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Research Article

Analytical Method Development and Validation for the simultaneous estimation of Theophylline and Etofylline in combined Dosage form by RP –HPLC method

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Abstract
A new method was established for simultaneous estimation of Theophylline and Etofylline byRP-HPLC method. The chromatographic conditions weresuccessfully developed for the separation of Theophylline and Etofylline by using Xterra C185µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH4.6: ACN (55:45%v/v) (pH was adjusted with orthophosphoric acid), detection wavelength was 255nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, and Empower-software version-2. The retention times were found to be 2.399mins and 3.907mins. The %purity of Theophylline and Etofylline wasfound to be100.7% and 101.4% respectively. The systemsuitability parameters for Theophylline and Etofylline suchas theoreticalplates and tailingfactor were found to be 1.3, 5117.5 and 1.4,3877.3 the resolution was found to be 8.0. The analytical method was validated according to ICH guidelines (ICH,Q2(R1)). The linearity study for Theophylline and Etofylline was found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient (r2) was found tobe 0.999 and 0.999, %mean recovery was found to be 100% and 100.5%, %RSD for repeatability was 0.2 and 0.4, %RSD for intermediate precision was 0.5 and 0.1 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.95 and 3.04, and LOQ value was 9.87 and 10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Theophylline and Etofylline inAPI and Pharmaceutical dosage form.

Keywords: XterraC18, Theophylline andEtofylline, RP-HPLC

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1. Introduction

A methylxanthine derivative from tea with diuretic, smooth muscle relaxant, bronchial dilation, cardiac and central nervous system stimulant activities. Mechanistically, theophylline acts as a phosphodiesterase inhibitor, adenosine receptor blocker, and histone

deacetylase activator. Theophylline is marketed under several brand names such as Uniphyl and Theochron, and it is indicated mainly for asthma, bronchospasm, and COPD.Etofylline nicotinate, a theophylline derivative, is a drug that causes vasodilation and relaxation of smooth muscle.

2. Methodology

Instrumentation: The instrument used was HPLC Alliance Waters model No. 2695 separation module. 2487 UV detector, Software- Empower. The stationary phase used was Agilent C18 column (4.6×150mm)5µ. Weighing balance -BSA224SCW, Sonicator (Enertech)-SE60US, pHmeter Lab India model No. AD102U, UV/VIS spectrophotometer UV3000 Lab India Software-UV Win5

Materials and reagents

Theophylline and Etofyllinewere gift samples provided by Dr. Reddy's Laboratories Hyderabad Water, Methanol, Acetonitrile and Potassium dihydrogen orthophosphate were supplied by Merck

Method development

Three trials were made by changing the mobile phase ratios and solvents Water:Methanol (40:60%v/v) Water:Methanol (40:60%v/v) Phosphate buffer (0.05m) pH5.0:Methanol (50:50%v/v) Phosphate buffer (0.05M) pH4.6:MeOH Phosphatebuffer (0.05M) pH4.6:ACN (30:70%v/v). Finally, the mobile phase optimized mobile phase ratio was Phosphatebuffer (0.05M)pH4.6: ACN (55:45%v/v).

Chromatographic conditions

The chromatographic conditions were successfully developed for the separation of Theophylline and Etofylline byusingXterraC185µm (4.6*250mm) column, flowrate was1ml/min, mobilephase ratio was Phosphatebuffer (0.05M) pH4.6: ACN (55:45%v/v) (pH was adjusted with orthophosphoric acid), detection wavelength was 255nm.

3. Results and Discussion

Table 1: Accuracy results of Etofylline

%Concentration (at specificationLevel)	Area	Amount added(mg)	Amount found(mg)	% Recovey	Mean Recovery
50%	2332744	5	5.10	101.8%	100.5%
100%	3132697	10	9.99	99.9%	
150%	3918997	15	14.9	99.1%	

Table 2: Accuracy results of Theophylline

%Concentration (at specification		Amount	Amount	% Recovery	Mean
50%	353867	5	5.0	101.3%	
100%	4735088	10	9.94	99.4%	100.0%
150%	5911798	15	14.8	99.2%	

Table 3:Repeatability results of Theophylline

Injection	Area
Injection-1	1501417
Injection-2	1486940
Injection-3	1490656
Injection-4	1487329
Injection-5	1490384
Average	1491345
Standard Deviation	5881.4
%RSD	0.39

Table 4: Repeatability results of Etofylline

Injection	Area
Injection-1	2235319
Injection-2	2240678
Injection-3	2249490
Injection-4	2245822
Injection-5	2251694

Average	2244601
Standard Deviation	6656.8
%RSD	0.32

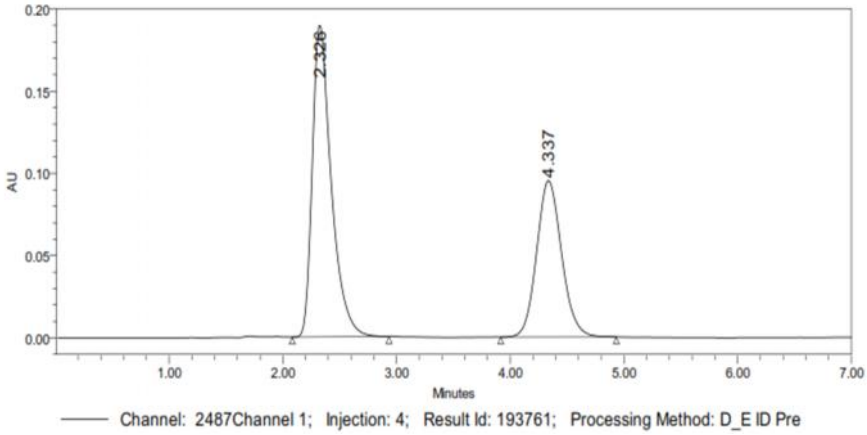

Figure 1:Chromatogram of Standard Inj

Table 5 Ruggedness results of Etofylline

Injection	Area
Injection-1	2194758
Injection-2	2195700
Injection-3	2196191
Injection-4	2195326
Injection-5	2200951
Average	2196585
Standard Deviation	2496.0
%RSD	0.11

Table 6 Ruggedness results of Theophylline

Injection	Area
Injection-1	1456296
Injection-2	1457422
Injection-3	1456513
Injection-4	1454579
Injection-5	1451483
Average	1455259
Standard Deviation	2347.6
%RSD	0.16

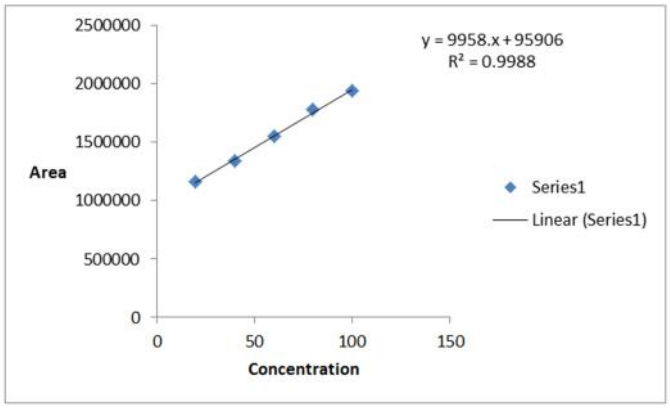

Figure 2 Calibration curve of Etofylline

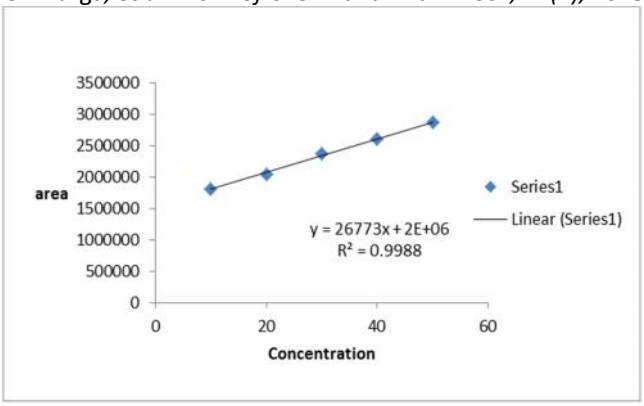

Figure 3 Calibration curve of Theophylline

Table 7: System suitability results for Etofylline (Flow rate)

S.No	FlowRate(ml/min)	System suitability results	
		USPPlatecount	USPTailing
1	0.8	1748.5	1.22
2	1.0	1548.2	1.2
3	1.2	1948.0	1.2

Table 8: System suitability results for Theophylline (Flow rate)

S.No	FlowRate(ml/min)	System suitability results	
		USPPlatecount	USPTailing
1	0.8	883.3	1.56
2	1.0	1234.0	1.1
3	1.2	969.2	1.6

4. Conclusion

A new method was established for simultaneous estimation of Theophylline and Etofylline by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Theophylline and Etofylline by using XterraC185µm (4.6*250mm) column, flowrate was1ml/min, mobilephase ratio wasphosphatebuffer (0.05M) pH4.6: ACN (55:45%v/v) (pH wasadjusted with orthophosphoric acid), detection wavelength was 255nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Em-power-softwareversion-2. The retention times were found to be 2.399 mins and 3.907mins. The %purity of Theophylline and Etofylline was found to be 100.7% and 101.4% respectively.The system suitability parameters for Theophylline and Etofylline such as theoreticalplates and tailing factor were found to be 1.3, 5117.5 and 1.4, 3877.3 the resolution was found to be 8.0. The analytical method was validated according to ICH guidelines (ICH,Q2(R1)). The linearity study for Theophylline and Etofylline was found inconcentration range of 1µg-5µg and 100µg-500µg and correlation coefficient (r2) was found to be 0.999 and 0.999, %mean recovery was found to be 100% and 100.5%, %RSD for repeatability was 0.2 and 0.4, %RSD for intermediate precision was 0.5 and 0.1 respectively. The precision study was precise, robust, andrepeatable. LOD value was 2.95 and 3.04, and LOQ value was 9.87 and 10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Theophylline and Etofylline in API and Pharmaceutical dosage form.

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