), accurate (overall average recovery yields: 99 % for salbutamol, 101.2 % for ambroxol and 99.8 % for theophylline) and selective. Due to its simplicity and accuracy, the proposed method can be used for routine quality control analysis of these drugs in combination tablets.

Key Words: Salbutamol, Ambroxol, Theophylline, HPLC.

INTRODUCTION

Salbutamol, [(*t*-butylamino)methyl]-4-hydroxy-*m*-xylene-1,2-diol is used as a bronchodilator[1,2]. Ambroxol, is a potent mucolytic and is chemically, 4{(2-amino-3,5-dibromophenyl)methyl]amino}cyclo-hexanol[1]. Theophylline, chemically 3,7-dihydro-1,3-dimethyl-1*H*-purine-2,6-dione is a CNS stimulant[1-3]. The combination of three drugs, salbutamol (2 mg), ambroxol (30 mg) and theophylline (100 mg) is available commercially as Ambrolite-ST tablets. This combination is used for the treatment of bronchial asthma. Many methods[1-6] have been described in the literature for the determination of salbutamol, ambroxol and theophylline individually. However, there is no HPLC method reported for the determination for these drugs either as active pharmaceutical ingredient or from dosage forms. The present work describes a simple, precise and accurate reverse phase HPLC method for the simultaneous estimation of salbutamol, ambroxol and theophylline in combined dosage forms.

EXPERIMENTAL

The drug samples, salbutamol, ambroxol and theophylline were obtained as gift samples from SUN Pharmaceutical Industries, Mumbai. Sodium dihydrogen ortho phosphate AR, orthophosphoric acid AR, acetonitrile and methanol of HPLC grade were supplied by S.D Fine Chemicals, Mumbai. Water of HPLC grade was obtained form a Milli-Q RO water purification system. An isocratic high pressure liquid chromatograph (Shimadzu HPLC class VP series) with LC-10 AT pump, variable wavelength programmable UV/Vis detector SPD-10 AVP, operating software-winchrome was used for the analysis.

The method was carried out on a Hypersil C_{18} (250 mm × 4.6 mm i.d., 5 μ) column as a stationary phase and methanol:acetonitrile: 25 mM phosphate buffer (pH adjusted to 7.0 with orthophosphoric acid) in the ratio of 30:30:40 (v/v/v) as the mobile phase at the flow rate of 1 mL/min. The mobile phase was filtered through a 0.45 μ membrane filter and degassed before analysis. A Rheodyne 7725 injector with a 20 μL loop was used for the injection of samples. Detection was done at 270 nm and separation was carried out at the room temperature of about 20 ºC.

Standard stock solution of salbutamol (20 μg/mL), ambroxol (300 μg/mL) and theophylline (1 μg/mL) were prepared in a mixture of methanol and acetonitrile (1:1 v/v). From the standard stock solutions, mixed standard solution was prepared containing 1.2 μg/mL of salbutamol, 18 μg/mL of ambroxol and 60 μg/mL of theophylline.

Ten marketed tablets each containing 2 mg of salbutamol, 30 mg of ambroxol and 100 mg of theophylline were weighed and finely powdered. A quantity of powder equivalent to 2 mg of salbutamol, 30 mg of ambroxol and 100 mg of theophylline was weighed and transferred to a sintered glass crucible. The drugs were extracted with three quantities, each of 20 mL of mixture of acetonitrile and methanol (1:1 v/v). The combined extracts were made up to 100 mL with mobile phase and further dilutions were made to get a concentration of 1.2 μg/mL of salbutamol, 18 μg/mL of ambroxol and 60 μg/mL of theophylline. The content was vortexed, filtered through a 0.45 μ membrane filter and injected in triplicate. The peak area of each drug was calculated. The mixed standard solution was subjected to proposed HPLC method of analysis for finding out intra and inter day variations. Linearity and range was also determined by analyzing mixed standard solutions. The calibration curve was plotted using peak area *vs.* concentration of the standard solutions. Recovery studies were carried out by adding known amount of standard drug to the pre-analyzed samples and reanalyzing them using the HPLC method of analysis, which is being developed.

RESULTS AND DISCUSSION

The present study was carried out to develop a simple and rapid HPLC method for the simultaneous estimation of salbutamol, ambroxol and theophylline using Thermohypersil C-18 column. The retention time of salbutamol, ambroxol and theophylline was found to be 3.07, 4.91 and 1.77 min. The assay concentration of 1.2 µg/mL salbutamol, 18 µg/mL of ambroxol and 60 µg/mL of theophylline was selected according to the labeled claim. The peaks were well resolved symmetrical in shape and asymmetry factor for all the peaks were found to be less than 1.20. There was good repeatability of the proposed method as the precision of the method was less than 2 % for all the three drugs. The coefficient of variance for salbutamol, ambroxol and theophylline were found to be 0.88, 0.63 and 0.49 %, respectively, that shows the method is highly precise.

Linearity experiment was performed thrice for all the three components and response was found to be linear in the concentration range of 1-5 µg/mL for salbutamol, 4-20 µg/mL for ambroxol and 20-100 µg/mL for theophylline. Regression lines were obtained at 95 % confidence interval using least square method. Correlation coefficient 'r' values for all three drugs were ≥ 0.999. Accuracy of method was determined by recovery studies (n = 3). The concentrations of standard spiked to the sample were 1-3 µg/mL for salbutamol, 4-12 µg/mL for ambroxol and 20-60 µg/mL for theophylline. Recovery data from the study are reported in Table-1. The mean % recovery was found to be 99.0 % for salbutamol, 101.2 % for ambroxol and 99.8 % for theophylline. The content of the drugs in the commercial dosage form was found to be 99.2 % for salbutamol, 99.5 % for ambroxol and 99.6 % for theophylline per tablet by this method. The estimated amount was within the acceptable limits of the labeled claim of the formulation.

TABLE-1
RECOVERY STUDIES

Drug	Amount added (µg/mL)	Amount recovered (µg/mL) n = 3	Recovery (%)	Average recovery (%)
Salbutamol	1	1.03	103.00	101.61
	2	2.05	102.50	
	3	2.98	99.33	
Ambroxol	4	4.11	102.75	101.34
	8	8.05	100.62	
	12	12.08	100.66	
Theophylline	20	20.12	100.60	100.14
	40	39.87	99.67	
	60	60.09	100.15	

Recovery studies data showing amount of drug recovered from sample solution and average percentage recovery.

The developed RP-HPLC method provides a convenient and efficient method for the separation and estimation of salbutamol, ambroxol and theophylline in combined dosage form. There was no interference from the excipients used in the tablet formulation and hence the method is suitable for analysis of tablets. The results of validation showed that the proposed method is simple, linear, precise, accurate and selective and can be employed in routine assay of salbutamol, ambroxol and theophylline in tablets.

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