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A NEW METHOD DEVELOPMENT FOR ETOPHYLLINE AND THEOPHYLLINE SIMULTANEOUS ESTIMATION BY RP-HPLC AND VALIDATION

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ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Etophylline and Theophylline, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Phenomenex Luna C18 (4.6×150mm, 5µ) column using a mixture of Acetonitrile: Water (10:90% v/v) as the mobile phase at a flow rate of 0.9ml/min, the detection was carried out at 240nm. The retention time of the Etophylline and Theophylline was 1.933, 3.396 ±0.02min respectively. The method produce linear responses in the concentration range of 16.5-82.5mg/ml of Etophylline and 5-25mg/ml of Theophylline. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

KEYWORDS: Etophylline, Theophylline, RP-HPLC, validation.

LITERATURE REVIEW

Nirav P. M et al (2011) Method development, validation and stability study for simultaneous estimation of Etofylline and Theophylline by RP-HPLC chromatography in marketed formulation. An approach of forced degradation study was successfully applied for the development of a stability-indicating assay method for simultaneous estimation of Etofylline and Theophylline in a formulation in the presence of its degradation products. The method showed adequate separation of Etofylline and Theophylline from their associated main impurities and degradation products. Separation was achieved on an YMC Pack-ODS-AQ, 150 x 4.6 mm the mobile phase 10mM Potassium Di-Hydrogen Phosphate : Acetonitrile (90:10) pH-4.5 with ortho phosphoric acid buffer flow rate of 1 mL/min and UV detection at 272 nm. Comprehensive stress testing of Etofylline and Theophylline Rt= 6.4 & 5.2 min was according to the International Conference on Harmonization (ICH) guideline Q1A (R2). The method was validated in terms of system suitability, precision, linearity, accuracy, robustness, ruggedness, LOD, LOQ and solution stability.[33]

Devang. N. Wadia et al (2012) Ultra Performance Liquid Chromatography (UPLC) Method Development and Validation for the Simultaneous Estimation Of etophylline and Theophylline in Pharmaceutical Dosage Form. A simple, precise, accurate and validated reverse phase UPLC method has been developed for the simultaneous estimation of Etophylline and Theophylline in injectables. The quantification was carried out using Silica gel column, packed with octadecylsilane , 2.1 mm × 100 mm, i.d, 1.7 µm particle size and separation was carried out in an isocratic mode, having a mixture of 0.05 M Sodium Acetate and Acetonitrile (90:10), pH 4.5 and mobile phase at a flow rate of 0.3 ml / min. The detection wavelength was 270 nm at ambient temperature. The retention time was 2.368 min and 3.129 min for Theophylline and Etophylline respectively. The results obtained showed a good agreement with the declared content. Recovery values for Theophylline and Etophylline were 99.84 - 100.89 %. The proposed method is reliable, rapid, precise, selective and may be used for the quantitative analysis of Theophylline and Etophylline in injectables.[34]

Ramakrishna V.S. Nirogi et al (2007) A simple and rapid HPLC/UV method for the simultaneous quantification of theophylline and etofylline in human plasma. A simple, sensitive and selective high performance liquid chromatography (HPLC) method with ultraviolet detection (272 nm) was developed and validated for the simultaneous quantification of theophylline and etofylline in human plasma. Following rapid sample preparation, the analytes and internal standard (hydrochlorothiazide) were separated using an isocratic mobile phase on a reverse phase C_{18} column. The lower limit of quantification was 100 ng/mL for both theophylline and etofylline with a relative standard deviation of less than 6%. A linear dynamic range of 100–10,000 ng/mL for both theophylline and etofylline was established. This HPLC method was validated with between-batch precision of 2.2–6.0 and 1.4–3.7% for

theophylline and etofylline, respectively. The between-batch accuracy was 94.3–98.0 and 95.4–98.2%, respectively. Stability of theophylline and etofylline in plasma was excellent, with no evidence of degradation during sample processing (autosampler) and 30 days storage in a freezer. This validated method is simple and rugged enough to be used in pharmacokinetic studies.[35]

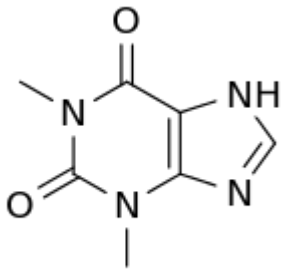
Supriya Shidhaye et al (2009) Validated stability indicating HPLC method for estimation of theophylline from a novel microsphere formulation. A new, simple, specific, precise and robust isocratic reversed-phase (RP) stability-indicating high-performance liquid chromatographic (HPLC) method was developed and validated for determination of theophylline from a novel formulation. The liquid chromatographic separation was achieved isocratically using a mobile phase of acetonitrile: 50 mM sodium acetate buffer (15:85) adjusted to pH 6.5 using dilute hydrochloric acid. The analysis was carried out using Hi-Q-Sil C18 column [250 mm x 4.6 mm, 5 µm] at flow rate of 1 ml/min and the UV detection at 270 nm. The method was validated for accuracy, precision, linearity, range, selectivity, and robustness. The linearity of the proposed method was investigated in the range of 1 to 24 µg/ml (r 2 = 0.9995). The drug was subjected to oxidation, hydrolysis, heat, and photolysis to apply stress conditions. The method provided good peak parameters with retention time of 8.6 ± 0.3 min. Degradation products resulting from stress studies did not interfere with the detection of theophylline and the assay can thus be considered as stability-indicating.[36]

Kowsar banu S et al (2013) A New RP-HPLC Method Development and Validation for Simultaneous Estimation of Salbutamol Sulphate and Theophylline in Pharmaceutical Syrup Dosage Form. A simple, rapid reverse phase high-performance liquid chromatographic method was developed and validated for the simultaneous estimation of salbutamol sulphate and theophylline in a cough syrup formulation marketed as Theoasthalin. Chromatographic separation was done using Phenomenex LunaC18 column having dimension of 4.6×250mm having particle size of 5µm, with mobile phase consisting of acetonitrile and water (40:60 %v/v), flow rate was adjusted to 1.0 ml/min and detection wavelength at 230nm. The retention times of salbutamol sulphate and theophylline was found to be 2.1 and 3.5mins. The Proposed method has been validated for linearity, range, precision, accuracy and robustness were within the acceptance limit according to ICH Q2B guidelines. Quantification of the components in actual syrup formulations was calculated against the responses of freshly prepared external standard solutions. Linearity for salbutamol sulphate and theophylline was found in range of0.25ppm-1.5ppm & 12.5ppm-75ppm and correlation coefficient was found to be 0.999 and 0.999, %RSD for intermediate precision was found to be 0.67 and 0.49 and for system precision 0.58 and 0.57 and for repeatability was 0.67 and 0.49.The percentage purity of salbutamol sulphate and theophylline was found to be 99.70and 99.54%v/v respectively.The method was found to be robust even by change in the mobile phase ±5% and in less flow condition.[37]

V Venkatesh et al (2014) A New RP-HPLC Method for Simultaneous Estimation of Etophylline and Theophylline in Tablets. A simple, accurate, economical and reproducible RP-HPLC method for simultaneous estimation of two component drug mixture of Etophylline (ETO) and Theophylline (THEO) in combined tablet dosage form have been developed. The reversed phase chromatography system was used with C18 column and the detection was made at 241 nm in the UV region. Mobile phase consisted of methanol: phosphate buffer (75:25) at a flow rate of 1 ml/min. The calibration curve was linear in the concentration range of 50-250 µg/ml for ETO and 15-75 µg/ml for THEO. The retention time of ETO and THEO was 2.78 and 5.08 min respectively. The developed method was validated for accuracy, precision, linearity, limit of detection and limit of quantification.[38]

Jain JK et al (2008) Simultaneous determination of multi drug components Theophylline, Etofylline, Guaiphenesine and Ambroxol Hydrochloride by validated RP-HPLC method in liquid dosage form. The RP-HPLC (reverse phase high performance liquid chromatography) method was developed and validated for simultaneous determination of Multi drug components i.e., Theophylline, Etofylline, Guaiphenesine and Ambroxol Hydrochloride in a liquid dosage form. Chromatographic separation of the four drugs was performed on a Hypersil Phenyl BDS (25cmX4.6mm, 5mm). The mobile phase constituted of triethylamine pH 3.0 buffer: methanol (85:15) v/v was delivered at the flow rate 1.5 mL/min. Detection was performed at 235 nm. The peak purity of Theophylline, Etofylline, Guaiphenesine and Ambroxol Hydrochloride were 0.99970, 0.99979, 0.99986 and 0.99949 respectively. Calibration curves were linear with correlation coefficient between 0.99995 to 0.99997 over a concentration range of 5 to 37 microg/mL for Theophylline, 19 to 140 microg/mL for Etofylline, 20 to 149 microg/mL for Guaiphenesine and 6 to 45 microg/mL for Ambroxol hydrochloride. The relative standard deviation (RSD) was found < 2.0%. The percentage recovery was found between the range of 98.6% and 100.5% at three different levels. Robustness and ruggedness were performed and result found within the RSD of 2%. All the parameters of validation were found in the acceptance range of ICH guideline.[39]

DRUG PROFILE
THEOPHYLLINE

Synonym	:	1,3-dimethyl-7H-purine-2,6-dione, 1,3-dimethylxanthine, Elixophyllin, Theophyllin, Theophylline Anhydrous,
Drug category	:	Phosphodiesterase Inhibitors, Purinergic P1 Receptor Antagonists, Bronchodilator Agents, Vasodilator Agents, Muscle Relaxants, Respiratory
Brand name (Single Drug)	:	Elixophyllin, Quibron-T, Respbid, Theodur G, Theolair, Theo 24.
Structure	:	
IUPAC Name	:	1,3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione
Molecular Formula	:	$C_7H_8N_4O_2$
Molecular Weight	:	180.164 gm/mole.
Official Pharmacopoeia	:	British *Pharmacopoeia*, United States *Pharmacopoeia*, Indian *Pharmacopoeia, European pharmacopoeia*

PHYSICOCHEMICAL PROPERTIES

Description	:	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.
Physical State	:	solid
Solubility	:	water solubility-7360 mg/L (at 25 °C)
Storage Conditions	:	Store at room temperature
Dosage	:	Solution(Intravenous); Tablet, extended release(oral)100mg,200mg solutions(oral)- 100 mg/15ml, liquids(oral) Elixir-80 mg/15ml
Melting point	:	273°C
pKa(strongest basic)	:	8.81
Log p value	:	-0.02

PHARMACOKINETICS

Bioavailability	:	100%
Half-life	:	8hrs
Absorption	:	Theophylline is rapidly and completely absorbed after oral administration in solution or immediate-release solid oral dosage form.
Volume of Distribution	:	0.3-0.7 L
Protein binding	:	40%, primarily to albumin.
Metabolism	:	Hepatic
Time of peak action	:	1-2 hr
Excretion	:	50% via Renal

PHARMACODYNAMICS

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. Theophylline competitively inhibits type III and type IV phosphodiesterase (PDE), the enzyme responsible for breaking down cyclic AMP in smooth muscle cells, possibly resulting in

bronchodilation. Theophylline 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.

Indications : For the treatment of the symptoms and reversible airflow obstruction associated with chronic asthma and other chronic lung diseases, such as emphysema and chronic bronchitis.

Adverse reactions : Symptoms of overdose include seizures, arrhythmias, and GI effects.

Contraindications : Tobacco Smoking, Stop Smoking, Poisoning by Breathing Drug Theophylline, Multiple Organ Failure, High Blood Pressure, Heart Attack, Angina, Cor Pulmonale, Chronic Heart Failure, Fluid in the Lungs, Ulcer from Stomach Acid, Active Inflammation of the Liver, Liver Problems, Seizures, Fever for Many Days, Fast Heartbeat, Shock, Serious Lack of Oxygen in the Blood, Sepsis Syndrome, Overactive Thyroid Gland, Underactive Thyroid, Diabetes, Cystic Fibrosis, Third Trimester of Pregnancy, Habit of Drinking Too Much Alcohol

INTERACTIONS

Drug interactions : Aciclovir-Acyclovir may increase the effect and toxicity of theophylline.
Amobarbital-The barbiturate, amobarbital, decreases the effect of theophylline.
Bromazepam-Theophylline may decrease the therapeutic effect of bromazepam. Monitor for changes in the therapeutic effects of bromazepam if theophylline is initiated, discontinued or dose changed.
Atracurium-Theophylline decreases the effect of the muscle relaxant
Clarithromycin-Clarithromycin may increase the therapeutic and adverse effects of theophylline.

Food interactions : Avoid alcohol, Avoid excessive quantities of coffee or tea (Caffeine), Take with food.

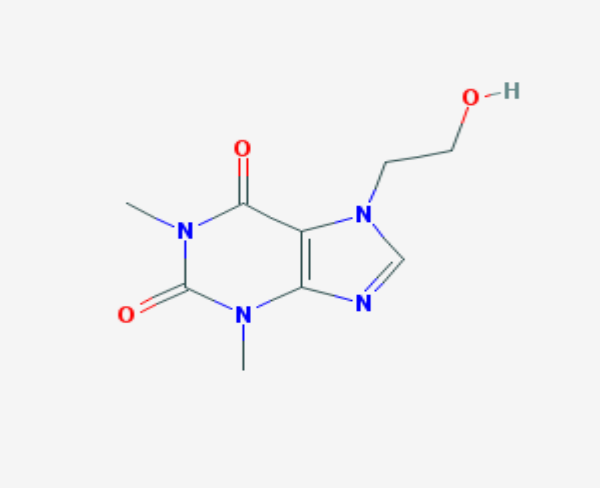
ETOPHYLLINE

Synonym : Etofyllinum(Latin),Etofyllin(German),Etofylline(French), Etofilina (Spanish),7-(2-Hydroxyethyl)theophylline, Aethophyllinum, Oxyphylline, Oxyethyltheophylline, 519-37-9, Ascorphylline

Drug category : Cardiac stimulant
Bronchodilator
Antiasthmatic agent
Non-selective phosphodiesterase inhibitor

Brand name (Single Drug) : Deriphyllin, Deriphyllin Retard , Oxyphyllin, Bronchilet

Structure :



IUPAC Name : 7-(2-hydroxyethyl)-1,3-dimethylpurine-2,6-dione

Molecular Formula : $C_9H_{12}N_4O_3$

Molecular Weight : 224.2 gram/mole

Official Pharmacopoeia : British *Pharmacopoeia*, United States *Pharmacopoeia*, Indian *Pharmacopoeia*,

European pharmacopoeia

PHYSICOCHEMICAL PROPERTIES		
Description	:	The applied Preparation is a combination of theophylline and etophylline in the ratio of 1:3. Etophylline is the hydroxy ethyl ester of theophylline (containing 80% of theopylline by weight). They belong to methyl xanthine group of drugs.
Physical State	:	A White crystalline powder.
Solubility	:	soluble in water, slightly soluble in alcohol.
Storage Conditions	:	Store in a cool, dark place.
Dosage	:	Tablet(oral)- 400 – 1600 mg/day in 2 –3 divided doses. I.V. infusion of 0.6 mg./kg/hour (in hepatic failure reduce to 0.3 mg/kg/hour).
Melting point	:	153-155°C
pKa(strongest basic)	:	13.39
PHARMACOKINETICS		
Bioavailability	:	80%.
Half-life	:	6-8hrs
Absorption	:	It is well absorbed orally, distributed in all tissuses, crosses the placentas and is secreted in milk
Volume of Distribution	:	0.60 liter/kg
Protein binding	:	60%
Metabolism	:	metabolized in the liver by demethylation and oxidation.
Time of peak action	:	1-3days
Excretion	:	20% of the drug is excreted unchanged in the urine.
PHARMACODYNAMICS		
Mechanism of action	:	It inhibits phosphodiesterase, which degrades cyclic nucleotides, hence increased amount of intra cellular CAMP molecules causing smooth muscle relaxation. · Blockade of adenosine receptors (which enhance release of histamine and other inflammatory mediator and bronchospasm). Overall effect of the drug is to produce. - Bronchodilation by bronichial muscle relaxation. - Suppression of response of airways to stimuli. - Cardiac stimulation (increases heart rate and cardiac output). - Respiratory stimulation it also induces diuresis.
Indications	:	Treatment of acute attacks or status asthamticus in conjunction with other drugs. · Suppression of attacks in chronic asthma. · Relief of dyspnoea, bronchospasm in other respiratory disorders like COPD, emphysema. · Acute left ventricular failure and pulmonary edema.
Adverse reactions	:	They are dose dependent. · GIT disturbances like nausea, vomiting, abdominal pain diarrhoea and GI bleeding. · CNS affects – insomnia, headache, restlessness. · Palpitation, diuresis. · Tachycardia
Contraindications	:	Hypersensitivity. · Uncontrolled arrhythmias. · Hyperthyroidism. · Active peptic ulcer. · Uncontrolled seizure disorders. · Prophyria
INTERACTIONS		
Drug interactions	:	Potentially fatal: · The following drugs increase theophylline levels inhibition of hepatic cytochrmma P450 and may cause toxicity of theophylline. - Ciprofloxacin, erythromycin, clarithromycin, fluconazole, ketoconazole. - Didulfiram, estogen contain oral contraceptive pills, pentoxphylline, ticlopidine, methotrexate, propofenone, diltiazem, and verapmil. Allopurinol,

fluvoxamine.

AIM AND OBJECTIVES

- Review of literature for Etophylline and Theophylline gave information regarding its physical and chemical properties, various analytical methods that were conducted alone and in combination with other drugs.
- Literature survey reveals that certain chromatographic methods were reported for simultaneous estimation of Etophylline and Theophylline and single method is available for such estimation by RP-HPLC.
- In view of the need for a suitable RP-HPLC method for routine analysis of Etophylline and Theophylline in formulations, attempts were made to develop simple, precise and accurate analytical method for simultaneous estimation of Etophylline and Theophylline and extend it for their determination in formulation.
- Validation is a necessary and important step in both framing and documenting the capabilities of the developed method.
- The utility of the developed method to determine the content of drug in commercial formulation was also demonstrated. Validation of the method was done in accordance with USP and ICH guideline for the assay of active ingredient. The method was validated for parameters like system suitability, linearity, precision, accuracy, specificity, ruggedness, robustness, limit of detection and limit of quantification. This method provides means to quantify the component. This proposed method was suitable for the analysis of Pharmaceutical dosage forms.

The primary objective of proposed work is

- To develop new simple, sensitive, accurate and economical analytical method for the simultaneous estimation of Etophylline and Theophylline.
- To validate the proposed method in accordance with USP and ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the Etophylline and Theophylline in dosage form.

5. EXPERIMENTAL WORK

INSTRUMENTS USED

Table: Instruments used.

S.No	Instruments And Glasswares	Model
1	HPLC	WATERS, software: Empower 2, Alliance 2695 separation module. 996 PDA detector.
2	pH meter	LabIndia
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

CHEMICALS USED

Table: chemicals used.

S.No	Chemical	Brand names
1	Etophylline	Sura labs
2	Theophylline	Sura labs
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

HPLC METHOD DEVELOPMENT TRAILS

Preparation of standard solution

Accurately weigh and transfer 10 mg of Etophylline and Theophylline working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.15ml of Theophylline and 0.49ml the above Etophylline stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization
Initially the mobile phase tried was Methanol: Water, Acetonitrile: water and Phosphate buffer pH 4.0: Methanol with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Water in proportion 10:90v/v respectively.

Optimization of Column
The method was performed with various columns like C18 and C8 columns, Symmetry and Xterra column. Luna C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 0.9ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used	: Waters Alliance 2695 HPLC with PDA Detector 996 model.
Temperature	: 35ºc
Column	: Phenomenex Luna C18 (4.6×150mm, 5µ)
Mobile phase	: Acetonitrile: Water (10:90v/v)
Flow rate	: 0.9ml/min
Wavelength	: 240 nm
Injection volume	: 10 µl
Run time	: 6min

VALIDATION
PREPARATION OF BUFFER AND MOBILE PHASE
Preparation of mobile phase
Accurately measured 100 ml (10%) of Acetonitrile and 900 ml of Water (90%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation
The Mobile phase was used as the Diluent.

VALIDATION PARAMETERS
SYSTEM SUITABILITY AND SPECIFICITY STUDY OF DRUG
Preparation of Standard Solution
Accurately weigh and transfer 10 mg of Etophylline and 10mg of Theophylline working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of Theophylline and 0.49ml the above Etophylline stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure: The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Preparation of Sample Solution
Take average weight of Ten Tablets and crush in a mortar by using pestle and weight 10 mg equivalent weight of Etophylline and Theophylline sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.15ml of Theophylline and 0.49ml the above Etophylline stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure
Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

PREPARATION OF DRUG SOLUTIONS FOR LINEARITY
Accurately weigh and transfer 10 mg of Etophylline and 10mg of Theophylline working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (16.5ppm of Etophylline&5ppm of Theophylline)
Pipette out 0.16ml of Etophylline and 0.05ml of Theophylline stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (33ppm of Etophylline&10ppm of Theophylline)
Pipette out 0.33ml of Etophylline and 0.1ml of Theophylline stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (49.5ppm of Etophylline&15ppm of Theophylline)
Pipette out 0.49ml of Etophylline and 0.15ml of Theophylline stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (66ppm of Etophylline&20ppm of Theophylline):
Pipette out 0.66ml of Etophylline and 0.2ml of Theophylline stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (82.5ppm of Etophylline&25ppm of Theophylline):
Pipette out 0.82ml of Etophylline and 0.25ml of Theophylline stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure
Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

PRECISION

REPEATABILITY

Preparation of Etophylline and Theophylline Product Solution for Precision

Accurately weigh and transfer 10 mg of Etophylline and 10mg of Theophylline working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of Theophylline and 0.49ml the above Etophylline stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

INTERMEDIATE PRECISION

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure

DAY 1

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

DAY 2

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy

For preparation of 50% Standard stock solution

Accurately weigh and transfer 10 mg of Etophylline and 10mg of Theophylline working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.075ml of Theophylline and 0.24ml the above Etophylline stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 100% Standard stock solution

Accurately weigh and transfer 10 mg of Etophylline and 10mg of Theophylline working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of Theophylline and 0.49ml the above Etophylline stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

For preparation of 150% Standard stock solution

Accurately weigh and transfer 10 mg of Etophylline and 10mg of Theophylline working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.74ml of Etophylline and 0.22ml of Theophylline from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Etophylline and Theophylline and calculate the individual recovery and mean recovery values.

ROBUSTNESS

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

For preparation of Standard solution

Accurately weigh and transfer 10 mg of Etophylline and 10mg of Theophylline working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of Theophylline and 0.49ml the above Etophylline stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Effect of Variation of flow conditions

The sample was analyzed at 0.8 ml/min and 1.0 ml/min instead of 0.9ml/min, remaining conditions are same. 10µl of the above sample was injected twice and chromatograms were recorded.

Effect of Variation of mobile phase organic composition

The sample was analyzed by variation of mobile phase i.e. Acetonitrile: Water was taken in the ratio and 5: 95, 15:85 instead 10:90, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

Trails

Trail 1.

Column : Symmetry C18 (4.6×250mm) 5µ
Column temperature : 30ºC
Wavelength : 240nm

Mobile phase ratio : Methanol: Water (80:20) V/V
Flow rate : 1ml/min
Injection volume : 20 µl
Run time : 9min

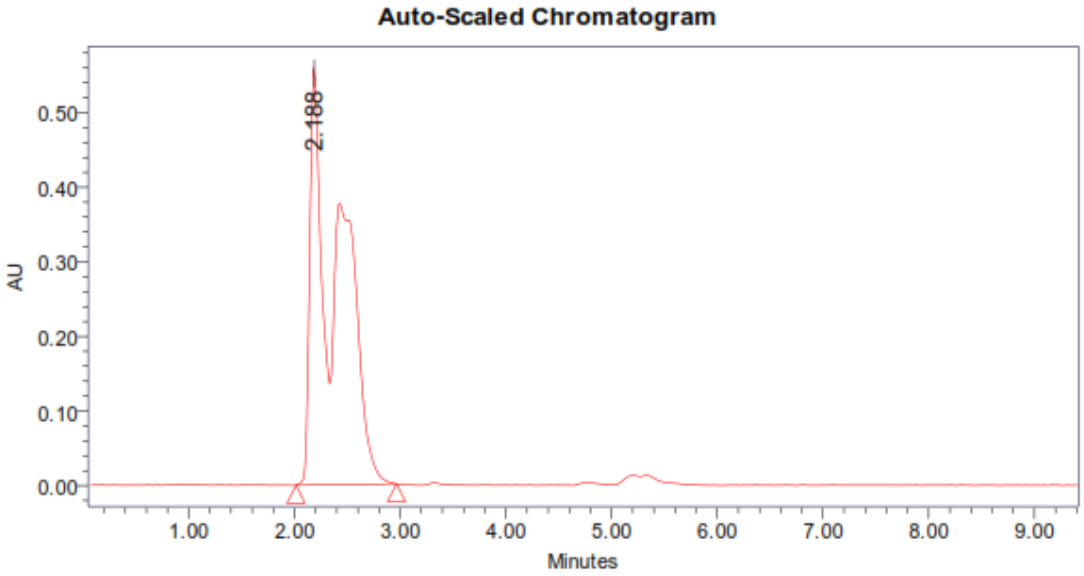


Figure: Chromatogram for trail 1.

Table: peak results for trail 1.

S.No	Peak Name	R_t	Area	Height	USP Tailing	USP Plate count
1	Etophylline	2.188	10200381	560888	3.71	1771

Observation

From the above chromatogram it was observed that the Etophylline and Theophylline peaks were not properly separated and show less plate count, more tailing and improper baseline in the chromatogram. Required more trails to get good peaks.

Column temperature : 30°C
Wavelength : 240nm
Mobile phase ratio : Acetonitrile: Water (60:40% v/v)
Flow rate : 1ml/min
Injection volume : 10µl
Run time : 10min

Trail 2

Column : X terra C18 5µm (4.6×150mm)

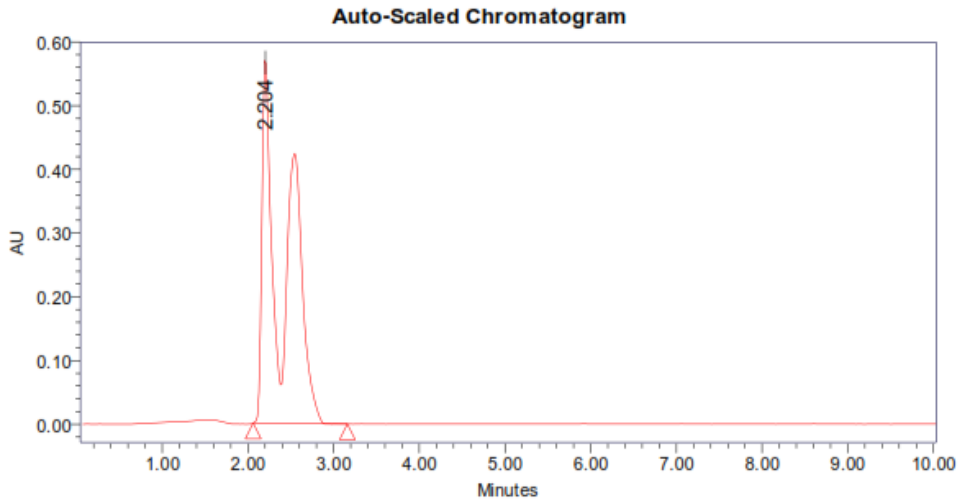


Figure: chromatogram for trail 2.

Table: peak results for trail 2.

S. No	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Etophylline	2.204	9711556	576049	4.0	1815

Observation: From the above chromatogram it was observed that the two sample peaks are properly not separated shows more tailing and less plate count in the chromatogram. So it's required more trails to obtained proper peaks.

Trail 3

Column : Xterra C18 (4.6 × 150mm) 5µm
Column temperature : 35ºC
Wavelength : 240nm
Mobile phase ratio : Phosphate buffer pH 4: Methanol (55:45%v/v)
Flow rate : 0.9ml/min
Injection volume : 10µl
Run time : 10min

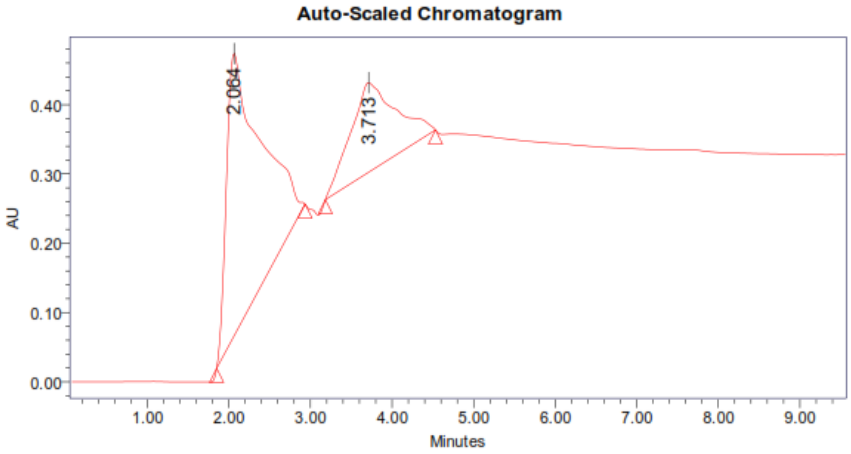


Figure- Chromatogram for trail 3.

Table: peak results for trail 3.

S. No	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Etophylline	2.064	11765665	408691	2.48	136
2	Theophylline	3.713	4945559	129571	1.29	183

Observation: From the above chromatogram, it shows improper separation of two peaks and less plate count and more tailing in the chromatogram. Required more trails to get proper peaks.

Optimized Chromatogram (Standard)

Mobile phase : Acetonitrile: Water (10:90%v/v)
Column : Phenomenex Luna C18 (4.6×150mm, 5µ)
Column temperature : 35ºC
Wavelength : 240nm
Flow rate : 0.9ml/min
Injection volume : 10µl
Run time : 6minutes

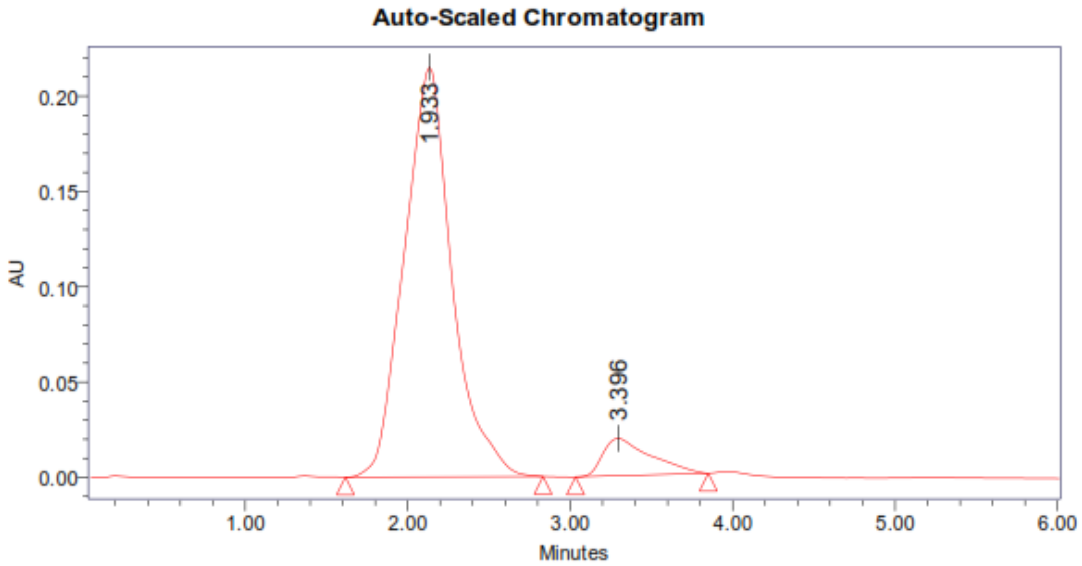


Figure: Optimized Chromatogram (Standard)

Table: Optimized Chromatogram (Standard).

S.No	Name	Rt (min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP Plate count
1	Etophylline	1.933	409905	214828		1.15	4242
2	Theophylline	3.396	392596	19612	4.9	1.78	6515

Observation

By the above chromatogram it shows that separation of two peaks is well, it shows proper plate count, tailing and shows proper resolution. So it was optimized chromatogram.

Optimized Chromatogram (Sample)

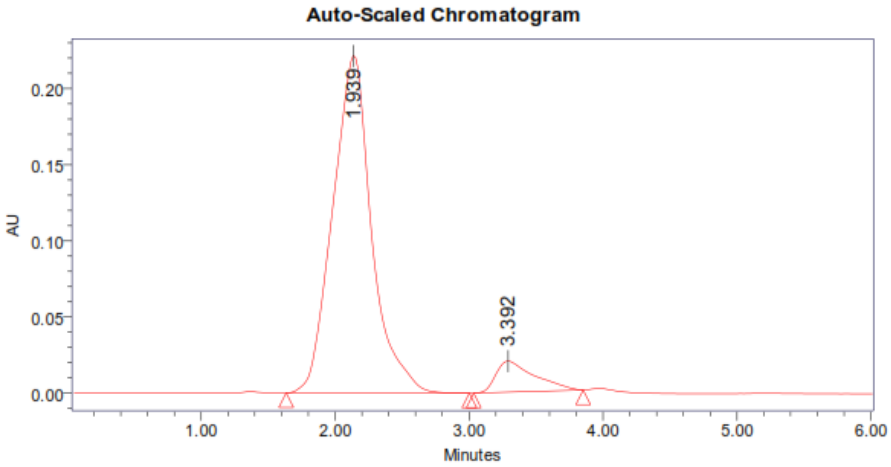


Figure: Optimized Chromatogram (Sample).

Table: Optimized Chromatogram (Sample)

S.No	Name	Rt (min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP Plate count
1	Etophylline	1.939	409003	221408		1.13	5253
2	Theophylline	3.392	323731	20385	4.9	1.79	7569

Acceptance criteria

- Resolution between two drugs must be not less than 2
- Theoretical plates must be not less than 2000
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

VALIDATION

Blank

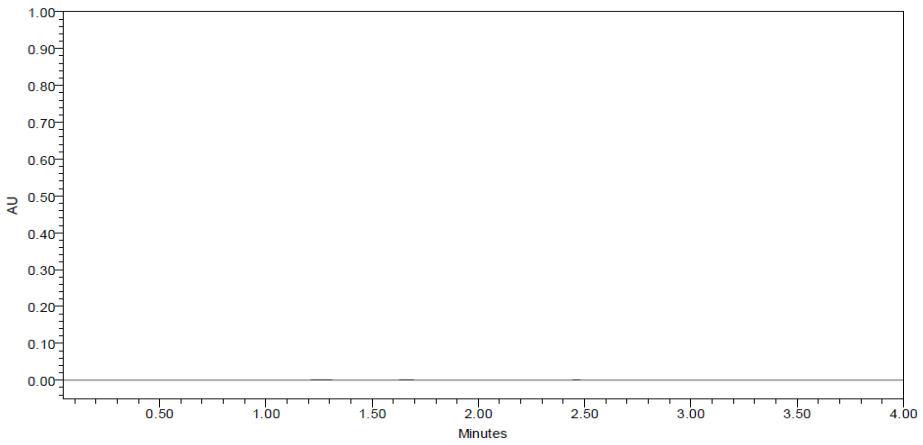


Fig: Chromatogram showing blank (mobile phase preparation).

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitate Etophylline and Theophylline in drug product.

Assay (Standard)

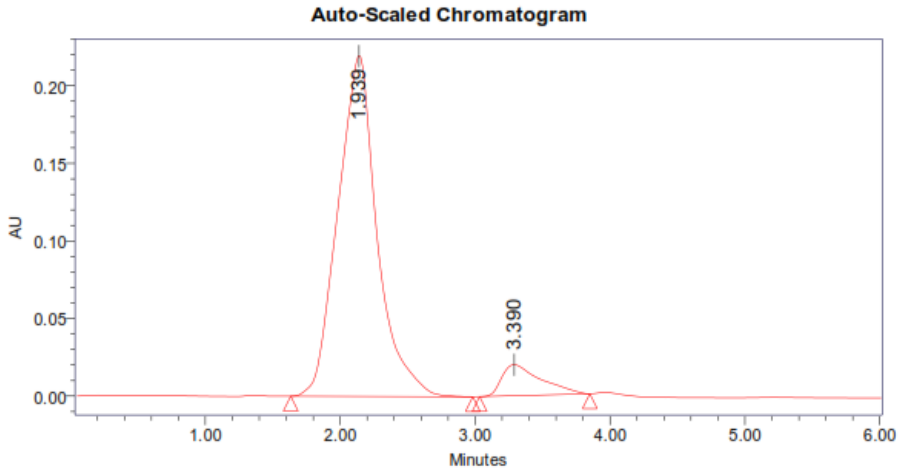


Fig. Chromatogram showing assay of standard injection -1.

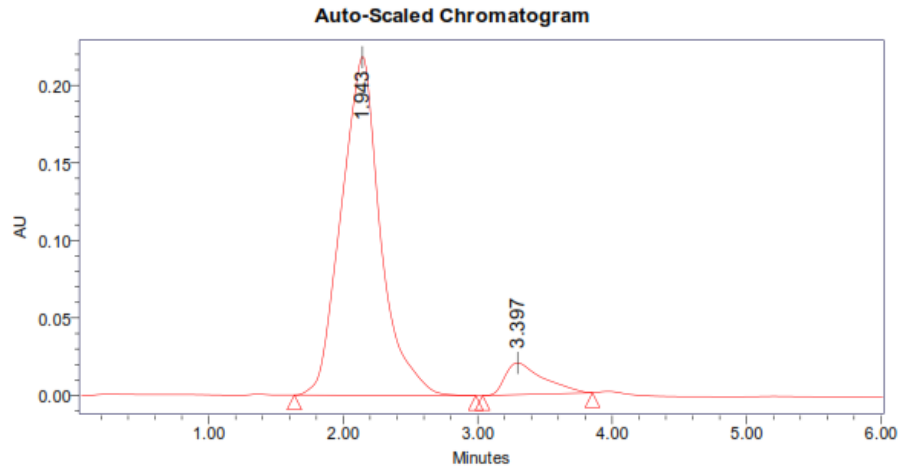


Fig. Chromatogram showing assay of standard injection -2.

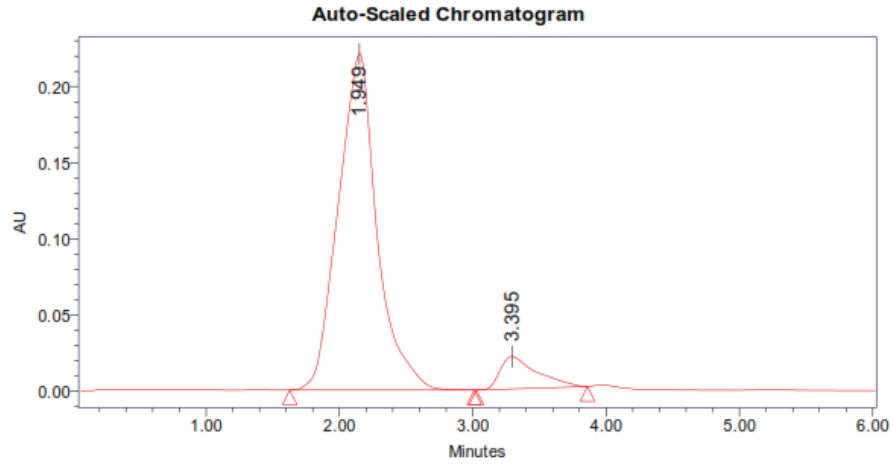


Fig. Chromatogram showing assay of standard injection -3.

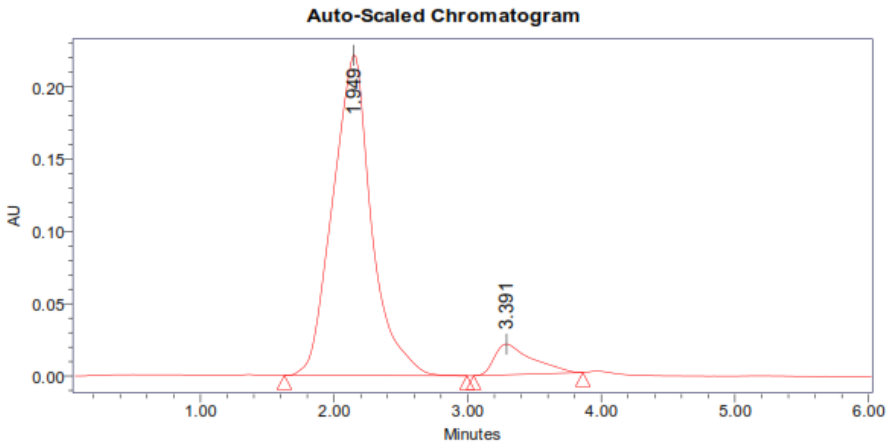


Fig. Chromatogram showing assay of standard injection -4.

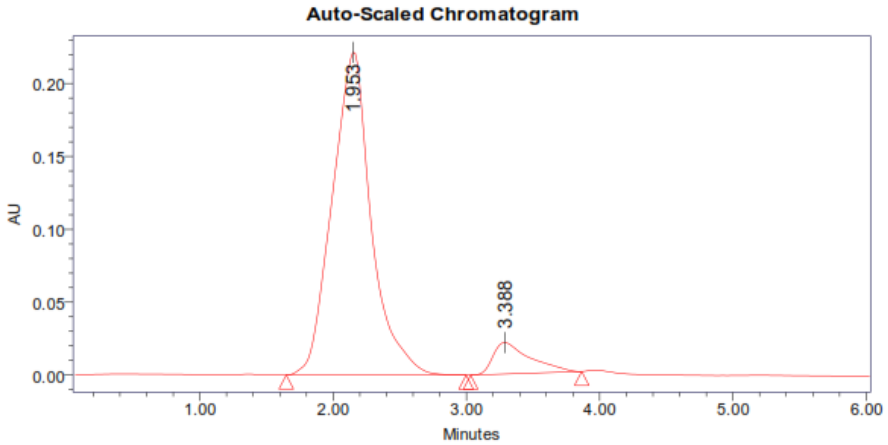


Fig. Chromatogram showing assay of standard injection -5.

Table: Peak results for assay standard of Etophylline

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Etophylline	1.939	407105	219674	5249	1.14
2	Etophylline	1.943	407333	218266	5248	1.14
3	Etophylline	1.949	409824	221080	5254	1.13
4	Etophylline	1.949	403182	221866	5255	1.12
5	Etophylline	1.953	407276	221578	5253	1.13
Mean			406944			
Std. Dev.			2384.036			
% RSD			0.585839			

Acceptance criteria

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

Table: Peak results for assay standard of Theophylline.

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Resolution
1	Theophylline	3.390	390942	20057	6569	1.23	4.9
2	Theophylline	3.397	392296	20602	6613	1.29	4.9
3	Theophylline	3.395	398056	21296	6672	1.29	4.9
4	Theophylline	3.391	393286	21242	6619	1.29	4.9
5	Theophylline	3.388	392284	21592	6672	1.22	4.9
Mean			393372.8				
Std. Dev.			2747.438				
% RSD			0.698431				

Acceptance criteria

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

Assay (Sample)

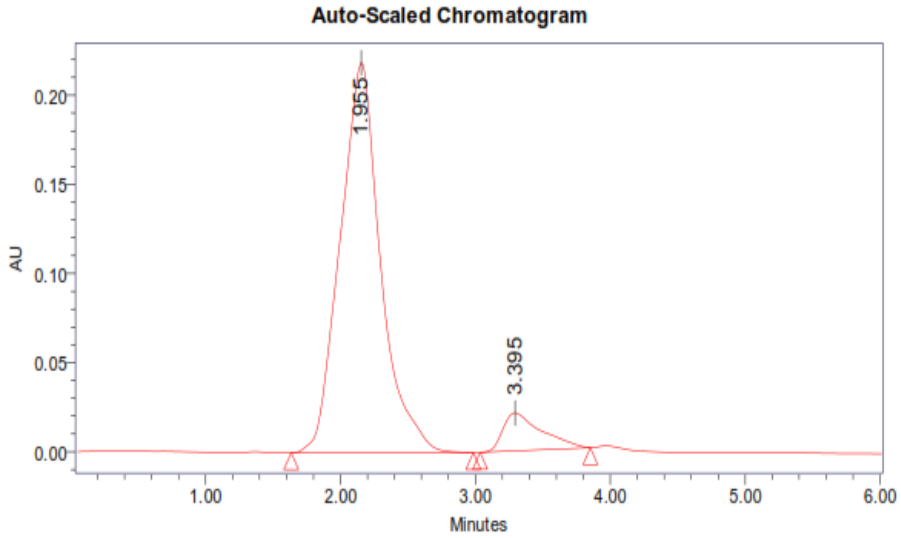


Fig: Chromatogram showing assay of sample injection-1.

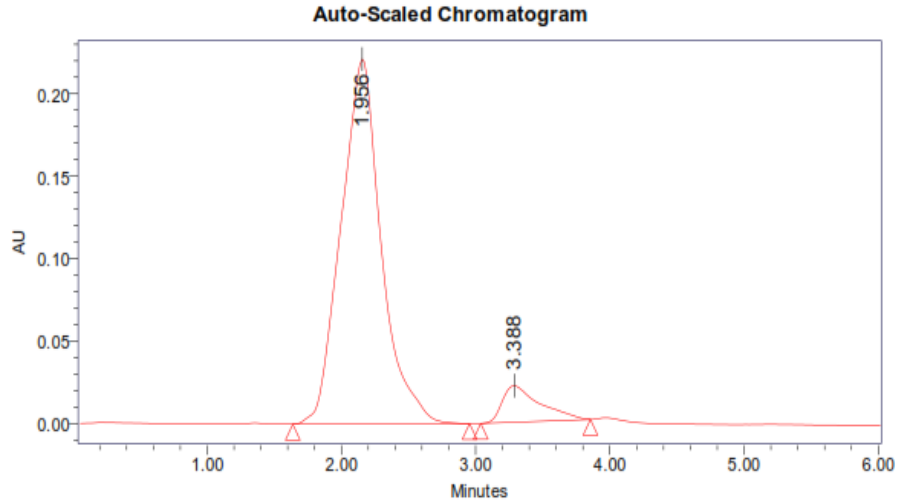


Fig. Chromatogram showing assay of sample injection-2.

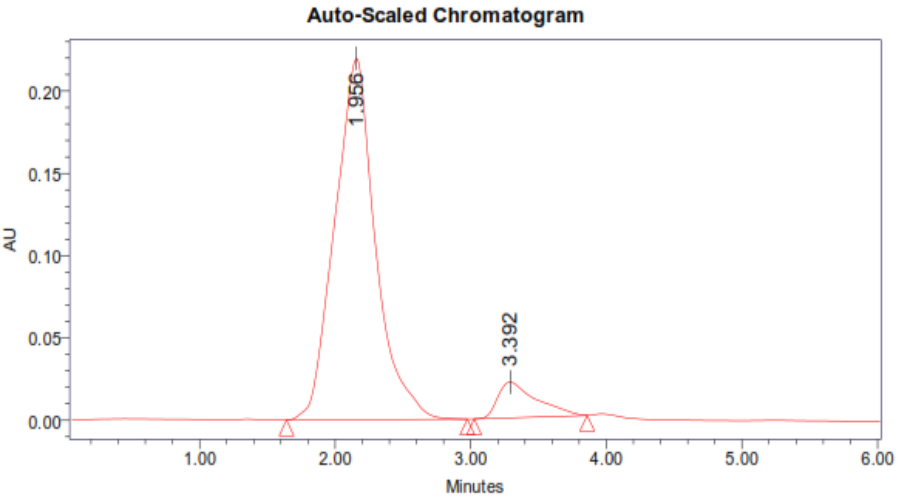


Fig: Chromatogram showing assay of sample injection-3.

Table: Peak results for Assay sample of Etophylline.

S.No	Name	Rt	Area	Height	USP Tailing	USP Plate Count
1	Etophylline	1.955	409895	218842	1.16	5218
2	Etophylline	1.956	409411	221359	1.14	5216
3	Etophylline	1.956	409066	219684	1.14	5427

Table: Peak results for Assay sample of Theophylline.

S.No	Name	Rt	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Theophylline	3.395	387469	21283	1.20	4612	4.9
2	Theophylline	3.388	387471	22171	1.25	4690	4.9
3	Theophylline	3.392	386604	21731	1.20	4640	4.9

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

=409457.3 / 406944 ×10/49.5×49.5/0.0315×99.7/100×0.3151/100×100

= 100.3%

The % purity of Etophylline and Theophylline in pharmaceutical dosage form was found to be 100.3%.

LINEARITY

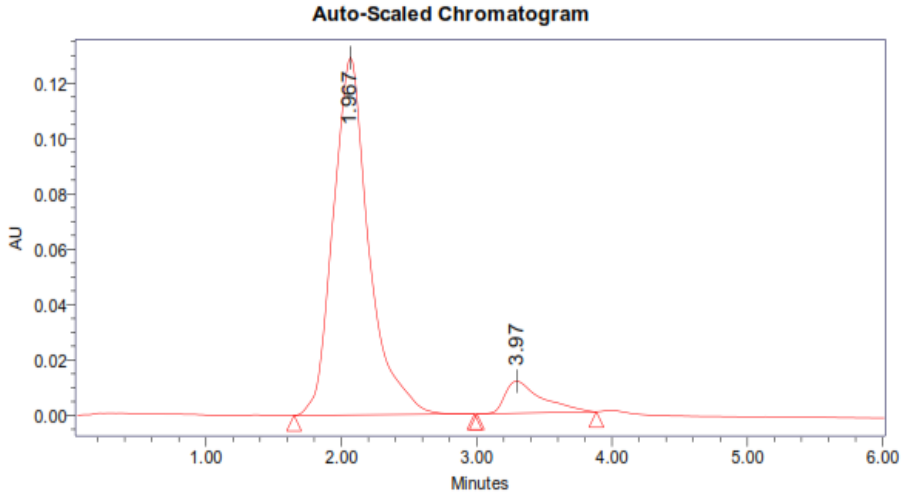


Fig. Chromatogram showing linearity level-1.

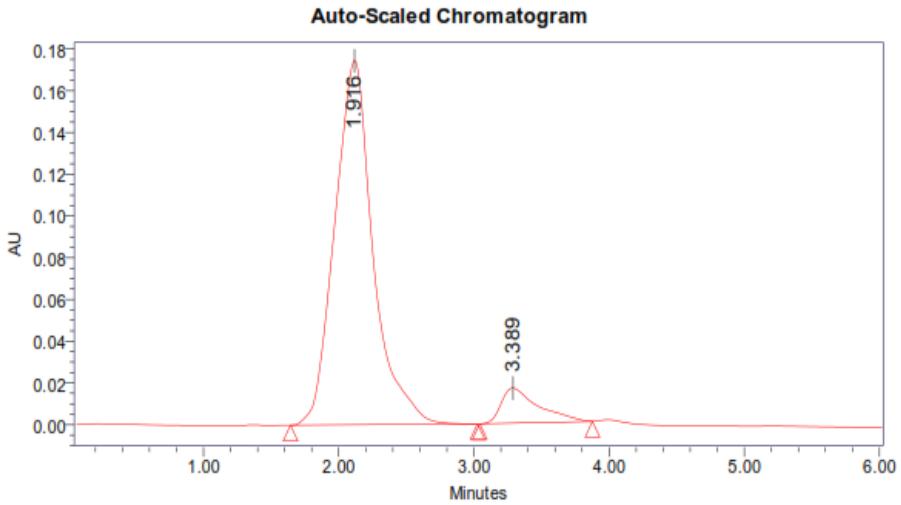


Fig. Chromatogram showing linearity level-2

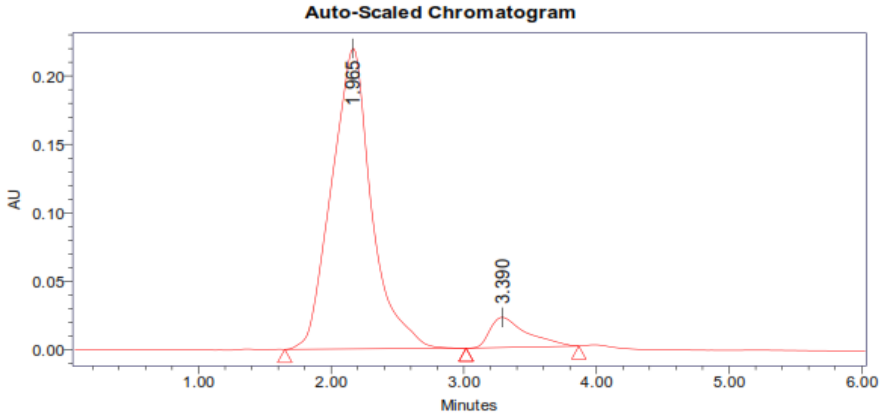


Fig. Chromatogram showing linearity level-3.

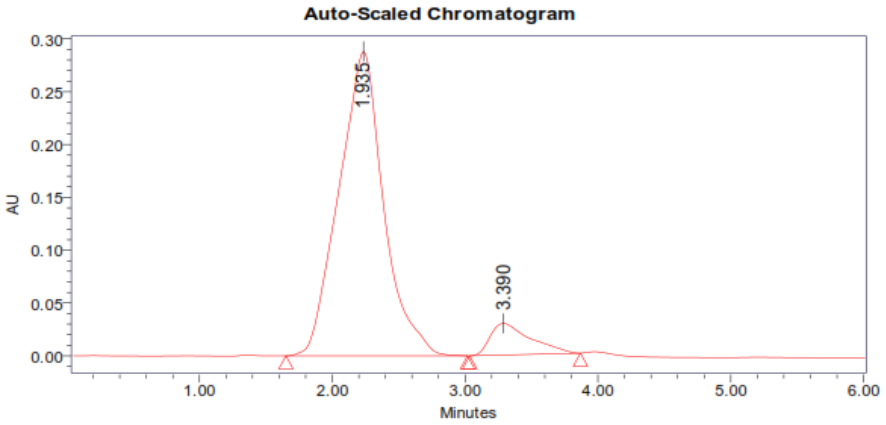


Fig. Chromatogram showing linearity level-4.

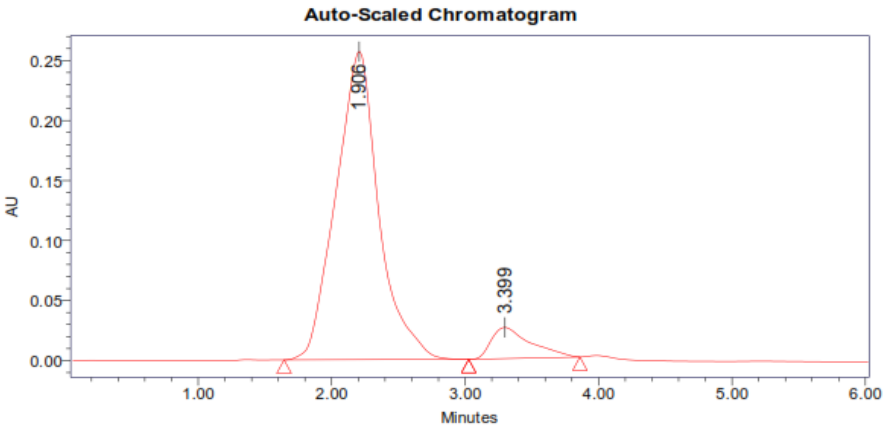
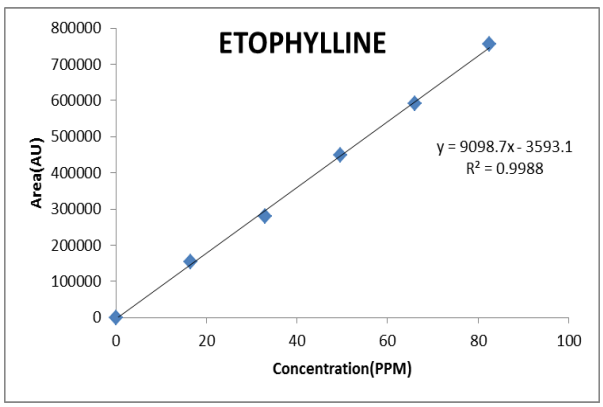


Fig. Chromatogram showing linearity level-5.

CHROMATOGRAPHIC DATA FOR LINEARITY STUDYOF ETOPHYLLINE

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	16.5	154449
66	33	280463
100	49.5	449653
133	66	590193
166	82.5	755619



LINEARITY PLOT

The plot of Concentration (x) versus the Average Peak Area (y) data of Etophylline is a straight line.

$$Y = mx + c$$

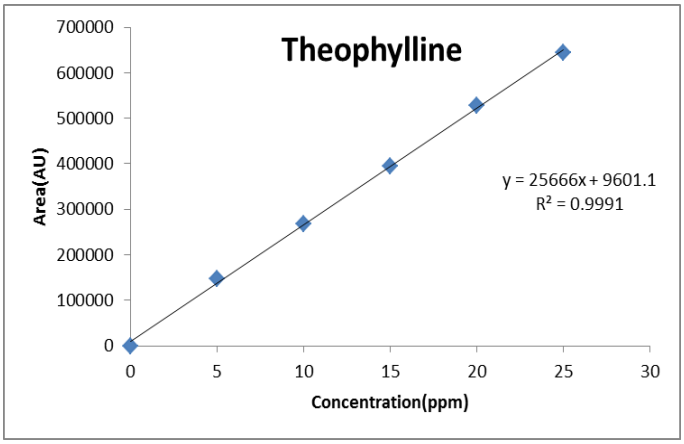
Slope (m) = 9098
Intercept (c) = 3593
Correlation Coefficient (r) = 0.99

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 3593. These values meet the validation criteria.

CHROMATOGRAPHIC DATA FOR LINEARITY STUDYOF THEOPHYLLINE

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	5	147581
66	10	267461
100	15	394576
133	20	528761
166	25	644180



LINEARITY PLOT

The plot of Concentration (x) versus the Average Peak Area (y) data of Theophylline is a straight line.

Y = mx + c
Slope (m) = 25666
Intercept (c) = 9601
Correlation Coefficient (r) = 0.99

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION

Correlation Coefficient (r) is 0.99, and the intercept is 13030. These values meet the validation criteria.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

REPEATABILITY

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

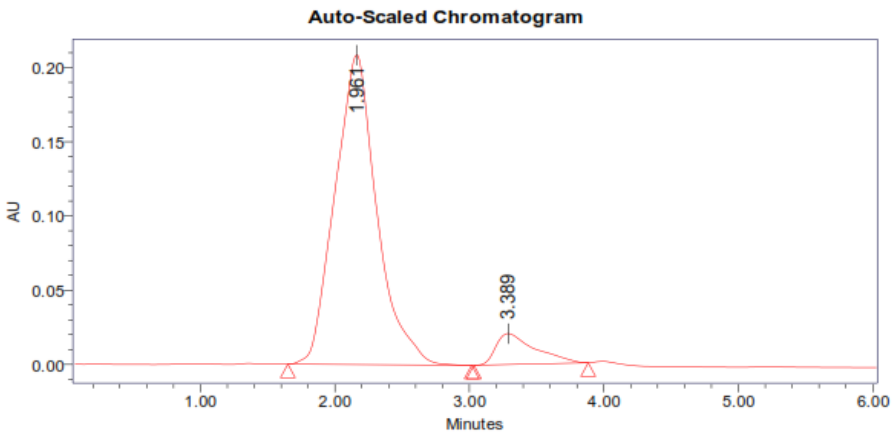


Fig. Chromatogram showing precision injection -1.

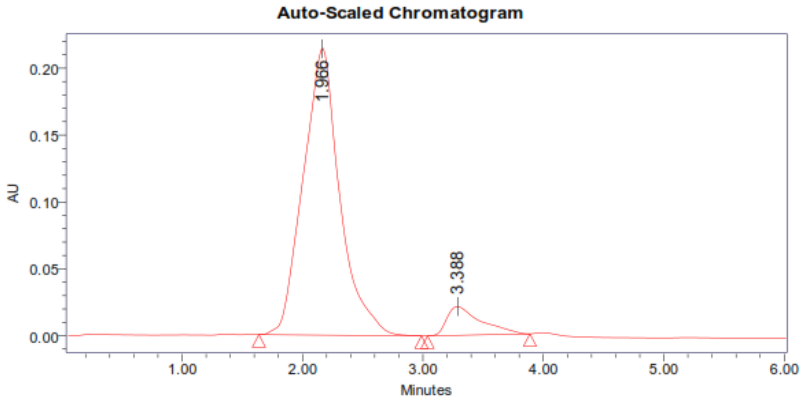


Fig. Chromatogram showing precision injection -2.

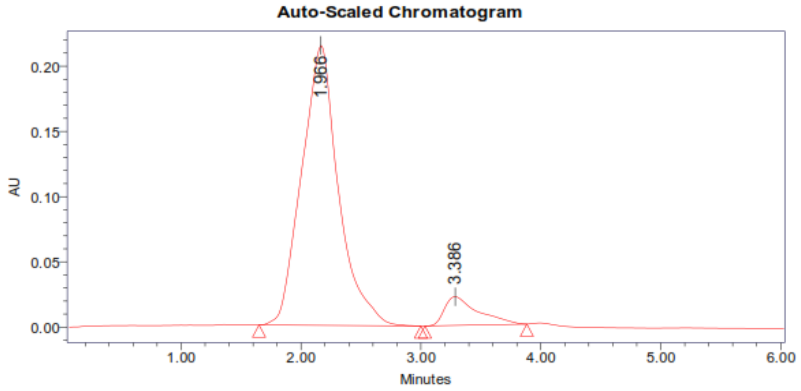


Fig. Chromatogram showing precision injection -3.

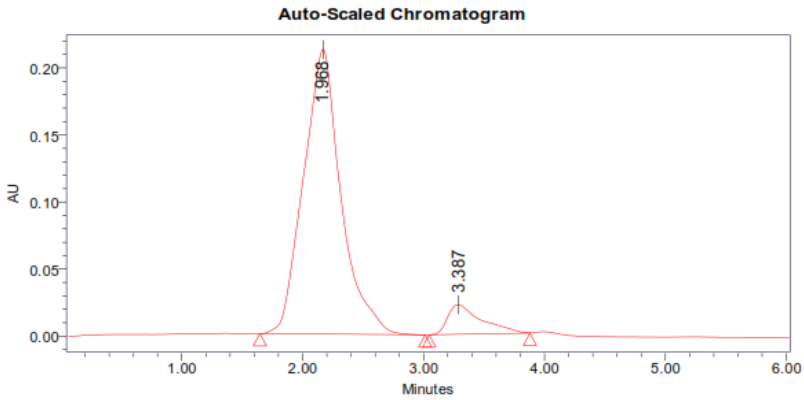


Fig. Chromatogram showing precision injection -4.

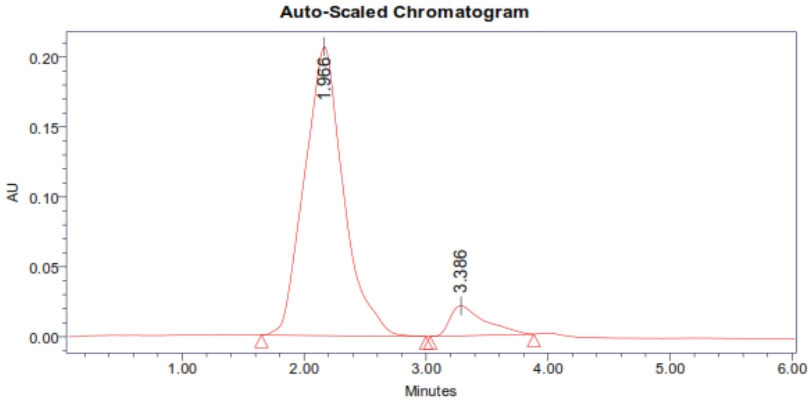


Fig. Chromatogram showing precision injection -5.

Table: Results of repeatability for Etophylline:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Etophylline	1.961	409349	208879	5200	1.18
2	Etophylline	1.966	409980	214656	5213	1.16
3	Etophylline	1.966	407839	214544	5208	1.17
4	Etophylline	1.968	409731	212354	5202	1.18
5	Etophylline	1.966	408042	218482	5193	1.16
Mean			408988.2			
Std.dev			985.0826			
%RSD			0.240858			

Acceptance criteria

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table: Results of repeatability for Theophylline.

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Theophylline	3.389	317876	20821	7639	1.28
2	Theophylline	3.388	320133	21502	6718	1.22
3	Theophylline	3.386	323930	22054	6762	1.21
4	Theophylline	3.387	324517	22022	6748	1.23
5	Theophylline	3.386	323107	21455	6878	1.21
Mean			321912.6			
Std.dev			2816.936			
%RSD			0.875062			

Acceptance criteria

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision

Day 1

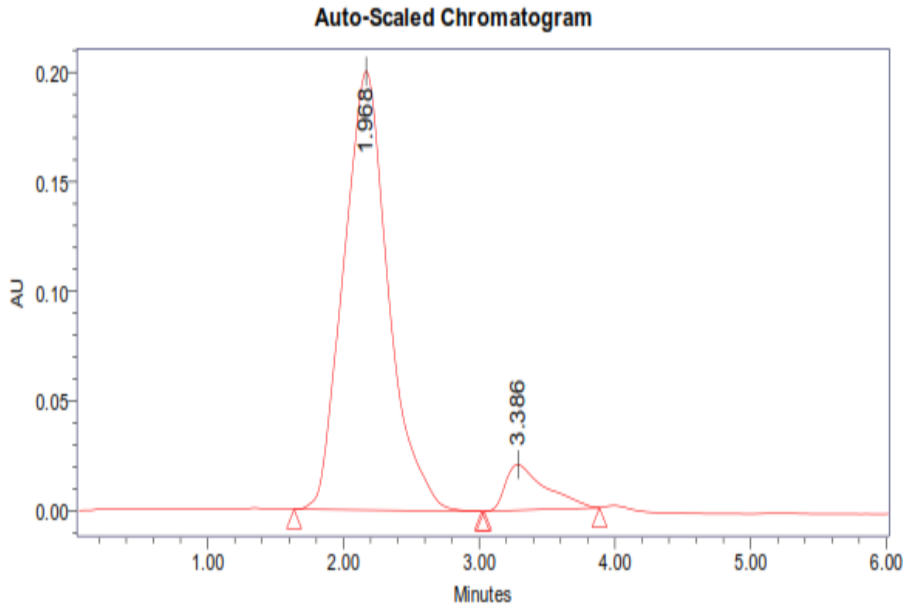


Fig: Chromatogram showing Day1 injection -1.

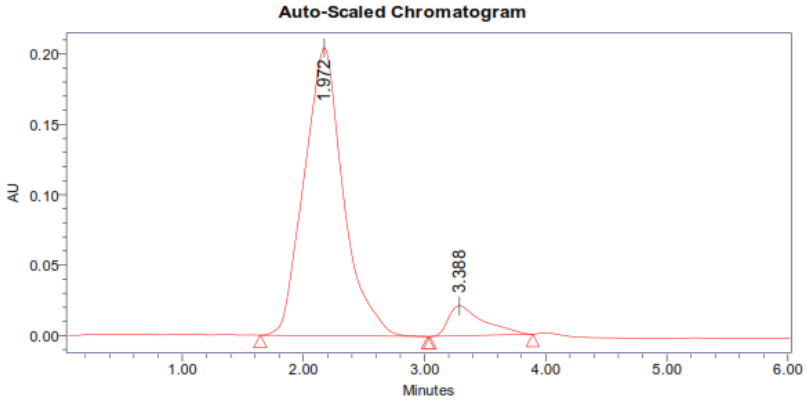


Fig: Chromatogram showing Day1 injection -2.

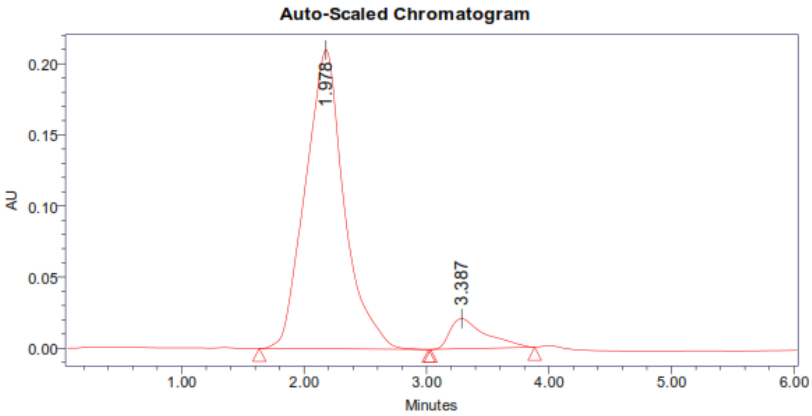


Fig: Chromatogram showing Day1 injection -3.

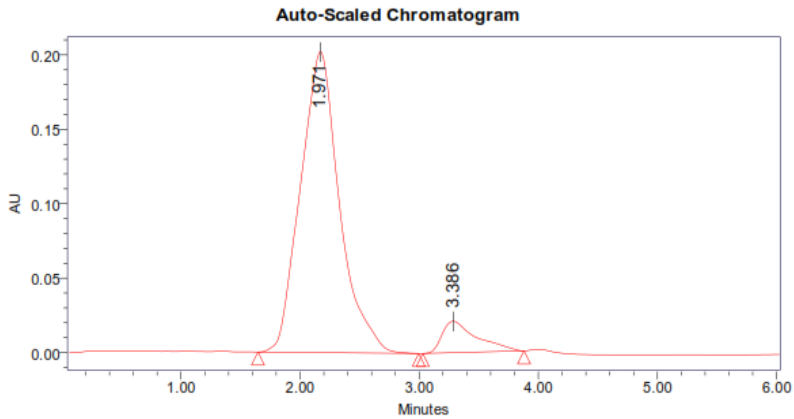


Fig: Chromatogram showing Day1 injection -4.

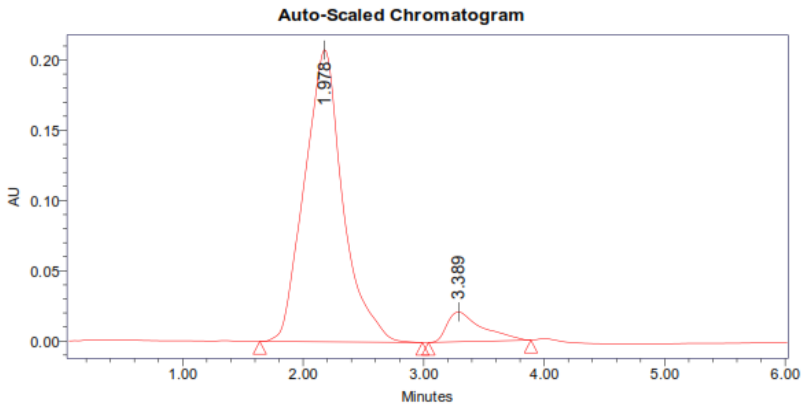


Fig: Chromatogram showing Day1 injection -5.

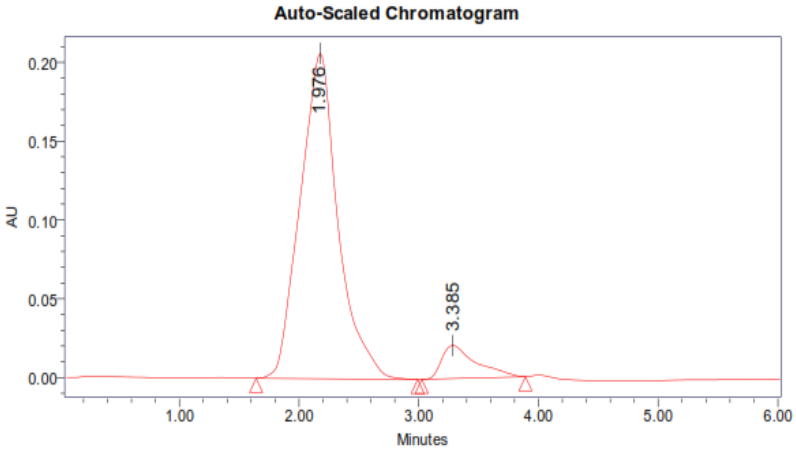


Fig: Chromatogram showing Day1 injection -6.

Table: Results of Intermediate precision for Etophylline.

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Etophylline	1.968	409600	200415	5192	1.1
2	Etophylline	1.972	409792	204737	5202	1.1
3	Etophylline	1.971	409710	202315	5198	1.1
4	Etophylline	1.978	408131	210538	5213	1.1
5	Etophylline	1.978	409596	208031	5213	1.1
6	Etophylline	1.976	409932	206543	5217	1.1
Mean			409460.2			
Std. Dev.			663.3016			
% RSD			0.161994			

Table: Results of Intermediate precision for Theophylline.

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Theophylline	3.386	323199	20851	6281	1.2	4.9
2	Theophylline	3.388	324588	21266	6392	1.2	4.9
3	Theophylline	3.386	321726	21070	6293	1.2	4.9
4	Theophylline	3.387	326955	21217	6039	1.2	4.9
5	Theophylline	3.389	323546	21257	6153	1.2	4.9
6	Theophylline	3.385	327755	20978	6293	1.2	4.9
Mean			324628.2				
Std. Dev.			2316.421				
% RSD			0.713561				

Acceptance criteria

- %RSD of five different sample solutions should not more than 2

Day 2

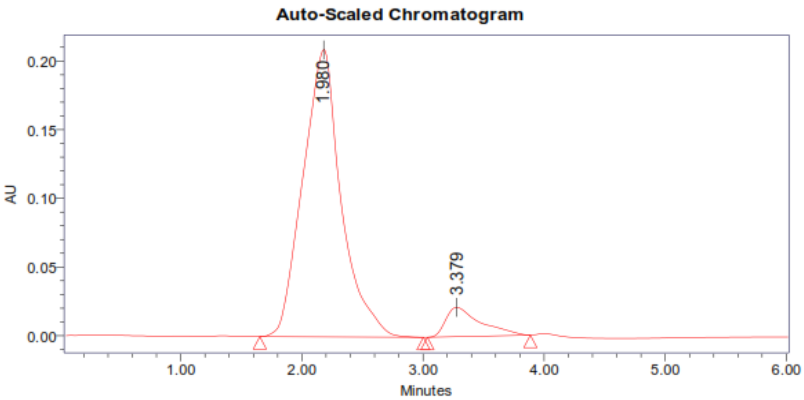


Fig: Chromatogram showing Day 2 injection -1.

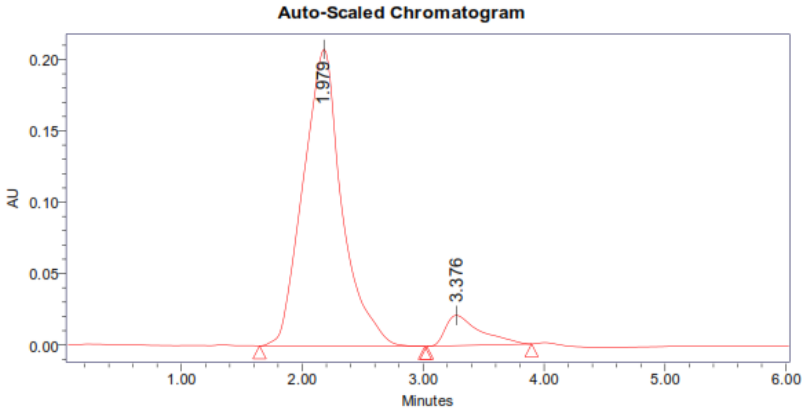


Fig: Chromatogram showing Day 2 injection -2.

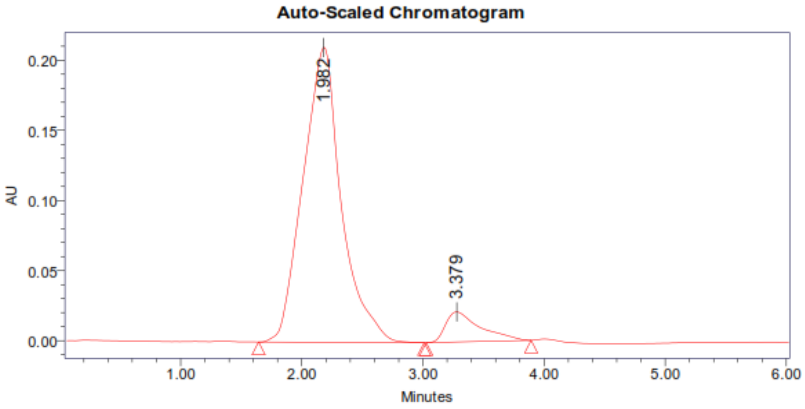


Fig: Chromatogram showing Day 2 injection -3.

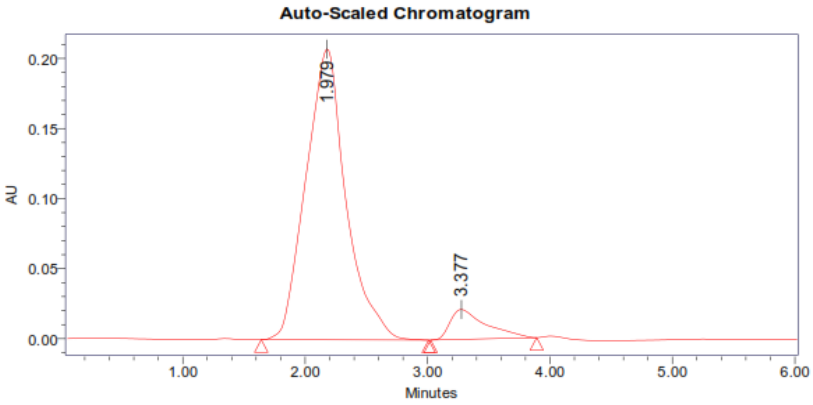


Fig: Chromatogram showing Day 2 injection -4.

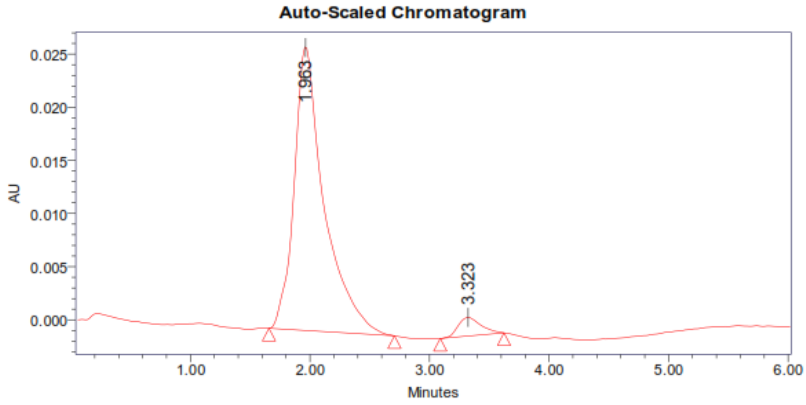


Fig: Chromatogram showing Day 2 injection -5.

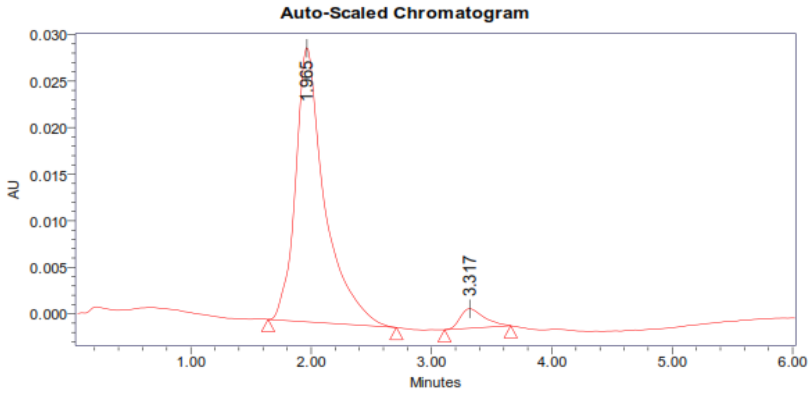


Fig: Chromatogram showing Day 2 injection -6.

Table: Results of Intermediate precision Day 2 for Etophylline.

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Etophylline	1.980	409042	209754	5237	1.1
2	Etophylline	1.982	409920	210411	5023	1.1
3	Etophylline	1.979	407912	208055	5983	1.1
4	Etophylline	1.979	409213	207720	5294	1.1
5	Etophylline	1.963	406475	206740	5819	1.1
6	Etophylline	1.965	409079	209516	5183	1.1
Mean			408606.8			
Std. Dev.			1227.327			
% RSD			0.300369			

Table: Results of Intermediate precision Day 2 for Theophylline.

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Theophylline	3.379	323744	21401	6173	1.2	4.9
2	Theophylline	3.379	325554	21446	6183	1.2	4.9
3	Theophylline	3.376	323154	21266	6103	1.2	4.9
4	Theophylline	3.377	331213	21312	6482	1.2	4.9
5	Theophylline	3.323	323263	21750	6831	1.2	4.9
6	Theophylline	3.317	328951	21602	6153	1.2	4.9
Mean			325979.8				
Std. Dev.			3369.293				
% RSD			1.033589				

Acceptance criteria

- %RSD of five different sample solutions should not be more than 2

6.3.4: ACCURACY

Accuracy at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Accuracy50%

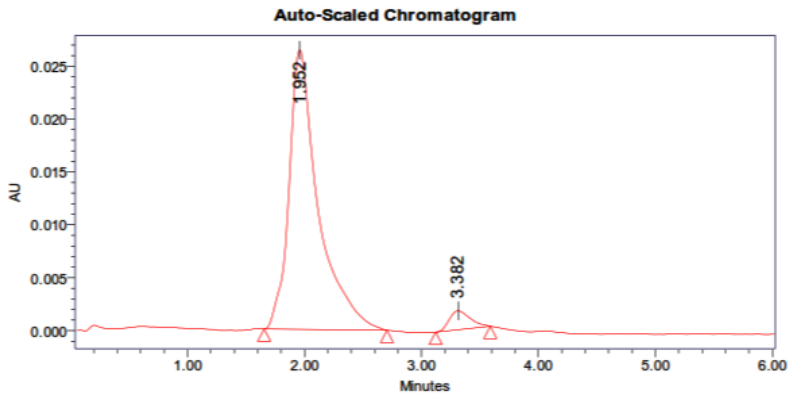


Fig. Chromatogram showing accuracy-50% injection-1.

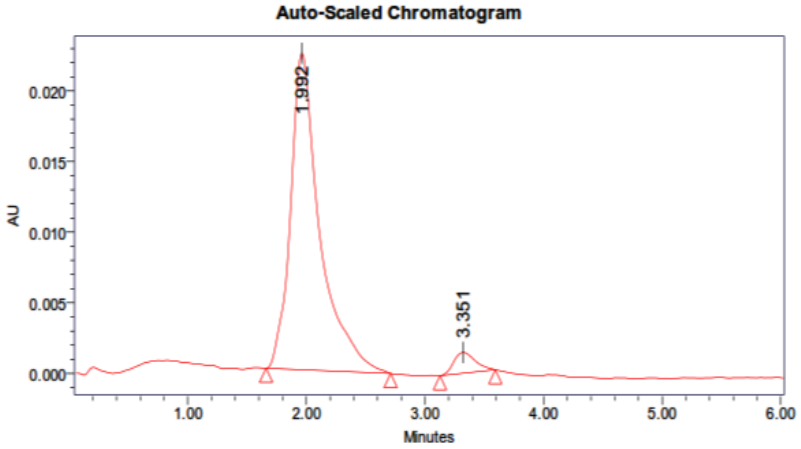


Fig. Chromatogram showing accuracy-50% injection-2.

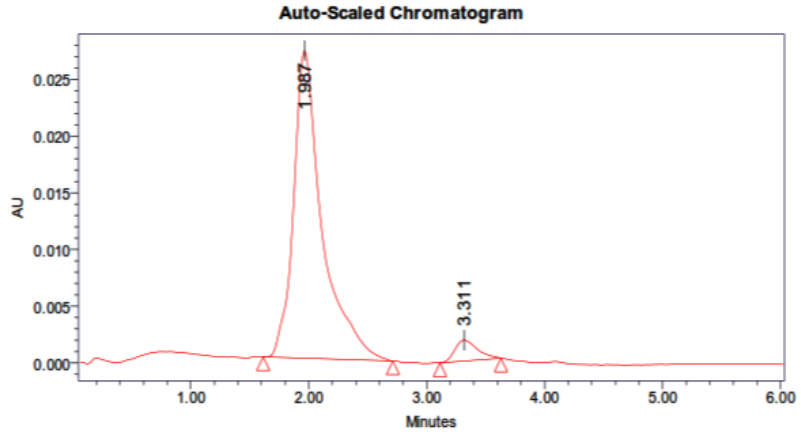


Fig. Chromatogram showing accuracy-50% injection-3.

Table: Results of Accuracy for concentration-50%.

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Etophylline	1.952	211010	11500	1.59	4389	
2	Theophylline	3.382	204421	1599	1.08	6993	4.9
3	Etophylline	1.992	211426	10504	1.68	4337	
4	Theophylline	3.351	198792	1543	1.11	6993	4.9
5	Etophylline	1.987	213644	22037	1.65	4386	
6	Theophylline	3.311	204077	1426	1.34	6326	4.9

Accuracy100%

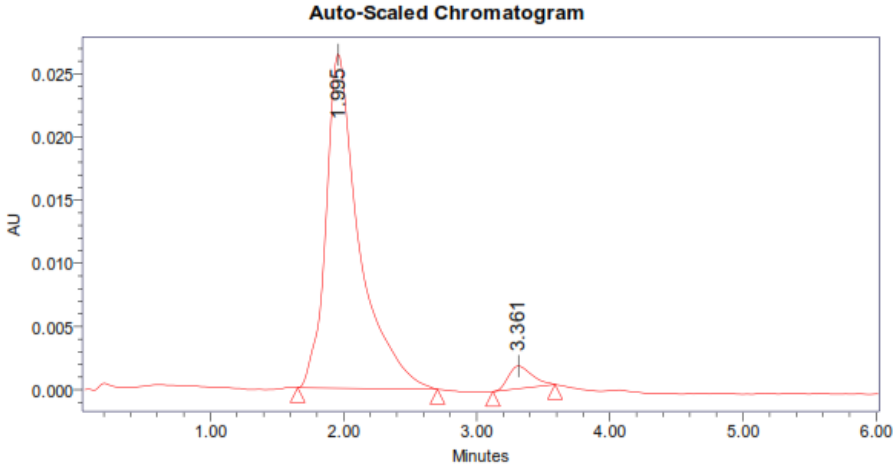


Fig. Chromatogram showing accuracy-100% injection-1.

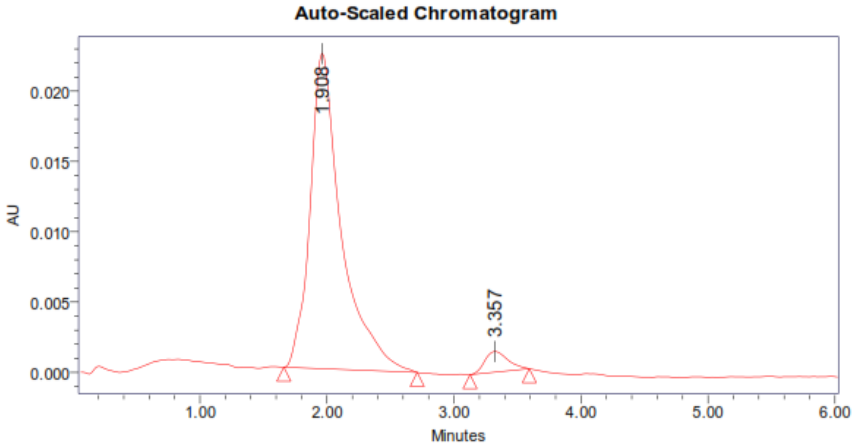


Fig: Chromatogram showing accuracy-100% injection-2.

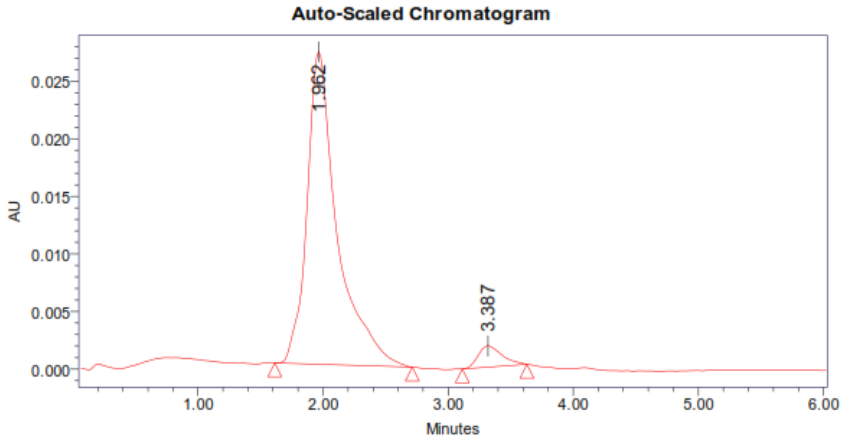


Fig: Chromatogram showing accuracy-100% injection-3.

Table Results of Accuracy for concentration-100%

S.No	Name	RT	Area	Height	USP Tailing	Resolution	USP Plate Count	Injection
1	Etophylline	1.961	409657	26502	1.1		5273	1
2	Theophylline	3.314	392084	1801	1.2	4.9	6932	1
3	Etophylline	1.963	405371	22464	1.1		5193	2
4	Theophylline	3.324	398580	1470	1.2	4.9	6038	2
5	Etophylline	1.965	405629	27150	1.1		5193	3
6	Theophylline	3.319	394317	1843	1.2	4.9	6391	3

Accuracy150%

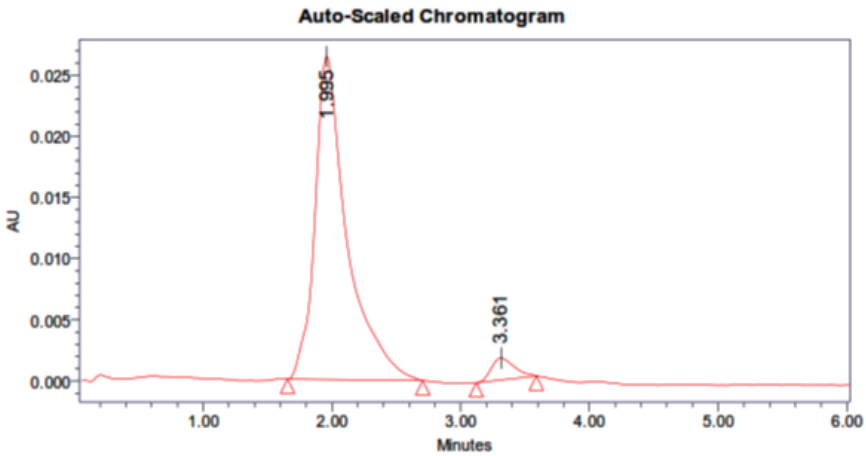


Fig. Chromatogram showing accuracy-150% injection-1.

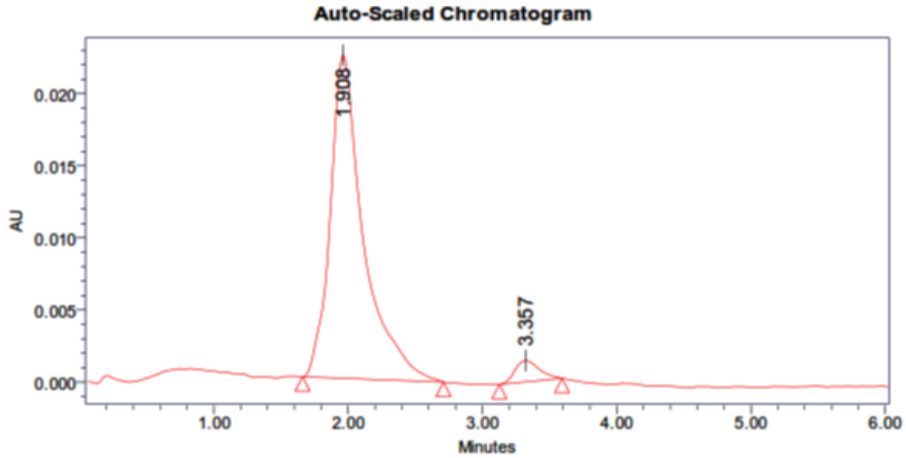


Fig. Chromatogram showing accuracy-150% injection-2.

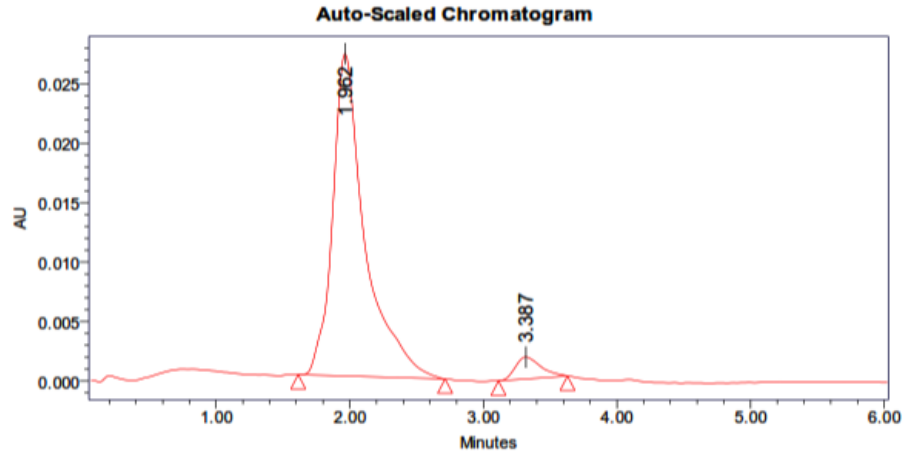


Fig. Chromatogram showing accuracy-150% injection-3.

Table Results of Accuracy for concentration-150%.

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution	Injection
1	Etophylline	1.995	617572	21235	1.1	5182		1
2	Theophylline	3.361	593673	1431	1.2	6821	4.9	1
3	Etophylline	1.908	610827	15704	1.1	5837		22
4	Theophylline	3.357	593703	1058	1.2	6937	4.9	2
5	Etophylline	1.962	615275	19447	1.1	5038		3
6	Theophylline	3.387	593301	1327	1.2	6183	4.9	3

The accuracy results for Etophylline.

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	222026.7	24.75	24.75	100	99.8%
100%	443552.3	49.5	49.2	99.2	
150%	674558	74.25	74.25	100	

Acceptance Criteria

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

The accuracy results for Theophylline.

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	202430	7.5	7.3	98.6	99.8%
100%	394993.7	15	14.9	99.8	
150%	593559	22.5	22.6	101.1	

LIMIT OF DETECTION

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

$$LOD = 3.3 \times \sigma / s$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

ETOPHYLLINE

Result

=3.3×12079.98/9098

=4.3µg/ml

THEOPHYLLINE

Result

=3.3×5074.9/25666

=0.6µg/ml

QUANTITATION LIMIT

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

$$LOQ = 10 \times \sigma / S$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

ETOPHYLLINE

Result

=10×12079.98/9098

=13.2µg/ml

THEOPHYLLINE

Result

= 10×5074.9/25666

= 1.9µg/ml

Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Etophylline and Theophylline. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Etophylline and Theophylline were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Variation in flow

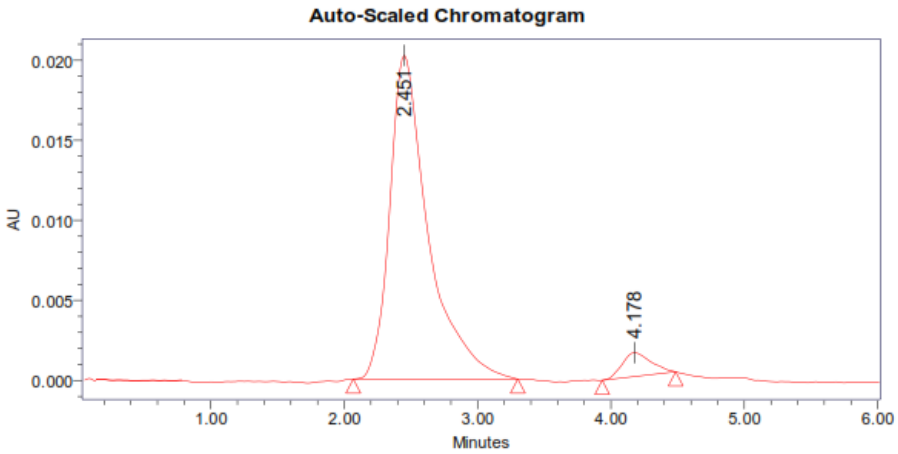


Figure: chromatogram showing less flow of 0.9ml/min.

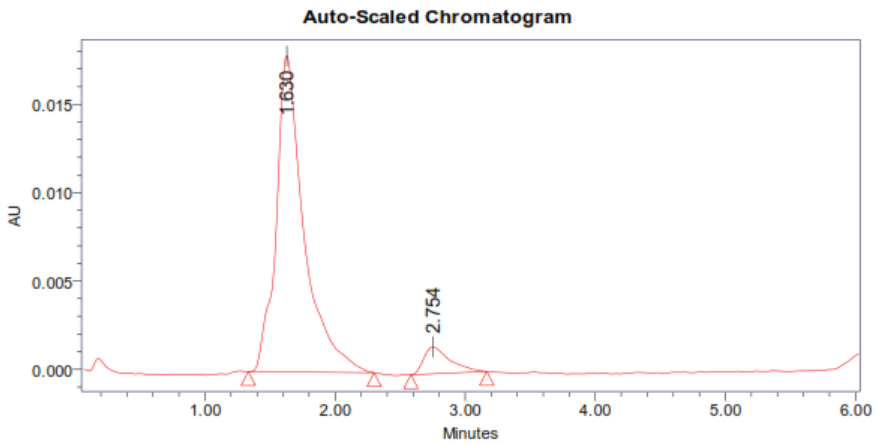


Figure: chromatogram showing more flow of 1.1 ml/min.

Variation of mobile phase organic composition

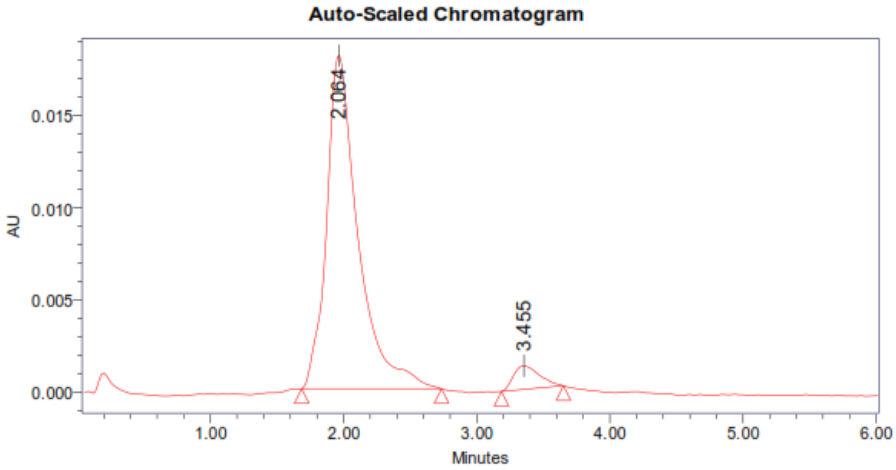


Figure: chromatogram showing less organic composition.

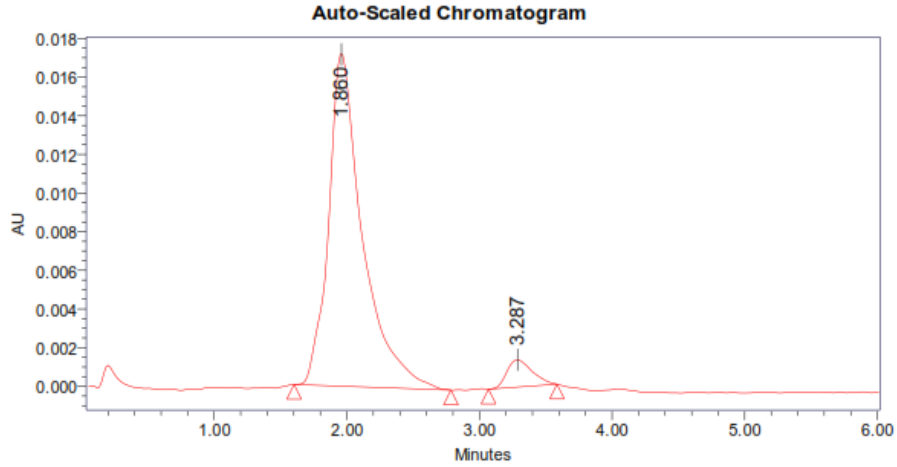


Figure: chromatogram showing more organic composition.

Table: Results for Robustness

ETOPHYLLINE

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	409905	1.933	4242	1.1
Less Flow rate of 0.9 mL/min	407262	2.451	5405	1.6
More Flow rate of 1.1 mL/min	409250	1.630	5365	1.5
Less organic phase	407722	2.064	4393	1.6
More Organic phase	406458	1.960	4358	1.5

Acceptance criteria

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

THEOPHYLLINE

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	392596	3.396	6515	1.7
Less Flow rate of 0.9 mL/min	322247	4.178	4698	1.1
More Flow rate of 1.1 mL/min	321244	2.754	7934	1.7
Less organic phase	317397	3.455	4368	1.4
More Organic phase	318735	3.287	5371	1.3

Acceptance criteria

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

SUMMARY

The analytical method was developed by studying different parameters.

First of all, maximum absorbance was found to be at 240nm and the peak purity was excellent.

Injection volume was selected to be 10µl which gave a good peak area.

The column used for study was Phenomenex Luna C18 (4.6×150mm, 5µ) because it was giving good peak.

35ºC temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 0.9ml/min because of good peak area and satisfactory retention time.

Mobile phase is Acetonitrile: Water (10:90% v/v) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study.

Run time was selected to be 6min because analyze gave peak around 1.9, 3.3 and also to reduce the total run time.

The persent recovery was found to be 98.0-102.0 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range.

The analytical method was found linearity over the range 16.5-82.5mg/ml of Etophylline and 5-25mg/ml of Theophylline of the target concentration.

The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Etophylline and Theophylline in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

Etophylline and Theophylline was freely soluble in ethanol, methanol and sparingly soluble in water.

Acetonitrile: Water (10:90% v/v) was chosen as the mobile phase. The solvent system used in this method was economical.

The %RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

This method can be used for the routine determination of Etophylline and Theophylline in bulk drug and in Pharmaceutical dosage forms.

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