

Simultaneous Estimation of Ambroxol Hydrochloride and Doxofylline in Pharmaceutical Formulation by HPTLC-Desitometric Method

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Abstract

A simple, precise, rapid, selective, and economic reversed phase high-performance thin layer chromatography (HPTLC) method has been established for simultaneous analysis of Ambroxol Hydrochloride and Doxofylline. The HPTLC method was performed on precoated silica gel G60 F254 plates with Diethyl ether:n-butanol:Ammonia (9:0.9:0.1 v/v/v) as mobile phase. The plates were developed in a 7.0 cm at ambient temperature. The developed plates were scanned and quantified at their absorption at approximately 254 nm for AMB and DOX. The drugs were satisfactorily resolved with R_f 0.29 ± 0.02 for and R_f 0.56 ± 0.02 for DOX. The calibration plot was linear between 100-600 ng /band for AMB and 200-1200 ng/band based for DOX. This HPTLC procedure is economic, sensitive, and less time consuming than other chromatographic procedures. It is a user-friendly and importance tool for analysis of combined dosage form.

Keywords: HPTLC; Precoated silica gel G60 F254 plates; Ambroxol Hydrochloride (AMB); Doxofylline (DOX)

Introduction

Ambroxol hydrochloride (AMB; (Trans-4-(2-amino-3,5-dibromobenzylamino) cyclohexanol hydrochloride); (Figure 1a) is a widely secretolytic agent used in the treatment of respiratory diseases associated with viscid or excessive mucus [1]. Doxofylline (DOX; 7-(1,3-dioxolan-2-yl)AMBhyl)-1,3-diAMBhylpurine-2,6-dione Figure 1b). It is used for maintenance therapy in patients suffering with Asthma and Chronic Obstructive Pulmonary Disease (COPD) [2].

Literature survey revealed that various analytical Methods like HPLC [3-11], UV [12-23] and HPTLC [24] have been reported for the determination of AMB and DOX either individually or combination with some other drugs. The review of literature prompted us to develop an accurate, selective and precise simultaneous Method for the estimation of AMB and DOX in combined dosage forms.

Experimental

Chemicals and materials

Ambroxol Hydrochloride and Doxofylline working standards were kindly gifted by Cadila Healthcare Limited, Ankleshwar, Gujarat. Methanol (HPLC Grade), Triethylamine (AR Grade), Isopropylalcohol (AR Grade), Ammonia and HPLC grade water were used as solvents to prepare the mobile phase. Tablet formulation Synasma-Ax (Ranbaxy Laboratories Ltd.) was procured from local market.

Chromatographic conditions

A Pre-coated silica gel G60-F254 aluminum sheet (100×100 mm, thickness layer 0.2 mm) pre washed with methanol was used as stationary phase. The linear ascending development was carried out in a CAMAG twin-trough glass chamber (20×20 cm) equilibrated with the mobile phase diethyl ether:n-butanol:ammonia (9:0.9:0.1, v/v/v) for 30 min at room temperature. The length of the chromatogram run was 70 mm. Quantitative evaluation of the plate was performed in absorbance mode at 254 nm. The slit dimensions were 5 mm length and 0.45 mm width, with a scanning rate of 20 mm/s with a computerized CAMAG TLC scanner -3 integrated with win CATS 4 software.

Sample preparation

Finalised HPTLC parameters were kept same as described earlier. Standard stock solution, sample solution and mobile phase were prepared in similar way as described earlier. Procedure for analysis of marketed preparation. 2 microlitre of the sample solutions (100 µg/ml Ambroxol Hydrochloride and 200 µg/ml Doxofylline) was applied on the TLC plate followed by development and scanning. Six replicate of sample solution was applied. From the peak area of the AMB and DOX, amounts of drugs in samples were computed.

Preparation of standard solution: A 25 mg of standard AMB and 50 mg of DOX were accurately weighed and transferred to two separate 25 ml volumetric flasks and dissolved in few ml methanol. The flask was sonicated for 10 min. The flask was shaken and volume was made up to the mark with methanol to yield a solution containing 1000 µg/ml of AMB and 2000 µg/ml DOX.

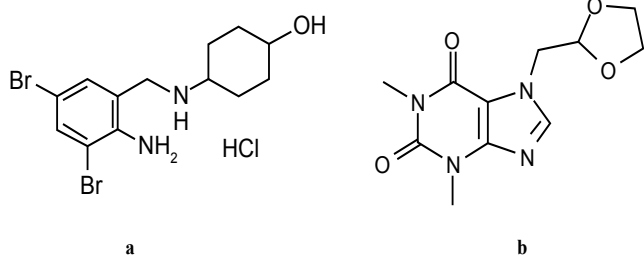


Figure 1: Structure of Ambroxol hydrochloride (a) and Doxofylline (b).

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Method validation

The developed method was validated for linearity and range, specificity, accuracy, precision, Limit of detection, Limit of quantitation, robustness and solution stability as per ICH guidelines.

Linearity and range: Stock solutions were applied containing (100 µg/ml AMB and 200 µg/ml DOX) in the range 1-6 µl on to HPTLC plate. The plate was developed and scanned. Each amount was analyzed and peak areas were recorded. Calibration plots of peak area against the respective amount of drug were established separately for AMB and DOX.

Specificity: Specificity of an analytical method is ability to measure specifically the analyte of interest without interferences from blank and placebo. The peak purity index for the main peaks and known impurities peaks in standard preparation and sample preparation should be equal to or more than 0.995. Peak purity for AMB and DOX was assessed by comparing spectra acquired at the start, apex and end of the peak obtained from the scanning of spot, i.e. $r(S, M)$ and $r(M, E)$. The high value of r indicates specificity of the method.

Accuracy (% Recovery): Accuracy is the closeness of the test results obtained by the method to the true value. To study the accuracy 20 tablets were weighed and powdered and analysis of the same was carried out. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels taking into consideration percentage purity of added bulk drug samples. Recovery of the drugs at 3 different levels in the formulation was studied by applying of 50%, 100%, and 150% of the standard drug solution of AMB and DOX; the mixtures were re-analyzed by the proposed method. At each level three analyses were performed.

Method precision (Repeatability): Method precision for assay was established by determining the assay of six sample preparations containing (400 µg/ml AMB and 800 µg/ml DOX) optimized as described earlier. Six replicates of sample were prepared at sample concentration and analyzed on same day.

Intermediate precision: The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day (intraday) and 3 on different days (interday), for that standard solutions containing (100 µg/ml AMB

and In the above changed conditions, stock solution was analyzed and results of robustness studies were expressed in term of %RSD of peak areas in each changed condition and were compared with similar results obtained in unchanged experimental conditions.

Limits of detection (LOD) and Limits of quantitation (LOQ): LOD and LOQ were calculated from the data obtained from the linearity studies. The slope of the linearity plot was determined. For each of the six replicate determinations, y intercept was calculated and the standard deviation of the y intercept was computed. From these values, the parameters Limit of detection (LOD) and Limit of quantitation LOD and (LOQ) were determined on the basis of response and slope of the regression equation. LOD and LOQ were calculated using following equation as per ICH guidelines. $LOD=3.3 \times \sigma / S$; $LOQ=10 \times \sigma / S$; Where σ is the standard deviation of y -intercepts of regression lines and S is the slope of the calibration curve.

Robustness: In order to establish the robustness of the method, small deliberated changes were made in the experimental conditions and chromatographic parameters like change in plate activation time, chamber saturation time ($\pm 20\%$ change from set time), volume of mobile phase ($\pm 10\%$ change from set volume) and development distance ($\pm 10\%$ change from set distance).

In the above changed conditions, stock solution was analyzed and results of robustness studies were expressed in term of %RSD of peak areas in each changed condition and were compared with similar results obtained in unchanged experimental conditions.

Application of validated method to pharmaceutical formulation

Finalised HPTLC parameters were kept same as described earlier. Standard stock solution, sample solution and mobile phase were prepared in similar way as described earlier. Procedure for analysis of marketed preparation. 2 microlitre of the sample solutions (100 µg/ml Ambroxol Hydrochloride and 200 µg/ml Doxofylline) was applied on the TLC plate followed by development and scanning. Six replicate of sample solution was applied. From the peak area of the AMB and DOX, amounts of drugs in samples were computed.

Results and Discussion

Method development and optimization of chromatographic conditions

The sensitivity of HPTLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. In the present study mixture of solution of 100 µg/ml of IBU and 200 µg/ml of DOX were prepared in Methanol. A mixed solution was filled in the syringe and under nitrogen stream; it was apply in form of band of having concentration of 100–600 ng/band and 200-1200 ng/band of DOX and DOX respectively on a single plate. Plate was developed using Diethyl ether:n-butanol:ammonia (9:0.9:0.1, v/v/v) and dried in air. Developed plate was subjected to densitometric measurements in scanning mode in the UV region of 200-400 nm and the overlain spectrum was recorded using Camag TLC Scanner 3. The overlain spectra of AMB and DOX revealed that at 254 nm the drug possess significant absorbance (Figure 2).

Validation of the method

Linearity: Linearity was obtained between the concentration range of 100-600 ng/spot for AMB and 200-1200 ng/spot for DOX (Figure 3).

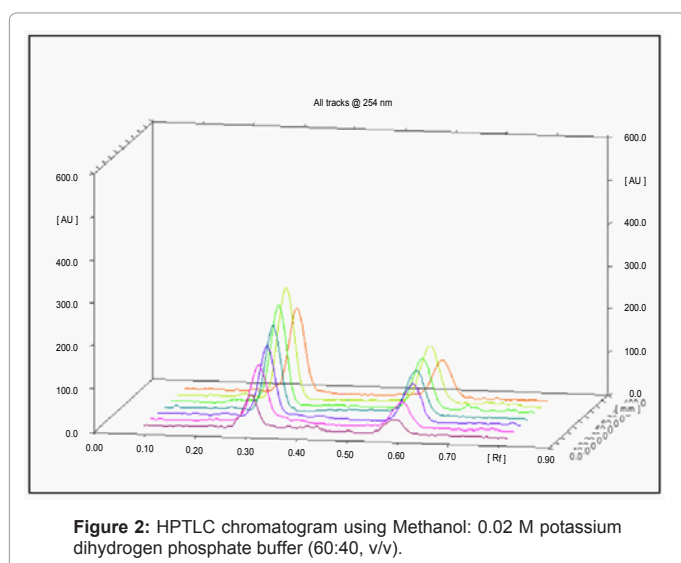
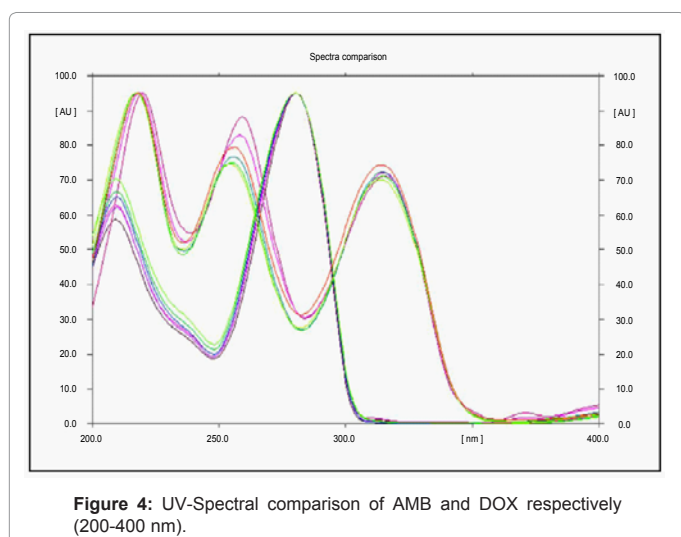
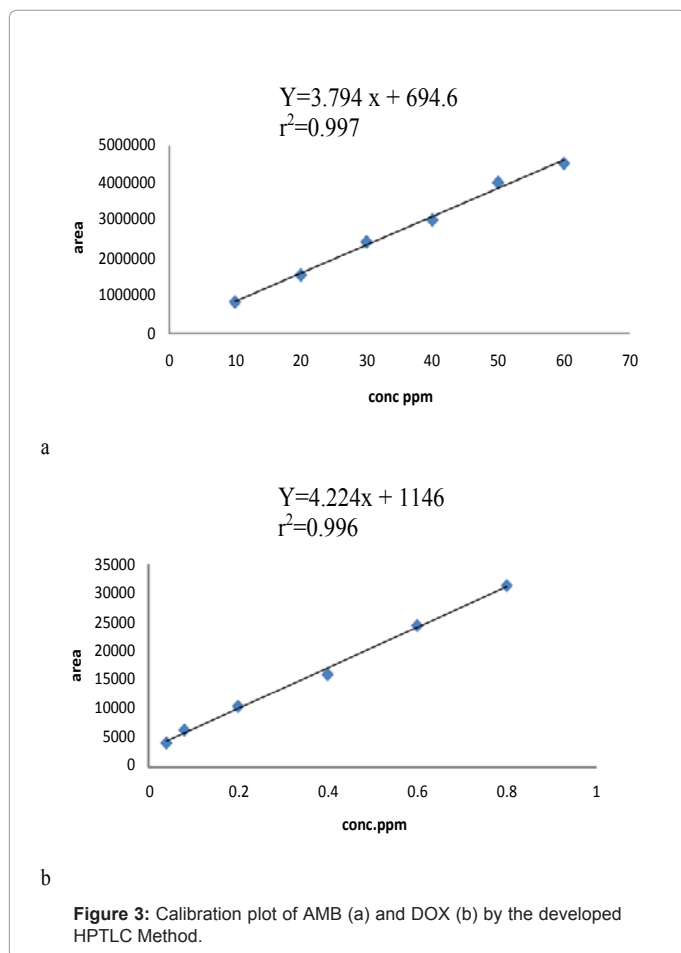


Figure 2: HPTLC chromatogram using Methanol: 0.02 M potassium dihydrogen phosphate buffer (60:40, v/v).



Specificity: Specificity is the ability of an analytical method to determine the analyte unequivocally in the presence of sample matrix. Specificity of the method for AMB and DOX was proved from the spectral scan and peak purity correlation (r) results for AMB and DOX in tablet formulations indicate that there is no co-eluting peak with AMB and DOX, so there is no interference from any excipients present in tablet formulation (Figure 4, Table 1).

Accuracy: Recovery was checked at three concentration level (50%, 100% and 150%). % Recovery for individual and mean value (n=3) at each level was recorded. It should be between 98.0% to 102.0% recovery as per ICH guidelines (Table 2).

Precision: The %RSD values of intraday precision are 0.48-0.74% for AMB and 0.26-0.47% for DOX, and interday 0.71-1.10% for AMB and 0.44-1.20% for DOX, the %RSD values of intraday and interday precision study are within the limit (i.e. <2) reveal that the proposed method is precise as per ICH guidelines Instrument precision was determined by performing injection repeatability test and the RSD values for AMB and DOX were found to be 1.2639% and 0.6226%, respectively. The %RSD for AMB and DOX within the prescribed ICH guideline limit (i.e. <2%) which indicates that the method is precise as per ICH guidelines (Table 3).

Limits of detection (LOD) and Limits of quantification (LOQ): LOD and LOQ for AMB was found to be 14.09 and 42.724 and for DOX the values of LOD and LOQ found to be 8.9005 and 26.9713 respectively (Table 3).

Robustness: Acceptable %RSD values obtained after making small deliberate changes in the developed HPTLC method indicate that the method is robust for the intended purpose (Table 4).

Method application

The proposed validated method was applied for the simultaneous estimation of AMB and DOX in tablet dosage form (Table 5).

Sample	Correlation of center and slope spectra	
	r (s, m)	r (m, e)
AMB	0.995	0.998
AMB in tablet formulation	0.997	0.997
DOX	0.998	0.996
DOX in tablet formulation	0.996	0.996

Table 1: Peak purity correlation results of AMB and DOX in formulation at peak start, middle and end using CAMAG TLC SCANNER.

Amount of sample Taken (µg/ml)		Amount of standard drug added (µg/ml)		Amount of drug recovered (µg/ml)		% recovery ± %RSD (n=3)	
AMB	DOX	AMB	DOX	AMB	DOX	AMB	DOX
0.3	4	0	0	0.3019	3.98	99.14 ± 1.56	99.18 ± 0.91
0.3	4	0.15	2	0.4419	5.99	99.10 ± 0.98	100.26 ± 1.17
0.3	4	0.3	4	0.594	7.84	100.96 ± 1.29	99.17 ± 0.97
0.3	4	0.45	6	0.764	10.21	100.58 ± 1.37	100.96 ± 0.67

Table 2: Results from accuracy study.

Parameters	AMB	DOX
Retention time (min)	5.86	3.60
Tailing factor	0.96	1.42
Resolution	3.23	
Theoretical Plates	9412	8722
Detection limit (µg/ml)	0.02694	0.0732
Quantitation limit (µg/ml)	0.08165	0.2219
Accuracy (%)	99.10-100.96%	99.17-100.96
Precision (%RSD)		
Intra-day (n=3)	1.06-1.69	0.67-1.18
Inter-day (n=3)	1.47-1.84	0.59-1.12
Instrument precision (%RSD)	1.0139	1.0604
Specificity	Specific	Specific

Table 3: Summary of validation parameters of developed HPTLC method.

Parameter	Method condition	% RSD of peak area	
		AMB	DOX
Flow rate	0.8 ml/min	0.87	1.16
	1.2 ml/min	0.94	1.28
Mobile phase ratio 0.02 M KH ₂ PO ₄ :Methanol	(66:34)	1.46	1.29
	(54:46)	1.09	1.56

Table 4: Results from the robustness study of Method.

Formulation TABLET	Label claim (mg)		% of lable claim (n=5) ± % RSD (n=5)	
	AMB	DOX	AMB	DOX
Synasma-Ax	30	400	100 ± 1.02	100 ± 0.63

n=number of determinations

Table 5: Results from analysis of AMB and DOX in the combined tablet dosage form.

Conclusions

This developed and validated method for simultaneous analysis of AMB and DOX in pharmaceutical preparations is very rapid, accurate, and precise. This method was successfully applied for determination of AMB and DOX in its pharmaceutical tablet formulations. Moreover it has advantages of short run time and the possibility of analysis of a large number of samples, both of which significantly reduce the analysis time per sample. Hence this method can be conveniently used for routine quality control analysis of AMB and DOX in its pharmaceutical formulations.

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