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Research Article

VALIDATED HPLC METHOD FOR SIMULTANEOUS QUANTITATION OF DOXOFYLLINE AND TERBUTALINE SULPHATE IN BULK DRUG AND FORMULATION

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ABSTRACT

A simple, precise and accurate HPLC method was developed for estimation of Doxofylline (DO) and Terbutaline Sulphate (TS) in formulation. HPLC separation was achieved with Thermo Hypersil BDS–C_{18} (250 mm × 4.6 mm, 5.0 µ) with isocratic conditions and simple mobile phase containing methanol: Aq. phosphate buffer (pH- 4.55) (90:10 v/v) at flow rate of 1 mL/min using UV detection at 282nm. The retention time of DO and TS were found to 2.925 and 4.233 min respectively. The developed method was validated as per ICH guidelines.

Keywords: Doxofylline, Terbutaline Sulphate, HPLC, Validation

INTRODUCTION

Doxofylline, (7-(1, 3-dioxalan-2-ylmethyl) theophylline [1] **(Fig 1)**, is a new generation long acting oral methyl xanthine derivative. It is bronchodilator and plays a direct role in bronchial relaxation of bronchial smooth muscle. Methyl xanthines are phosphodiesterase inhibitors. It is mainly used for maintenance therapy in patients suffering with Asthma and Chronic Obstructive Pulmonary Disease (COPD) [2]. Doxofylline by inhibiting the phosphodiesterase within the smooth muscle cells and cause smooth muscle relaxation, thus achieving suppression of asthma. It is a novel bronchodilator xanthine that differs from theophylline because the presence of a dioxalane group in position C-7. Similarly to theophylline, its mechanism of action is related to the inhibition of phosphodiesterase activities, but in contrast it appears to have decreased affinities towards adenosine A_1 and A_2 receptors However, differently from theophylline, doxofylline appears to have decreased affinities toward adenosine A1 and A2 receptors which may account for the better safety profile of the drug[6] .

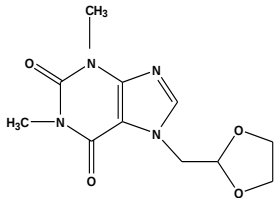

Figure1: Structure of Doxofylline

Terbutaline Sulphate, 2-tert-Butylamino-1-(3, 5-dihydroxyphenyl) ethanol sulphate **(Fig 2)**, is a Bronchodilator; relatively selective, short-acting β_2-adrenergic receptor agonist[3]. It produces bronchodilation by relaxing bronchial smooth muscle through β_2 receptor stimulation[4]. Terbutaline is used as a fast-acting bronchodilator and as a tocolytic to delay premature labor. Terbutaline sulfate is a directacting sympathomimetic with mainly beta-adrenergic activity and a selective action on $beta_2$ receptors. Terbutaline is given as the sulfate for its bronchodilating properties in reversible airways obstruction, as occurs in asthma and in some patients with chronic obstructive pulmonary disease. It also decreases uterine contractility and may be used to arrest premature labour. Current asthma guidelines recommend that inhaled short-acting $beta_2$ agonists such as Terbutaline be used on an 'as-required', not regular, basis. In those patients requiring more than occasional use of Terbutaline, anti-inflammatory therapy is also needed. An increased requirement for, or decreased duration of effect of, Terbutaline indicates deterioration of asthma control and the need for increased anti-inflammatory therapy[5].

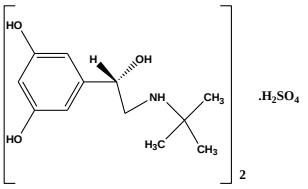

Figure 2: Structure of Terbutaline Sulphate

Literature review reveals that methods have been reported for analysis of Doxofylline and Terbutaline Sulphate, Doxofylline exerts a prophylactic effect against bronco constriction and pleurisy induced by PAF[7], spectrophotometric determination of Doxofylline in Tablet Formulation[8,-11], development and validation of a sensitive LCMS/MS method with Electro spray Ionization for quantitation of Doxofylline in human serum[12], method development and degradation studies of Doxofylline by RP-HPLC and LC- MS/MS[13-14], development and validation of a stability-indicating RP-HPLC method for analysis of Doxofylline in Human Serum[15], non-extraction HPLC method for simultaneous measurement of Dyphylline and Doxofylline in serum[16], simultaneous estimation of Doxofylline and its combinations by RP-HPLC method from solid dosage forms [17-21], HPTLC methods for determination of Doxofylline in bulk and formulations[22-23], stability and compatibility of Doxofylline with Phentolamine Mesilate in 0.9% sodium chloride or 5% dextrose injection for intravenous infusion[24] .

Spectrophotometric simultaneous analysis of Ambroxol Hydrochloride, Guaifenesin and Terbutaline Sulphate in liquid dosage form such as syrup[25], RP-HPLC and stability indicating HPLC methods for simultaneous determination of Terbutaline alone and with combinations[26-29], simultaneous determination of Terbutaline Sulphate with other drugs in tablet formulation by UV Spectrophotometry[30], stability indicating HPTLC method for determination of Terbutaline Sulphate in bulk and from submicronised dry powder inhalers[31].

To date, there have been no published reports about the simultaneous quantitation of Doxofylline and Terbutaline Sulphate by HPLC in bulk drug and in tablet dosage form. This present study reports for the first time simultaneous quantitation of Doxofylline

and Terbutaline Sulphate by HPLC in bulk drug and in tablet dosage form. The proposed method is validated as per ICH guidelines.

EXPERIMENTAL

Materials

Cipla Ltd. Kurkumbh (Pune). India, kindly supplied pure drug sample of Doxofylline as a gift sample of and Blue Cross Pharmaceuticals Pvt. Ltd Nasik supplied pure drug sample of Terbutaline Sulphate. They were used without further purification. Double Distilled water was generated in house. All chemicals and reagents used were of HPLC grade and were purchased from Merck Chemicals, India.

Instrumentation

The HPLC system consisted of a Pump (model Jasco PU 2080), Intelligent LC pump with sampler programmed at 20 µL capacity per injection was used. The detector consisted of UV/ VIS (Jasco UV 2075) model operated at a wavelength of 282nm. Data was integrated using Jasco Borwin version 1.5, LC-Net II/ADC system. The column used was Thermo Hypersil BDS–C_{18} (250 mm × 4.6 mm, 5.0 µ) from Germany.

Preparation of standard stock solutions

Standard stock solution of concentration 1000 µg/mL of Doxofylline and 1000 µg/mL of Terbutaline Sulphate was prepared using methanol. From the standard stock solution, the mixed standard solutions were prepared using to contain 10 µg/mL of Doxofylline and 10 µg/mL of Terbutaline Sulphate. The stock solution was stored at 2-8 °C protected from light.

Optimization of HPLC method

The HPLC procedure was optimized with a view to develop a simultaneous assay method for Doxofylline and Terbutaline Sulphate respectively. The mixed standard stock solution (10 µg/mL of Doxofylline and 10 µg/mL of Terbutaline Sulphate) was injected in HPLC. For optimization of this method different ratios of methanol and aqueous phosphate buffer (10mM of Potassium Dihydrogen Phosphate of pH-4.55) were tried but it was found that methanol: aqueous phosphate buffer in the ratio 90: 10 v/v, at flow rate 1 mL/min gives acceptable retention time (t_R), plates and good resolution for Doxofylline and Terbutaline Sulphate Fig 3.

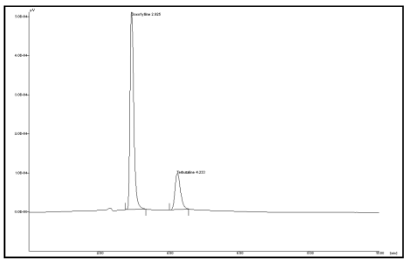

Figure 3: HPLC chromatogram of standard Doxofylline and Terbutaline Sulphate (10 µg/mL and 10 µg/mL)

VALIDATION OF THE METHOD:

Validation of the optimized HPLC method was carried out with respect to the following parameters.

Linearity and Range

The mixed standard stock solution (10µg/mL of Doxofylline and 10 µg/mL of Terbutaline Sulphate) was further diluted to get Doxofylline and Terbutaline Sulphate concentration in the range of 3-8 µg/mL and 4-9 µg/mL respectively. Linearity of the method was studied by injecting six concentrations of the drug prepared in the mobile phase in triplicate into the LC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

Precision

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations 3, 5, 7 µg/mL for Doxofylline and 4, 6, 8 µg/mL for Terbutaline Sulphate six times on the same day. The intermediate precision of the method was checked by repeating studies on three different days.

Limit of detection and Limit of quantitation

Limits of detection (LOD) and quantification (LOQ) represent the concentration of the analyte that would yield signal-to-noise ratios of 3 for LOD and 10 for LOQ, respectively. To determine the LOD and LOQ, serial dilutions of mixed standard solution of Doxofylline and Terbutaline Sulphate was made from the standard stock solution. The samples were injected in LC system and measured signal from the samples was compared with those of blank samples.

Robustness

To evaluate robustness of a HPLC method, few parameters were deliberately varied. The parameters included variation of flow rate, percentage of methanol in the mobile phase and solvents from different lot were taken. Robustness of the method was done at three different concentration levels 3, 5, 7 µg/mL and 4, 6, 8 µg/mL for Doxofylline and Terbutaline Sulphate respectively.

Specificity

The specificity of the method towards the drug was established through study of resolution factor of the drug peak from the nearest resolving peak. The peak purity of Doxofylline and Terbutaline Sulphate was determined by comparing the spectrum at three different regions of the spot i.e. peak start (S), peak apex (M) and peak end (E). Effect of excipients of formulation was studied for whether it interfered with the assay.

Accuracy

Accuracy of the method was carried out by applying the method to drug sample (Doxofylline and Terbutaline Sulphate combination tablet) to which know amount of Doxofylline and Terbutaline Sulphate standard powder corresponding to 80, 100 and 120 % of label claim had been added (Standard addition method), mixed and the powder was extracted and analyzed by running chromatogram in optimized mobile phase.

Analysis of a marketed formulation

To determine the content of Doxofylline and Terbutaline Sulphate in conventional tablet (Brand name: Mucosma-T, Label claim: 400 mg Doxofylline and 5 mg Terbutaline Sulphate per tablet), twenty tablets were weighed, their mean weight determined and finely powdered. The weight of the tablet triturate equivalent 400 mg Doxofylline and 5 mg Terbutaline Sulphate was transferred into a 100 mL volumetric flask containing 60 mL methanol, sonicated for 30 min and diluted up to 100 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min and the drug content of the supernatant was determined (4000 µg/mL and 50 µg/mL for Doxofylline and Terbutaline Sulphate respectively). Supernatant was taken and after suitable dilution the sample solution was then filtered using 0.45-micron filter (Millipore, Milford, MA). The above stock solution was further diluted to get sample solution of 4 µg/mL and 5 µg/mL for Doxofylline and Terbutaline Sulphate respectively. A 20 µl volume of sample solution was injected into HPLC, six times, under the conditions described above. The peak areas were measured at 282 nm and concentrations in the samples were determined using multilevel calibration developed on the same HPLC system under the same conditions using linear regression equation.

RESULTS AND DISCUSSION

The results of validation studies on simultaneous estimation method developed for Doxofylline and Terbutaline Sulphate in the current study involving methanol: aqueous phosphate buffer (90: 10, v/v) (10Mm Potassium Dihydrogen Phosphate pH- 4.55) are given below.

Linearity

Doxofylline and Terbutaline Sulphate showed good correlation coefficient (r^2 = 0.9994 for Doxofylline and 0.9998 for Terbutaline Sulphate) in given concentration range (3-8 µg/mL for Doxofylline and 4-9 µg/mL for Terbutaline Sulphate). The mean values of the slope and intercept were 31148 ± 1.02 and 30631 ± 1.12 for Doxofylline and 3041 ± 1.01 and 32082 ± 1.25 for Terbutaline Sulphate respectively.

Precision

The results of the repeatability and intermediate precision experiments are shown in Table 1 and Table 2. The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were < 2 %, respectively as recommended by ICH guidelines.

LOD and LOQ

Signal-to-noise ratios of 3:1 and 10:1 were obtained for the LOD and LOQ respectively. The LOD and LOQ were found to be 1 µg/mL and 2 µg/mL for Doxofylline and 2 µg/mL and 3 µg/mL Terbutaline Sulphate respectively.

Robustness

Each factor selected (except columns from different manufacturers) was changed at three levels (−1, 0 and 1). One factor at the time was changed to estimate the effect. Thus, replicate injections (n = 6) of mixed standard solution at three concentration levels were performed under small changes of three chromatographic parameters (factors). Insignificant differences in peak areas and less variability in retention time were observed in Table 3 and Table 4.

Specificity

The peak purity of Doxofylline and Terbutaline Sulphate was assessed by comparing their respective spectra at the peak start, apex and peak end positions i.e., r (S, M) = 0.9992 and r (M, E) = 0.9999. A good correlation (r = 0.9996) was also obtained between the standard and sample spectra of Doxofylline and Terbutaline Sulphate respectively. Also, excipients from formulation were not interfering with the assay.

Recovery

As shown from the data in Table 5 and Table 6 good recoveries of the Doxofylline and Terbutaline Sulphate in the range from 99.33 to 100.14 % were obtained at various added concentrations.

Analysis of a formulation

Experimental results of the amount of Doxofylline and Terbutaline Sulphate in tablets, expressed as a percentage of label claims were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients which are normally present. The drug content was found to be 99.96 % for Doxofylline and 99.60 % for Terbutaline Sulphate. Two different lots of Doxofylline and Terbutaline Sulphate combination tablets were analyzed using the proposed procedures as shown in Table 7 and Table 8.

Table 1: Precision study of Doxofylline

Concentration (µg/mL)	Repeatability (n=6)			Intermediate precision (n=6)		
	Measured conc. ±SD	(%) RSD	Recovery (%)	Measured conc. ±SD	(%)RSD	Recovery (%)
3	2.94 ± 0.87	0.98	98.00	3.03 ± 0.96	1.02	101.0
5	4.99 ± 2.78	1.13	99.80	4.97 ± 2.71	1.11	99.40
7	7.02 ± 6.01	1.45	100.28	6.99 ± 5.94	1.30	99.85

Table 2: Precision study of Terbutaline Sulphate

Concentration (µg/mL)	Repeatability (n=6)			Intermediate precision (n=6)		
	Measured conc. ±SD	(%) RSD	Recovery (%)	Measured conc. ±SD	(%)RSD	Recovery (%)
4	3.97 ± 2.39	0.84	99.25	4.02 ± 0.72	0.91	100.5
6	6.05 ± 1.85	1.14	100.83	5.95 ± 1.77	1.23	99.16
8	7.99 ± 2.65	1.17	99.87	7.94 ± 2.34	1.15	99.25

Table 3: Robustness testing[a] of Doxofylline

Factor[a]	Level	Retention time	Retention factor	Asymmetry
A: Flow rate (mL/min)				
0.9	-1	2.89	0.20	1.42
1.0	0	2.92	0.25	1.46
1.1	+1	2.95	0.30	1.50
Mean ± SD (n = 3)		2.92 ± 0.03	0.25 ± 0.05	1.46 ± 0.04
B: % of methanol in the mobile phase (v/v)				
89	-1	2.87	0.23	1.43
90	0	2.92	0.25	1.46
91	+1	2.97	0.27	1.49
Mean ± SD (n = 3)		2.92 ± 0.05	0.25 ± 0.02	1.46 ± 0.03
C: Solvents of different lots				
First lot		2.92	0.25	1.46
Second lot		2.94	0.26	1.50
Mean ± SD (n = 3)		2.92 ± 0.02	0.25 ± 0.01	1.46 ± 0.04

[a] Three factors were slightly changed at three levels (-1, 0, 1)

Table 4: Robustness testing[a] of Terbutaline Sulphate

Factor[a]	Level	Retention time	Retention factor	Asymmetry
A: Flow rate (mL/min)				
0.9	-1	4.20	0.35	1.26
1.0	0	4.23	0.37	1.30
1.1	+1	4.26	0.39	1.34
Mean ± SD (n = 3)		4.23 ± 0.03	0.37 ± 0.02	1.30 ± 0.04
B: % of methanol in the mobile phase (v/v)				

89	-1	4.19	0.35	1.23
90	0	4.23	0.38	1.30
91	+1	4.27	0.41	1.37
Mean ± SD (n = 3)		4.23 ± 0.04	0.38 ± 0.03	1.30 ± 0.07
C: Solvents of different lots				
First lot		4.23	0.39	1.30
Second lot		4.29	0.40	1.35
Mean ± SD (n = 3)		4.23 ± 0.06	0.39 ± 0.01	1.30 ± 0.05

[a] Three factors were slightly changed at three levels (-1, 0, 1)

Table 5: Recovery study of Doxofylline (n = 6)

Label claim (mg/tablet)	Amount added (mg)	Total amount (mg)	Amount Recovered (mg) ± % RSD	% Recovery
400	320 (80%)	720	719.87 ± 0.96	99.98
400	400 (100%)	800	799.86 ± 1.01	99.98
400	480 (120%)	880	881.26 ± 0.78	100.14

Table 6: Recovery study of Terbutaline Sulphate (n=6)

Label claim (mg/tablet)	Amount added (mg)	Total amount (mg)	Amount Recovered (mg) ± % RSD	% Recovery
5	4 (80%)	9	8.94 ± 1.16	99.33
5	5 (100%)	10	10.03 ± 1.40	100.30
5	6 (120%)	11	11.01 ± 0.98	100.09

Table 7: Analysis of commercial formulation for Doxofylline

Doxofylline (400 mg)	Doxofylline found (mg per tablet)	
	Mean ± SD (n= 6)	Recovery (%)
1st Lot	399.85 ± 1.02	99.96
2nd Lot	400.03 ± 0.95	100.00

Table 8: Analysis of commercial formulation for Terbutaline Sulphate

Terbutaline Sulphate (5 mg)	Terbutaline Sulphate found (mg per tablet)	
	Mean ± SD (n= 6)	Recovery (%)
1st Lot	4.98 ± 1.06	99.60
2nd Lot	5.02 ± 1.01	100.40

CONCLUSION

HPLC method was developed and validated as per ICH guidelines. UV detection allowed an accurate quantitation of chromophoric compounds. The drug was analysed by HPLC method using Thermo Hypersil BDS–C_{18} (250 mm × 4.6 mm, 5.0 µ) from Germany with isocratic conditions and simple mobile phase containing methanol: Aq. Phosphate Buffer (1mM of Potassium Dihydrogen Phosphate adjusted to pH - 4.55 with OPA) (90:10 v/v) at flow rate of 1 mL/min using UV detection at 282 nm. The procedure has been evaluated for the linearity, accuracy, precision and robustness in order to ascertain the suitability of the analytical method. The method was also applied to marketed samples. It has been proved that the method is selective and linear between concentration range 3-8 µg/mL for Doxofylline and 4-9 µg/mL for Terbutaline Sulphate. LOD and LOQ were found to be 1 µg/mL and 2 µg/mL for Doxofylline. LOD and LOQ were found to be 2 µg/mL and 3 µg/mL for Terbutaline Sulphate respectively. Statistical analysis proves that the method is suitable for the analysis of Doxofylline and Terbutaline Sulphate as bulk drug and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of Doxofylline and Terbutaline Sulphate and also for its estimation in plasma and other biological fluids.

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