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Quality By Design (QbD) Based Approach to Analytical RP-HPLC Method Development and Validation of Combined Etophylline & Theophylline in Bulk and Tablets.

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KEYWORDS

RP-HPLC, QbD, EtophyllineTheophylline, Method Development, Validation.

ABSTRACT:

Introduction: A convenient, accurate, rapid, precise, and sensitive RP-HPLC method involving UV detection was developed and validated for the determination and quantification of Etophylline and Theophylline in tablet dosage form.

Objective: To develop and validate a simple, precise and reliable, economical method using RP-HPLC as per ICH guidelines.

Method: The determination was carried out on a Kromasil C18 (250 x 4.6 mm, 5 µm) column using a filtered and degassed mixture of methanol: Buffer pH 4.5 (94: 06 v/v) as mobile phase at a flow rate of 1 ml/min and effluent was monitoredat 272 nm.

Results: The retention time found for Etophylline was 1.535 min and for Theophylline 2.477 min. Etophylline and Theophylline showed a linear response in the concentration range of 10-60µg/mland 20-120µg/ml individually. The correlation coefficient for Etophylline and Theophylline was0.9993 and 0.9998, respectively.

Conclusion: The method was validated in terms of linearity, precision, accuracy, specificity, robustness, and system suitability. The proposed method can be useful inthe quality control of bulk manufacturing and pharmaceutical dosage forms.

1. Introduction

Etophylline(EP) and Theophylline(TP) is a combination of two bronchodilators. The action of the drug is to relax the muscles in the airways and widen the airways. This makes breathing easier. Thus this combination can be promising for treatment and prevention of asthma and chronic obstructive pulmonary disease (COPD). Etophylline, 7-(β-Hydroxyethyl) Theophylline) Etophylline (7-(β-Hydroxyethyl) Theophylline) is a N-7-substituted derivative of Theophylline[1]. Etophylline is a bronchodilator that can be used for the prevention of asthma. Etophylline is also an anticholesteremic and reduces total cholesterol levels in the blood. It improves the patient's condition by inhibiting the phosphodiesterase enzymes in the body. Theophylline, also known as 1,3-dimethylxanthine, is a phosphodiesterase-inhibiting drug used in therapy for respiratory diseases such as chronic obstructive pulmonary disease (COPD) and asthma under a variety of brand names [1,2]. As a member of the xanthine family, it bears structural and pharmacological similarities to Theophyllinebromine and caffeine. The literature reveals that no methods have been reported. Structures of EP and EP are shown in Fig. 1 & 2.

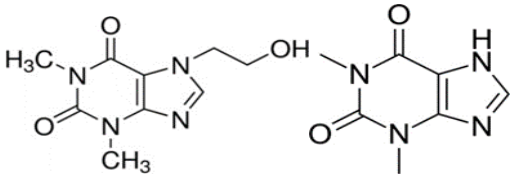

Figure 1: Structure of EP **Figure 2:** Structure of TP

2. Methods

2. Material and methods:

2.1 Chemicals and reagents: The pure EP and TP were supplied as gift samples by Medipol Pharmaceutical

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India Private Limited, Chennai, India. HPLC water and methanol were purchased from Qualigen Ltd (India), and Orthophosphoric acid and acetate Buffer from Merck Life Science. Throughout the experiment calibrated class A-grade volumetric glassware was used. For Filtration of the mobile phase Millipore membrane filters with a pore size of 0.45µ were used.

2.2 Instrumentation: Various equipment like HPLC of model Series LC2030, pump PU2030, sample injection used was autosampler, UV detector UV 2075 plus, software used lab solution (Shimadzu (I prominence plus). pH meter 101 by chemiline, Balance AY-120 (Shimadzu) and sonicator UCB-40(Rolex).

3. Design of Experiment (DOE)

Central Composite Design (CCD)

Central Composite Design (CCD) was used by using Design Expert Software version 8.

Central Composite design is an experimental matrix that has limited application in Response surface methodology (RSM) when number of factors is higher than 2 because the number of experiments required for this design (calculated by expression N = 3k, where N is experiment number and k is factor number) is very large, thereby losing its efficiency in the modeling of quadratic functions[3]. Because a complete Central Composite design for more than two variables requires more experimental runs than can usually be accommodated in practice, designs that present a smaller number of experimental points, such as the Box-–Behnken, 3-level factorial design, and Doehlert designs, are more often used [5]. However, for two variables, the efficiency is comparable with designs such as three-level factorial design[4]. The majority of applications of Central Composite factorial designs are in the area of chromatography. The selection of parameters is given in Table 1.

Table 1. Selection of Parameter

S.No.	Selection of factors	Selection of Responses	Columns used	Mobile phase selected	Change pH of buffer	Change Mobile phase proportion Range
1	Mobile Phase	Retention Time	C18 Column	Buffer : Methanol	Range: 3. 50 to 5.50 mmol/L.	70-90% (Consider Organic Phase)
2	pH of Mobile phase	Area		Water : Methanol		
3		Theoretical Plate		Water : Acetonitrile		
5		Asymmetry				

3. Methods:

3.1 Preliminary Analysis of Drug

The color and texture of etophylline (EP)& theophylline (TP) were compared with the reported characters mentioned in the drug bank. Solubility of drugs was carried out in various solvents like water, NaOH, HCl, and ethanol.

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3.2 UV Analysis

UV analysis was carried out by scanning the solution of API at 200-400 nm.

3.3 Preparation of stock solutions

The stock solution was prepared by dissolving 10 mg EP and TP in 10 ml methanol and then diluted further to obtain a concentration of 10 µg/ml, and labeled as standard stock

3.4 Preparation of sample solutions

A tablet weighing 100 mg containing 77% EP and 23% TP was powdered, and weighed equivalent to 10 mg to obtain a concentration of 10 µg/ml, and labeled as a sample solution.

3.5 Preparation of mobile phase

Methanol and water in a ratio of 94: 06 v/v proportions were used to prepare the mobile phase. The solution was filtered through a 0.45µ membrane filter and then sonicated in a sonicator bath for 10 min.

3.6 Selection of detection wavelength

From the standard stock solution further dilutions were made using water and scanned over the range of 200-400 nm and the spectra were overlain. It was observed that the drug showed considerable absorbance at 272 nm.

4. Method Validation

The proposed RP-HPLC method was validated as per ICH guidelines[19,20].

4.1 Linearity: The aliquots of standard solutions of EP & TP were made in different 10 ml volumetric flasks with a mobile phase such that the final concentration would be between 10-60 µg/ml and 20-120 µg/ml respectively at 272 nm, and peak area was recorded. The calibration curve was plotted as concentration against peak area.

4.2 Specificity: The specificity of the RP-HPLC method was determined by comparison of the chromatogram of mixed standards and sample solutions. The parameters like retention time (t R), resolution (RS), and tailing factor (Tf) were calculated.

4.3 Precision: To determine the precision of the method, six replicates of the sample prepared from the commercial tablets were injected and the assay was calculated to measure the repeatability of retention times and peak area of the standard and sample. The precision of the method was verified by using a tablet stock solution. Intraday and interday precision were determined by repeating the assay six times on the same day for intraday precision and on different days for interday precision studies.

4.4 Accuracy: The accuracy of the method was calculated by recovery studies at three levels (80%, 100%, and 120%) by the standard addition method. The accuracy was expressed as the percentage of the analyte recovered. For EP, tablet powder equivalent to 5 mg was weighed individually and added in three different 25 ml volumetric flasks respectively, followed by the addition of 8 mg (80%), 10 mg (100%), and 12 mg (120%) of standard EP in each of the volumetric flasks. 25 ml of the mobile phase [Methanol: Water (94:06 v/v)] was added to each volumetric flask and sonicated for 5 min. The solutions were then filtered and 1 ml of the filtrate from each was taken in 10 ml volumetric flasks individually and diluted up to the mark with mobile phase. The solutions were injected in triplicates into the chromatographic system and the peak area was evaluated to give percent recovery and standard deviation. A similar procedure was repeated for Theophylline.

4.4 Robustness: Robustness was performed by doing a small deliberate change by varying the solvent ratio in the mobile phase, flow rate, pH of the mobile phase, and wavelength range. Sample solutions were injected as 10 µl injection into the chromatographic system. The Peak area and standard deviation were observed.

4.5 Limit of detection and Limit of quantification (LOD, LOQ): The LOD and LOQ of the proposed method were determined by progressively injecting lower concentrations of the standard solutions under the set chromatographic conditions.

L.O.D. = 3.3(SD/S)

L.O.Q. = 10(SD/S)

Where, SD = Standard deviation of the response,

S =Slope of the calibration curve.

The slope S may be estimated from the calibration curve of the analyte.

5. Result and Discussion

Central Composite Design (CCD)

When CCD data is inserted into the software. It gave 13 runs at different pH and mobile phase proportions. Followed the same procedure for each selected mobile phase. 26 runs were given by software. After completion of all trials software gave one optimized best value for given chromatographic conditions. CCD was utilized for

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method development to evaluate the effects of the amount of buffer, buffer pH, and flow rate on responses. Total 13 runs were suggested by the software. Factors and responses considered for the study are shown in Table 2. The ranges considered were based on previous univariate chromatographic separation studies.

Table 2 : Runs were suggested by the software

Sr.No	Mobile Phase Composition (Organic Phase)	pH of Buffer
1	80.00	4.50
2	80.00	4.50
3	70.00	5.50
4	65.86	4.50
5	94.14	4.50
6	90.00	3.50
7	80.00	4.50
8	80.00	3.09
9	80.00	4.50
10	70.00	3.50
11	90.00	5.50
12	80.00	5.91
13	80.00	4.50

Optimization

Screening design for suitable chromatographic condition:

By using a suitable column and solvent system based on peak parameters. Methanol: water, ACN: Water, and Methanol: Buffer, were selected as mobile phase. The pH selected was 3.50 to 5.50 mmol/L. These mobile phases were screened by varying the organic phase composition from 70 to 90 % v/v and flow rate 1.0 mL/ min·

Results of various trials, having Organic phase composition 94.14 % v/v are shown in the following tables.

Table 3 : Trials performed at mobile phase (94.14: 5.86 v/v) with a pH range of 4.50

Sr. no.	Composition	Observation	Remarks
1	Methanol: water (94.14: 5.86 v/v)	More retention time with greater peak asymmetry	Very Dissatisfactory
2	ACN: water (94.14: 5.86 v/v)	Greater peak Asymmetry and lower theoretical plates	Not satisfactory

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3	Methanol: Buffer (94.14: 5.86 v/v)	Good peak properties, less retention time with Lower theoretical plates	Extremely Satisfactory

Results of various trials, having organic phase composition 90 % v/v are shown in following tables.

Table 4: Trials performed at mobile phase (90:10 v/v) with pH of Buffer 3.50 mmol/L

Sr.No.	Composition	Observation	Remarks
1	Methanol: water (90:10 v/v)	Good peak properties, less retention time with Lower theoretical plates	Satisfactory
2	ACN: water (90:10 v/v)	More retention time	Not satisfactory
3	Methanol: Buffer (94: 10 v/v)	Less retention time with Lower theoretical plates	Satisfactory

Table 5: Trials performed at mobile phase (94:10 v/v) with pH of Buffer 4.50 mmol/L

Sr.No.	Composition	Observation	Remarks
1	Methanol: water (94: 10 v/v)	Less peak asymmetry with more theoretical plates and more retention time	Satisfactory
2	ACN: water (94: 10 v/v))	Good Peak Properties but More retention time	Not satisfactory
3	Methanol: Buffer (94: 10 v/v)	Less retention time with Lower theoretical plates	Satisfactory

Results of various trials, having Organic phase composition 80 % v/v are shown in following tables

Table 6: Trials performed at mobile phase (80:20 v/v) with pH of Buffer 3.09 mmol/L

Sr. No.	Composition	Observation	Remarks
1	Methanol: water (80:20 v/v)	Greater peak asymmetry and Less Theoretical Plates	Very Dissatisfactory
2	ACN: water (80:20 v/v)	Greater peak Asymmetry and Very Small Peak Height	Not satisfactory
3	Methanol: Buffer (80:20 v/v)	Less Peak height and good theoretical plates	Satisfactory

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Table 7: Trials performed at mobile phase (80:20 v/v) with pH of Buffer 4.50 mmol/L

Sr. no.	Composition	Observation	Remarks
1	Methanol: water (80:20 v/v)	Broad Peak Appeared	Not satisfactory
2	ACN: water (80:20 v/v)	Very Small Peak appeared	Not satisfactory
3	Methanol: Buffer (80:20 v/v)	Less Peak height and good theoretical plates	Satisfactory

Table 8 : Trials performed at mobile phase (80:20 v/v) with pH of Buffer 5.91 mmol/L

Sr.No.	Composition	Observation	Remarks
1	Methanol: water (80:20 v/v)	Lower theoretical plates	Dissatisfactory
2	ACN: water (80:20 v/v)	Less Peak height and good theoretical plates	Satisfactory
3	Methanol: Buffer (80:20 v/v)	Broad Peak Appeared	Satisfactory

Results of various trials, having Organic phase composition 70 %v/v are shown in following tables.

Table 9 : Trials performed at mobile phase (70: 30 v/v) with with pH of Buffer 3.50 mmol/L

Sr.No	Composition	Observation	Remarks
1	Methanol: water (70: 30 v/v)	Lower theoretical plates	Dissatisfactory
2	ACN: water (70: 30 v/v)	Less Peak height and good theoretical plates	Satisfactory
3	Methanol: Buffer (70: 30 v/v)	Not Good Peak Properties	Partly Satisfactory

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Table 10 : Trials performed at mobile phase (70: 30 v/v) with pH of Buffer 5.50 mmol/L

Sr. No	Composition	Observation	Remarks
1	Methanol: water (70: 30 v/v)	More Retention Time	Not satisfactory
2	ACN: water (70: 30 v/v)	More Retention Time	Not satisfactory
3	Methanol: Buffer (70: 30 v/v)	Broad Peak Appeared	Satisfactory

Conclusion of Trials performed at mobile phase (94.14: 5.86 v/v) with pH range 4.50 mmol/L are extremely satisfactory.

Table 11: Design expert has optimized the following chromatographic conditions with respect to desirability value.

Sr. No	Mobile Phase Composition (Organic Phase)	pH of Buffer	R.T EP	R.T TP	Asymmentry(EP)	Asymmetry(TP)	Theoretical Plates (EP)	Theoretical Plates (TP)
1	80.00	4.50	3.1	4.3	1.54	1.35	4021	2014
2	80.00	4.50	3.1	4.3	1.54	1.35	4021	2014
3	70.00	5.50	6.4	5.2	0.91	1.1	6548	8457
4	65.86	4.50	7.65	8.95	2.24	2.18	4521	3265
5	94.14	4.50	1.54	2.478	1.1	1.2	10080	9587
6	90.00	3.50	2.81	3.87	1.24	1.89	7681	2017
7	80.00	4.50	3.12	4.31	1.52	1.32	4025	2016
8	80.00	3.09	3.18	4.56	1.66	1.64	3647	8024
9	80.00	4.50	3.65	4.87	1.21	1.34	5567	9874
10	70.00	3.50	4.22	5.32	1.35	1.21	6398	8647
11	90.00	5.50	3.64	4.52	2.65	2.14	2114	3265
12	80.00	5.91	3.65	4.75	2.21	2.81	6598	9841
13	80.00	4.50	3.1	4.3	1.54	1.35	4021	2015

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This methodology was initially based on constructing a desirability function for each response. The scale of the individual desirability function ranges between i= 0, for a completely undesirable response and i =1, for a fully desired response. The selection of the trial was based on the maximum desirability value. Therefore, first trial which was having desirability one (i=1) selected for method optimization. Fig 3 shows desirability.

Table 12 : Optimized trials suggested by software based on desirability value

Sr. No	Mobile Phase Composition (Organic Phase)	pH of Buffer	Retention Time(EP)	Retention Time(TP)	Asym Metry(EP)	Asym Metry(TP)	Theoretical Plates(EP)	Theoretical Plates(TP)	Desirability
1	94.14	4.50	1.54	2.478	1.1	1.2	10080	9587	**0.896**

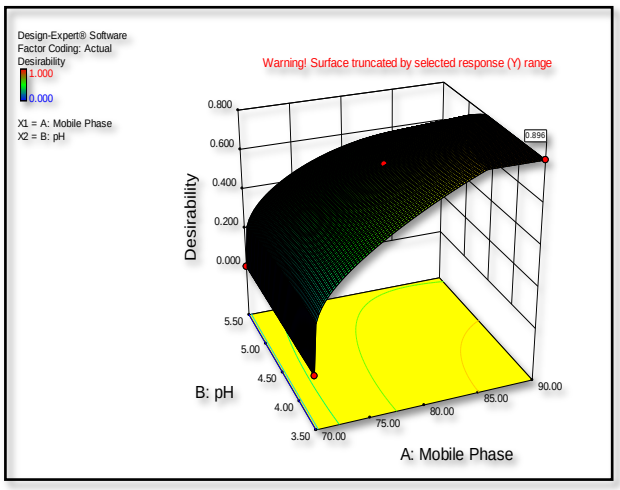

Figure 3: Desirability value

Optimized chromatographic conditions

Mobile phase: Methanol: Phosphate Buffer (94.64: 5.36 v/v), with pH range 4.50 mmol/L Analytical column: C_{18} column Waters XBridge (4.6× 250mm id. particle size 5µm), UV detection: 260nm, Injection volume: 10 µL, Flow rate: 1.00 mL min^{-1}, Temperature: Ambient, Run time: 10 min

Effect of independent variables on retention time (X):

After applying experimental design, suggested Response Surface Linear Model was found to be significant with model F value of 10.48 & 8.87, *p* value less than 0.0038 & 0.0061 and R^2 value of 0.8822 & 0.6395. There is only a 0.38% & 0.61% chance that a "Model F-Value" this large could occur due to noise. A value of % C.V is 18.86. Adjusted R^2 were 0.7980 Etophyline and Theophyline respectively. The model for response X (Retention time) is as follows:

The equation for response surface quadratic model is as follows

Retention Time (Etophyline) = +3.21 -1.6 * A +0.46 * B -0.34* A * B +0.76 * A^2 +0.17 * B^2

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Fig 4 (a) & (b) shows a graphical representation of the pH of Buffer (B) and the amount of Methanol (A), As in pH of Buffer does not show a drastic change in retention time (X), also decrease in the amount of Methanol showed increases the retention time.

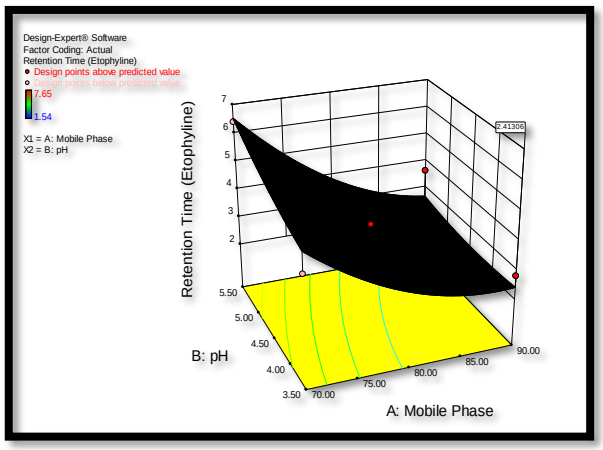

Figure 4 a: Three-dimensional plot for retention time as a function of pH of buffer and amount of buffer. Constant factor (flow rate- 1mL min^{-1})

Fit summary: Surface Quadratic model was suggested by the software.

ANOVA: ANOVA of developed Full three level factorial model for retention time (Y_1).

Values of "Prob > F" (p- value) less than 0.0500 indicate model terms are significant. In this case A value was Significant however B was found to be Insignificant model terms.

Table 13: Significance of *p* value on model terms of retention time

Model terms	*p* value	Effect of factor	Remarks
A	0.0004	1.00	Significant
B	0.1114	1.00	Insignificant
AB	--	0.46	Insignificant
A^2	--	3.98	Insignificant
B^2	--	0.19	Insignificant
Overall model	0.0038	-	**Significant**

Retention Time (Theophyline)= +4.75 -1.41 *A +0.100 * B

Fig.3 (b) shows a graphical representation of pH of Buffer (B) and amount of Methanol (A), As in pH of Buffer does not show drastic change in retention time (X), also decrease in amount of Methanol showed increases the retention time.

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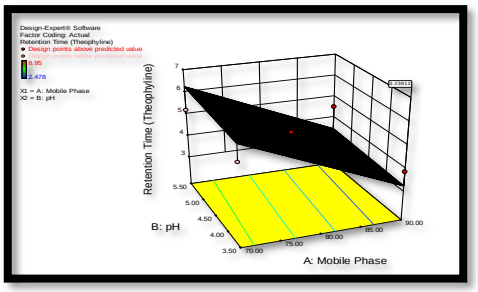

Figure 4 b: Three-dimensional plot for retention time as a function of pH of buffer and amount of buffer. Constant factor (flow rate- 1mL min^{-1})

Fit summary: Surface Quadratic model was suggested by the software.

ANOVA: ANOVA of developed Full three level factorial model for retention time (Y_1).

Values of "Prob > F" (p- value) less than 0.0500 indicate model terms are significant. In this case A and B are significant model terms.

Table 14: Significance of *p* value on model terms of retention time

Model terms	*p* value	Effect of factor	Remarks
A	*0.0018*	*15.91*	Significant
B	*0.7723*	*0.080*	Significant
Overall model	0.0061		**Significant**

Effect of independent variables on tailing factor (Y):

After applying experimental design, suggested Response Surface Linear Model was found to be significant with model F value of 2.14 & 1.03, *p* value less than 0.1656 & 0.4679 and R^2 value of 0.4160 & 0.4237. There is only a 16.56 % & 46.79 % chance that a "Model F-Value" this large could occur due to noise. A value of % C.V is 31.31 & 38.29. Adjusted R^2 were 0.0121 & 0.0590 Etophyline and Theophyline respectively.

Asymetric Factor (EP)= +1.59 +2.225E-00* A +0.22 * B +0.46 * A * B

Fig 5 (a) shows a graphical representation of pH of Buffer (B) and amount of Methanol (A), As maintain flow rate at 1mL/min decrease the tailing factor, it is synergistic effect on response (Y) while gradually decreases the amount of Methanol showed decreases in the asymmetry.

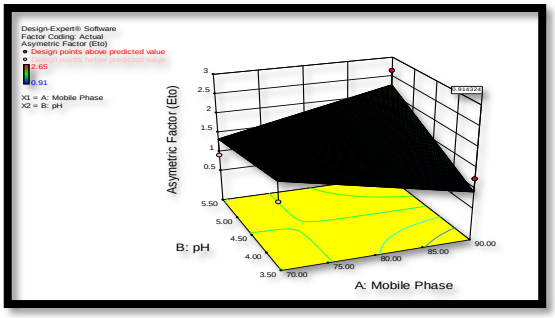

Figure 5 a: Three-dimentional plot for tailing factor as a function of pH of buffer and % v/v of buffer. Constant factor (flow rate- 1mL min^{-1})

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Fit summary: Surface Quadratic model was suggested by the software.

ANOVA : ANOVA of developed CCD model for tailing factor (Y_2).

Values of "Prob > F" (p- value) less than 0.0500 indicate model terms are significant. In this case B is Insignificant model terms.

Table 15: Significance of *p* value on model terms of tailing factor

Model terms	*p* value	Effect of factor	Remarks
A	*0.9889*	*3.959E-005*	Insignificant
B	*0.1931*	*0.38*	Insignificant
Overall model	0.1656	-	**Insignificant**

Asymetric Factor (TP) = +1.34 +0.042 * A +0.22 * B +0.090 * A * B +0.081 * A^2+0.35* B^2

Fig 5.(b) shows a graphical representation of pH of Buffer (B) and amount of Methanol (A), As maintain flow rate at 1mL/min decrease the tailing factor, it is synergistic effect on response (Y) while gradually decreases the pH of Buffer showed decreases in the asymmetry.

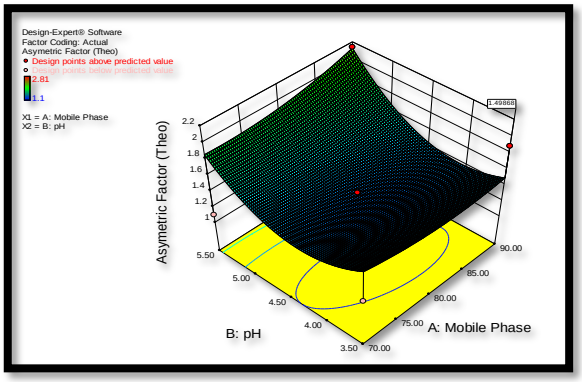

Figure 5 b: Three-dimentional plot for tailing factor as a function of pH of buffer and % v/v of buffer. Constant factor (flow rate- 1mL min^{-1})

Fit summary: Surface Quadratic model was suggested by the software.

ANOVA: ANOVA of developed Full three level factorial model for retention time (Y_1).

Values of "Prob > F" (p- value) was not found to be less than 0.0500 indicate model terms are In significant. In this case A and B was determined as Insignificant model terms.

Table 16: Significance of *p* value on model terms of retention time

Model terms	*p* value	Effect of factor	Remarks
A	*0.8211*	*0.014*	Insignificant
B	*0.2475*	*0.40*	Insignificant

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AB	--	1.00	Insignificant
A^2		1.00	Insignificant
B^2		1.00	Insignificant
Overall model	0.4679		**Insignificant**

Effect of independent variables on theoretical plates (Z):

After applying experimental design, suggested Response Surface Linear Model was found to be significant with model F value of 1.15 & 1.02, *p* value less than 0.4169 & *0.5297* and R^2 value of 0.4511 & 0.8438 & . There is only a 41.69 % & 52.97% chance that a "Model F-Value" this large could occur due to noise. . A value of % C.V is 38.29 & 64.79. Adjusted R2 were 0.0590 & 0.070 Etophyline and Theophyline respectively

Theoretical Plates (EP) = +4331.0 +588.83* A -155.46 * B -1429.25 * A * B +1353.19 * A^2 +264.19 * B^2

Fig 6 (a) shows a graphical representation of amount of Methanol (A) and Flow rate (B), A decrease in pH of buffer showed not a significant effect on number of theoretical plates (Z), while increase in amount of Methanol showed increases response

.

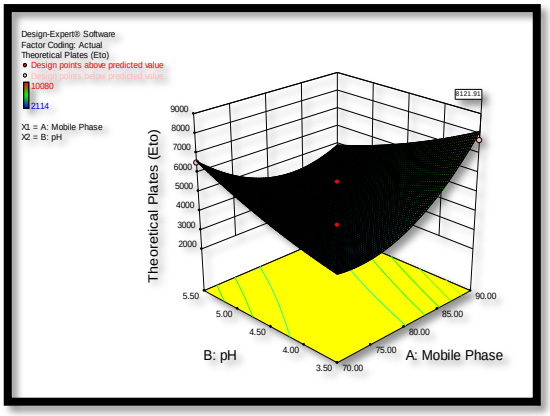

Figure 6 a: Three-dimentional plot for theoretical plates as a function of pH of buffer and % v/v of buffer. Constant factor (flow rate- 1 mL min^{-1})

Fit summary: Linear model was suggested by the software

ANOVA : ANOVA of developed CCD model for theoretical plates (Y_3).

Values of "Prob > F" (p- value) less than 0.0500 indicate model terms are significant. In this case A value was indicated as In significant model terms.

Table 17: Significance of *p* value on model terms of theoretical plates

Model terms	*p* value	Effect of factor	Remarks
A	*0.4411*	*2.774E+006*	Insignificant
B	*0.8355*	*1.933E+005*	Insignificant

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AB		1.00	Insignificant
A^2		1.00	Insignificant
B^2		1.00	Insignificant
Overall model	0.4169		**Insignificant**

Theoretical Plates (TP) = +5464.31

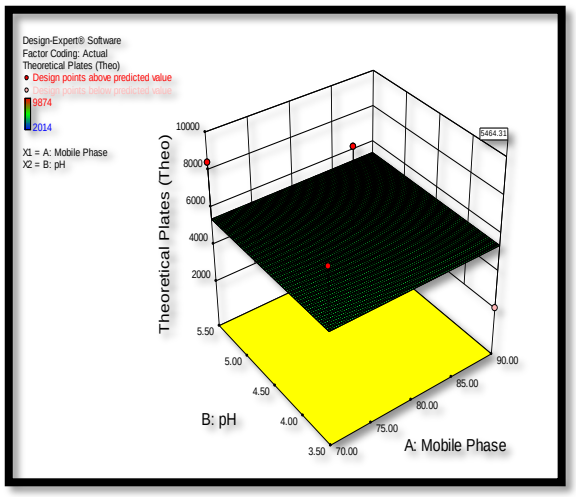

Figure 6 b: Three-dimentional plot for theoretical plates as a function of pH of buffer and % v/v of buffer. Constant factor (flow rate- 1 mL min^{-1})

6. Method Validation

6.1 Linearity

The R.T of EP was found to be 1.5 min whereas TP was at 2.4 min at 272 nm Standard Peak observed in figure 8. Linearity data indicated in Table 18 , and plotted in fig. 7(a) and 7 (b),7 (c).

Table 18: Linearity Data

Linearity				
Sr. No	**Concentration (µg/mL)**	**Peak Area (EP)**	**Concentration (µg/mL)**	**Peak Area (TP)**
1	10	4279016	20	396666
2	20	8558032	40	793332
3	30	12787048	60	1139998
4	40	17116064	80	1586664
5	50	21395080	100	1983330
6	60	25674096	120	2379996

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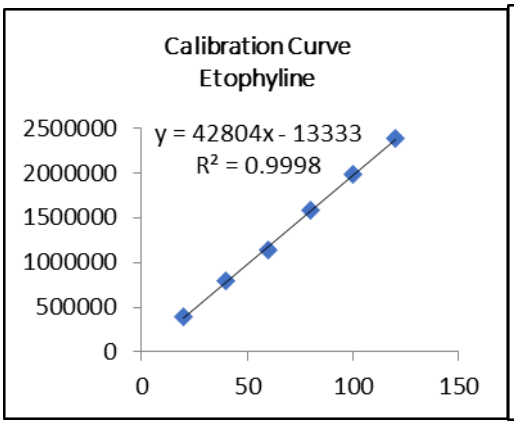

Figure 7a: Calibration curve of EP

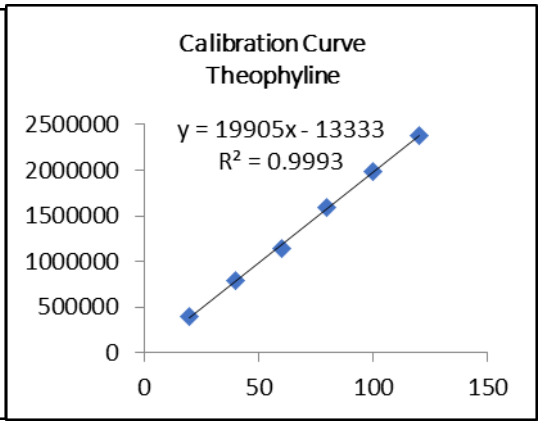

Figure 7b: Calibration curve of TP

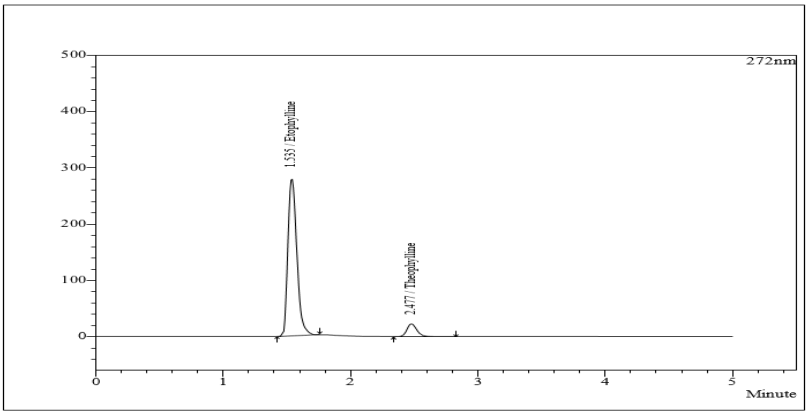

Figure 7 c: Standard Chromatogram of EP and TP

6.2 Specificity: For EP and TP recovery was found to be 99.88 and 99.60 % , which is found to be good between the results of mixed standards and sample solutions and no interference of any other peak was found.Fig 8 a,b,c shows specificity.

Table 19 : Specificity of EP and TP

Specificity				
Etophyline				
Sample	**Label Claim (mg)**	**Amount Found**	**Recovery**	**Retention Time**
Tablet	77	76.91	99.88312	1.608
Theophyline				
Tablet	23	22.91	99.6087	2.483

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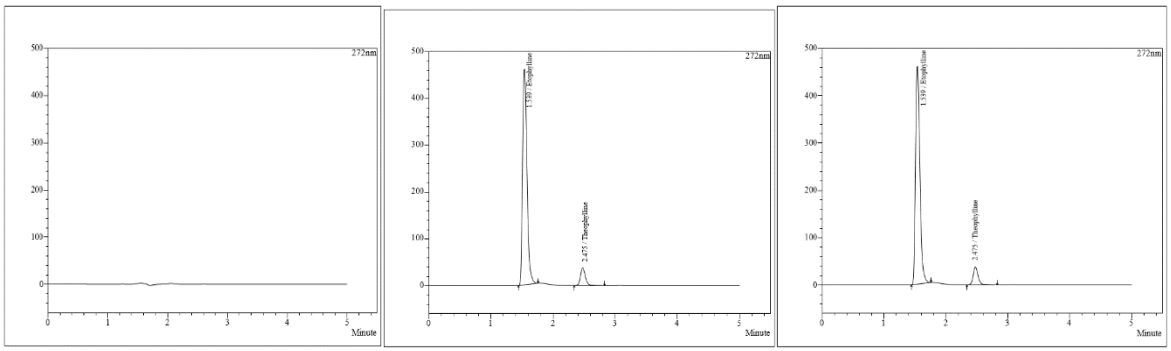

(a) **(b)** **(c)**

Figure 8: Chromatogram of Blank(a), standard solution(b) sample solution (c)

6.3 Precision: The results of precision after interday and interday as indicated in Table:20. %RSD was found to be less than 2 indicating good resolution found.

Table 20: Precision Study

Precision					
Sr. No	**Concentration (µg/mL)**	**Intraday EP**	**Interday EP**	**Intraday TP**	**Interday TP**
1	40	17121569	17171569	1592169	1642169
2	40	17114420	17181770	1585020	1652370
3	40	17119099	17242984	1589699	1713584
4	40	17100392	17258454	1570992	1729054
5	40	17171311	17268352	1641911	1738952
6	40	17119052	17278233	1589652	1748833
Average		17124307.2	17233560	1594907.2	1704160
Standard Deviation		22140.80	41712.85	22140.80	41712.85
RSD%		0.1293	0.242	1.3882	1.998

6.4 Accuracy: For finding accuracy peak area was evaluated to give percent recovery and standard deviation. Table 21 indicate % Recovery,fig 9 a, b, c shows recovery peak at 80%, 100%, 120%

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Table 21: Recovery study of EP and TP

Accuracy				
Etophyline				
Sr. No	**Concentration (µg/mL)**	**Peak area**	**Found Concentration (µg/mL)**	**% Recovery**
1	32	13680732.8	31.99	99.96
2	40	17100916	39.99	99.98
3	48	10260549.6	48.09	100.18
Theophyline				
1	64	1257212.8	63.99	99.98
2	80	1571516	79.99	99.99
3	96	471454.8	96.09	100.09

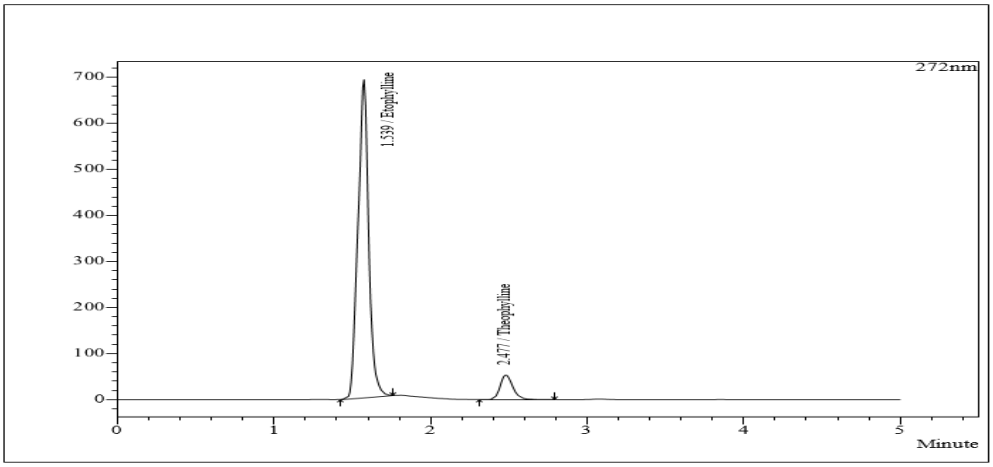

Figure 9 a: Chromatogram of Recovery Study at 80%

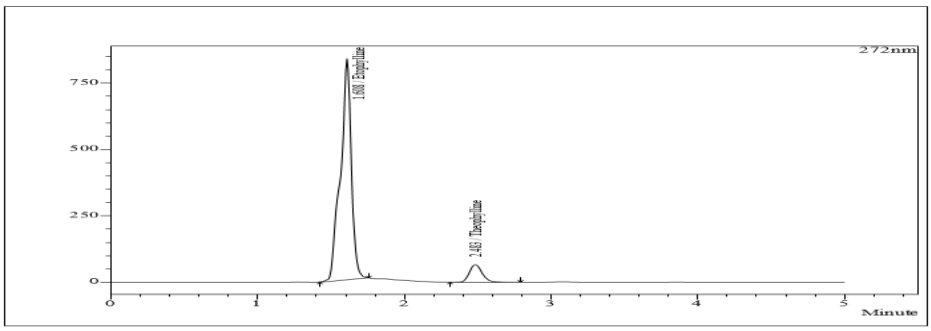

Figure 9 b: Chromatogram of Recovery Study at 100%

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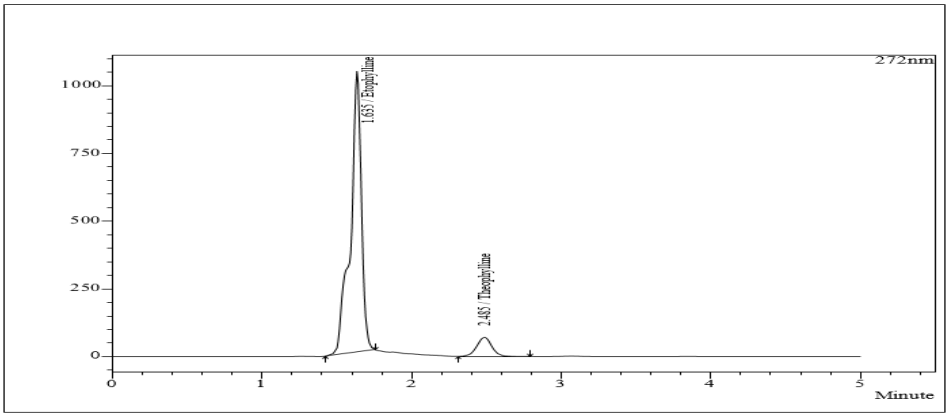

Figure 9 c : Chromatogram of Recovery Study at 150%

6.5 Robustness: Sample solutions were injected as 10 µl injection into the chromatographic system. The parameters studied were peak area and found their standard deviation & % RSD.

Table 22: Robustness study of EP and TP

Robustness					
EP			**TP**		
Sr. No	**Parameter**		**Response**	**Parameter**	**Response**
Methanol : Water			**Retention Time (min)**	**Detection Wavelength**	**Peak Area**
(V/V)				(nm)	
1	93.14	6.86	1.602	270	17057937
2	94.14	5.86	1.608	272	17116252
3	95.14	4.86	1.665	274	17149516
Average			1.625	Average	17107902
Standard Deviation			0.02839	Standard Deviation	37850.36
RSD%			1.747085	**RSD%**	0.221
Flow Rate			**Retention Time (min)**	**pH of Buffer**	**Peak Area**
(mL/min)				(mmol/L)	
1	0.9		1.598	4.3	17144324
2	1		1.608	4.5	17116425
3	1.1		1.668	4.7	17040804

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Average	1.624667	Average	17100518
Standard Deviation	0.030912	Standard Deviation	43733.13
RSD%	1.902671	**RSD%**	0.2557

6.6 Limit of detection and Limit of quantification (LOD, LOQ): The slope S may be estimated from the calibration curve of the analyte.

Table 23: LOD and LOQ Results

LOD & LOQ			
		EP	TP
1	LOD (µg/mL)	0.1744	3.7510
2	LOQ (µg/mL)	0.5286	11.3668

Summary:

Table 24: Summary Table

Parameter	**Result**	
	EP	**TP**
Calibration range (µg/ml)	10-60	20-120
Detection wavelength (nm)	272 nm	
Solvent (Buffer:Methanol)	6: 94 v/v	
Regression equation (y*)	y = 5E-05x - 0.1239	y = 3E-05x - 0.1259
Correlation coefficient(r2)	0.9993	0.9998
Retention time	1.365 ± 0.023	2.477 ± 0.057
Area	76.09%	23.91%
Asymmetry	1.35	1.30
Theoretical plate	7864	6305
Precession		
Interday Precession	Precise	Precise
Interday Precession	Precise	Precise

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Accuracy (Recovery)		
80 %	99.92	99.98
100 %	99.98	99.99
120 %	100.02	100.09
Robustness		
Robustness for flow rate 0.9 ml	Robust	Robust
Robustness for flow rate 1.1 ml	Robust	Robust

Conclusion:

To maintain the safety and efficacy of any formulation it is important to ensure its quality. Any unstable product can be lethal to the patient hence proper quality control is essential. This study developed a single method for dual drugs in combination, which is found to reduce the cost of analysis. Thus method was found to be economical, simple, accurate, rapid, and precise and gave good resolution between Etophylline and Theophylline with short analysis time. The method can be easily used for routine analysis of bulk drugs and tablets and nanoparticles without involving any complicated sample preparation.

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Conflict of Interests

No conflicts of interest

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