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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE DETERMINATION OF ETOPHYLLINE AND THEOPHYLLINE IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

An HPLC-PDA method was developed and validated for the determination of hydrochlorothiazide in bulk and pharmaceutical formulation. The method was optimized selecting chromatographic conditions of 70: 30 acetonitrile : phosphate buffer, C18 column (ODS-3 150 mm × 4.6 mm, 5 µm), 20µL injection volume, flow rate of 1 mL/min at ambient temperature (30°C), and 272 nm. The retention time was found to be 1.539 Min and 2.475 Min of Etophylline and Theophyllline was According To International Conference of Harmonization (ICH) Q1A (R2). The method was compared with official BP and other reported methods. The proposed method is economic, simple, and rapid and hence can be employed for routine analysis in quality control laboratories.

KEYWORDS: Linearity plot, Theophylline, Etophylline, ambient temperature, histamine, Coefficient.

INTRODUCTION ETOPHYLLINE

The chemical name is 7-(2-hydroxyethyl)-1,3- dimethylpurine-2,6-dione. It has a molecular formula C9H12N4O3 and molecular weight of 224.22 g/mol. It is practically insoluble (0.583 mg/L) in water. It has the chemical structure as shown in (Fig.1).

Mechanism of action: Etophylline is the ethyl salt of Theophylline. It inhibits phosphodiesterase enzyme which degrades cyclic nucleotides intracellularly and it results the cyclic AMP accumulation in the cell.[1] This causes bronchodialatation, cardiac stimulation and vasodilatation. It causes release of calcium from sarcoplasmic reticulum, especially in cardiac muscles and results increased cardiac muscle contraction.

Theophylline

The chemical name is 1,3-dimethyl-2,3,6,7-tetrahydro-1H- purine-2,6-dione.. It has a molecular formula $C_7H_8N_4O_2$ and molecular weight of 180.167 g/mol. It is slightly soluble in alcohol; more soluble in hot water; soluble in alkaline solutions. It has a chemical structure as shown in (Fig.2).

Mechanism of action: Theophylline relaxes the smooth muscle of the bronchial airways and pulmonary blood vessels and reduces airway responsiveness to histamine, methacholine, adenosine and allergen. It competitively inhibits type III and type IV phosphodiesterase (PDE), the enzyme responsible for breaking down cyclic AMP in smooth muscle cells, possibly resulting in bronchodilator.[2] It also binds to the adenosine A2B receptor and blocks adenosine mediated bronchoconstriction. In inflammatory states, theophylline activates histone deacetylase to prevent transcription of inflammatory genes that require the acetylation of histones for transcription to begin.

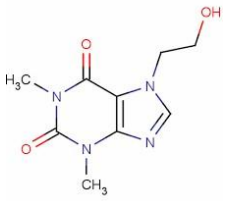

Figure 1: Chemical structure of Etophylline.

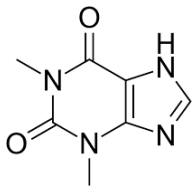

Figure 2: Chemical structure of Theophylline.

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The literature survey reveals that there are a very few HPLC methods available for the determination individual Etophylline and Theophylline in bulk and dosage forms.[3-7] There were no reported analytical methods for simultaneous estimation of Etophylline and Theophylline in bulk and their combined dosage forms in presence of their degradation products. Hence an attempt was made to develop stability indicating specific, sensitive, accurate and precise RP-HPLC method for simultaneous estimation of these drugs with isocratic elution mode.

MATERIAL AND METHODS

Chemicals and reagents

API of Etophylline and Theophylline were obtained as gift samples from Suven Pharmaceutical Pvt Ltd. Hyderabad, India. HPLC grade Acetonitrile was procured from Merck (Mumbai, India), HPLC grade water (Milli Q or equivalent). Other all chemicals used were f AR grade.

Instrumentation

HPLC experiment was carried out on a Scimadzu Model LC- 2030 PLUS (IND) System Consisting of Thermo – Hypersil ODS. The analytical column used for the preparation was C18. Other equipments used were ultra-sonicator (Remi), Analytical balance, pH meter.

Preparation of solutions

Buffer: Mix 0.1ml acetic acid in 100ml of water

Mobile phase: Mix Acetonitile and Phosphate buffer in proportion of 70:30. The pH 4.5 was adjusted using NaOH.

Chromatographic condition: Flow rate 1.0ml/min; detection wavelength 272nm; injection volume 10µl; column used thermo-hypersil ODS-C18 (5µm, 150x4.6mm); column temperature: 25^{O}C; mobile phase: Acetonitrile: Phosphate Buffer (70: 30).

Method development: Working standard of various **Preparation of standard solution**: 375 mg of Etophylline and 10 mg of Theophylline Drug was weighed and dissolved in 50ml with methanol and taken in 50 ml of volumetric flask. The mixture was sonicated for 10 mins. 2 ml

of this solution was further mixed with 50 ml methanol concentrations was prepared by taking aliquots of standard solution and diluted to get required concentration for calibration plot and which was injected.

Method validation: A Standard solution was prepared by using Etophylline& Theophylline working standard as Pretest method and was injected five times into the HPLC system.

The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Etophylline& Theophylline, retention times and peak areas. The % RSD for retention times and peak areas were found to be within the limits as shown in table: 1.

Specificity[3]

Solution of standard and sample were prepared as per the test method and were injected into chromatographic system. The chromatograms of standard and sample were identical with same retention time as shown in table 2.

Table 1: Data of system suitability.

Sample Name	Etophylline				Theophylline			
Injection	RT	Peak Area	Theoretical Plates	Tailing Factor	RT	Peak Area	Theoretical Plates	Tailing Factor
1	1.540	2304890	12620	1.318	2.477	209959	28167	1.232
2	1.541	2285275	12628	1.287	2.477	205746	28147	1.215
3	1.539	2282646	12697	1.326	2.477	204355	28186	1.212
4	1.541	2305849	12648	1.311	2.478	205298	27685	1.215
5	1.539	2292325	12686	1.329	2.477	204309	28170	1.211
Mean	1.540	2294197	12656	1.314	2.477	205933	28071	1.217
SD	0.001	10800.942	-------	-------	0.000	2333.14	-------	--------
%RSD	0.06	0.47	-------	-------	0.02	1.13	-------	--------

Table 2: Retention time of Etophylline and.

Sr.No	Name of the peak	Retention time (min)	Area	Area%	Tailing factor	Resolution	Plates
1	Etophylline	1.539	2270568	91.82	1.321	--	12669
2	Theophylline	2.475	202141	8.17	1.210	6.380	28217
Total			2472709	100.00			

Table 3: Retention time of Etophylline and.

Sr.No	Name of the peak	Retention time (min)	Area	Area%	Tailing factor	Resolution	Plates
1	Etophylline	1.538	2260767	91.89	1.329	--	12474
2	Theophylline	2.475	199303	8.10	1.204	6.367	28211
Total			2460070	100.00			

Precision

Repeatability

System Precision: Standard solution was prepared as per test method and injected five times

Method Precision: Six samples were prepared individually using single as per test method and injected each solution.[4]

Table 4: System Precision.

	RT of Etophylline	Peak area of Etophylline	RT of Theophylline	Peak area of Theophylline
Mean	1.540	2294197	2.477	205933
SD	0.001	10800.94	0.000	2333.14
%RSD	0.06	0.47	0.02	1.13

Table 5: Method Precision.

	Peak area of Etophylline	% Assay	Peak area of Theophylline	% Assay
Mean	2283220	99.52	198951	96.61
SD	18384.19	0.80	786.909	0.382
%RSD	0.81	0.81	0.40	0.40

Accuracy (Recovery)

Drug assay was performed in triplicate with equivalent amount of Etophylline & Theophylline into each volumetric flask for each spike level to get the concentration of Etophylline & Theophylline equivalent to 50%, 100% and 150% of the labelled amount as per the test method. The average % recovery of Etophylline & Theophylline was calculated.[5]

Theophylline for sample

Table 6: Accuracy.

	Concentration % of spiked level	% Recovery (Mean)
Etophylline	50% Sample	94.46
	100% Sample	93.00
	150% Sample	91.59
Theophylline	50% Sample	96.29
	100% Sample	96.36
	150% Sample	94.87

Linearity

A series of solutions were prepared using Etophylline and Theophylline working standard at concentration levels from 75 ppm to 375 ppm of target concentrations.

Table 7: Linearity data.

	Linearity (Mean)					Statistical Analysis
	75 ppm	**150 ppm**	**187.5 ppm**	**300 ppm**	**375 ppm**	
Etoph	6021	11535	17458	2290	28155	Linearity
ylline	33	45	19	187	39	Equation=
						Y= 556345x
						– 503937
						Correlation
						Coefficient=
						0.9996
Theo	5106	99925	14880	2008	25008	Linearity
phylli	1		1	16	6	Equation=
ne						Y= 49894x –
						49439
						Correlation
						Coefficient=
						0.9999

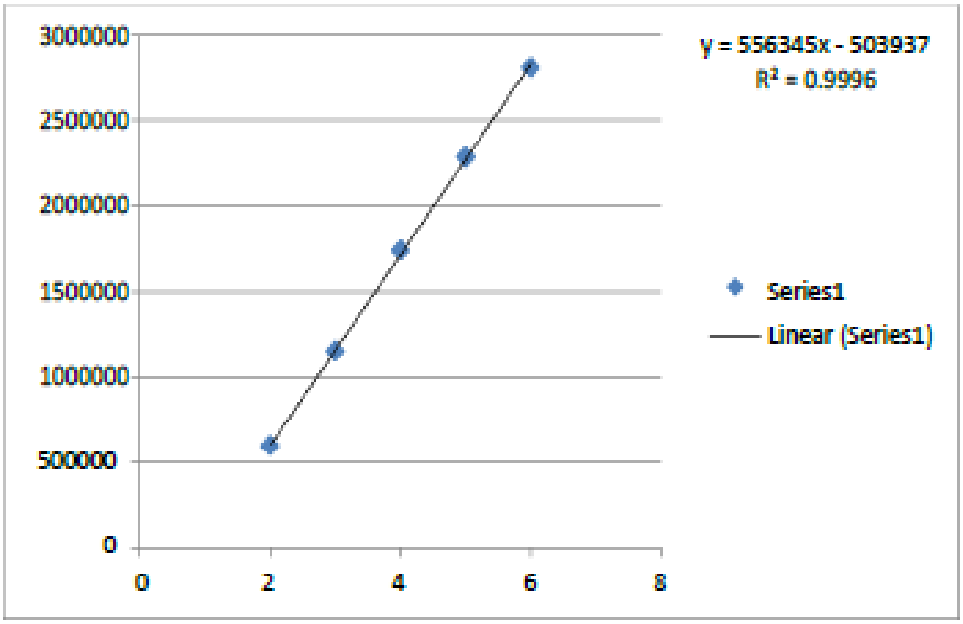

Figure 3: Linearity Plot (Concentration Vs Response) of Etophylline.

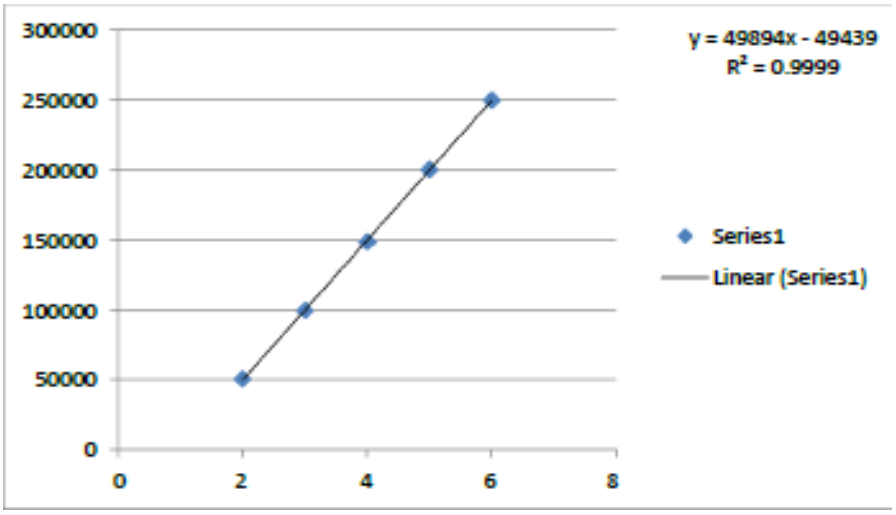

Figure 4: Linearity plot (Concentration Vs Response) of Theophylline.

Ruggedness

System to system variability study was conducted. Six samples were prepared and each was analyzed on different HPLC systems under similar conditions, at different times. Comparison of both the results obtained on two different HPLC systems, shows that the assay test method are rugged for System to system variability.[6]

Table 8: System variability.

	Peak area of Etophylline	% Assay	Peak area of Theophylline	% Assay
Mean	41808	100.00	2034	100.00
SD	788.663	1.886	35.788	1.76
%RSD	1.89	1.89	1.76	1.76

Robustness

The study was conducted to determine the effect to variation in flow rate. Standard solution prepare as per the test method was injected in to the HPLC system using flow rates 0.8 mL/min, 1.0 mL /min and 1.2 mL/min .The system suitability parameters were evaluated and found to be within the limits for 0.8 mL/min, 1.0 mL /min and 1.2 mL/min flow.[7]

Table 9: Robustness data.

Flow rate	Etophylline						Theophylline					
	Standard area			Tailing factor			Standard area			Tailing factor		
	Avg	SD	% RSD	Avg	SD	% RSD	Avg	SD	% RSD	Avg	SD	% RSD
0.8 ml	2853800	6154.4	0.22	1.185	0.009	0.78	170452	489.5	0.29	1.04	0.002	0.17
1.0 ml	2293497	25617.8	1.12	1.258	0.004	0.30	134512	1050.9	0.78	1.04	0.002	0.19
1.2 ml	1948849	25133.4	1.29	1.378	0.005	0.33	109767	136.2	0.12	1.04	0.008	0.76

Limit of Detection and Limit of Quantitation

From the linearity plot LOD and LOQ were calculated: For Etophylline:

$$LOD = \frac{3.3\sigma}{S} = \frac{3.3 \times 10800.942}{556345} = 0.064067$$

$$LOQ = \frac{10\sigma}{S} = \frac{10 \times 10800.942}{556345} = 0.194141$$

For Theophylline.

$$LOD = \frac{3.3\sigma}{S} = \frac{3.3 \times 2333.149}{49894} = 0.15415$$

$$LOQ = \frac{10\sigma}{S} = \frac{10 \times 2333.149}{49894} = 0.467621$$

(σ = standard deviation of the response, S = slope of the calibration curve of the analyte.)

CONCLUSION

A simple, fast, accurate and precise stability- indicating HPLC analytical method has been developed and validated for the quantitative analysis of Sofosbuvir and Ledipasvir in bulk drugs and combined dosage forms. Stress testing is a significant part of drug development process and the pharmaceutical industry has a lot interest in this area. The results of stress testing undertake according to the ICH guidelines reveal that the method is specific and stability-indicating. The proposed method has the ability to separate these drugs from their degradation products in tablet dosage forms and hence can be applied to the analysis of routine quality control samples and samples obtained from stability studies.

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