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HPTLC Method for the Simultaneous Estimation of Etophylline and Theophylline in Tablet Dosage Form

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A simple, precise, accurate and rapid high performance thin layer chromatographic method has been developed and validated for the estimation of etophylline and theophylline in combined dosage form. The stationary phase used was precoated silica gel $60F_{254}$. The mobile phase used were a mixture of toluene:isopropyl alcohol:acetic acid (12:12:1 v/v/v). The detection of spots was carried out at 261 nm. The method was validated in terms of linearity, accuracy, precision and specificity. The calibration curve was found to be linear between 200 to 400 ng for etophylline and 60-80 ng for theophylline with regression coefficient of 0.9997 and 0.9994. The proposed method can be successfully used to determine the drug content of marketed formulation.

Key Words: Etophylline, Theophylline, HPTLC.

INTRODUCTION

Etophylline is almost white crystalline powder and it is mainly used for the xanthine bronchodilator[1]. The chemical name is 7-(2-hydroxyethyl)-1,3-dimethyl-3,7dihydro-1*H* - purine-2,6-dione. Theophylline indicated for the chronic obstructive diseases of the air ways, chronic obstructive pulmonary diseases (COPD), bronchial asthma, infant apnea[2]. Its chemical name is 1,3-dimethyl-7*H*-purine-2,6-dione. Many methods[3-7] have been described in the literature for the determination of etophylline with other drugs individually and in combination. The techniques include serum RP-HPLC[8], TLC methods[9,10] derivative spectrophotometric[11]. However there is no HPTLC method reported for the simultaneous estimation of these drugs in combined dosage forms. Fixed dose combination containing etophylline and theophylline is available in the tablet form in the market. The aim of this work is to develop an HPTLC method for the simultaneous determination of etophylline and theophylline in pharmaceutical dosage forms.

Etophylline and theophylline standard were procured as a gift sample from ATOZ Pharma Ltd., Chennai, India. Silica gel $60F_{254}$ TLC plates (10 cm × 10 cm, layer thickness 0.2 mm, E. Merck, Mumbai, India) were used as a stationary phase. All chemicals and reagents used were of analytical grade. Tablets containing etophylline (77 mg) and theophylline (23 mg) were procured from the local pharmacy (Deriphylline, Zydus Cadila Pharmaceutical Ltd., Ahmedabad, India). A Camag HPTLC system comprising of Camag Linnomate V automatic sample applicator, Hamilton syringe (100 µL), Camag TLC Scanner 3, Camag WinCATS software, Camag Twin-trough chamber (10 cm × 10 cm) and ultrasonicator were used during study.

EXPERIMENTAL

Etophylline and theophylline (10 mg) each were weighed accurately, dissolved and diluted with isopropyl alcohol to obtain the final concentration of 100 µg/mL of each drug. Twenty tablets were weighed accurately and ground to a fine powder. Weight equivalent to 10 mg of etophylline and theophylline were transferred to conical flask and mixed with isopropyl alcohol. The solution was sonicated for 15 min. The extracts were filtered through Whatman filter paper No. 41. Required dilutions were made to get 100 µg/mL of etophylline and theophylline.

TLC plates were prewashed with isopropyl alcohol. Activation of plates was done in an oven at 50 ºC for 5 min. The chromatographic conditions maintained were precoated silica gel $60F_{254}$ aluminum sheets (10 cm × 10 cm) as stationary phase, toluene:isopropyl alcohol:acetic acid (12:12:1 v/v/v) as mobile phase, chamber and plate saturation time of 0.5 h, migration distance allowed was 90 mm, wavelength scanning was done at 261 nm keeping the slit dimensions at 5 × 0.45 mm. A deuterium lamp provided the source of radiation. Standard

solutions of etophylline and theophylline were spotted and developed. Photometric measurements were performed at 261 nm in reflectance mode with Camag TLC scanner 3 using Win CATS software.

Aliquots of 2.0, 2.5, 3.0, 3.5, 4.0, µL of standard solution of etophylline and 6.0, 6.5, 7.0, 7.5, 8.0, µL of theophylline were applied on the TLC plate (100 µg/mL of drug). TLC plate was dried, developed and analyzed photometrically. The standard calibration curve was generated using regression analysis with Microsoft excel. Sample solutions of the marketed formulation were spotted on to the same plate followed by development scanning. The analysis was repeated in triplicate. The content of the drug was calculated from the peak areas recorded. The developed method was validated in terms of linearity, accuracy, limit of detection, limit of quantification, intra-day and inter-day precision and repeatability of measurement as well as repeatability of sample application[12].

A solvent system that would give dense and compact spots with significant R_f values were desired for quantification of etophylline and theophylline in pharmaceutical formulations. The mobile phase consisting of toluene:isopropyl alcohol: acetic acid (12:12:1 v/v/v) gave R_f values of 0.63 ± 0.25 and 0.75 ± 0.03 for etophylline and theophylline, respectively. The linear regression data (n = 5, Table-1) showed a good linear relationship over a concentration range of 200-400 ng/spot and 60-80 ng/spot for etophylline and theophylline, respectively. The limit of detection and limit of quantification for etophylline was found to be 100 and 200 ng/spot and for theophylline, 30 and 60 ng/spot, respectively.

TABLE-1
METHOD VALIDATION PARAMETERS OF PROPOSED METHOD

Parameter	Etophylline	Theophylline
Linearity range (ng/spot)	200-400	60-80
Correlation coefficient (r)	0.9997	0.9994
Regression equation (y = mx + c)		
Slope (m)	10.596	23.814
Intercept (c)	91.914	163.41
Limit of detection (LOD)	100 ng/spot	30 ng/spot
Limit of quantification (LOQ)	200 ng/spot	60 ng/spot
Precision (CV)	0.27-1.19 %	0.12-1.05 %
Repeatability of application (n=5)	0.98	0.83
Repeatability of measurement (n=5)	0.43	0.49

The intra-day precision was determined by analyzing standard solutions in the concentration range of 200 ng/spot to 400 ng/spot for etophylline and 60 ng/spot to 80 ng/spot for theophylline for 3 times on the same day. The inter-day precision was determined by analyzing corresponding standards daily for 3 day over a period of one week. The intra-day and inter-day coefficients of variation for both drugs were found to be in the range of 0.27-1.19 and 0.12-1.05 %, respectively. These values indicate that the method is precise.

Repeatability of sample application was assessed by spotting 4 µL of drug solution 5 times on a TLC plate followed by development of plate and recording the peak area for 5 spots. The % RSD for peak area values of etophylline and theophylline were found to be 0.98 and 0.83, respectively. Repeatability of measurement of peak area was determined by spotting 4 µL of etophylline and theophylline solution on a TLC plate and developing the plate. The separated spot was scanned five times without changing the position of the plate and % RSD for measurement of peak area of etophylline and theophylline were found to be 0.43 and 0.49, respectively. To confirm the specificity of the proposed method, the solution of the formulation was spotted on the TLC plate, developed and scanned. It was observed that the excipients present in the formulation did not interfere with the peaks of etophylline and theophylline.

Recovery studies of the drugs were carried out for the accuracy parameter. These studies were carried out at three levels *i.e.* multiple level recovery studies. Sample stock solutions from tablet formulation of 100 µg/mL were prepared. Dilutions were made and recovery studies were performed. Percentage recovery was found to be within the limits as listed in Table-2. The assay value for the marketed formulation was found to be within the limits as listed in Table-2. The low RSD value indicated the suitability of the method for routine analysis of etophylline and theophylline in pharmaceutical dosage forms.

RESULTS AND DISCUSSION

The developed HPTLC method for the simultaneous estimation of etophylline and theophylline in combined dosage form utilizing toluene:isopropyl alcohol:acetic acid in ratio of (12:12:1 v/v/v) as a mobile phase. The detection of eluent was carried out at 261 nm. The excipients in the formulation did not interfere in the accurate estimation. The method was validated as per ICH guidelines. The developed HPTLC technique is simple, precise, specific and accurate and the statistical analysis proved that method is reproducible and selective for the analysis of etophylline and theophylline in bulk drug and tablet formulations.

TABLE-2
ASSAY AND RECOVERY STUDIES OF ETOPHYLLINE AND THEOPHYLLINE

Brand name	Label claim (mg/tablet)	Total amount added (mg)	Amount* recovered mg SD	% Recovery ± SD	% Assay*
Deriphylline	Etophylline 77	40.0	39.83	99.59 ± 0.98	100.78 ± 0.43
		50.0	49.28	99.10 ± 1.50	
		60.0	60.70	100.87 ± 0.79	
	Theophylline 23	6.5	6.57	100.87 ± 1.69	99.81 ± 0.85
		13.0	12.82	99.92 ± 0.95	
		18.5	19.12	102.06 ± 0.87	

*Each value is a mean of ± standard deviation of three determinations

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