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Development and Validation of HPTLC Method for Simultaneous Estimation of Doxofylline and Terbutaline sulphate in Combined dosage form

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ABSTRACT

A simple and sensitive high performance thin layer chromatographic method has been developed and validated for the simultaneous estimation of the Doxofylline and Terbutaline sulphate in combined dosage forms. The stationary phase used was precoated silica gel 60F254 plate. The mobile phase used was a mixture of Chloroform:methanol:ethyl acetate (5:3:2 %v/v/v). The detection of spots was carried out densitometrically using a UV detector at 254 nm in absorbance mode. This system was found to give compact spots for Doxofylline (R_f value of 0.76) and Terbutaline sulphate (R_f value of 0.61). The method was validated in terms of linearity, accuracy, precision, limit of detection, limit of quantification and specificity. The calibration curve was found to be linear between 8000 - 48000 and 100 - 600 ng/spot for Doxofylline and Terbutaline sulphate, respectively with significantly high value of correlation coefficient ($r^2 > 0.99$). The limits of detection and quantitation were found to be 154.41 and 348.61 ng/spot, respectively for Doxofylline and 15.35 and 46.51 ng/spot, respectively for Terbutaline sulphate. The proposed method was found to be accurate, precise, reproducible and specific and can be applicable for the simultaneous determination of Doxofylline and Terbutaline sulphate in tablet dosage form.

Keywords: *Doxofylline, Terbutaline sulphate, HPTLC method, Validation, Specificity.*

INTRODUCTION

HPTLC is a well-known and versatile separation method which shows a lot of advantages in comparison to other separation techniques. A number of enhancements can be made to the basic method of thin layer chromatography to automate the different steps, to increase the resolution achieved and to allow more accurate quantitative measurements. HPTLC is the most simple separation technique today available to the analyst. It can be considered a time machine that can speed your work and allows you to do many things at a time usually not possible with other analytical techniques. HPTLC layer is more homogeneous and thinner resulting in improved resolution, shorter analysis time and suitable for in situ quantification [1, 2].

A fixed dose combination of Doxofylline and Terbutaline sulphate is available for the treatment of asthma. Chemically DOXO known as 7-(1,3-dioxolan-2-ylmethyl)-1,3-dimethyl- 3,7-dihydro-1H-purine-2,6-dione and TBS known as 5-[2-(tert-butylamino)-1-hydroxyethyl]- benzene-1,3-diol sulfate (2:1) (salt). Doxofylline is a new methyl xanthine derivative used in obstructive airway diseases.

Doxofylline has significantly fewer side effects, making the drug immensely beneficial to the patients. Terbutaline sulphate is widely used as a bronchodilator for the treatment of bronchial asthma, chronic bronchitis, and emphysema. Terbutaline sulphate stimulates the α-adrenergic receptors of the sympathetic nervous system and has little or no effect on the adrenergic receptors [3].

In february 2009, a fixed-dose combination of Doxofylline and Terbutaline sulphate was approved by DCG (I) in India. Co-administration of Doxofylline with Terbutaline sulphate gives better bronchodilation with a lower degree of skeletal muscle tremor than a higher dose of terbutaline sulphate by mouth alone. Therefore, a fixed-dose combination of Doxofylline and Terbutaline sulphate is a better alternative for the treatment of acute and chronic asthma.

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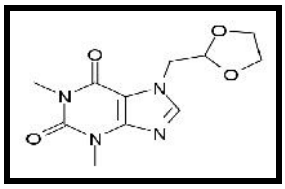

Fig. 1: Structure of Doxofylline

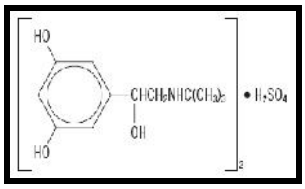

Fig. 2: Structure of Terbutaline sulphate

As per literature review RP-HPLC method, HPLC method and simultaneous equation method using UV spectroscopy have been reported for the estimation of Doxofylline and Terbutaline sulphate in combination of other drugs. No any other HPTLC method published and reported for simultaneous estimation of Doxofylline and Terbutaline sulphate combined dosage form as per the literature survey [4-7].

So, best attempt will be to achieve a simple, sensitive, accurate and cost-effective HPTLC method of Doxofylline and Terbutaline sulphate in their combined dosage form and to validate the as per ICH guidelines [8].

MATERIALS AND METHODS

Materials:

Pure Doxofylline was obtained from Ami pharmaceutical, vadodara and pure Terbutaline sulphate was obtained from Brundavan Laboratories, Hyderabad as a gift sample. Methanol, sodium hydroxide, Chloroform, trietylamine, ethyl acetate were purchased from Merck Chemicals, India. Tablet formulations, namely OXOBIT-TR and PHYLEX-TR (Lexus) were purchased from a local market. The marketed formulations have a composition of 400 mg of Doxofylline, 5 mg of Terbutaline sulphate and excipients (q.s).

Instrument:

A Camag HPTLC system (Switzerland) comprising of Camag Linomat V semiautomatic sample applicator, Camag TLC Scanner 3, Camag (Muttenz, Switzerland) flat bottom and twin-trough developing chamber (10x10 cm), UV cabinet with dual

wavelength UV lamp, Camag win CATS software, Hamilton syringe (100 µl), analytical balance (Shimadzu), Ultrasonic bath (Frontline FS-4, Mumbai, India) were used in the study.

Preparation of mobile phase: [9]

A mixture of Chloroform:methanol:ethyl acetate (5:3:2 v/v/v) previously filtered through 0.45 µm filter paper in a flask was used as mobile phase.

Preparation of standard solution:

800 mg of DOXO (16000 µg/ml) and 10 mg TBS (200 µg/ml) were dissolved and diluted with 50 ml mixture of methanol (25 ml) and water (25 ml)

Preparation of sample solution:

20 capsules (OXOBIT-TR) were weighed and powdered. Powder equivalent to 800 mg of DOXO and 10 mg of TBS transferred into 50 ml volumetric flask. Methanol (25 ml) and water (25 ml) were added to adjust level up to mark and sonicated for 30 min. The solution was filtered through whatman filter paper no. 42, discard in first few ml of filtrate and this sample solution used for estimation.

Determination of Wavelength for measurment:

The sensitivity of HPTLC method that uses UV detector depends upon the wavelength of detection. An ideal wavelength is the one that gives measurable response for the drugs. In the present study individual standard drug solution of DOXO (8000-48000 ng/spot) and TBS (100-600 ng/spot) were prepared in water and methanol mixture. These drug solutions were then scanned in the UV region of 200 - 400 nm and the overlain spectrums were recorded . At 254 nm both drugs have considerable absorbane. So, 254 nm was selected as a detection wavelength for estimation of DOXO and TBS.

HPTLC Calibration curve of DOXO and TBS:

Analysis was performed on pre-coated silica gel aluminium plate 60F254 (20 ×10 cm with 0.2 mm thickness) pre-washed with methanol and then dried for 30 minutes at 50°C. From the standard solution (0.5, 1, 1.5, 2, 2.5, and 3µl) were spotted on TLC plate under nitrogen stream using Linomat-V Applicator.
The plate was dried in air and then the plate was developed in twin through developing chamber (20 ×10 cm) with stainless lead previously saturated with the mobile phase with 20 min. The plate was removed from chamber and dried in air and was scanned from 400-200 nm in excitation mode with Camag TLC scanner having software Proquant. The spectra were overlayed. Both the drugs have significant absorption at 254 nm so it was selected as wavelength for measurement. The peak areas were quantified at 254 nm in excitation mode for each lane at R_f 0.76 for DOXO and 0.61 for TBS. Calibration curve of peak areas vs concentrations were plotted and correlation coefficient and regression line equations were determined for DOXO and TBS.

Chromatographic conditions:

The experiment was performed on silica gel 60F254 aluminum sheets (10 x 10 cm) as stationary phase, using mobile phase comprised of Chloroform : methanol : ethyl acetate (5:3:2 v/v/v). TLC plates were prewashed with methanol and activated in an oven at 50°C for 5 min prior to chromatography. The solutions were applied on TLC plate in the form of bands of 6 mm width under a stream of nitrogen gas using a Camag Linomat V automatic sample applicator. A constant application rate of 0.1 ml/s was employed and space between two bands was fixed at 5mm. Ascending development to 80 mm was performed in 10 cm x 10 cm Camag twin trough glass chamber (Muttenz, Switzerland) saturated with the mobile phase for 30 min at room temperature. The developed TLC plate was air dried and then scanned between 200 to 400 nm using Camag TLC scanner 3 using Win CATS software. Both components show reasonably good response at 254 nm keeping the slit dimension of 5 x 0.45 mm and scanning speed of 10 mm/s. The monochromatic band width was set at 20 nm, each track was scanned thrice and baseline correction was used. 6µl of standard and sample solutions of DOXO and TBS were spotted and developed.

Chromatographic separation:

6µl of mixed standard solution of DOXO and TBS was applied on TLC plate under nitrogen stream using semiautomatic spotter. The experiment was performed on silica gel 60F254 aluminum sheets (10 x 10 cm) as stationary phase, using mobile phase comprised of Chloroform:methanol:ethyl acetate (5:3:2 v/v/v). TLC plates were prewashed with methanol and activated in an oven at 50° for 5 min prior to chromatography. The sample solutions were applied on TLC plate in the form of bands of 6 mm width under a stream of nitrogen gas using a Camag Linomat V automatic sample applicator. A constant application rate of 0.1 ml/s was employed and space between two bands was fixed at 5 mm. Ascending development to 80 mm was performed in 10 cm x 10 cm Camag twin trough glass chamber (Muttenz, Switzerland) saturated with the mobile phase for 30 min at room temperature. The developed TLC plate was air dried and then scanned between 200 to 400 nm using Camag TLC scanner 3 using WinCATS software. Both components show reasonably good response at 254 nm keeping the slit dimension of 5 x 0.45 mm and scanning speed of 10 mm/s. The monochromatic band width was set at 20 nm, each track was scanned thrice and baseline correction was used.

Method validation:

Linearity and range:

The linearity of response in the concentration range of 8000-48000 ng/spot of DOXO and 100-600 ng/spot of TBS were determined as plot of spot area vs. concentration.

Precision:

Variations of results within the same day (intraday), variation of the results between days (inter day) were analyzed and its %RSD for each observation was calculated.
For Intraday and Interday precision, Synthetic mixture of drugs containing DOXO (ng/spot) and TBS (ng/spot) equivalent to 24000 DOXO:300 TBS, 32000 DOXO:400 TBS, 40000 DOXO :500 TBS were determined 3 times a day interval of 1 hour and different day, simultaneously and %RSD was calculated.
For Intraday and Interday precision, three levels of assay were selected. At each level three times assay was carried out and result was expressed as %RSD.

Accuracy:

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. Percentage recovery for DOXO and TBS were found out.

Recovery between 98%-102% justifies the accuracy of the method.

Accurately weighed quantity of pre analysed tablet 1600 mg of DOXO and 20 mg of TBS was taken in 25 volumetric flask.
To above flask API of both drug in 50%, 100%, and 150% were added and continued assay procedure.

Limit of Detection:

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value particularly important for limit tests.

$$\text{LOD: } 3.3\ \sigma/S$$

Where,
σ = standard deviation of intercept and it was calculated from the equation,
S = Slope obtained from calibration curve

Limit of Quantification:

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy

$$\text{LOQ: } 10\ \sigma/S$$

Specificity:

Specificity is carried out by taking peak purity of standard and sample of each drug and standard and sample peak spectra were overlain to check specificity of each individual drug peak. The peak purity for DOXO and TBS was tested by correlation of spectra acquired at the peak start (s), peak maximum (m), and peak end (e) positions which found pass for DOXO and TBS. The correlation spectra of sample were compered with that of standard.

RESULTS AND DISCUSSION

A simple, economic, precise, accurate method for simultaneous estimation of Doxofylline and Terbutaline sulphate was developed. This developed method was validated according to ICH guidelines.

The TLC procedure was optimized with a view to develop

an assay method for the simultaneous estimation of DOXO and TBS. The standard solutions of both the drugs were spotted on the TLC plates and run in different solvent systems. The mobile phase consisting of Chloroform:methanol:ethyl acetate (5:3:2 v/v/v) gave sharp and symmetrical peaks with the R_f values 0.76 of and 0.61 for DOXO and TBS, respectively. Well defined spots were obtained when the chamber was saturated with mobile phase for 30 min at room temperature (27 ± 3^{0}C). A combined densitogram of mixed standards and 3-D chromatogram showing peaks of DOXO and TBS in different concentrations at 254 nm are depicted in Fig. 3 & 4, respectively.

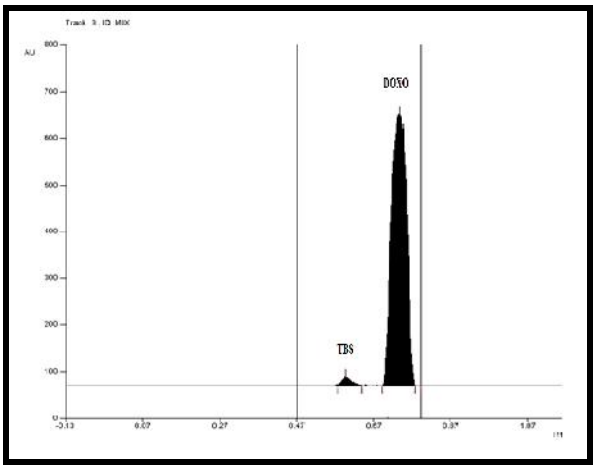

Fig. 3: Densitogram of Doxofylline (24000 ng/spot) and Terbutaline sulphate (300 ng/spot)

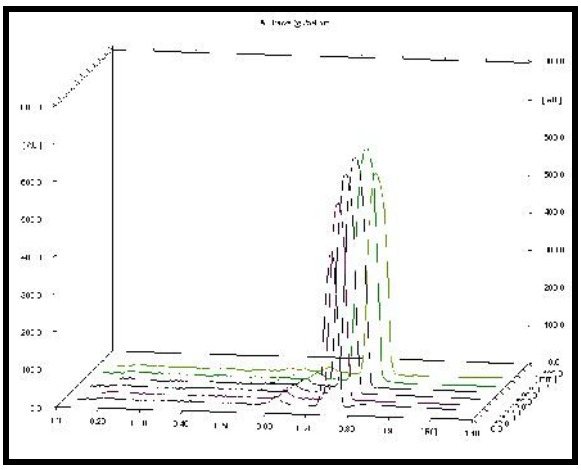

Fig. 4: Overlain 3D spectra of all tracks

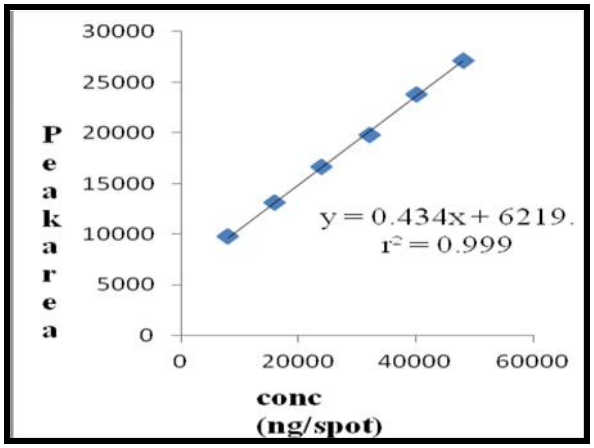

Fig. 5: Calibration curve of DOXO (8000 – 48000 ng/spot) in mobile phase

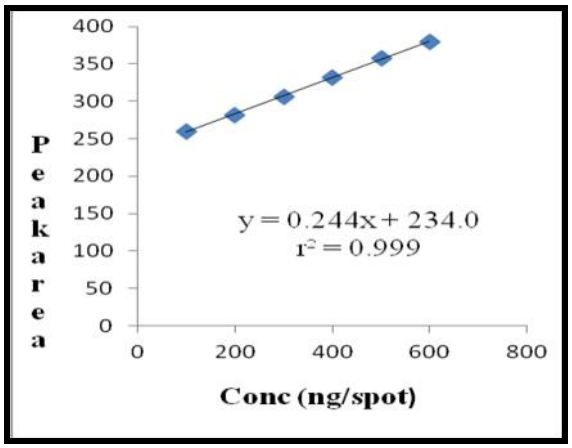

Fig. 6: Calibration curve of TBS (100 - 600 ng/spot) in mobile phase

Table No. 1: Results of Accuracy

		DOXO			TBS		
Amount Taken (ng/spot)			16000			200	
Amount Added	**%**	50	100	150	50	100	150
	ng/spot	8000	16000	24000	100	200	300
% Recovery*		100.80± 2.8	100.4± 2.5	100.3± 0.37	100.4± 2.1	101.7± 0.8	101.7± 1.4
Mean Recovery ±S.D.			100.5±0.1			101.23±0.866	

*Average of three determinations

Table No. 2: recovery data of marketed formulation

Formulation	Tablet content taken(ng/spot)		Amount found (ng/spot)		Assay %estimated (n=3 MEAN±SD)	
	HPTLC Method					
Oxobit-tr	**DOXO**	**TBS**	**DOXO**	**TBS**	**DOXO**	**TBS**
	16000	200	15897.6	204.06	99.36±1.5	102.03±1.14

Table No. 3: Results of validation parameters for HPTLC method

PARAMETER	DOXOFYLLINE	TERBUTALINE SULPHATE
LINEARITY AND RANGE	8000-48000 ng/spot	100-600 ng/spot
r^2 DATA	0.999	0.999
Regression equation	y=0. 434x + 6219	y= 0.244x + 234.0
R_f value	0.76	0.61

PRECISION (% RSD)		
INTRADAY PRECISION	1.28	0.43
INTERDAY PRECISION	1.52	1.49
ACCURACY	98-103.3%	98.27-102.5%
LOD (ng/spot)	154.41	15.35
LOQ (ng/spot)	348.61	46.51
SPECIFICITY	Pass	Pass
CO-RELATION R(S,M)	0.999893	0.999821
CO-RELATION R(M,E)	0.999459	0.998320
ASSAY	99.36±1.5	102.03±1.14

The proposed HPTLC method was validated in terms of linearity, precision, accuracy, LOD, LOQ and specificity. The calibration plot was found to be linear over the concentration range 8000- 48000 and 100-600 ng/spot for DOXO and TBS, respectively with a correlation coefficient of 0.999 and 0.999 for DOXO and TBS, respectively. LOD for DOXO and TBS were found to be 154.41 ng/spot and 15.31 ng/spot, respectively. LOQ for DOXO and TBS were found to be 348.61 ng/spot and 46.51 ng/spot, respectively indicate the sensitivity of the method. The low % RSD values of intraday (1.28 for DOXO and 0.43 for TBS) and interday (1.52 for DOXO and 1.49 for TBS) precision reveals that the proposed method is precise. Relative standard deviation for repeatability of measurements is less than 2%, which indicates that the proposed method is repeatable. To study the accuracy of the method, recovery studies were performed. The percent average recoveries obtained were 98-103.3%and 98.27-102.5% for DOXO and TBS, respectively indicating that the proposed HPTLC method is highly accurate. The proposed validated method was successfully applied to determine DOXO and TBS in tablet dosage forms. The percent average assay was found to be 99.36±1.5 and be 102.03± 1.14 for DOXO and TBS, respectively (Table 2). The low values of standard deviation indicate the suitability of this method for routine analysis of DOXO and TBS in pharmaceutical dosage forms. To confirm the specificity of the proposed method, the solution of formulation was spotted on TLC plate, developed and scanned. It was observed that the excipients present in the formulation did not interfere with the sample peak.

CONCLUSION

The proposed HPTLC method is simple, rapid, accurate, precise, and economic and validated in terms of linearity, accuracy, precision, specificity and reproducibility. This method can be successfully used for simultaneous estimation of Doxofylline and Terbutaline sulphate in pure and tablet dosage form.

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