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Original Article

Analytical Method Mevelopment and Validation of Doxofylline and Terbutaline Sulfate by RP-HPLC Method

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ABSTRACT

The objective of present work was to develop and validate a simple, accurate, precise HPLC method for the estimation of doxofylline and terbutaline sulfate. The chromatographic separation was achieved on a Hypersil BDSC 18column(4.6x250 mm,5µmparticlesize).Different mobile phase systems in different proportions were tried. For HPLC method a mobile phase consisting of Methanol and Acetonitrile (80:20) produced symmetric peak shape with good resolution for both the drugs. Next, the drugs were chromatographed under different flow rates from which a flow rate of 1.0 ml/min was selected. The retention timesof Doxofylline and Terbutaline sulfate were found to be 2.869 min and 3.942 min, respectively.The proposed method was found to have excellent linearity in the concentration range of 20-80mg/ml with correlation coefficient r2=0.999 and 0.999 for Doxofylline and Terbutaline sulfaterepectively.The method was validated for linearity, precision, LOD, LOQ and robustness. The proposed method optimized and validated as per ICH guidelines.

Keywords: Doxofylline, Terbutaline, chromatographed.

1. INTRODUCTION

Doxofylline and Terbutaline sulfate are anti-asthmatic drugs. Doxofylline is chemically:7-(1,3-dioxolan-2-ylmethyl)-1,3-dimethylpurine-2,6-dione.It has a molecular weight of 266g/mol .Doxofylline is solid and it is soluble in methanol. Terbutaline sulfate is1, 3-

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Benzenediol,5-[2-[(1,1-dimethylethyl)amino]-1-hydroxyethyl]-,sulfate(2:1)(salt). (±)-a-[(tert-Butylamino)methyl]-3,5-dihydroxybenzyl alcohol sulfate (2:1)(salt).It has a molecular weight of 548g/mol.Terbutaline sulfate in low doses acts relatively,selectively at beta-adrenergic receptors to cause branchodilation and relax the pregnant uterus. Terbutaline sulfate is white to gray, crystalline powder and it is soluble in methanol. [1, 2]

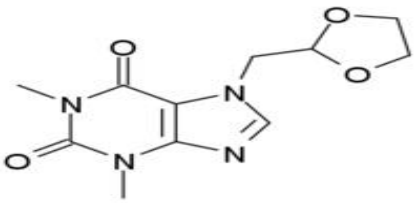

Fig 1: Doxofylline

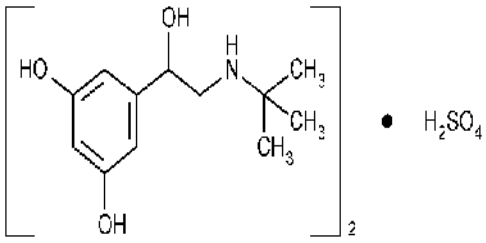

Fig 2: Terbutaline sulfate

Literature survey revealed that several methods were reported for Doxofylline and Terbutaline sulfate individually and in combination sk.vsurendranath,gananadhamusamanthula,ShuklaD,ChakrabortyS, singh s vidhya t bhwari et al.Therefore, the main objective of this study was to attempttodevelopasimpleandrapidanalyticalmethodforsimultaneousestimationofcefiximetrihydrateandclavulanatepotassiuminasingledosageformandvalidatethepropose dassay. [3, 4]

2. MATERIALS AND METHODS

Apparatus: The HPLC waters 2690/5 liquid chromatograph equipped with a PDA detector , the software installed was Empower, with 20µl loop, Hypersil-BDS C18 coloumn (250mmx4.6mm,5µl).The other instrument included are(SARTORIOUS) electronic balance and a sonicator (Fast clean).

Chemicals and reagents

Active pharma ingredient of Doxofylline and Terbutaline sulfate was obtained as a gift sample from Aurobindo Pharma Ltd,purified water HPLC grade was prepared by triple glass distillation and filtered through a 0.45µ membrane filter.Methanol HPLC grade and Acetonitrile. HPLC was run at a flow rate of 1.0ml/min, 20µl of the sample was injected in the chromatographic system.Mobile phase comprising of Methanol:Acetonitrile at the ratio (80:20).The coloumn temperature was ambient with a detection wavelength of 282nm.

Preparation of standard solution

Stock solutions were prepared by dissolving 10mg of Doxofylline and 10mg of Terbutaline sulfate in mobile phase seperatly.Aliquots of standard solution ofDoxofylline and Terbutaline sulfate were transferred into 10ml volumetric flasks and solutions were made upto the volume to yeid concentrations of Doxofylline and Terbutaline sulfate.[5-7]

Pharmaceutical formulation

Formulation DOXOLL-TL manufactured by FLOREAT PHARMA LTD was purchased from the local pharmacy in Hyderabad.

Preparation of sample solution

For analysis of commercial formulation, 10 tablets of DOXOLL-TL of Doxofylline 400 mg and Terbutaline sulfate 5mg were weighed the average weight was calculated and powdered. A quantity equivalent to 400mg of Doxofylline and 5mg of Terbutaline sulfate was weighed and transferred to a 100ml volumetric flask which contain mobile phase and then shake it for 10mins and sonicate it for 20mins.The solution was allowed to stand at a room temperature for 20-30mins and filterd it through a whatmann filter paper.Then suitable aliquots of formulation solution were prepared

and injected into HPLC to obtain concentration in linearity range. [8-10]

Validation of analytical method

ACCURACY: Accuracy is the closeness of results obtained by a method to the true value. It is the measure of exactness of the method. Recovery studies of the drug were carried out for determining accuracy parameter. Accuracy is the closeness of results obtained by a method to the true value. It is the measure of exactness of the method. It was done by mixing known quantity of standard drugs with the analyzed sample formulation and the contents were reanalyzed by the proposed method. This was carried out in 50% 100% and 150% levels. [11, 12]

PRECISION: The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The Precision expressed as standard deviation or relative standard deviation.

a.System precision: Standard solution prepared as per test method and injected five times.

b.Method precision: Prepare five sample preparations individually using the single as per test method and injected each solution. [13, 14]

LINEARITY: The linearity of amalytical ,method is the ability to clicit test results that are directly proportional to the concentration of analyte in the sample within the given range.The linearity was performed by seven different concentrations,which were injected and calibration curve were plotted.The linearity of Doxofylline and Terbutaline sulfate was found to be in the range of 20-80µg/ml respectively The chromatograms of the resulting solutions were recorded. The plot showing linearity and range study for Doxofylline and Terbutaline sulfate is shown in figure.

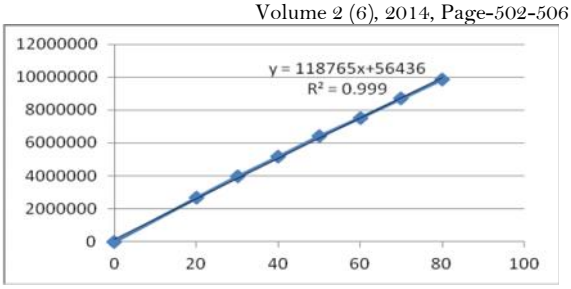

Fig 3: Plot of linearity and range study for Doxofylline

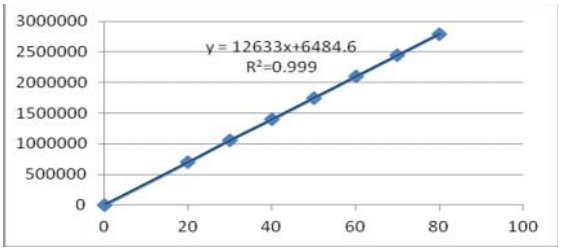

Fig 4: Plot of linearity and range study for Terbutaline sulfate

Ruggedness:

a) System to System variability: System to system variability study was conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared and each was analyzed as per test method. A comparison of both the results obtained on two different HPLC systems, shows that the assay test method is rugged for System to system variables. [15]

Robustness: The robustness of an analytical procedure are a measure of its capacity to remain unaffected by small, but deliberate changes in the method parameters and provides an indication of its reliability during normal usage. Robustness of the method was investigated under a variety of conditions including changes of composition of buffer in the mobile phase and flow rate. % RSD of assay was calculated. [16-18]

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD of an analytical procedure is the lowest concentration of an analyte in a sample which can be detected but not necessarily quantitated as an exact value where as LOQ is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. [19]

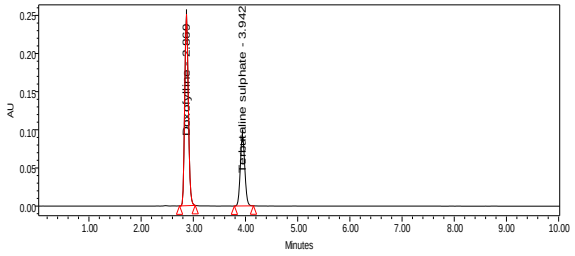

Fig 5: Optimized chromatogram

Table 1: Results of validation parameters of RP-HPLC

Sl. no	Validation Parameter	Doxofylline	Terbutaline sulfate	Acceptance Criteria
1	Linearity (in µg)	20 – 80	20 – 80	Correlation coefficient
2	Regression Line Equation	y=118765x+5643	y=12633x+6484.6	(R^2 =0.999 or 1)
3	R^2 Value	0.999	0.999	
4	Precision System Precision (%RSD)	0.08	0.21	RSD<2%
	Method Precision(%RSD)	0.07	0.19	
5	LOD	2.47	1.69	
6	LOQ	7.50	5.13	-
7	Analysis of marketed formulation	99%	95%	95-105%
8	% Recovery	99-101	99-101	95-105%
9	Ruggedness	0.016	0.18	RSD<2%

3. RESULTS AND DISCUSSION

- The slope, intercept and correlation coefficient values were found to be 118765, 56436 and 0.999 and 12633, 6484.6 and 0.999 for Doxofylline and Terbutaline sulfate respectively.
- The LOD of Doxofylline and Terbutaline sulfate were found to be 2.47µg/ml and 1.69µg/ml respectively. The LOQ of Doxofylline and Terbutaline sulfate found to be 7.50µg/ml and 5.13µg/ml respectively.
- Precision of the developed method was studied. Low % RSD values indicate that the method is precise.

4. CONCLUSION

The proposed RP-HPLC method for the estimation of the Doxofylline and Terbutaline sulfate in the pharmaceutical dosage form were simple, reliable and selective providing satisfactory accuracy and precision with lower limits of detection and quantification. The recoveries achieved was good by RP-HPLC method. The methods can be recommended for routine and quality control analysis of these drugs in the pharmaceutical dosage forms. In this proposed method symmetrical peaks with good resolution were obtained.

Table 2: summury of analysis of doxofylline and terbutaline sulfate by RP-HPLC method

Drugs	Labeled amount, mg/ tablet	Estimated Amount , mg/tablet	% Label claim	% *RSD
DOXO	400	397.56	99.39	1.53
TER	5	4.79	95.80	1.13

5. ACKNOWLEDGEMENT

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