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Development and validation of UV Spectroscopic Method for Simultaneous Estimation of Doxofylline and Terbutaline sulphate in combined Dosage form

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ABSTRACT

Two simple spectrophotometric methods have been developed for simultaneous estimation of Doxofylline and Terbutaline sulphate in tablet dosage form. The methods is developed using 0.1M NaOH as a solvent. Method (I) is the Q- absorbance ratio method, two wavelengths are selected, one being the isoabsorptive point 294.1 nm and other being the absorption maxima of Doxofylline at 273 nm. Method (II) is the Absorbance correction method, two wavelengths are selected, one being absorption maxima of Doxofylline at 273 nm and other being the corrected absorbance at 302.96 nm. the Linearity was observed in the concentration range of 10-50 µg/ml for Doxofylline and 2-8 µg/ml for Terbutaline sulphate respectively, correlation coefficient ($r^2 < 1$). The accuracy and precision were determined and found to comply with ICH guidelines. Both the methods showed good reproducibility and recovery with % RSD in the desired range. The proposed methods can be successfully applied for the routine analysis of both the drugs from tablet dosage form.

Keywords: *Doxofylline, Terbutaline sulphate, Q- absorbance ratio method, Absorption correction, Beer–Lambert's law, UV visible spectrophotometric.*

INTRODUCTION

Spectrophotometric methods are a large group of analytical methods that are based on atomic and molecular spectroscopy. Spectroscopy is a branch of science dealing with the study of interaction of electromagnetic radiation with matter. The kind and amount of radiation absorbed by the molecule depends on the number of molecule interacting with the radiation [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].

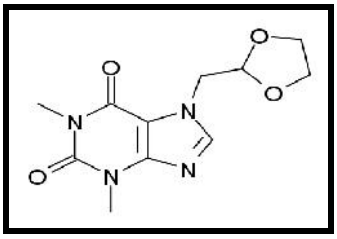

Fig. 1: Structure of Doxofylline

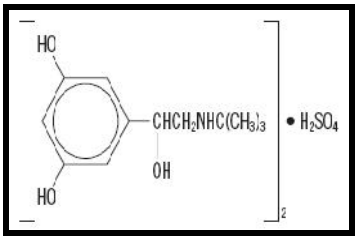

Fig. 2: Structure of Terbutaline sulphate

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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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 spectroscopic 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 UV spectrophotometric methods (Q-absorbance ratio method and absorbance correction 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 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:

UV-visible double beam spectrophotometer (Shimadzu, 1800, Japan) with matched quartz cells corresponding to 1 cm path length.

Preparation of 0.1N NaOH: [9]

0.1N NaOH was prepared by dissolving 4 gm Sodium Hydroxide pellets in 1000 ml volumetric flask using distilled water and then volume was make up by distilled water.

Preparation of standard stock solution:

Standard stock solutions (1000 µg/ml) of DOXO and TBS were prepared separately by dissolving 100 mg of DOXO and TBS, respectively in 100 ml 0.1N NaOH.

Preparation of working standard solution of Doxofylline and Terbutaline sulphate:

100 µg/ml of DOXO and TBS solution was prepared by diluting 10 ml stock solution up to 100 ml with 0.1 N NaOH.

The solutions were prepared by pipetting out 1, 2, 3, 4, 5 ml of the working standard solution of DOXO (100 µg/ml) and 0.2, 0.4, 0.6, 0.8, 1 ml of the working standard solution of TBS (100 µg/ml) into a series of 10.0 ml volumetric flasks.

This series consisted of five concentrations of standard DOXO solution ranging from 10 – 50 µg/ml and TBS solution ranging from 2- 10 µg/ml.

Preparation of sample solution:

Twenty tablets were weighed and powdered. Powder equivalent to 80 mg of DOXO was weighed and transferred into a 100 ml of volumetric flask, volume adjusted up to mark with 0.1 N NaOH.

The mixture sonicated for 10 minutes and filtered through Whatman filter paper no.42, discarding first few ml of filtrate. 1ml of this filtrate diluted to 10 ml with 0.1 N NaOH Further 1 ml diluted to 10 ml of 0.1 N NaOH.

The solution was scanned against 0.1 N NaOH as blank in a range of 200-400 nm with medium scan speed. The spectrum was obtained. The concentration of DOXO and TBS can be obtained by using equation of straight line.

Calibration Curve Procedure:

Aliquots of standard stock solutions of DOXO and TBS were taken in volumetric flasks and diluted with 0.1N NaOH to get final concentrations in range of 10-50 µg/ml for DOXO and 2-8 µg/ml of TBS. The solution was scanned in the range of 200 to 400 nm against 0.1N NaOH as blank, the wavelengths were found to be 273 nm λmax of DOXO and iso-absorptive point of DOXO and TBS at 294.1nm for Method I and 273 nm λmax of DOXO and corrected absorbance of TBS at 302.96 nm for method Method II

Method I:

Q- absorbance ratio method:

Absorbance ratio method uses the ratio of absorbances at two selected wavelengths one at iso-absorptive point and other being the λmax of one of the two components. In Absorbance ratio method overlain spectra showed that,

DOXO and TBS shown isobestic absorbance point at 294.1 nm wavelength. Weres TBS and DOXO has same absorbance at 273 nm which is λmax of DOXO, at this wavelength, both drugs shows considerable absorbance. So selected wavelength were 273 nm and 294.1 nm.

Relative concentration of two drugs in the sample was calculated using following equations.

$$Cx = [(Q_M - Qy)/ (Qx - Qy)] \times A_1/ax_1$$
$$Cy = [(Q_M - Qx)/ (Qy - Qx)] \times A_1/ay_1$$

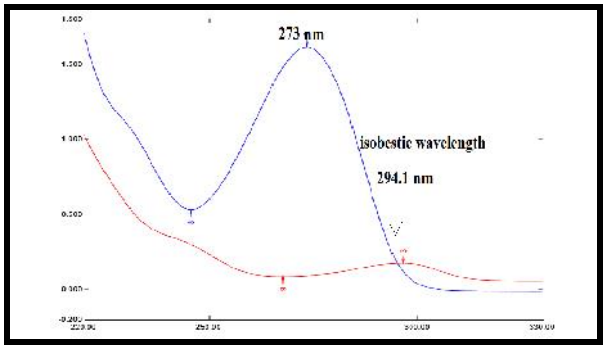

Fig. 3: Overlain uv spectra of Doxofylline (50 µg/ml) and Terbutaline sulphate (10 µg/ml)

Method II:

Absorbance correction method:

In absorbance correction method overlain spectra showed that,

DOXO has zero absorbances at 302.96 nm Weres TBS has substantial absorbance.Thus TBS was estimated directly at 302.96 nm without interference of DOXO.

At 273 nm which is λmax of DOXO, at this wavelength, both drugs shows considerable absorbance. So selected wavelength were 273 nm and 302.96 nm

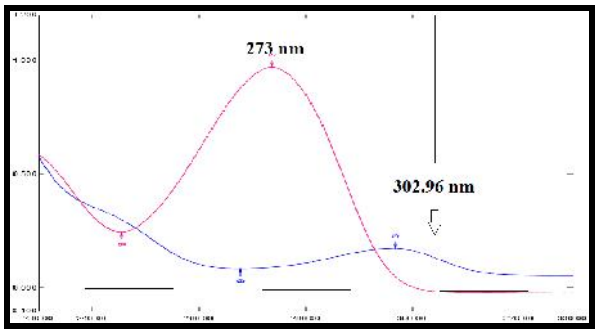

Fig. 4: Overlain uv spectra of Doxofylline (50 µg/ml) and Terbutaline sulphate (10 µg/ml)

Method validation:

Linearity and range:

The linearity of response was determined in concentration range of 10-50 µg/ml DOXO and 2-10 µg/ml TBS. The calibration curve of absorbance vs concentration plotted, correlation coefficient and regression line equations for DOXO and TBS were obtained using Microsoft excel. Linearity is expressed in terms of correlation co-efficient of linear regression line.

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 (µg/ml) and TBS (µg/ml) equivalent to 20 DOXO:4 TBS, 30 DOXO:6 TBS, 40 DOXO:8 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 80 mg of DOXO and 1 mg of TBS was taken in 100 volumetric flask.To above flask API of both drug in 80%, 100%, and 120% were added and continued assay procedure.

LOD & LOQ:

The sensitivity of the method was determined with

respect to limit of detection (LOD) and limit of quantitation (LOQ). The LOD was calculated by below eq.

LOD: 3.3 σ/S
LOQ: 10 σ/S

Linearity and range:

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.

For Method I

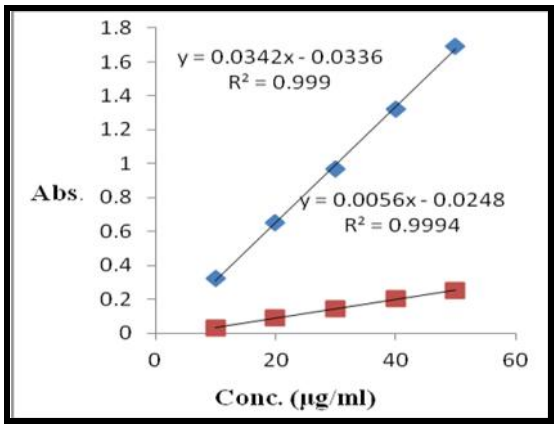

Fig. 5: Calibration curve for Doxofylline (10-50µg/ml) at 273 nm and 294.1 nm

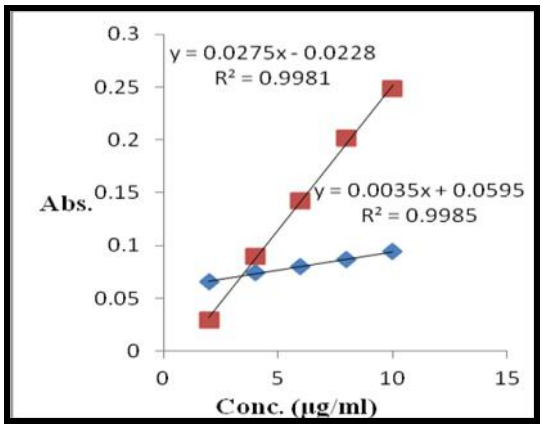

Fig. 6: Calibration curve for Terbutaline sulphate(2-10 µg/ml) at 273 nm and 294.1 nm

For Method II

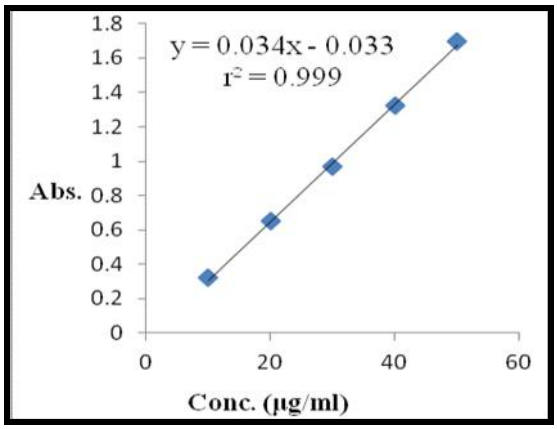

Fig. 7: Calibration curve for Doxofylline (10-50 µg/ml) at 273nm

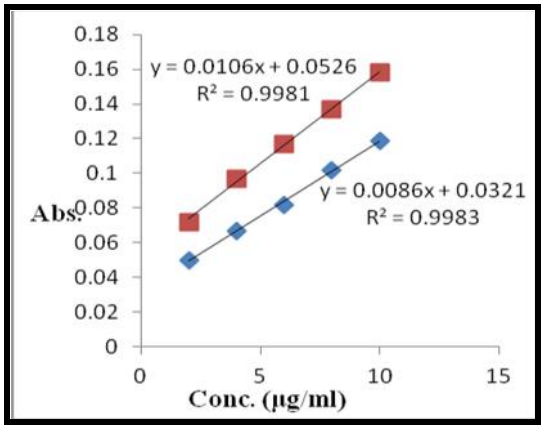

Fig. 8: Calibration curve for Terbutaline sulphate (2-10 µg/ml) at 273 nm and 302.93 nm

Table No. 1: Optical parameters & regression characteristic for Doxofylline and Terbutaline sulphate

Parameters	Method I				Method II		
	DOXO		TBS		DOXO	TBS	
	273 nm	294.1 nm	273 nm	294.1 nm	273 nm	273 nm	302.93nm
r^2	0.999	0.999	0.998	0.998	0.999	0.998	0.998
Equation	Y=0.034X -0.033	Y=0.005X+ 0.024	Y=0.003X +0.059	Y=0.027X+ 0.022	Y=0.034 X-0.033	Y=0.008X +0.032	Y=0.010X+ 0.05
Absorptivity	321.4	45.37	171	229.01	321.43	162.43	218.84

Table No. 2: Results of Accuracy

		Method I						Method II					
		DOXO			TBS			DOXO			TBS		
Amount Taken (µg/ml)		80			1			80			1		
Amount Added	%	80	100	120	80	100	120	80	100	120	80	100	120
	µg/ml	64	80	96	0.8	1	1.2	64	80	96	0.8	1	1.2
% Recovery*		100.± 0.9	100.90 ±1	100.37 ±2	100.08 ±1	101.93 ±1	100.66 ±1	101.41 ±1	99.9± 2	100.8 ±2	101± 1.75	100.5 ±1	100.1 ±2
Mean Recovery ±S.D.		100.42.±0.2			100.89.±1.2			100.56.±1.6			100.53.±1.1		

*Average of three determinations

Table No. 3: Intraday Precision and Interday Precision

Method	Drug	Precision %R.S.D.	
		Intraday n=3	Interday*
I	DOXO	1.28	1.36
	TBS	1.43	1.35
II	DOXO	1.12	1.52
	TBS	1.13	1.49

*Average of three determinations

Table No. 4: LOD and LOQ

	Method I		Method II	
	DOXO	TBS	DOXO	TBS
LOD	0.16 µg/ml	0.41µg/ml	0.16µg/ml	0.19 µg/ml
LOQ	0.48 µg/ml	1.27 µg/ml	0.48 µg/ml	0.58 µg/ml

Table 5: recovery data of marketed formulation

Formulation	Tablet content taken(mg)		Amount found(mg)		Assay % estimated (n=3 MEAN±SD)	
	Method I					
	DOXO	TBS	DOXO	TBS	DOXO	TBS
Oxobit-tr	80	1	81.32	1.02	101.65 ±0.95%	102.3 ±0.74%
	Method II					
Oxobit-tr	80	1	79.81	1.02	99.76 ±0.94%	102 ±0.84%

CONCLUSION

The proposed spectrophotometric methods are simple, rapid, accurate, precise, and economic and validated in terms of linearity, accuracy, precision, specificity and reproducibility. These two methods can be successfully used for simultaneous estimation of Doxofylline and Terbutaline sulphate in pure and tablet dosage form.

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