ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ACETYLCYSTEINE AND TAURINE IN TABLET DOSAGE FORM BY USING RP-HPLC

Dissertation submitted to

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI

In partial fulfillment of the requirements for the award of the degree of

MASTER OF PHARMACY

(PHARMACEUTICAL ANALYSIS)

By

(Reg.No:261530351)

Under the Guidance of

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DEPARTMENT OF PHARMACEUTICAL ANALYSIS

ARULMIGU KALASALINGAM COLLEGE OF PHARMACY

ANAND NAGAR, KRISHANKOIL-626126

OCTOBER-2017

CERTIFICATE

This is to certify that the investigation described in the dissertation entitled **"ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ACETYLCYSTEINE AND TAURINE IN TABLET DOSAGE FORM BY USING RP-HPLC"** submitted by **Reg.No:261530351** was carried out in the **Department of Pharmaceutical Analysis, Arulmigu Kalasalingam College of Pharmacy, Anand Nagar, Krishnankoil -626126,** which is affiliated to **The Tamil Nadu Dr. M.G.R. Medical University, Chennai** under my supervision and guidance for the partial fulfillment of **Degree of Master of Pharmacy in Department of Pharmaceutical Analysis.**

Place: Krishnankoil

Date:

Department of Pharmaceutical Analysis

Arulmigu Kalasalingam College of Pharmacy

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Place: Krishnankoil

Date:

Principal

Arulmigu Kalasalingam College of Pharmacy

Krishnankoil

EVALUATION CERTIFICATE

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Centre: Arulmigu Kalasalingam College of Pharmacy, Krishnankoil

Date:

Examiners:

1.

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This is to certify that the research project work entitled "**ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ACETYLCYSTEINE AND TAURINE IN TABLET DOSAGE FORM USING RP-HPLC**" is the bonafied work of **Ms.DEVI.M, (Reg.No: 261530351) Arulmigu Kalasalingam College Of Pharmacy, Krishnankoil 626126**, carried out in the department of **Analytical Research and Development, Fourrts (India) Laboratories Pvt. Limited**, during the year 2016-2017 under my direct guidance and supervision in partial fulfillment for the award of degree of **"MASTER OF PHARMACY** in **PHARMACEUTICAL ANALYSIS".**

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DEDICATED

TO

“MY PARENT”

“MY BROTHERS”

AND

“MY FRIENDS”

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I pray our profound gratitude to the almighty god for this invisible help and blessing for the fulfillment of this work.

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M.Devi

TABLE OF CONTENTS


Introduction

INTRODUCTION

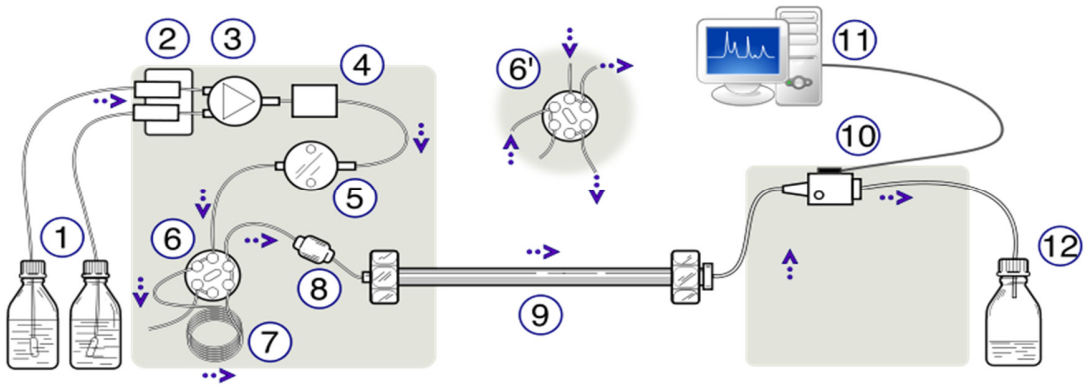
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

High performance liquid chromatography is a very sensitive analytical technique most widely used for quantitative and qualitative analysis of pharmaceutical. The principle advantage of HPLC compared to classical column chromatography is improved resolution of the separated substance, faster separation times and the increased accuracy, precision and sensitivity.

PRINICIPLE OF HPLC

The principle of separation in normal phase mode and reverse phase mode is adsorption. The component which has more affinity towards the adsorbent travels slower. The component which has less affinity towards the stationary phase travels faster. Since 2 components have the same affinity towards the stationary phase, the components are separated.

INSTRUMENTATION OF HPLC



1) Solvent reservoirs
2) Solvent degasser
3) Gradient valve
4) Mixing vessel for delivery of the mobile phase

5) High-pressure pump

6) Switching valve in "inject position" switching valve in "load position"

7) Sample injection loop

8) Pre-column

9) Analytical column

10) Detector (i.e. IR, UV)

11) Data acquisition

12) Waste or fraction collector

Solvent delivery system

- Ability to mix solvents and vary polarity of mobile phase during run
- Unlimited solvent reservoir
- Generation of pressure to 6000 psi
- Flow rates ranging from 0.1 to 10 mL/min
- Flow reproducibility of 0.5% or better
- Resistance to corrosion by a variety of solvents
- Plus free out put

Degasser

- Vacuum pumping systems
- Distillation system
- A system for heating and stirring the system
- Spraying system bubbles an inert gas of low solubility through the solvent

Three basic types of **pumps** are used

- Pneumatic pumps
- Motor driven syringe type pumps
- Reciprocation pumps

Gradient controller

- The gradient controller is the device that allows you to create a gradient program.
- Gradient are produced differently for different types of pumping system.

Injectors

- Sample injection systems
- Rubber stopper injector or syringe
- Sample value

Guard column

Remove impurities from solvent saturates mobile phase with liquid of stationary phase before the analytical column, straight: 15 to 150 cm in length; 2 to 3 mm and packing - silica gel, alumina, celite.

Packing

Originally these were irregular silica and alumina a range synthetic regular shape is available now,

- **Porous:** Channels through packing.
- **Superficial porous:** Rough surface.
- **Smooth:** Like bead.

Common reverse phase solvents

- Methanol, acetonitrile, tetrahydrofuran, water

Normal phase solvents

- Amino (-NH2), Cyano (-CN), diol (glycidoxy-ethyl methoxide)

Detector

- Mostly optical
- Equipped with a flow cell
- Focus light beam at the center for maximum energy transmission
- Cell ensures that the separated bands do not wide

Types of detector

- UV/Visible
  - Fixed wavelength
  - Variable wavelength
- Photo idode array
- Refractive index
- Fluorescence
- Evaporative light scattering
- Conductivity
- Electrochemical
- Chiral detectors

Application of HPLC in the pharmaceutical industry

Manufacturing, content uniformity, degradation products and related substances, dissolution and stability studies.

Definition of validation (As per USP)

Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for the intended analytical applications.

Methodology

The real goal of the validation process is to challenge the method and determine the limits or allowed deriability for the condition needed to the method.

Types of analytical procedure to be validated

1. Identification test
2. Quantitative test for impurities contents
3. Limit test for the control of impurities
4. Quantitative test of the active molecule in samples of drug substance or drug product

Specificity

An investigation of specificity should be conducted during the validation of identification test, determination of impurities and assay.

Linearity

The linearity of an analytical procedure is of ability (within a given range) to obtain test result while are directly proportional to the concentration of analyte in the dam pole for establishment of linearity. A minimum of 5 concentrations is recommended other approaches would justify.

Range

- The range of analytical procedure is introduced between the higher and lower concentration of analyte in the sample.
- For assay of drug substance: Normally from so to use of the test concentration.
- For content uniformity :- 70-80% of the concentration

- For dissolution release product 20% another one hour up to 40% after 24 hour for the validating range would be 0-100% of the label claim.

Accuracy

Accuracy on the method was determined by relative and absolute recovery experiments.

Precision

Validation of test for assay and quantitative determination of impurities includes an included an investigation of precision.

Detection limit

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

Quantification limit

The quantitative limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be quantitative determined with suitable precision and accuracy.

Robustness

The robustness of the method was assessed by altering the some experimental conditions such as, by changing the flow rate from 0.9 to1.1 mL/min, amount of diluents (10% to 15%) the temperature of the column (28°C to 32°C) and pH of the mobile phase

System stability testing

It is an integral of many analytical procedures. This based on the concept that the equipment electronics analytical operation and samples.

LITERATURE REVIEW

LITERATURE REVIEW

Geetha Susmita A *et al.,* 2015 established the simultaneous estimation of acebrophylline and acetylcysteine in tablet dosage form by RP-HPLC method. An enable Hypersil BDS, C_{18}, 100 x 4.6 mm5µ particle size column was used as stationary phase. The mobile phase consisting of a mixture of buffer solution and acetonitrile (90:10) was pumped isocratically at a flow rate of 1 mL/min with detection at 260nm. The retention time of acebrophylline and N-acetylcysteine were found to be 5.5 min and 2.3 min respectively. The calibration curves were linear over a concentration range of 25-150 µg/mL with coefficient regression (r^2) = 0.9995 and (r^2) = 0.9996 for acebrophylline and N-acetylcysteine. The limits of detections were 0.18 µg/mL and 1.50 µg/mL for acebrophylline & N-acetylcysteine respectively. The selectivity, specificity, system suitability, ruggedness and robustness were performed as per ICH guidelines. In quantitative and recovery studies was 100.37% and 100.8% for acebrophylline and N-acetylcysteine respectively.

Shukla Khushboo N *et al.,* 2015 propose of this development of new analytical methods and their validation for the determination of acetylcysteine in bulk and marketed formulation. Method IA, NAC gives light brown colour with ninhydrine in alkaline medium, which showed λ^{max} at 485.2nm. The drug was reacted with fehling's solutions (A&B in equal volume) and ferric chloride, which produce yellow colour chromogen which showed λ_{max} at 537.2 nm. The linearity range of 50-300µg/mL for both method IA and method IB. The correlation coefficient is 0.9990 and 0.9991. The LOD and LOQ for estimation of NAC were found as 0.0773, 0.2343 for method IA and 0.0667, 0.2021 for method IB respectively formulations.

Tvinkal P *et al.,* 2015 have reported developed and validated for the simultaneous estimation of acebrophylline and acetylcysteine in tablet dosage form by absorbance ratio method. Acebrophylline and acetylcysteine were found to have absorbance maxima at 274 and 195 nm respectively in distilled water and iso absorptive point was found to be 210nm. Acebrophylline was found to be linear in the concentration range of 5 to 11 µg/ mL at 274 nm and acetylcysteine was found to be

linear in the concentration range of 30 to 66 µg/mL at 195 nm. The assay of marketed tablet formulation (Pulmo clear tablet) was found to be 103.4% and 101.58% for acebrophylline and acetylcysteine respectively. The method was validated statistically as per ICH guidelines. The method showed good reproducibility and recovery with % RSD less than 2.

Nitin S *et al.,* 2014 reported validated RP-HPLC method development for the simultaneous estimation of acetylcysteine and acebrofylline in capsule formulation a new simple, precise, rapid and accurate reverse phase high performance liquid chromatographic method had been developed or the simultaneous estimation of acetylcysteine (ACST) and acebrofylline (ACBF) in capsule dosage form. The chromatographic separation was achieved on a Hypersil BDS, C_{18}, 100 x 4.6 mm, 5µm particle size column was used with PDA detemine by using mobile phase containing mixture of 0.02M potassium dihydrogen orthophosphate (KH_2PO_4) buffer: acetonitrile (90:10 % v/v pH 3.2) was used. The flow rate was 0.9mL /min and effluents were monitored at 260 nm. Chromatogram showed two main peaks corresponding to acetylcysteine and acebrofylline at retention time 2.365 and 5.505 min respectively. The method was linear over the concentration range of 150-900µg/mL for acetylcysteine and 25-150 µg/mL for acebrofylline respectively.

Mangelings D *et al.,* 2014 reported development and validation of an HPLC method with post column derivatisation for assay of n-acetylcysteine in plasma determination of both low endogenous and high therapeutic concentrations of N-acetylcysteine (NAC) in plasma. The compound is detected fluorimetrically after derivatisation with ortho-phthalaldehyde in the presence of a primary amine. Validation of the method revealed injection and method repeatability was good. The linear range was adequate and the limit of quantification was between 0.4 and 0.6µM. Recovery of N-acetylcysteine from plasma samples was also acceptable. This method was applied to plasma samples from patients. Six samples were taken at different times after administration of N-acetylcysteine.

Asiya Begum *et al.,* 2014 to established a development and validation of acetylcysteine and taurine tablet dosage form by using RP-HPLC using the isocratic separation method Inertsil ODS (250 x 4.6 mm, 5 µm) column and potassium using dihydrogen OPA: acetonitrile (60:40) Mobile phase as pumped at a rate of 1mL/min the detection was carried out 248nm. The retention time is found in the range of 3.186 and 4.142 minutes. The percentage assay of acetylcysteine and taurine were 99% and 100%.The linear regression analysis data for the calibration plots showed a good linear was found to be in the range of acetylcysteine and taurine over a concentration range of 1000-3000 µg/mL and 300-900µg/mL with correlation co-efficient of 0.999 for acetylcysteine and 0.999 for taurine. The limit of detection and quantitation were found to be 2.93, 2.76 & 9.75, 9.20 respectively.

Mogili Swetha *et al.,* 2013 have reported RP-HPLC method for estimation of acetylcysteine and taurine in API and pharmaceutical dosage form development and validation for the simultaneous estimation of acetylcysteine and taurine in tablets using a Agilent C_{18}4.6×150mm.5µ column, mobile phase comparied of 0.01N KH_2PO_4 and methanol in the ratio of 60:40v/v and flow rate is 1mL/min. The linearity was in the range of 300-900µg/mL; hence the RP-HPLC method developed and validated can be used routinely for the simultaneous estimation of acetylcysteine and taurine in tablets.

Tessier Neirinick *et al.,* 2013 reported novel sensivity ursodeoxycholic acid and its glycine and taurine conjugates in human plasma. solid phase extraction of UDCA, GDCA, TDCA and the internal standard, 23- nordeoxycholic acid from human plasma on a C_{18}.Chromatography was performed by isocratic reverse phase separation with methanol/25 mM ammonium acetate (40/60, v/v) containing 0.05% acetic acid C_{18}column with embedded polar functional group. Linearity of glycine and taurine of 10–3000 µg/mL average correlation coefficient of 0.9992. The absolute recovery for UDCA, GDCA, TDCA and the internal standard was 87.3, 83.7, 79.5 and 95.8%, respectively.

Marine De Person *et al.*, 2012 have reported development and validation of a hydrophilic interaction chromatography-mass spectrometry assay for taurine and methionine in matrices rich in carbohydrates developed and validated for the

simultaneous determination of underivatised taurine and methionine rich in carbohydrates such as energy drinks column Astecap Hera NH_2 (150 mm × 4.6 mm; 5 µm) methanol–water (60/40) as mobile phase. Threonine as the internal standard for specifity, detection limits, linearity, accuracy, precision and stability limit of detection 20 µg L^{-1} for taurine to 50 µg L^{-1} for methionine.

Athawale Rajani *et al.,* 2012 have reported phase development and validation of RP-HPLC method for the estimation of N-acetylcysteine in wet cough syrup. C_{18} (150mm X 4.6 mm i.d., particle size 3.5µm) column. The gradient flow system was used. Mobile phase consisting of acetonitrile and 0.05M phosphate buffer (pH was adjusted to 3.0+0.05 by using orthrophosphoric acid), flow rate is 0.8 mL/min maintain the ambient temperature. Detection was carried out at 214nm. The retention time of about 4.6 minutes was recorded. Linear over the concentration range of 400-600 µg/mL (R=0.999). The proposed method was applicable to routine analysis of acetylcysteine in wet cough syrup dosage form.

Paraskevas D *et al.,* 2012 reported HPLC method for determination of N-acetylcysteine using post-column derivatization with methyl -propiolate a new, green analytical method is proposed for the determination of N-acetylcysteine (NAC) in pharmaceutical formulations. The analyte was separated from the samples matrix using a 100% aqueous mobile phase [0.05% v/v CH_3COOH+1 mol L^{-1} ethylenediaminetetraacetic acid (EDTA) in water] and a suitable analytical column (Prevail reversed phase column). Detection was carried out at 285nm after on-line post column derivatization (PCD) with methyl- propiolate (MP) in alkaline medium. Method development included both chromatographic and reaction parameters, while validation was based on international recommendations.

Aline Ferreira Ourique *et al.,* 2011 have reported the method involves the use of phase column, and a mixture of 0.05 M the reversed KH_2PO_4 and acetonitrile (95 : 5v/v) containing 0.095% (v/v) of phosphoric acid, as the mobile phase. UV detection was performed at 214 nm linear response was observed over the concentration range between 10 and 50 µg mL^{-1} of NAC. Validated with the official guidelines for specificity,

linearity (r = 1), detection and quantification limits (0.70 and 2.11 µg mL^{-1}), precision (RSD < 2%), accuracy (recovery > 97%), and robustness (RSD < 4%). Therefore, NAC can be assayed in granules and effervescent tablets using the same chromatographic conditions without derivatization.

Emillia Marchei *et al.*, 2011 have reported development and validation of a high-performance liquid chromatography–mass spectrometry assay for methylxanthines and taurine in dietary supplements a C_{18} reversed-phase column using water:methanol:acetic acid 75:20:5 as a Mobile phase analytes were determined in LC–MS single ion monitoring mode with atmospheric pressure ionization-electrospray (ESI) interface. Sample specimens were extracted with 4 mL of hexane/isopropanol (9:1). Validated in the taurine and caffeine range is 0.1–500 and 0.06–500 µg/mL or µg/g and caffeine, respectively; 0.06–100 µg/mL or µg/g for theobromine and theophylline. Mean recoveries ranged between 70.1 and 94.4% for different analytes. The quantification limits were 0.1 µg/mL or µg/g for taurine and 0.06µg/mL or µg/g for methylxanthines either in liquid samples or solid samples. 100 to 1000µg/mL amount of taurine present in the energetic drinks.

Zhi Chen *et al.,* 2006 reported HPLC/ESI-MS method for the simultaneous determination of taurine and 10 water-soluble vitamins including vitamin B_1 (thiamine), B_2 (riboflavin), B_5 (pantothenic acid), B_6 (pyridoxine and pyridoxal), B_8 (biotin), B9 (folic acid), C (ascorbic acid) and PP (nicotinamide and nicotinic acid) in multivitamin tablets was developed and validated. The separation was accomplished on a Johnson Spherigel C_{18} (250 mm×4.6 mm) reversed phase column with methanol in an aqueous solution of heptafluorobutyric acid (5 mm) as mobile phase under gradient elution mode. Detection of target components was by ESI-MS switching continuously from positive ion mode to negative ion mode hippuric acid was used as an internal standard for quantification. Sensitivity, precision and accuracy were determined.

Vander Heyden Y *et al.,* 2004 reported that developed enables determination of both low endogenous and high therapeutetic concentrations of N-acetylcysteine (NAC) in plasma. The linear range was adequate and the limit of quantification was between 0.4

and 0.6 μM. Recovery of N-acetylcysteine from plasma samples was also acceptable. This method was applied to plasma samples from patients with a clinical septic shock who had received very high doses of N-acetylcysteine. Six samples were taken at different times after administration of N-acetylcysteine. The blood-concentration profiles obtained indicate the method is suitable for following the evolution of NAC in plasma under these conditions and can therefore be used for pharmacokinetic profiling. N-Acetylcysteine (NAC) is mainly used as a mucolytic in bronchitis or pulmonary diseases. By depolymerising mucopolysaccharides it reduces the viscosity of pulmonary secretions.

AIM OF WORK

AIM AND PLAN OF WORK

The drug analysis plays an important role in the development of drugs, their manufacture and the therapeutic use. Pharmaceutical industries rely upon quantitative chemical analysis to ensure that the raw materials used and final product obtained meets the required specification. The number of drugs and drug formulations introduced in to the markets has been increased at a disturbing rate. These drugs or formulation may be either in the new entities in the market or partial structure modification of the existing drugs or novels dosage forms or multi component dosage forms.

AIM OF WORK

The present work aims at developing newer analytical methods for acetylcysteine and taurine in tablet dosage forms by using RP-HPLC, that are simple, accurate, rapid, precise, sensitive and reliable.

PLAN OF WORK

To validated a method for analytical quantitation of assay in acetylcysteine and taurine in tablets dosage form.

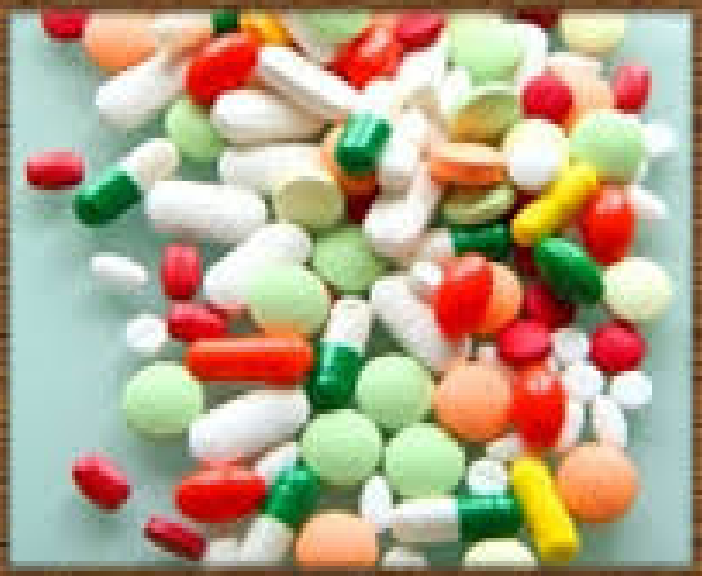
To give a general ICH guidelines for the validation of methods aim for the quantitation of acetylcysteine and taurine in tablets dosage form.

Obtaing results with improved accuracy and precision.

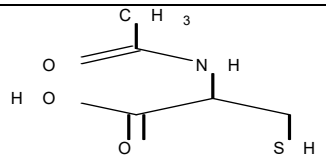
OBJECTIVE OF WORK

To develop analytical and validation method for the estimation of of acetylcysteine and taurine in tablet dosage form by using RP-HPLC method.

DRUG PROFILE

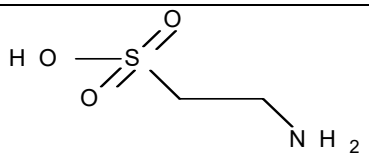


DRUG PROFILE OF ACETYLCYSTEINE

Structure	
Molecular weight	163.5
Molecular formula	$C_5H_9NO_3S$
Chemical name	(2R)-2-acetamido-3-sulfanylpropanic acid
Description	It is a white with light yellow cast powder has a pKa of 9.5 at 30 °C. N- acetylcysteine is derived from the sulfur-containing amino acid
Adverse effects	Rash, urticaria, itchiness, anaphylaxis reaction, hypotension, wheezing, shortness of breath, lower rates of anaphylactoid reaction, stomatitis and rhinorrhea
Pharmacodynamic	Acetylcysteine serves a prodrug to L- cysteine. Acetylcysteine also known as *N*-acetylcysteine or *N*-acetyl-L-cysteine (NAC) is a medication use treatment of paracetamol overdose (acetaminophen)
Solubility	Soluble in water and alcohol. Insoluble in chloroform and ether
Route of administration	By oral, inhalation and injection.
Uses	Mucolytic theraphy, hemorrhagic cystitis, nephroprotective agent, obstructive lung disease and psychiatry
ATC Code	R05CB01(WHO)S01×A08(WHO)V03B23
Bioavailability	10% (oral)
Protein binding	50 to 83%

Metabolism	Liver
Biological half life	5-6 hours
CAS number	616-91-1
Dosage form	Solution for inhalation – inhaled mucolytic therapy or ingested for nephroprotective effect Intravenous injection – treatment of acetaminophen overdose Oral solution – various indications. Ocular solution - for mucolytic therapy.
Sensitivity reaction	Anaphylactoid reactions other allergic reactions Generalized urticaria reported rarely
Warning	Encephalopathy due to hepatic failure, respiratory effects, observes asthmatic patients closely. When administered intra veneous caution in patients with asthma or history of bronchospasm.
Pharmacokinetics	Extensively liver metabolized, CYP450 a minimal. Urine excretion 22-30%, half-life of 5.6 hours in adults and 11 hours in neonates

DRUG PROFILE OF TAURINE

Structure	
Chemical name	2- amino ethane-1-sulphonic acid
Molecular formula	$C_2H_7NO_3S$
molecular weight	125.14
density	1.734g/cm^3
Melting point	305.11°C
Therapeutic uses	Treatment of cardiac and metabolic disease
Brand name	Bestoxol, Genferon and Taufon
Biological role	Congication of bile acids, antioxidation, osmoregulation, membrane stabilitilization, modulation of calcium signaling, cardiovascular function and skeletal muscle function.
Solubility	Soluble in water
Shelf life	Shelf-life of 36 months at 25°C 100 Shelf-life was measured under accelerated conditions at 40°C and no loss was observed for six months parts of 95% alcohol dissolves 0.004 parts at 17°C; insoluble in absolute alcohol
Mechanism	Reduces oxidative stress on cardiac and muscle tissue in the presence oxidative stress can protect against damage from ischemia-reperfusion injury in cardiac tissue.

MATERIAL AND METHODS

REQUIRED INSTRUMENT AND REAGENT

The following table lists the instruments that were used in this study.

S.No	Name of the Instrument	Ref. Number	Make	Model
1.	Electronic balance	I/RD/OEB/EB/01	Adventurer OHAUS	AR2140
2.	HPLC	I/RD/HPC/03	Shimadzu	LC-2010C HT
3.	HPLC	I/RD/HPC/03	Agilent	1260 Series

Materials

The following table lists the materials that were used in this study.

S.No	Name	Grade	Supplier	Lot No or B.No	Potency / Purity	Expiry
1.	Potassiumdihydrogen ortho phosphate	AR	Rankem	J037C16	100.0%	Feb 20
2.	Phosphoric acid	AR	Rankem	G025J15	88.0%	Sep 18
3.	Sodium metabisulphite	AR	Rankem	J042F09	95.0%	Jun 18
4.	Sodium acetate anhydrous	AR	Fisher	22217301-7	98.0%	Jan 2018
5.	Sodium acetate anhydrous	AR	Rankem	G517L18	98.0%	Oct 2017
6.	Triethylamine	AR	Rankem	R013H14	99.5%	Aug 2019

7.	Tetrahydrofuran	HPLC	Fisher	4568920815	99.8%	Jul 2020
8.	Glacial acetic acid	AR	Rankem	W015E13	99.8%	May 2018
9.	Boric acid	AR	Rankem	N009L12	99.5%	Jan 2018
10.	O-Phthaldehyde	AR	SRL	8740689	99.0%	May 2021
11.	Sodium hydroxide	AR	Fisher	2611860815	98.0%	Jul 2020
12.	Potassium hydroxide	AR	Rankem	P14C100872	85.0%	Feb 2019
13.	Mercapto ethanol	AR	Loba	D257F1253	95.0%	Oct 2018
14.	Methanol	HPLC	Fisher	1572151116	99.9%	Oct 2021
15.	Acetonitrile	HPLC	Finar	2124611248 2	99.9%	Jan 2022

Working standard

The following table lists the standards that were used in this study.

S.No	Name	Grade	Lot No./B.No	Potency/Purity	Date of expiry
1.	Acetylcysteine	USP	WS023/10	99.67%	14/09/2017
2.	Taurine	USP	WS075/09	99.80%	14/09/2017

Column details

The following table lists the column that was used in this study.

Column	Ref. Number	I.D. Number	Make	Specification
1.	RD/COL/76	K63402	Nacalai Tesque,Inc.	Cosmosil,5C_{18}-MS-II (250 X 4.6 mm, 5µm)
2.	RD/COL/58	H15-005968	Phenomenex	Phenomenex, Hyperclone ODS (C_{18}),120A 250 X 4.6mm, 5µm


Analytical Method
Development

ANALYTICAL METHOD DEVELOPMENT

Introduction

Analytical method development work is carried out to ensure that the API used and dosage forms that are developed and manufactured for human consumption are meeting the regulated quality norms before starting the analytical reference will be taken based on PDP/Pharmacopoeia /tech peak/PMF and tentative specification.

For chromatographic method development following points shall be considered

a) Literature review

b) Chemical structure and nature of component

c) Solubility of component

d) Any other relevant

Method development of HPLC

The following parameters are usually considered during method development by HPLC

- Selection of mobile phase
- Selection of stationary phase
- Selection of pH
- Selection of other parameters

Selection of mobile phase

Mobile phase A

Weigh accurately about 2.72 g of anhydrous sodium acetate and dissolve it in 1000 mL of water. Add 0.18 mL of triethylamine and adjust the pH to 7.2 using dilute acetic acid and then add 3.0 mL of tetrahydrofuran. Mix well, filter and degas.

Mobile phase B

Weigh accurately about 2.72 g of anhydrous sodium acetate and dissolve it in 200mL of water. Adjust the pH to 7.2 using dilute acetic acid and then add 400 mL of acetonitrile and 400 mL of methanol. Mix well, filter and degas. Mobile phase A and mobile phase B mix with 50:50 give satisfactory result of taurine. Various mobile phase systems containing phosphate buffer and pH was adjusted to 3 with phosphoric acid, that it was observed resolution was satisfactory gives satisfactory result and two well resolved peaks for acetylcysteine and taurine were recorded.

Effect of pH

It was observed that the retention of the analyte increase with pH and decrease with in pH it was also observed that the shape of peak becomes in basic pH from the chromatographic conditions, it was that the pH 3 is suitable for acetylcysteine and pH 7.2 is suitable for taurine.

Selection of phase

On the basis of HPLC mode and number of carbon atom present in molecule C_{18}bonded stationary phase was tried. Cosmosil, C_{18}, 250 X 4.6 mm, 5 µm or equivalent, Phenomenex, Hyperclone ODS (C_{18}), 120A 250 X 4,6mm, 5µm for this two stationary phase was selected as it gives good result in case of system suitability parameter i.e. resolution, USP tangent, USP tailing.

Selection of other parameter

Other parameter like mobile phase, flow rate, column, temperature and wavelength of detector can be selected on the basis of chemical properties of components present in the sample sensitivity and system suitability requirement of the analytical method.

TRIAL METHODS

Initial condition were from taurine in tablet dosage form

Parameter	Trial method 1	Trial method 2	Trial method 3
Flow rate	1 mL/ mins	1 mL/ mins	1 mL/ mins
Mobile phase	Mobile phase A and B (40:60)	Mobile phase A and B (30:70)	Mobile phase A and B (50:50)
Buffer pH	7.2	7.2	7.2
Diluent	Mix equal volume of mobile phase A and B	Mix equal volume of mobile phase A and B	Mix equal volume of mobile phase A and B
Column	Cosmosil,C_{18},250 X 4.6 mm,5 µm or equivalent	Cosmosil,C_{18},250 X 4.6 mm, 5 µm or equivalent	Cosmosil,C_{18},250×4.6mm5µm or equivalent

Preparation of mobile phase

Mobile phase A

Weigh accurately about 2.72 g of anhydrous sodium acetate and dissolve it in 1000 mL of water. Add 0.18 mL of triethylamine and adjust the pH to 7.2 using dilute acetic acid and then add 3.0 mL of tetrahydrofuran. Mix well, filter and degas.

Mobile phase B

Weigh accurately about 2.72 g of anhydrous sodium acetate and dissolve it in 200 mL of water. Adjust the pH to 7.2 using dilute acetic acid and then add 400 mL of acetonitrile and 400 mL of methanol. Mix well, filter and degas.

Standard solution

Weigh accurately about 250 mg of taurine working standard into clean, 50 mL volumetric flask. Add 30 mL of diluent and sonicate for 10 minutes. Make up the volume to 50 mL with the diluent and mix well. Dilute 5 mL of this solution to 50 mL with the diluent.

Test solution

Weigh and crush 20 tablets to a fine powder. Weigh accurately about 420mg of powdered tablet into clean, 50 mL volumetric flask. Add 30 mL of diluent and sonicate for 10 minutes. Make up the volume to 50 mL with the diluent, mix well and filter. Dilute 5 mL of the filtrate to 50 mL with the diluent.

Separately inject 10µL of standard and test solution into the chromatogram and measure the response for taurine.

Conclusion

Trial method 1: Peak was not split so the column was not suitable.

Trial method 2: Peak was not split so the column was not suitable.

Trial method 3: The peak was well separated and this method has satisfactory resolution. Therefore this method was suitable for analysis.

OPTIMIZED METHOD

Initial condition were from taurine in tablet dosage form

Preparation of mobile phase

Mobile phase A

Weigh accurately about 2.72 g of anhydrous sodium acetate and dissolve it in 1000 mL of water. Add 0.18 mL of triethylamine and adjust the pH to 7.2 using dilute acetic acid and then add 3.0 mL of tetrahydrofuran. Mix well, filter and degas.

Mobile phase B

Weigh accurately about 2.72 g of anhydrous sodium acetate and dissolve it in 200 mL of water. Adjust the pH to 7.2 using dilute acetic acid and then add 400 mL of acetonitrile and 400 mL of methanol. Mix well, filter and degas.

Chromatographic conditions

Column	: Cosmosil, C_{18}, 250 X 4.6 mm, 5 µm or equivalent
Flow rate	: 1.0 mL / min
Detection wavelength	: 338 nm
Injection volume	: 10 µL (Online derivatisation method)
	Borate buffer: OPA reagent: Sample / Standard
	In the ratio 5: 3: 3 (Use injector program)
Oven temperature	: 40°C
Mobile phase	: Mobile phase A: Mobile phase B (50: 50)
Diluent	: Mix each 200 mL of mobile phase A and B in a 500 mL stopper flask.

Standard solution

Weigh accurately about 250 mg of taurine working standard into clean, 50 mL volumetric flask. Add 30 mL of diluent and sonicate for 10 minutes. Make up the volume to 50 mL with the diluent and mix well. Dilute 5 mL of this solution to 50 mL with the diluent.

Test solution

Weigh and crush 20 tablets to a fine powder. Weigh accurately about 420mg of powdered tablet into clean, 50 mL volumetric flask. Add 30 mL of diluent and sonicate for 10 minutes. Make up the volume to 50 mL with the diluent, mix well and filter. Dilute 5 mL of the filtrate to 50 mL with the diluent.

Separately inject 10µL of standard and test solution into the chromatogram and measure the response for taurine.

Conclusion

The peak was well separated and this method has satisfactory resolution. Therefore this method was suitable for analysis.

System stability testing

It is an integral of many analytical procedures. This based on the concept that the equipment electronics analytical operation and samples.

Specification limit

98% to 102% w/w of the label claim.

TRIAL METHODS

Initial condition were from acetylcysteine in tablet dosage form

Parameter	Trial method 1	Trial method 2	Trial method 3
Flow rate	1 mL/ mins	1 mL/ mins	1 mL/ mins
Mobile phase	100% buffer	100% buffer	100% buffer
Buffer pH	7.2	4.5	3
Diluent	Sodium metabisulfite(6.8g/L)	Sodium metabisulfite(6.8g/L)	Sodium metabisulfite(6.8g/L)
Column	Cosmosil,C_{18},250X4.6 mm, 5µm or equivalent	Cosmosil,C_{18},250X4.6 mm 5µm or equivalent	Cosmosil,C_{18},250X4.6 mm 5µm or equivalent

Buffer preparation

Dissolve 6.8g of monobasic potassium phosphate in 1000mL of water, filter and degas. Adjust the pH to 4.5 with phosphoric acid.

Sodium metabisulfite solution

Weigh accurately about 500 mg of sodium metabisulfite and dissolve in 1000mL of water.

Standard solution

Weigh accurately about 50 mg of acetylcysteine working standard in 100 mL volumetric flask, add 50 mL of sodium metabisulfite solution, dissolve and make up the volume with sodium metabisulfite solution.

Test solution

Weigh and powder 20 tablets. Weigh accurately about 275 mg of powdered tablets into a clean, 100mL volumetric flask. Add 50mL of sodium metabisulfite solution and sonicate for 60 minutes. Make up the volume to 100mL with sodium metabisulfite solution, mix well and filter. Use the filtrate.

Conclusion

Trial method 1: The peak was not split so the column was not suitable.

Trial method 2: The peak was not split so the column was not suitable.

Trial method 3: The peak was well separated and this method has satisfactory resolution. Therefore this method was suitable for analysis.

OPTIMIZED METHOD

Initial condition were from acetylcysteine in tablet dosage form

Chromatographic conditions

Column : Cosmosil, C_{18}, 250 X 4.6 mm, 5 µm or equivalent

Flow rate : 1.0 mL / min

Detection wavelength : 214 nm

Injection volume : 10 µL

Oven temperature : 30°C

Mobile phase : 100% Buffer

Buffer preparation

Dissolve 6.8g of monobasic potassium phosphate in 1000mL of water, filter and degas. Adjust the pH to 3.0 with phosphoric acid.

Sodium metabisulfite solution

Weigh accurately about 500 mg of sodium metabisulfite and dissolve in 1000mL of water.

Standard solution

Weigh accurately about 50 mg of acetylcysteine working standard in 100 mL volumetric flask, add 50 mL of sodium metabisulfite solution, dissolve and make up the volume with sodium metabisulfite solution.

Test solution

Weigh and powder 20 tablets. Weigh accurately about 275 mg of powdered tablets into a clean, 100mL volumetric flask. Add 50mL of sodium metabisulfite solution

and sonicate for 60 minutes. Make up the volume to 100mL with sodium metabisulfite solution, mix well and filter. Use the filtrate.

Conclusion

The peak was well separated and these methods have satisfactory resolution. Therefore this method was suitable for analysis.

System stability testing

It is an integral of many analytical procedures. This based on the concept that the equipment electronics analytical operation and samples.

Specification limit

98% to 102%w/w of the label claim.


validation

ACETYLCYSTEINE

Reference

USP and ICH Guidelines

Chromatographic conditions

Column	: Cosmosil, C_{18}, 250 X 4.6 mm, 5 µm or equivalent
Flow rate	: 1.0 mL / min
Detection wavelength	: 214 nm
Injection volume	: 10 µL
Oven temperature	: 30°C
Mobile phase	: 100% Buffer

Buffer preparation

Dissolve 6.8g of monobasic potassium phosphate in 1000mL of water, filter and degas. Adjust the pH to 3.0 with phosphoric acid.

Sodium metabisulfite solution

Weigh accurately about 500 mg of sodium metabisulfite and dissolve in 1000mL of water.

Standard solution

Weigh accurately about 50 mg of acetylcysteine working standard in 100 mL volumetric flask, add 50 mL of sodium metabisulfite solution, dissolve and make up the volume with sodium metabisulfite solution.

Test solution

Weigh and powder 20 tablets. Weigh accurately about 275 mg of powdered tablets into a clean, 100mL volumetric flask. Add 50mL of sodium metabisulfite solution

and sonicate for 60 minutes. Make up the volume to 100mL with sodium metabisulfite solution, mix well and filter. Use the filtrate.

Procedure

Separately inject the standard solution (6 injections) and test solution (2 injections) into the chromatograph and record the major responses. Ensure the following system suitability parameter.

System suitability parameter	Accepted criteria
Tailing factor (standard solution)	NMT 2.0
Column effiencency (standard solution)	NLT 2000 theoretical plate
Relative standard deviation	NMT 2.0

Calculation

% Assay of acetylcysteine

$$= \frac{\text{Area of sample X Standard wt (mg) X 100 X Purity of std. X}}{\text{Avg wt. of tablet (mg) X 100}}$$

$$= \frac{}{\text{Area of standard X 100 X Sample wt (mg) X 100 X 150}}$$

= ---------- % of acetylcysteine

Validation program and acceptance criteria

Specificity

Analyze the *placebo* (excipients with taurine) and acetylcysteine separately.

***Placebo* solution**

Weigh accurately about 225.0 mg of *placebo* into a clean, 100 mL volumetric flask. Add 50 mL of sodium metabisulfite solution and sonicate for 60 minutes. Make up the volume to 100 mL with sodium metabisulfite solution, mix well and filter. Use the filtrate (concentration: 2.25 mg/mL of *placebo*).

Standard solution

Weigh accurately about 50 mg of acetylcysteine working standard in 100 mL volumetric flask, add 50 mL of sodium metabisulfite solution, dissolve and make up the volume with sodium metabisulfite solution (concentration: 0.50 mg/mL of acetylcysteine).

Standard + *placebo* solution

Weigh accurately about 225mg of *placebo* and 50mg of acetylcysteine working standard into a clean, 100 mL volumetric flask. Add 50 mL of sodium metabisulfite solution and sonicate for 60 minutes. Make up the volume to 100 mL with sodium metabisulfite solution, mix well and filter. Use the filtrate (concentration: 2.25 mg/mL of *placebo* and 0.50 mg/mL of acetylcysteine).

Procedure

Prepare the above solution and inject each solution as per the test method and report the results.

Acceptance criteria

The *placebo* chromatogram should not show any peak at the retention time of acetylcysteine.

System precision

Standard solution

Prepare the concentration of acetylcysteine 0.50 mg/mL.

Procedure

Inject the standard solution (6 injections). Ensure the following system suitability criteria

System suitability parameter	Accepted criteria
Tailing factor (standard solution)	NMT 2.0
Column effiencency (standard solution)	NLT 2000 theoretical plate
Relative standard deviation	NMT 2.0

Linearity and Range

Level - I (50%):

Prepare the concentration of 0.25mg/mL of acetylcysteine.

Level - II (80%):

Prepare the concentration of 0.40mg/mL of acetylcysteine.

Level - III (100%):

Prepare the concentration of 0.50mg/mL of acetylcysteine.

Level - IV (120%):

Prepare the concentration of 0.60mg/mL of acetylcysteine.

Level - V (150%):

Prepare the concentration of 0.75mg/mL of acetylcysteine.

Procedure

Prepare the above solutions ranging from 50% to 150% and inject each level in duplicate. Perform the correlation co-efficient by covering at least five points and report the linearity as the range for determining the assay.

Acceptance criteria

The plot of concentration versus peak area should be linear with a correlation coefficient not less than 0.995.

Accuracy

Level - I (50%) – 1:

Weigh accurately about 225 mg of *placebo* and 25 mg of acetylcysteine working standard into a clean, 100 mL volumetric flask. Add 50 mL of sodium metabisulfite solution and sonicate for 60 minutes. Make up the volume to 100 mL with sodium metabisulfite solution, mix well and filter. Use the filtrate (concentration: 2.25 mg/mL of *placebo* and 0.25 mg/mL of acetylcysteine).

Level - I (50%) – 2:

Prepare the concentration 2.25 mg/mL of *placebo* and 0.25 mg/mL of acetylcysteine.

Level - I (50%) – 3:

Prepare the concentration 2.25 mg/mL of *placebo* and 0.25 mg/mL of acetylcysteine.

Level - II (100%) – 1:

Weigh accurately about 225.0 mg of *placebo* and 50 mg of acetylcysteine working standard into a clean, 100 mL volumetric flask. Add 50 mL of sodium metabisulfite solution and sonicate for 60 minutes. Make up the volume to 100 mL with sodium metabisulfite solution, mix well and filter. Use the filtrate (concentration: 2.25 mg/mL of *placebo* and 0.50 mg/mL of acetylcysteine).

Level - II (100%) – 2:

Prepare the concentration 2.25 mg/mL of *placebo* and 0.50 mg/mL of acetylcysteine.

Level - II (100%) – 3:

Prepare the concentration 2.25 mg/mL of *placebo* and 0.50 mg/mL of acetylcysteine.

Level - III (150%) – 1:

Weigh accurately about 225 mg of *placebo* and 75 mg of acetylcysteine working standard into a clean, 100 mL volumetric flask. Add 50 mL of sodium metabisulfite solution and sonicate for 60 minutes. Make up the volume to 100 mL with sodium metabisulfite solution, mix well and filter. Use the filtrate (concentration: 2.25 mg/mL of *placebo* and 0.75 mg/mL of acetylcysteine).

Level - III (150%) – 2:

Prepare the concentration 2.25 mg/mL of *placebo* and 0.75 mg/mL of acetylcysteine.

Level - III (150%) – 3:

Prepare the concentration 2.25 mg/mL of *placebo* and 0.75 mg/mL of acetylcysteine.

Procedure

Prepare the above solutions in the range of from 50%, 100% and 150% and inject. Each solution in duplicate as per the test method. Calculate the recovery in each level by calculating the measured concentration against theoretical concentration.

Acceptance criteria

The recovery should be in the range of 98.0-102.0%.

Method precision

The system precision test as per the test methods.

Test solution

Weigh and powder 20 tablets. Weigh accurately about 275mg of powdered tablets into a clean, 100 mL volumetric flask. Add 50 mL of sodium metabisulfite solution and sonicate for 60 minutes. Make up the volume to 100 mL with sodium metabisulfite solution, mix well and filter. Use the filtrate (concentration: equivalent to

0.50 mg/mL of acetylcysteine). Prepare the equivalent concentration of 0.50 mg/mL of acetylcysteine test solution in 6 times.

Procedure

Prepare the test solution of tablet dosage form as per the test method and inject each solution. Calculate the precision of the method by calculating % assay of each solution against standard solution. Report the % RSD of all individual assay values.

Acceptance criteria

The percentage relative standard deviation for the assay values should be less than 2.

Ruggedness (Intermediate precision)

The ruggedness of the method is to be performing by analyzing the test solution of tablet dosage form with the following varying parameters.

Parameter	Set I	Set II
Instrument to instrument	Instrument - 1	Instrument - 2
Column to column	Column – 1	Column - 2
Reagent to reagent	Reagent – 1	Reagent - 2
Analyst to analyst	Analyst – 1	Analyst - 2
Day to day	Day – 1	Day - 2

Standard solution

Proceed the system precision test as per the test methods.

Test solution - 1

Weigh and powder 20 tablets. Weigh accurately about 275 mg of powdered tablets into a clean, 100 mL volumetric flask. Add 50 mL of sodium metabisulfite solution and sonicate for 60 minutes. Make up the volume to 100 mL with sodium metabisulfite solution, mix well and filter. Use the filtrate (concentration: equivalent to

0.50 mg/mL of acetylcysteine). Prepare the equivalent concentration of 0.50 mg/mL of acetylcysteine test solution in 6 times.

Procedure

Prepare the test solution of tablet dosage form by different analyst with different reagent on different day as per the test method. Inject each solution with different instrument using different column. Calculate the ruggedness of the method by calculating % assay of each solution against standard solution. Report the overall % RSD of all individual assay values in set-I and set–II.

Acceptance criteria

The overall % RSD should not be more than 2.0%.

Robustness

The robustness of the method is to be determined by analyzing the standard solution six times with varying HPLC conditions as described below:

Parameter / Condition	Actual	Low	High
Flow rate	1.00 mL/min	0.90 mL/min	1.10 mL/min
Mobile phase	100% Buffer conc.: 6.8 g / L	100% Buffer conc.: 6.7 g / L	100% Buffer conc.: 6.9 g / L
Buffer Ph	3.0	2.9	3.1
Column oven temperature	30°C	28°C	32°C

Acceptance criteria

System suitability parameter	Accepted criteria
Tailing factor (standard solution)	NMT 2.0
Column effiencency (standard solution)	NLT 2000 theoretical plate
Relative standard deviation	NMT 2.0

Solution stability

Measure the stability of the tablet dosage form test solution against 100% of the standard concentration by keeping the solution up to 48 hours at 15°C. Inject the sample at different time intervals (e.g. Initial, 6,12,18,24,36 and 48 hours) and calculate the percentage relative standard deviation of acetylcysteine in tablet dosage form at different interval of time.

Acceptance criteria

The overall % RSD should not be more than 2.0%.

TAURINE

Reference

USP and ICH Guidelines

Chromatographic conditions

Column	: Cosmosil, C_{18}, 250 X 4.6 mm, 5 µm or equivalent
Flow rate	: 1.0 mL / min
Detection wavelength	: 338 nm
Injection volume	: 10 µL (Online derivatisation method)
	Borate buffer: OPA reagent: Sample / Standard
	In the ratio 5: 3: 3 (Use injector program)
Oven temperature	: 40°C
Mobile phase	: Mobile phase A: Mobile phase B (50: 50)
Diluent	: Mix each 200mL of mobile phase A and B in a 500mL stopper flask.

Preparation of mobile phase

Mobile phase A

Weigh accurately about 2.72 g of anhydrous sodium acetate and dissolve it in 1000mL of water. Add 0.18mL of triethylamine and adjust the pH to 7.2 using dilute acetic acid and then add 3mL of tetrahydrofuran. Mix well, filter and degas.

Mobile phase B

Weigh accurately about 2.72 g of anhydrous sodium acetate and dissolve it in 200mL of water. Adjust the pH to 7.2 using dilute acetic acid and then add 400mL of acetonitrile and 400mL of methanol. Mix well, filter and degas.

Preparation of derivatisation reagents

45% solution of potassium hydroxide

Weigh accurately 4.5 g of potassium hydroxide pellets and dissolve in 10mL of water.

40% solution of sodium hydroxide

Weigh accurately 4 g of sodium hydroxide pellets and dissolve in 10mL of water.

O-phthalaldehyde (OPA) reagent

Weigh accurately about 2.47 g of boric acid into 100mL volumetric flask and add 75mL of water. Shake well to dissolve and adjust the pH of this solution to 10.4 ± 0.1 with a 45% solution of potassium hydroxide. Make up the volume to 100mL with water.

Weigh accurately about 1.0 g of O- phthalaldehyde in a clean 250 mL beaker and add 5mL of methanol. Sonicate to dissolve and add 95 mL of borate buffer and 2 mL of mercapto ethanol and adjust the pH of this solution to 10.4 ± 0.1 with a 40% solution of sodium hydroxide.

Borate buffer pH 10.4

Weigh accurately about 2.47 g of boric acid into 100 mL volumetric flask and add 75mL of water. Shake well to dissolve and adjust the pH of this solution to10.4 ± 0.1 with a 40 % solution of sodium hydroxide. Make up the volume to 100 mL with water.

Standard solution

Weigh accurately about 250 mg of taurine working standard into clean, 50 mL volumetric flask. Add 30 mL of diluent and sonicate for 10 minutes. Make up the volume to 50 mL with the diluent and mix well. Dilute 5 mL of this solution to 50 mL with the diluent.

Test solution

Weigh and crush 20 tablets to a fine powder. Weigh accurately about 420mg of powdered tablet into clean, 50 mL volumetric flask. Add 30 mL of diluent and sonicate for 10 minutes. Make up the volume to 50 mL with the diluent, mix well and filter. Dilute 5 mL of the filtrate to 50 mL with the diluent.

Procedure

Separately inject the standard solution (6 injections) and test solution (2 injections) into the chromatograph and record the major responses. Ensure the following system suitability parameter.

System suitability parameter	Accepted criteria
Tailing factor (standard solution)	NMT 2.0
Column effiencency (standard solution)	NLT 2000 theoretical plate
Relative standard deviation	NMT 2.0

Calculate the % assay of the taurine using the following expression.

% Assay of taurine

$$= \frac{\text{Area of sample X Standard wt (mg) X 5 X 50 X 50 X Purity of std. X}}{\text{Avg wt. of tablet (mg) X 100}}$$

$$= \frac{\text{Area of standard X 50 X 50 X Sample wt (mg) X 5 X 100 X 500}}{\text{}} \text{ \% of taurine}$$

Validation program and acceptance criteria

Specificity

Analyze the *placebo* (excipients with acetylcysteine) and taurine separately.

***Placebo* solution**

Weigh accurately about 167 mg of *placebo* into clean, 50 mL volumetric flask. Add 30 mL of diluent and sonicate for 10 minutes. Make up the volume to 50 mL with the

diluent, mix well and filter. Dilute 5 mL of the filtrate to 50 mL with the diluents (concentration 0.33 mg/mL of *placebo).*

Standard solution (taurine)

Weigh accurately about 250 mg of taurine working standard into clean, 50 mL volumetric flask. Add 30 mL of diluent and sonicate for 10 minutes. Make up the volume to 50 mL with the diluent and mix well. Dilute 5 mL of this solution to 50 mL with the diluents (concentration 0.50 mg/mL of taurine).

Standard + *placebo* solution

Weigh accurately about 167 mg of *placebo* and 250 mg of taurine working standard into clean, 50 mL volumetric flask. Add 30 mL of diluent and sonicate for 10 minutes. Make up the volume to 50 mL with the diluent, mix well and filter. Dilute 5 mL of the filtrate to 50 mL with the diluents (concentration 0.33 mg/mL of *placebo* and 0.50 mg/mL of taurine).

Procedure

Prepare the above solution and inject each solution as per the test method and report the results.

Acceptance criteria

The *placebo* chromatogram should not show any peak at the retention time of taurine.

System precision

Standard solution

Weigh accurately about 250 mg of taurine working standard into clean, 50 mL volumetric flask. Add 30 mL of diluent and sonicate for 10 minutes. Make up the volume to 50 mL with the diluent and mix well. Dilute 5 mL of this solution to 50 mL with the diluent (concentration: 0.50 mg/mL of taurine).

Procedure

Inject the standard solution (6 injections). Ensure the following system suitability criteria.

System suitability parameter	Accepted criteria
Tailing factor (standard solution)	NMT 2.0
Column effiencency (standard solution)	NLT 2000 theoretical plate
Relative standard deviation	NMT 2.0

Linearity and range

Standard stock solution

Weigh accurately about 250 mg of taurine working standard into clean, 50 mL volumetric flask. Add 30 mL of diluent and sonicate for 10 minutes. Make up the volume to 50 mL with the diluent and mix well.

Level - I (50%):

Dilute 2.5 mL of standard stock solution to 50 mL with the diluents (concentration: 0.25 mg/mL of taurine).

Level - II (80%):

Dilute 4.0 mL of standard stock solution to 50 mL with the diluents (concentration: 0.40 mg/mL of taurine).

Level - III (100%):

Dilute 5.0 mL of standard stock solution to 50 mL with the diluents (concentration: 0.50 mg/mL of taurine).

Level - IV (120%):

Dilute 6.0 mL of standard stock solution to 50 mL with the diluent (concentration: 0.60 mg/mL of taurine).

Level - V (150%

Dilute 7.5 mL of standard stock solution to 50 mL with the diluent (concentration: 0.75 mg/mL of taurine).

Procedure

Prepare the above solutions ranging from 50% to 150% and inject each level in duplicate. Perform the correlation co-efficient by covering at least five points and report the linearity as the range for determining the assay.

Acceptance criteria

Linear with a correlation coefficient NLT 0.995.

Accuracy

Level - I (50%) – 1:

Weigh accurately about 167 mg of *placebo* and 125 mg of taurine working standard into clean, 50 mL volumetric flask. Add 30 mL of diluent and sonicate for 10 minutes. Make up the volume to 50 mL with the diluent, mix well and filter. Dilute 5 mL of the filtrate to 50 mL with the diluents (concentration: 0.33 mg/mL of *placebo* and 0.25 mg/mL of taurine).

Level - I (50%) – 2:

Prepare the concentration 0.33 mg/mL of *placebo* and 0.25 mg/mL of taurine.

Level - I (50%) – 3:

Prepare the concentration 0.33 mg/mL of *placebo* and 0.25 mg/mL of taurine.

Level - II (100%) – 1:

Weigh accurately about 167 mg of *placebo* and 250 mg of taurine working standard into clean, 50 mL volumetric flask. Add 30 mL of diluent and sonicate for 10 minutes. Make up the volume to 50 mL with the diluent, mix well and filter. Dilute 5 mL

of the filtrate to 50 mL with the diluent (concentration: 0.33 mg/mL of *placebo* and 0.50 mg/mL of taurine).

Level - II (100%) – 2:

Prepare the concentration 0.33 mg/mL of *placebo* and 0.50 mg/mL of taurine.

Level - II (100%) – 3:

Prepare the concentration 0.33 mg/mL of *placebo* and 0.50 mg/mL of taurine.

Level - III (150%) – 1:

Weigh accurately about 167 mg of *placebo* and 375 mg of taurine working standard into clean, 50 mL volumetric flask. Add 30 mL of diluent and sonicate for 10 minutes. Make up the volume to 50 mL with the diluent, mix well and filter. Dilute 5 mL of the filtrate to 50 mL with the diluents (concentration: 0.33 mg/mL of Placebo and 0.75 mg/mL of taurine).

Level - III (150%) – 2:

Prepare the concentration of 0.33 mg/mL of *placebo* and 0.75 mg/mL of taurine.

Level - III (150%) – 3:

Prepare the concentration of 0.33 mg/mL of p*lacebo* and 0.75 mg/mL of taurine.

Procedure

Prepare the above solutions in the range of from 50%, 100% and 150% and inject each solution in duplicate as per the test method. Calculate the recovery in each level by calculating the measured concentration against theoretical concentration.

Acceptance criteria

The recovery should be in the range of 98.0-102.0%.

Method precision

Standard solution

Proceed the system precision test as per the test methods.

Test solution

Weigh and crush 20 tablets to a fine powder. Weigh accurately about 420 mg of powdered tablet into clean, 50 mL volumetric flask. Add 30 mL of diluent and sonicate for 10 minutes. Make up the volume to 50 mL with the diluent, mix well and filter. Dilute 5 mL of the filtrate to 50 mL with the diluent (concentration: equivalent to 0.50 mg/mL of taurine). Prepare the equivalent concentration 0.50 mg/mL of taurine test solution in 6 times.

Procedure

Prepare the test solution of tablet dosage form as per the test method and inject each solution. Calculate the precision of the method by calculating % assay of each solution against standard solution. Report the % RSD of all individual assay values.

Acceptance criteria

The percentage relative standard deviation for the assay values should be less than 2.0.

Ruggedness (Intermediate precision)

The ruggedness of the method is to be performing by analyzing the test solution of tablet dosage form with the following varying parameters.

Parameter	Set I	Set II
Instrument to instrument	Instrument – 1	Instrument - 2
Column to column	Column – 1	Column - 2
Reagent to reagent	Reagent – 1	Reagent - 2
Analyst to analyst	Analyst – 1	Analyst - 2
Day to day	Day – 1	Day - 2

Standard solution

Proceed the system precision test as per the test method.

Test solution

Weigh and crush 20 tablets to a fine powder. Weigh accurately about 420 mg of powdered tablet into clean, 50 mL volumetric flask. Add 30 mL of diluent and sonicate for 10 minutes. Make up the volume to 50 mL with the diluent, mix well and filter. Dilute 5 mL of the filtrate to 50 mL with the diluent (concentration equivalent to 0.50 mg/mL of taurine). Prepare the equivalent concentration of 0.50 mg/mL of taurine test solution in 6 times.

Procedure

Prepare the test solution of tablet dosage form by different analyst with different reagent on different day as per the test method. Inject each solution with different instrument using different column, different reagent, different analyst and different days. Calculate the ruggedness of the method by calculating % assay of each solution against standard solution. Report the overall % RSD of all individual assay values in set-I and set–II.

Acceptance criteria

The overall % RSD should not be more than 2.0%.

Robustness

The robustness of the method is to be determined by analyzing the standard solution six times with varying HPLC conditions as described below.

Parameter / Condition	Actual	Low	High
Flow rate	1.00 mL/min	0.90 mL/min	1.10 mL/min
Mobile phase	Mobile phase A : Mobile phase B 50 : 50	Mobile phase A : Mobile phase B 52 : 48	Mobile phase A : Mobile phase B 48 : 52
Buffer pH	7.20	7.10	7.30
Column oven temperature	40°C	38°C	42°C

Acceptance criteria

System suitability parameter	Accepted criteria
Tailing factor (standard solution)	NMT 2.0
Column effiencency (standard solution)	NLT 2000 theoretical plate
Relative standard deviation	NMT 2.0

Solution stability

Measure the stability of the tablet dosage form test solution against 100% of the standard concentration by keeping the solution up to 48 hours at 15°C. Inject the sample at different time intervals (eg: Initial, 6 hours, 12 hours, 18 hours and 24 hours) and calculate the percentage relative standard deviation of taurine in tablet dosage form at different interval of time.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Specificity

Placebo solution was prepared separately at a concentration of 2.25 mg/mL of excipients blend. A solution of *placebo* was spiked with the acetylcysteine at its working concentration. The solution was analyzed as per the RP-HPLC method described. Table 1 summarizes the retention time (RT), relative retention time (RRT) values obtained for *placebo* and acetylcysteine.

Acceptance criteria

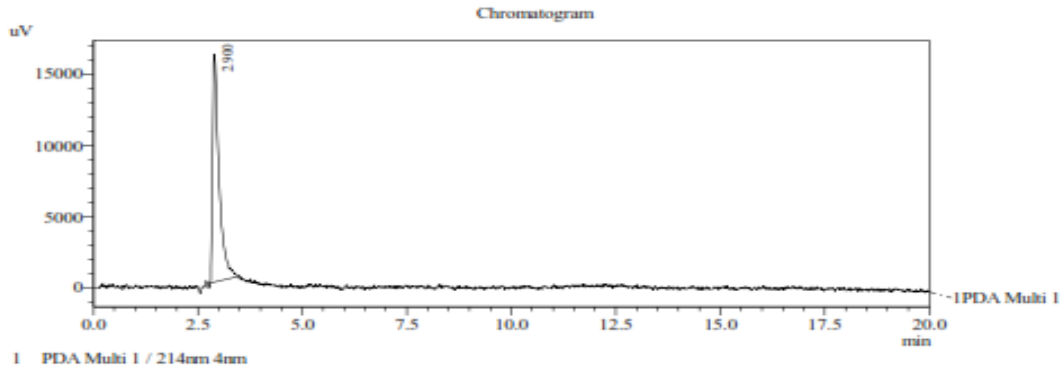
The *placebo* chromatogram should not show any peak at the retention time of acetylcysteine.

Table 1: Summary of retention time and relative retention time values for *placebo* and acetylcysteine

Peak name	Retention time (mins)	Relative retention time
Blank peak	2.90	0.22
Acetylcysteine	13.01	1.00

No peak was observed at the retention time of acetylcysteine in the chromatogram of *placebo*

Sample Name : Nefrosave Tablets
Sample ID : Blank
Method Filename : Nefrosave_Acetyl cysteine.lcm
Vial : 1
Injection Volume : 10 uL

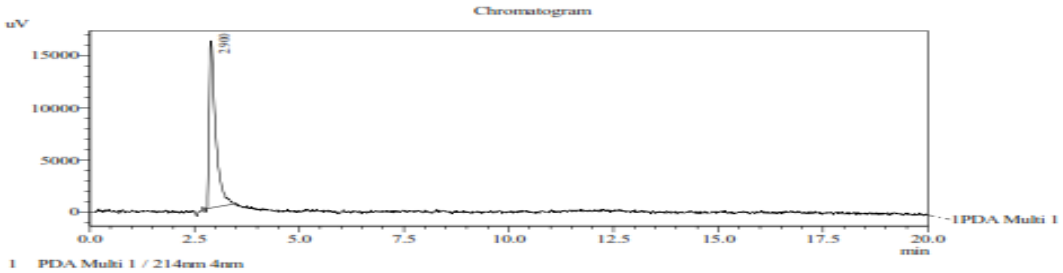


Quantitative Results

PDA

ID#	Name	Ret. Time	Area
1	Blank Peak	2.900	171424

Sample Name : Nefrosave Tablets
Sample ID : Blank
Method Filename : Nefrosave_Acetyl cysteine.lcm
Vial : 1
Injection Volume : 10 uL



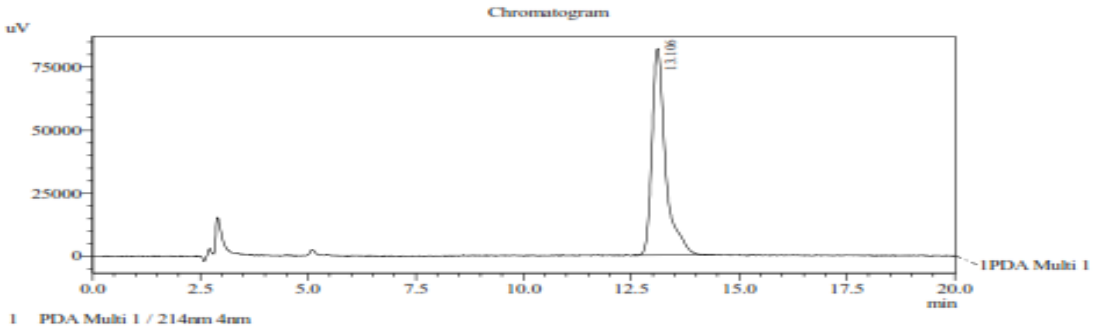
1 PDA Multi 1 / 214nm 4nm

Quantitative Results

PDA

ID#	Name	Ret. Time	Area
1	Blank Peak	2.900	171424

Sample Name : Acetyl Cysteine
Sample ID : Standard
Method Filename : Nefrosave_Acetyl cysteine.lcm
Vial : 3
Injection Volume : 10 uL



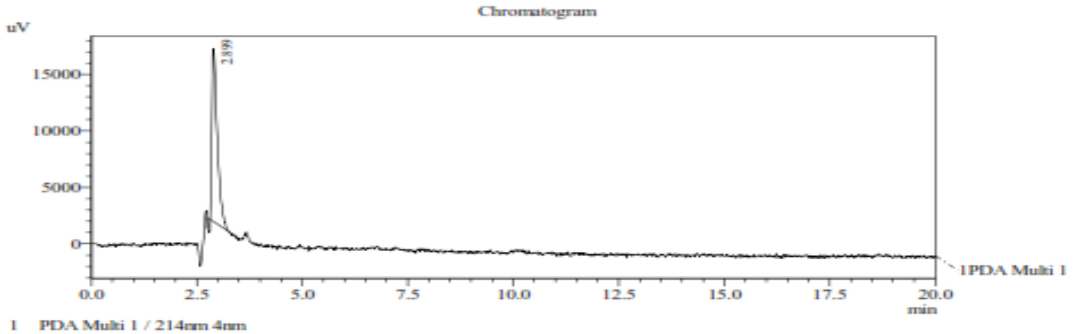
1 PDA Multi 1 / 214nm 4nm

Quantitative Results

PDA

ID#	Name	Ret. Time	Area
1	Acetylcysteine	13.106	1807908

Sample Name : Nefrosave Tablets
Sample ID : Placebo
Method Filename : Nefrosave_Acetyl cysteine.lcm
Vial : 2
Injection Volume : 10 uL

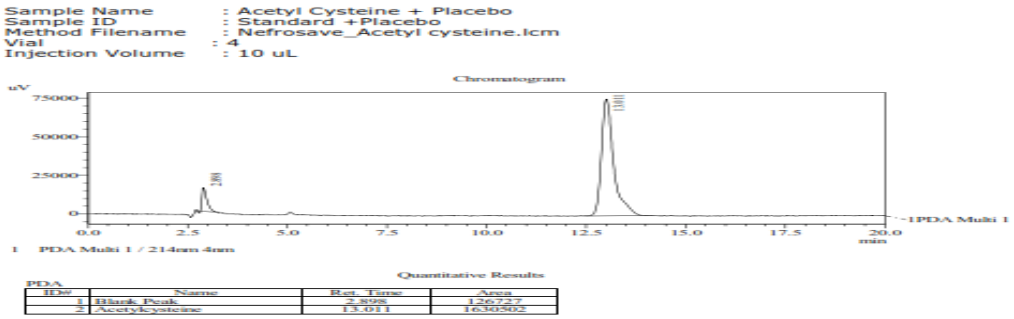


1 PDA Multi 1 / 214nm 4nm

Quantitative Results

PDA

ID#	Name	Ret. Time	Area
1	Blank Peak	2.899	131666



System precision

A standard solution of 0.50 mg/mL of acetylcysteine was prepared and analyzed as per the method. Table 2: summarizes system suitability results.

Acceptance criteria

(i) The tailing factor for the acetylcysteine peak should not more than 2.0 in standard solution.

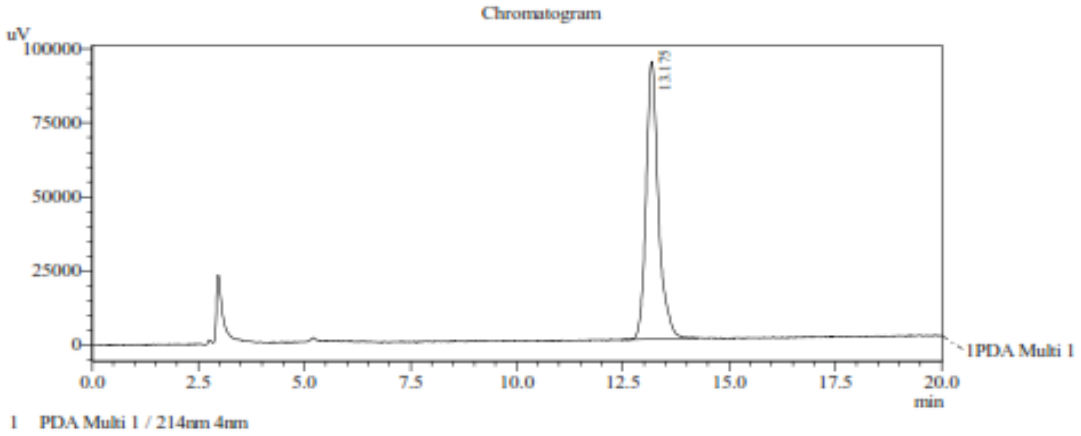
(ii) The number of theoretical plates for the acetylcysteine peak should not less than 2000 in standard solution.

Table 2: summary of retention time, % RSD of peak area, tailing factor and theoretical plates of the acetylcysteine peak

S.No	Retention time (Minutes)	Area	Tailing factor	Theoretical plates	Average	% RSD
1	13.18	1900436	1.29	10788	**1891268**	**0.27**
2	13.18	1890556				
3	13.19	1884890				
4	13.18	1892288				
5	13.23	1891080				
6	13.24	1888358				

The percentage relative standard deviation of peak area of acetylcysteine was 0.27 with the tailing factor and theoretical plates of 1.29 and 10788 respectively.

Sample Name : Standard
Sample ID : System Precision
Method Filename : Nefrosave_Acetyl cysteine.lcm
Vial : 2
Injection Volume : 10 uL



Quantitative Results

PDA

ID#	Name	Ret. Time	Area	Tailing Factor	Theoretical Plate
1	Acetylcysteine	13.175	1900436	1.28	10788

Linearity and range

The linearity of the HPLC method was demonstrated for acetylcysteine ranging from 0.2500 mg/mL to 0.7500 mg/mL, which is equivalent to 50% to 150% of the acetylcysteine working strength. Five standard solutions at the concentrations within the mentioned range were prepared and analyzed as per the method. The linearity results obtained are shown in Table 3. Figure 1 shows the line of best fit for concentration versus peak area of acetylcysteine.

Acceptance criteria

The plot of concentration versus peak area should be linear with a correlation coefficient (R^2) not less than 0.995.

Table 3: Linearity of acetylcysteine

Level	% of acetylcysteine	Concentration(mg/mL)	Peak area
50%	0.251	50.2	993424
80%	0.403	80.6	1583987
100%	0.508	101.6	1996797
120%	0.608	121.6	2436183
150%	0.754	150.8	2980211
		Correlation coefficient	0.9995

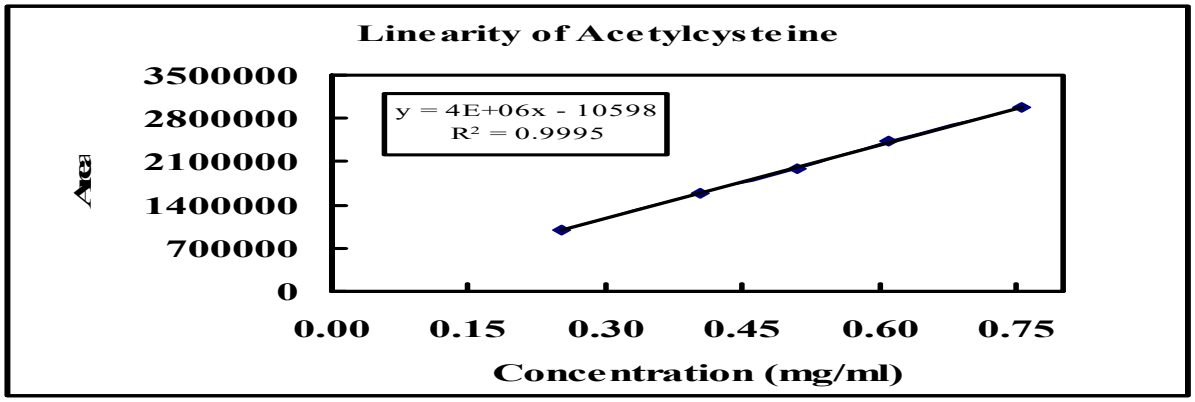
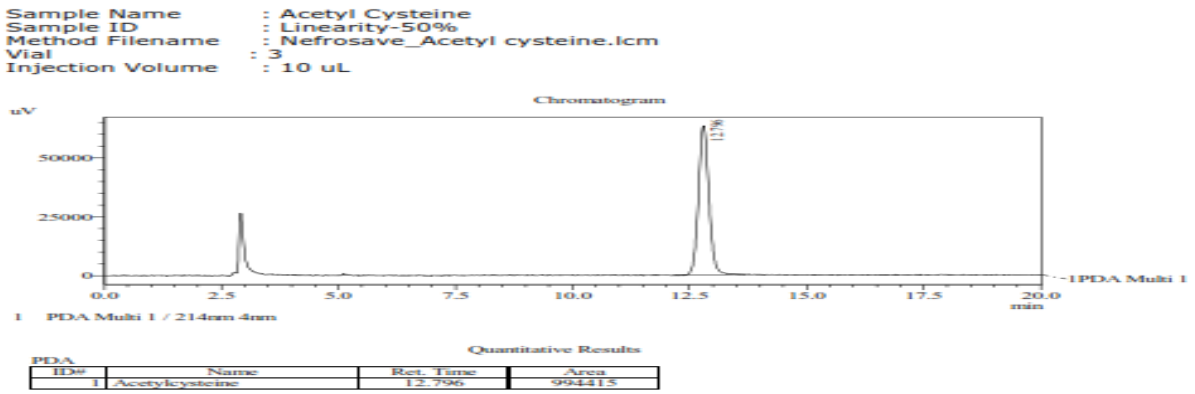
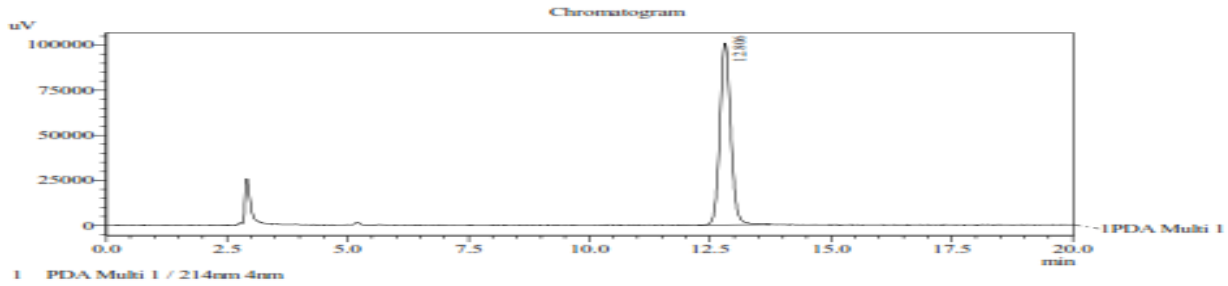


Figure 1: Linearity graph for acetylcysteine

Thus, the HPLC method for the estimation of acetylcysteine in tablet dosage form was shown to be linear in the range of 50% to 150% of the working concentration with a correlation coefficient of 0.9995. The range of the HPLC method for determining the assay of acetylcysteine in tablet dosage form is 50% to 150% of the working strength.



Sample Name : Acetyl Cysteine
Sample ID : Linearity-80%
Method Filename : Nefrosave_Acetyl cysteine.lcm
Vial : 4
Injection Volume : 10 uL

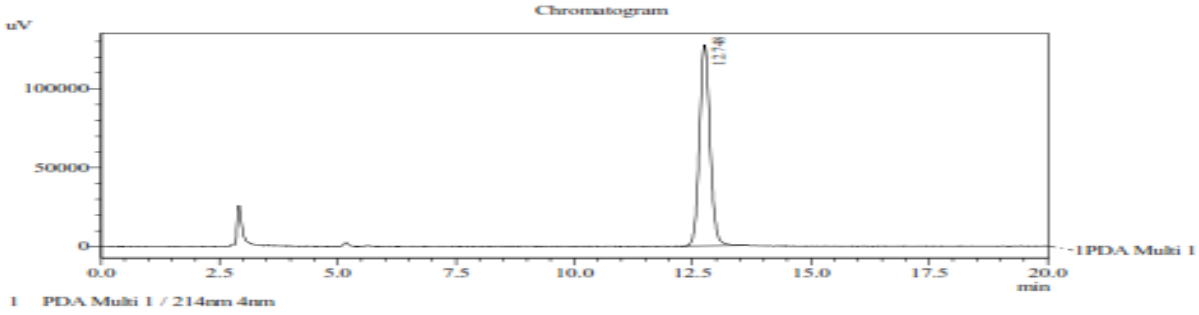


Quantitative Results

PDA

ID#	Name	Ret. Time	Area
1	Acetylcysteine	12.806	1587068

Sample Name : Acetyl Cysteine
Sample ID : Linearity-100%
Method Filename : Nefrosave_Acetyl cysteine.lcm
Vial : 5
Injection Volume : 10 uL

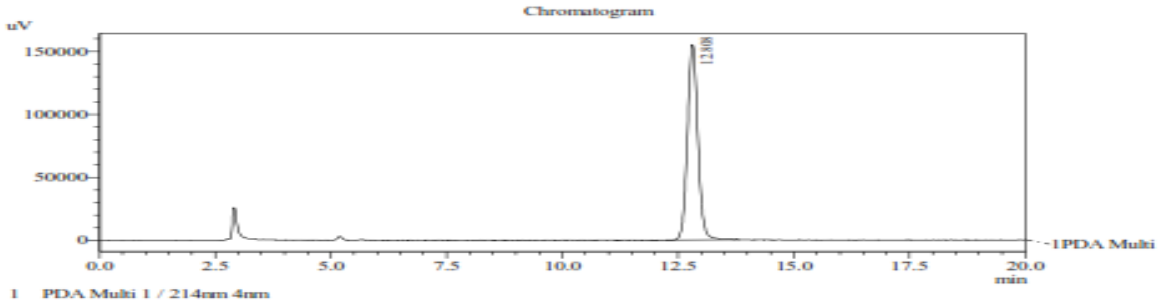


Quantitative Results

PDA

ID#	Name	Ret. Time	Area
1	Acetylcysteine	12.748	1996074

Sample Name : Acetyl Cysteine
Sample ID : Linearity-120%
Method Filename : Nefrosave_Acetyl cysteine.lcm
Vial : 6
Injection Volume : 10 uL

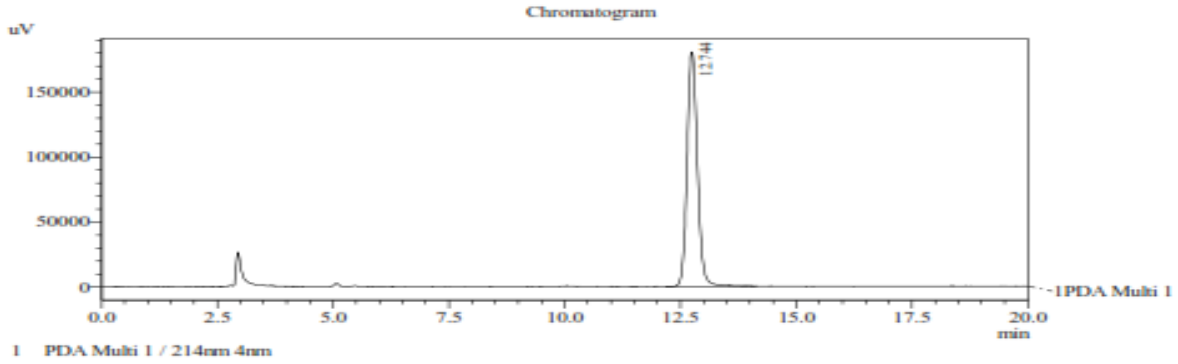


Quantitative Results

PDA

ID#	Name	Ret. Time	Area
1	Acetylcysteine	12.808	2437536

Sample Name : Nefrosave Tablets
Sample ID : Accuracy_150%_1
Method Filename : Nefrosave_Acetyl cysteine.lcm
Vial : 9
Injection Volume : 10 uL



Quantitative Results

PDA

ID#	Name	Ret. Time	Area
1	Acetylcysteine	12.744	2782946

Accuracy

The accuracy of the method was determined by using three solutions containing *placebo* spiked with acetylcysteine at approximately 50%, 100% and 150% of its working strength. Each level was analyzed. The percentage recovery results obtained are listed in Table 4.

Acceptance criteria

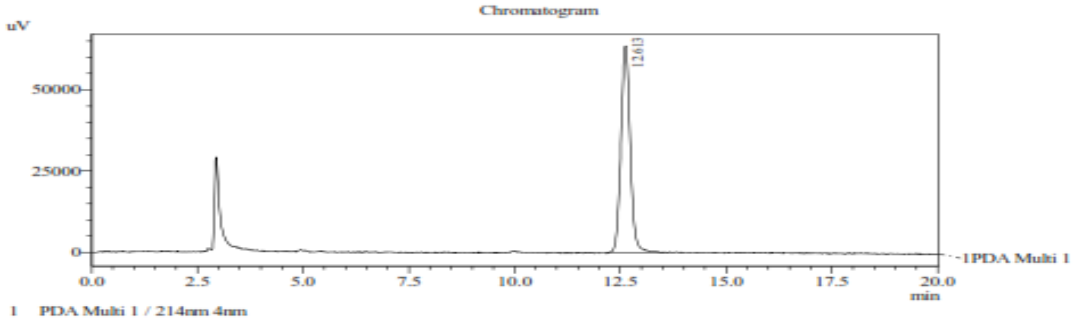
The recovery should be in the range of 98.0% – 102.0%.

Table 4: Accuracy of acetylcysteine

Level	%acetylcysteine working strength	Theoretical conc.(mg/mL)	Measured conc.(mg/mL)	% recovery
50%	51.0	0.25500	0.25822	101.26
	51.4	0.25700	0.25852	100.59
	50.8	0.25400	0.25772	101.46
100%	99.8	0.49900	0.50264	100.73
	100.2	0.50100	0.50134	100.07
	100.4	0.50200	0.49767	99.14
150%	149.4	0.74700	0.74422	99.63
	149.0	0.74500	0.73956	99.27
	150.2	0.75100	0.74264	98.89

The percentage recovery values were in the range of 98.89%- 101.46% which is within the acceptance criteria.

Sample Name : Nefrosave Tablets
Sample ID : Accuracy_50%_1
Method Filename : Nefrosave_Acetyl cysteine.lcm
Vial : 3
Injection Volume : 10 uL

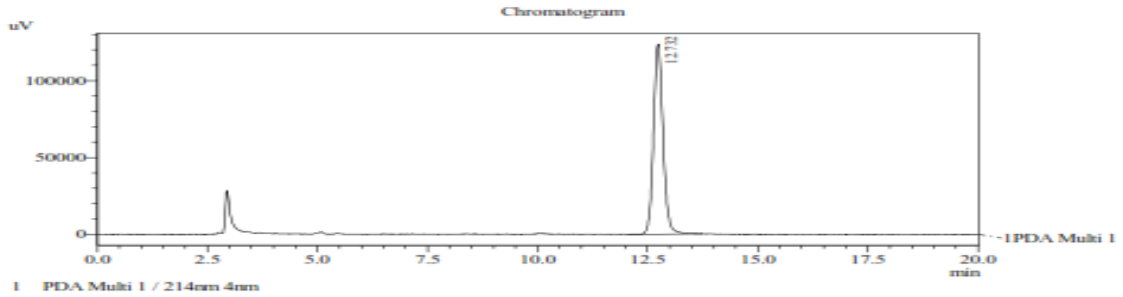


Quantitative Results

PDA

ID#	Name	Ret. Time	Area
1	Acetylcysteine	12.613	973922

Sample Name : Nefrosave Tablets
Sample ID : Accuracy_100%_1
Method Filename : Nefrosave_Acetyl cysteine.lcm
Vial : 6
Injection Volume : 10 uL

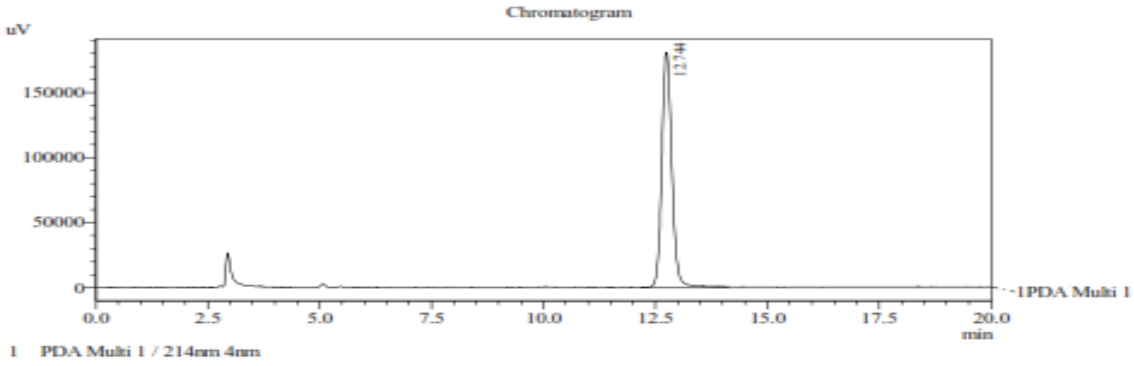


Quantitative Results

PDA

ID#	Name	Ret. Time	Area
1	Acetylcysteine	12.732	1883589

Sample Name : Nefrosave Tablets
Sample ID : Accuracy_150%_1
Method Filename : Nefrosave_Acetyl cysteine.lcm
Vial : 9
Injection Volume : 10 uL



Quantitative Results

PDA

ID#	Name	Ret. Time	Area
1	Acetylcysteine	12.744	2782946

Method precision

The method precision was performed by analyzing a sample solution of tablet dosage form (six replicate sample preparation). Table 5 summarizes the results of precision for the method.

Acceptance criteria

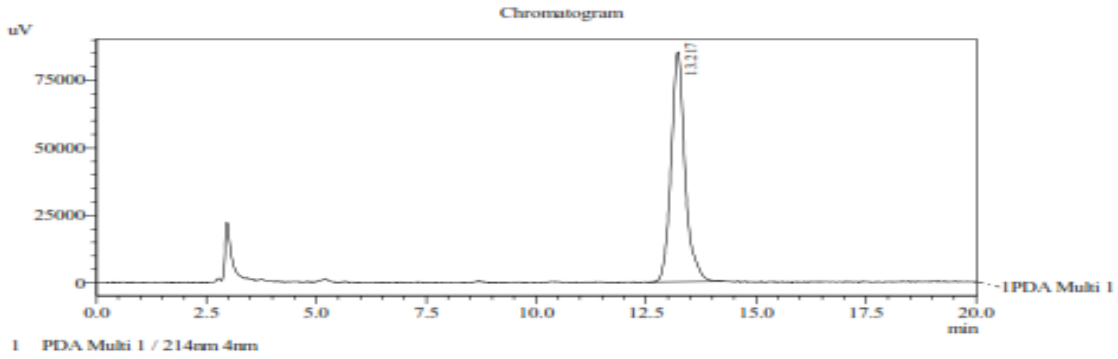
The percentage relative standard deviation for the assay values should be less than 2.0.

Table 5: Summary of results for precision of the method

Sample	Level	% Assay
1	100%	99.92
2	100%	100.03
3	100%	99.01
4	100%	99.58
5	100%	101.03
6	100%	100.15
	Average	**99.95**
	% RSD	**0.67**

The %RSD for the assay values of acetylcysteine in tablet dosage form was 0.67 %

Sample Name : NefroSave Tablets
Sample ID : Method Precision-1
Method Filename : Nefrosave_Acetyl cysteine.lcm
Vial : 3
Injection Volume : 10 uL



Quantitative Results

PDA

ID#	Name	Ret. Time	Area
1	Acetylcysteine	13.217	1882245

Ruggedness (Intermediate precision)

The ruggedness of the method was performed by analyzing a sample solution of tablet dosage form as per the test method (six replicate samples preparation) and injected each solution in duplicate using different instrument, column, reagent, and analyst on different days. The results of set I were compared with the results of set II. Table 6 shows the ruggedness of the method.

Acceptance criteria

The overall % RSD should not be more than 2.0%

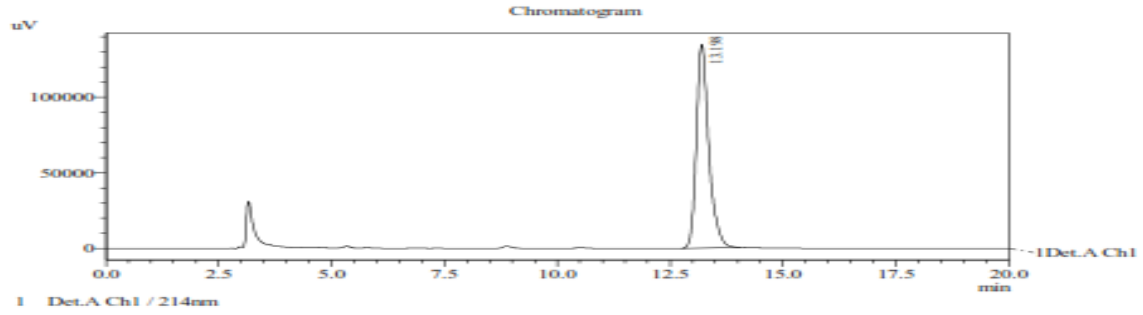
Table 6: summary of results for ruggedness

Sample	% Assay (acetylcysteine)	
	Set - I	**Set - II**
1	99.92	100.85
2	100.03	99.23
3	99.01	99.95
4	99.58	99.71
5	101.03	101.19
6	100.15	99.73
Average	**99.95**	**100.11**
% RSD	**0.67**	**0.75**
Overall average	**100.03**	
Overall % RSD	**0.68**	
Set	**Set I**	**Set II**

Instrument	I/RD/HPC/04	I/RD/HPC/03
Column	Cosmosil, 5C$_{18}$-MS-II (250 X 4.6 mm, 5µm), RD/COL/77	Cosmosil, 5C$_{18}$-MS-II (250 X 4.6 mm, 5µm), RD/COL/76
Reagent	Potassium dihydrogen orthophosphate (Rankem)	Potassium dihydrogen orthophosphate (Finar)
Analyst	A.Baskar Palraj	S.Vijay
Day	28/02/2017	03/03/2017

The above result indicates that the test method is rugged for instrument to instrument, column to column, reagent to reagent, analyst to analyst and day to day variation. The overall % RSD for the assay value of acetylcysteine in tablet dosage form was 0.68.

Sample Name : Nefrosave Tablets
Sample ID : Ruggedness_1
Method Filename : NEFROSAVE_ACETYL CYS.lcm
Vial : 3
Injection Volume : 10 uL



Quantitative Results

Detector A

ID#	Name	Ret. Time	Area
1	Acetyl Cysteine	13.198	2578963

Robustness

The following table (Table 7) shows the parameters of the method that were altered to test the robustness of the method. System suitability test was carried out to asses if these changes had a significant effect on the chromatography.

Table 7: Parameters altered for robustness test

Parameter / Condition	Actual	Low	High
Flow rate	1.00 mL/min	0.90 mL/min	1.10 mL/min
Mobile phase	100% Buffer Conc.: 6.8 g / L	100% Buffer Conc.: 6.7 g / L	100% Buffer Conc.: 6.9 g / L
Buffer pH	3.0	2.9	3.1
Column oven temperature	30°C	28°C	32°C

The acetylcysteine results obtained for robustness tests are summarized in Table 8.

Acceptance criteria

(i) The tailing factor for the acetylcysteine peaks should not more than 2.0 in standard solution.

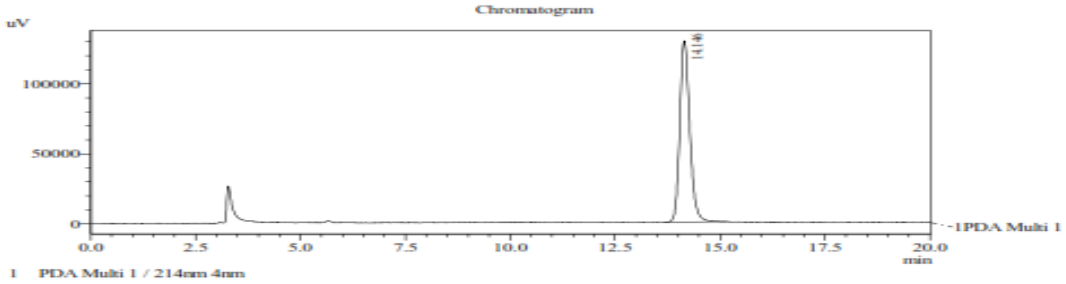
(ii) The number of theoretical plates for the acetylcysteine peaks should not less than 2000 in standard solution.

(iii) The relative standard deviation for the peak area of acetylcysteine should not be more than 2.0% in standard solution.

Table 8: Summary of robustness results of acetylcysteine (Flow)

Flow	Actual	Low	High
Retention time (min)	13. 18-13.24	14.14-14.18	11.55-11.65
Area	1888358-1900436	2145603-2153669	1733324-1754218
Tailing factor	1.29	1.21	1.20
Theoretical plates	10788	16721	15359
Average (Area)	1891268	2152542	1740558
%RSD (Area)	0.27	0.24	0.41

Sample Name : Standard
Sample ID : Robustness_Flow Low
Method Filename : Acetyl cysteine_Flow Low.lcm
Vial : 2
Injection Volume : 10 uL

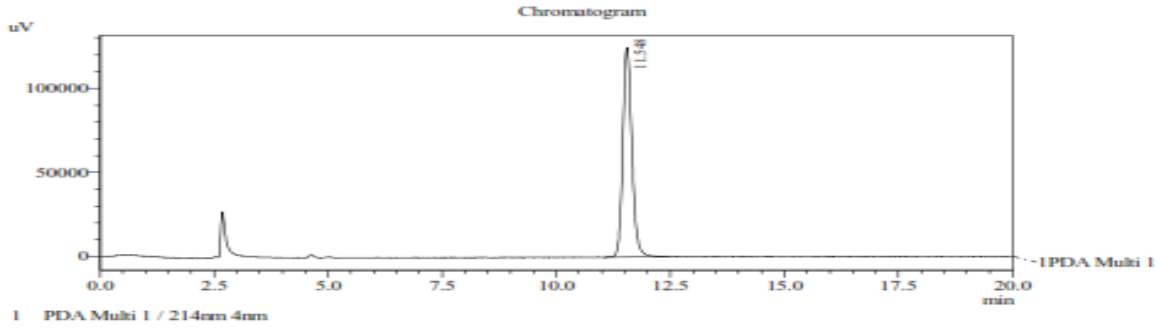


Quantitative Results

PDA

ID#	Name	Ret. Time	Area	Tailing Factor	Theoretical Plate
1	Acetylcysteine	14.146	2158056	1.21	16721

Sample Name : Standard
Sample ID : Robustness_Flow High
Method Filename : Acetyl cysteine_Flow High.lcm
Vial : 2
Injection Volume : 10 uL

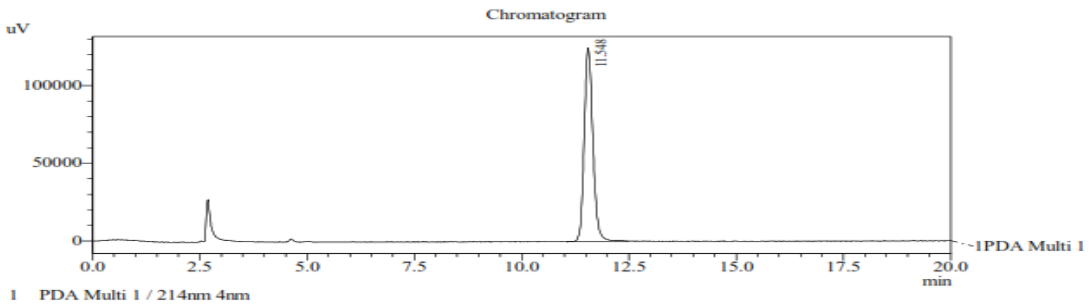


Quantitative Results

PDA

ID#	Name	Ret. Time	Area	Tailing Factor	Theoretical Plate
1	Acetylcysteine	11.548	1754218	1.20	15359

Sample Name : nefrosave
Sample ID : Robustness_Flow High
Method Filename : Acetyl cysteine_Flow High.lcm
Vial : 2
Injection Volume : 10 uL

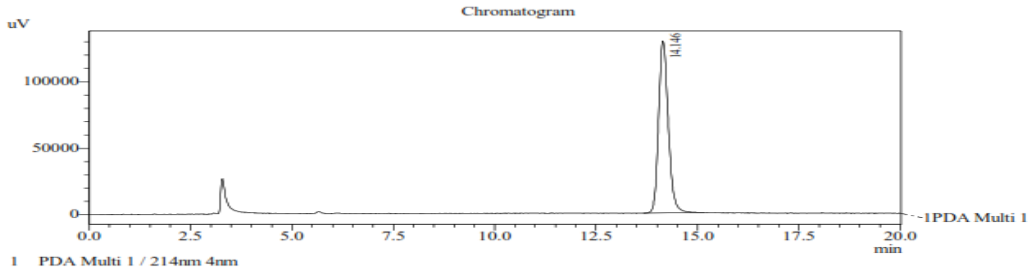


Quantitative Results

PDA

ID#	Name	Ret. Time	Area	Tailing Factor	Theoretical Plate
1	Acetylcysteine	11.548	1754218	1.20	15359

Sample Name : nefrosave
Sample ID : Robustness_Flow Low
Method Filename : Acetyl cysteine_Flow Low.lcm
Vial : 2
Injection Volume : 10 uL



Quantitative Results

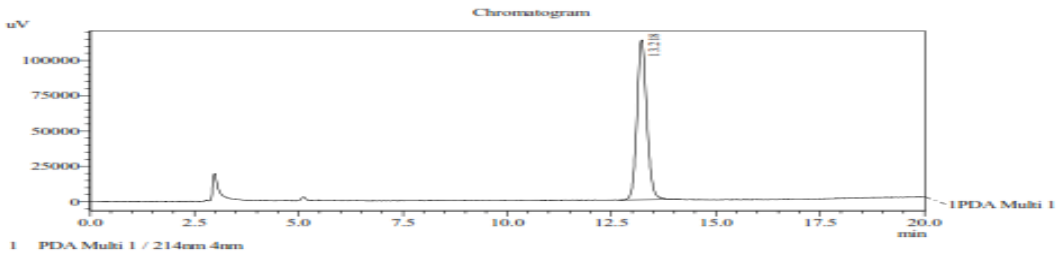
PDA

ID#	Name	Ret. Time	Area	Tailing Factor	Theoretical Plate
1	Acetylcysteine	14.146	2158056	1.21	16721

Table 9: Summary of robustness results of acetylcysteine (Mobile phase)

Mobile phase	Actual	Low	High
Retention time (min)	13.18_13.24	11.99-12.05	13.25-13.30
Area	1888358-1900436	1948798-1975414	1852245-1863360
Tailing factor	1.29	1.131	1.20
Theoretical plates	10788	15064	15359
Average (Area)	1891268	1962783	1858564
%RSD (Area)	0.27	0.57	0.24

Sample Name : Standard
Sample ID : Robustness_Mobile Phase High
Method Filename : Nefrosave_Acetyl cysteine.lcm
Vial : 2
Injection Volume : 10 uL

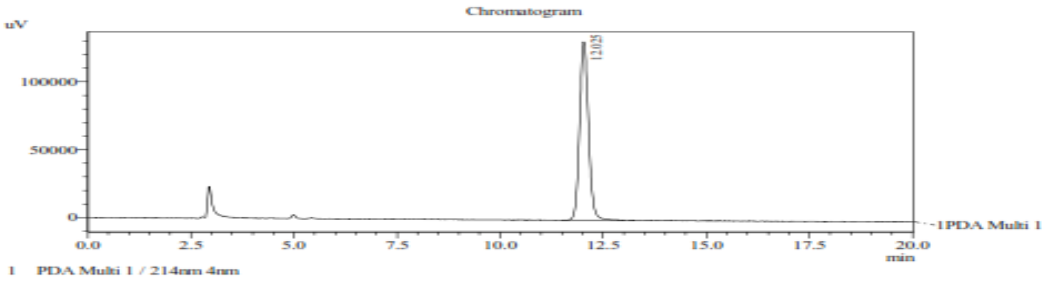


Quantitative Results

PDA

ID#	Name	Ret. Time	Area	Tailing Factor	Theoretical Plate
1	Acetylcysteine	13.218	1863360	1.13	14778

Sample Name : Standard
Sample ID : Robustness_Mobile Phase Low
Method Filename : Nefrosave_Acetyl cysteine.lcm
Vial : 2
Injection Volume : 10 uL

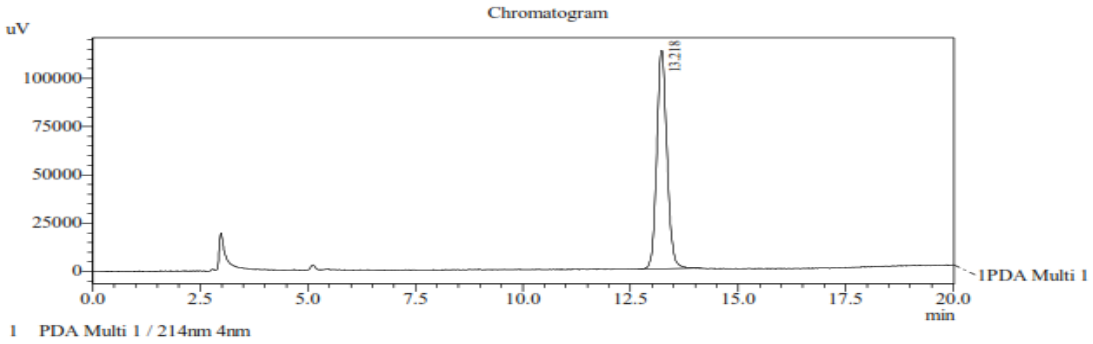


Quantitative Results

PDA

ID#	Name	Ret. Time	Area	Tailing Factor	Theoretical Plate
1	Acetylcysteine	12.025	1952364	1.13	15064

Sample Name : Nefrosave_
Sample ID : Robustness_Mobile Phase High
Method Filename : Nefrosave_Acetyl cysteine.lcm
Vial : 2
Injection Volume : 10 uL

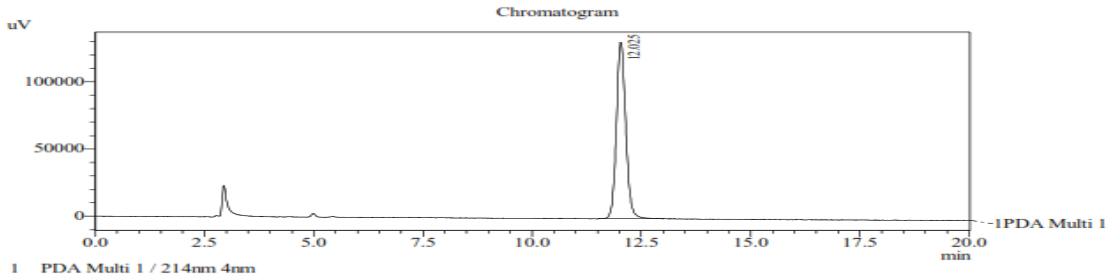


Quantitative Results

PDA

ID#	Name	Ret. Time	Area	Tailing Factor	Theoretical Plate
1	Acetylcysteine	13.218	1863360	1.13	14778

Sample Name : Nefrosave
Sample ID : Robustness_Mobile Phase Low
Method Filename : Nefrosave_Acetyl cysteine.lcm
Vial : 2
Injection Volume : 10 uL



Quantitative Results

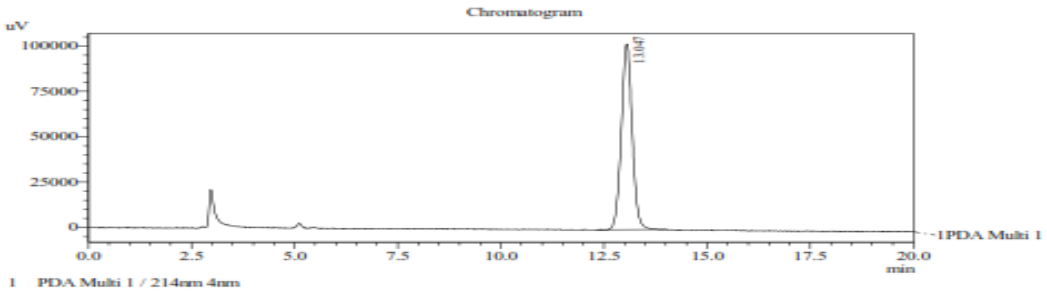
PDA

ID#	Name	Ret. Time	Area	Tailing Factor	Theoretical Plate
1	Acetylcysteine	12.025	1952364	1.13	15064

Table10: Summary of robustness results of acetylcysteine (Buffer pH)

Buffer pH	Actual	Low	High
Retention time (min)	13.18-13.24	12.97-13.05	11.66-11.75
Area	1892288-1900436	1879427-1885303	2018685-2034826
Tailing factor	1.29	1.06	1.09
Theoretical plates	10788	11501	13554
Average (Area)	**1891268**	**1890268**	**2024317**
%RSD (Area)	**0.27**	**0.41**	**0.30**

Sample Name : Standard
Sample ID : Robustness_Buffer pH Low
Method Filename : Nefrosave_Acetyl cysteine.lcm
Vial : 2
Injection Volume : 10 uL



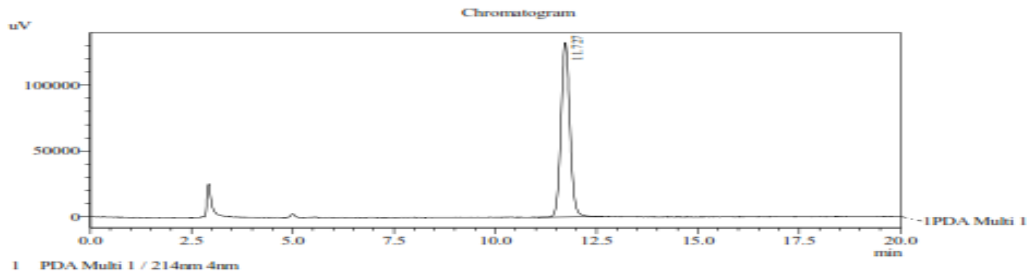
1 PDA Multi 1 / 214nm 4nm

Quantitative Results

PDA

ID#	Name	Ret. Time	Area	Tailing Factor	Theoretical Plate
1	Acetylcysteine	13.047	1879427	1.06	11501

Sample Name : Standard
Sample ID : Robustness_Buffer pH High
Method Filename : Nefrosave_Acetyl cysteine.lcm
Vial : 2
Injection Volume : 10 uL



1 PDA Multi 1 / 214nm 4nm

Quantitative Results

PDA

ID#	Name	Ret. Time	Area	Tailing Factor	Theoretical Plate
1	Acetylcysteine	11.727	2018685	1.09	13554

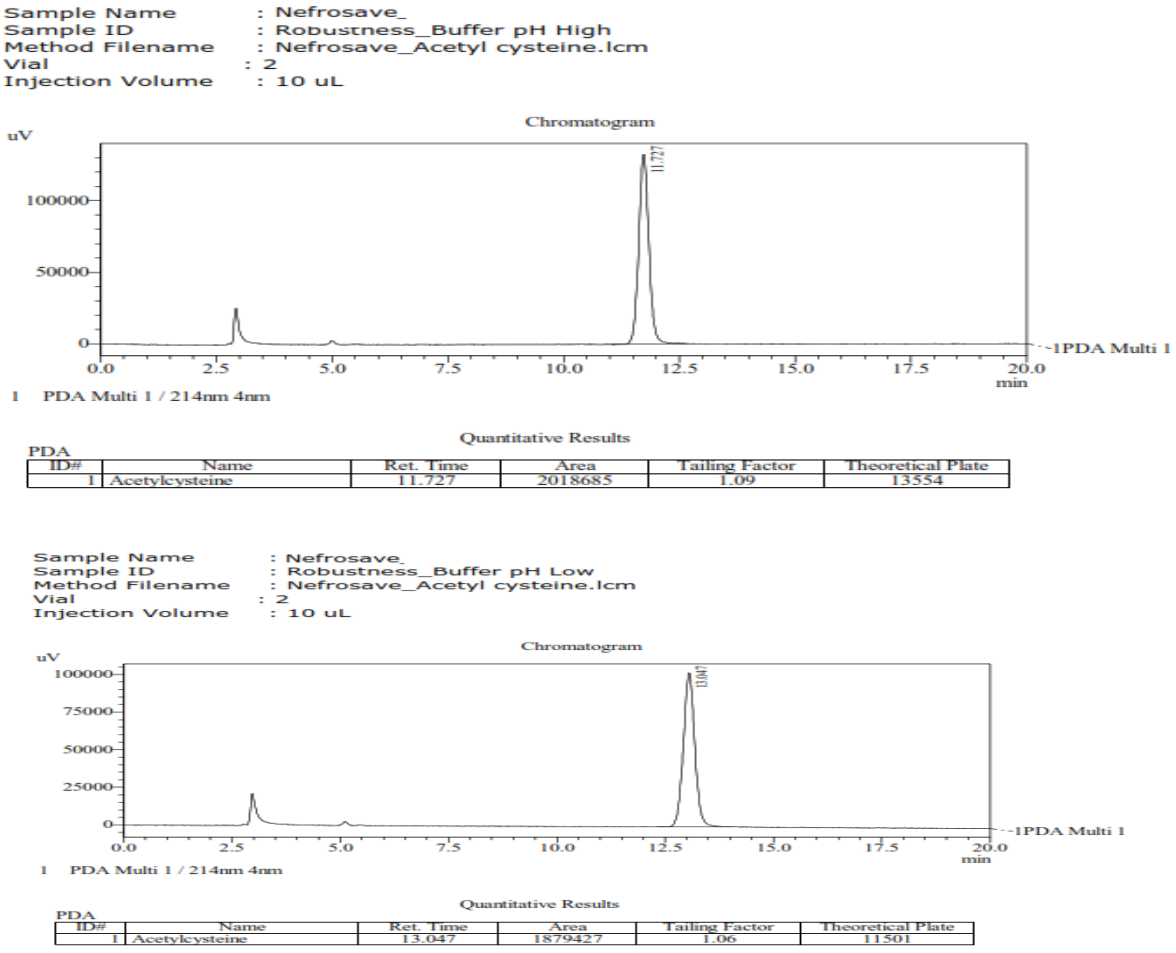
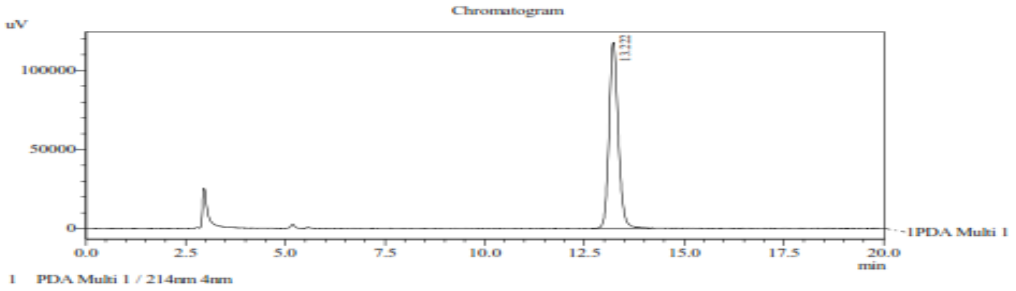


Table 11: Summary of robustness results of acetylcysteine (Column temperature)

Column temperature	Actual	Low	High
Retention time (min)	13.18-13.24	13.20-13.22	12.48-12.49
Area	1888358-1900436	1893207-1900998	1896528-1900962
Tailing factor	1.29	1.16	1.12
Theoretical plates	10788	15532	1 4663
Average (Area)	**1891268**	**1895568**	**1899345**
%RSD (Area)	**0.27**	**0.18**	**0.09**

Sample Name : Standard
Sample ID : Robustness_Column Temp Low
Method Filename : Acetyl cysteine_Col Temp_Low.lcm
Vial : 2
Injection Volume : 10 uL



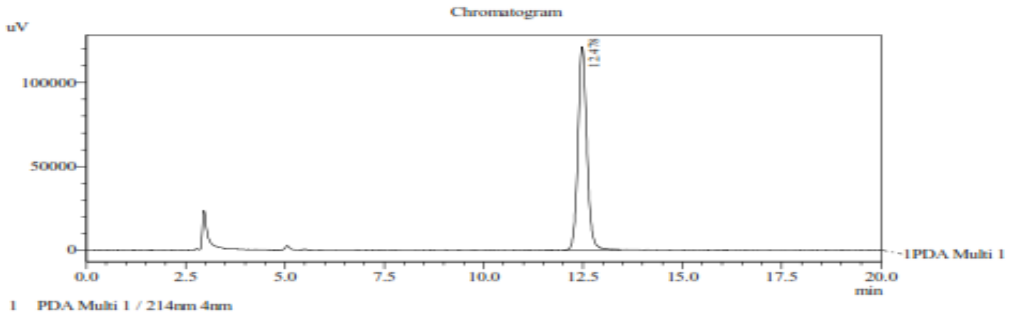
1 PDA Multi 1 / 214nm 4nm

Quantitative Results

PDA

ID#	Name	Ret. Time	Area	Tailing Factor	Theoretical Plate
1	Acetylcysteine	13.222	1900998	1.16	15532

Sample Name : Standard
Sample ID : Robustness_Column Temp High
Method Filename : Acetyl cysteine_Col Temp_High.lcm
Vial : 2
Injection Volume : 10 uL



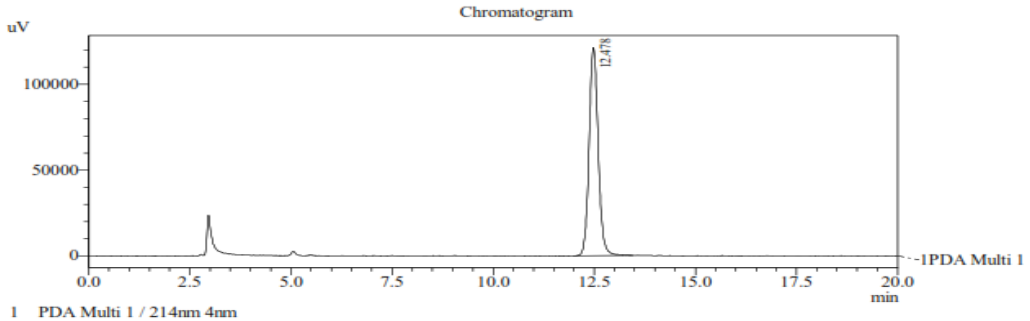
1 PDA Multi 1 / 214nm 4nm

Quantitative Results

PDA

ID#	Name	Ret. Time	Area	Tailing Factor	Theoretical Plate
1	Acetylcysteine	12.478	1900962	1.11	14663

Sample Name : Nefrosave
Sample ID : Robustness_Column Temp High
Method Filename : Acetyl cysteine_Col Temp_High.lcm
Vial : 2
Injection Volume : 10 uL

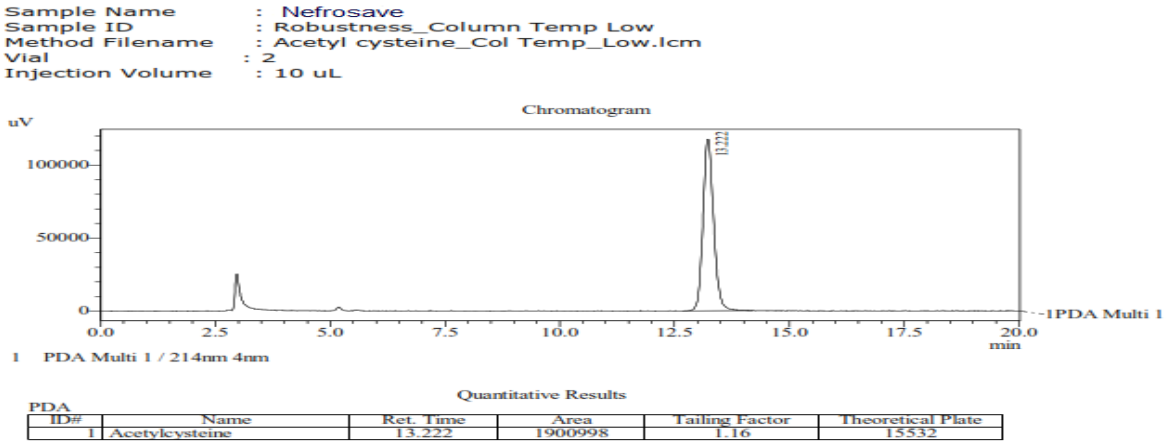


1 PDA Multi 1 / 214nm 4nm

Quantitative Results

PDA

ID#	Name	Ret. Time	Area	Tailing Factor	Theoretical Plate
1	Acetylcysteine	12.478	1900962	1.11	14663



Quantitative Results

PDA

ID#	Name	Ret. Time	Area	Tailing Factor	Theoretical Plate
1	Acetylcysteine	13.222	1900998	1.16	15532

Solution stability

A solution of tablet dosage form at 100% of working concentration was kept at 15°C. The solution stability was monitored at different time interval (Initial, 6 hours, 12 hours, 24 hours, 36 hours and 48 hours). The results are summarized in Table 12.

Acceptance criteria

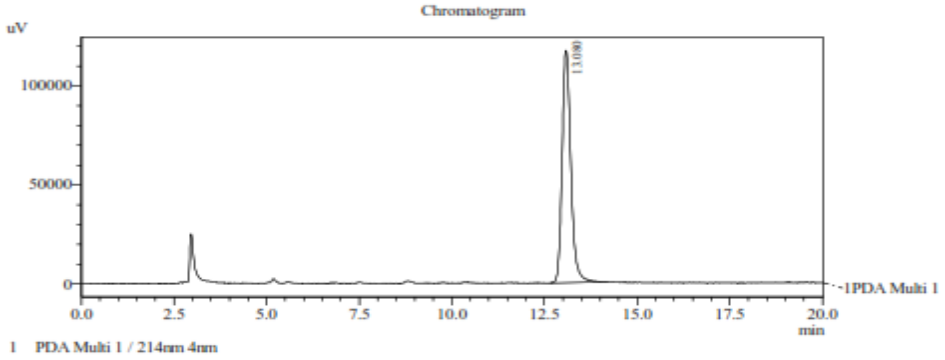
Record the results and assign the stability of solutions based on the experimental data. The relative standard deviation of assay results should not vary by more than 2.0% for a stable solution.

Table 12: summary of solution stability results

Time Intervals (hours)	Initial	6	12	18	24	36	48	**% RSD**
% acetylcysteine	100.43	100.07	101.15	100.63	100.55	100.95	100.7	**0.35**

The assay values of acetylcysteine in tablet dosage form was in the range of 100.07% to 101.15%.The %RSD between the assay results from the initial to 48hours was 0.35, which is within the acceptance limit of 2.0%. Therefore, the tablet dosage form sample solutions are stable up to 48 hours and 15^{o}C.

Sample Name : Nefrosave Tablets
Sample ID : Solution Stability_0 Hr
Method Filename : Nefrosave_Acetyl cysteine.lcm
Vial : 3
Injection Volume : 10 uL

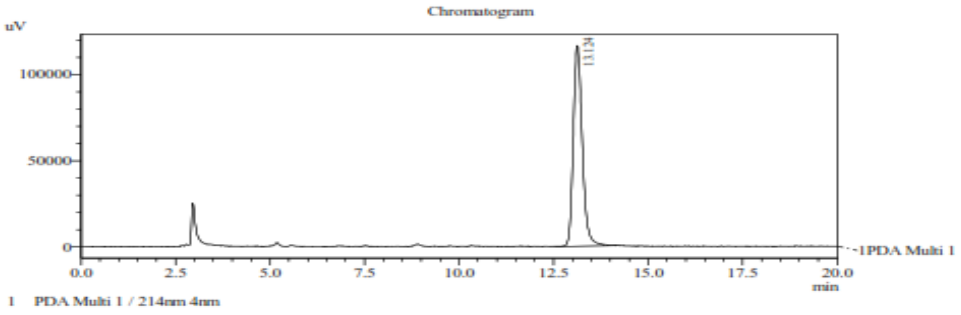


Quantitative Results

PDA

ID#	Name	Ret. Time	Area
1	Acetylcysteine	13.080	1927723

Sample Name : Nefrosave Tablets
Sample ID : Solution Stability_6 Hr
Method Filename : Nefrosave_Acetyl cysteine.lcm
Vial : 3
Injection Volume : 10 uL

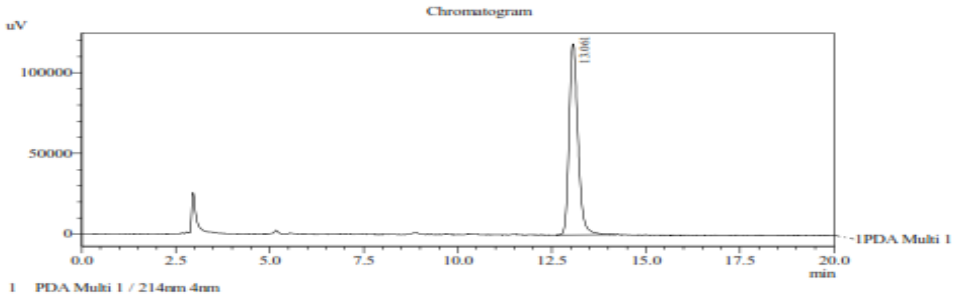


Quantitative Results

PDA

ID#	Name	Ret. Time	Area
1	Acetylcysteine	13.124	1914599

Sample Name : Nefrosave Tablets
Sample ID : Solution Stability_12 Hr
Method Filename : Nefrosave_Acetyl cysteine.lcm
Vial : 3
Injection Volume : 10 uL

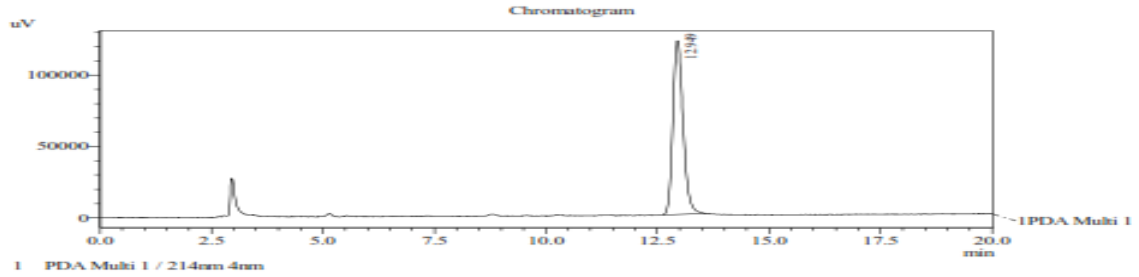


Quantitative Results

PDA

ID#	Name	Ret. Time	Area
1	Acetylcysteine	13.061	1935836

Sample Name : Nefrosave Tablets
Sample ID : Solution Stability_18 Hr
Method Filename : Nefrosave_Acetyl cysteine.lcm
Vial : 3
Injection Volume : 10 uL



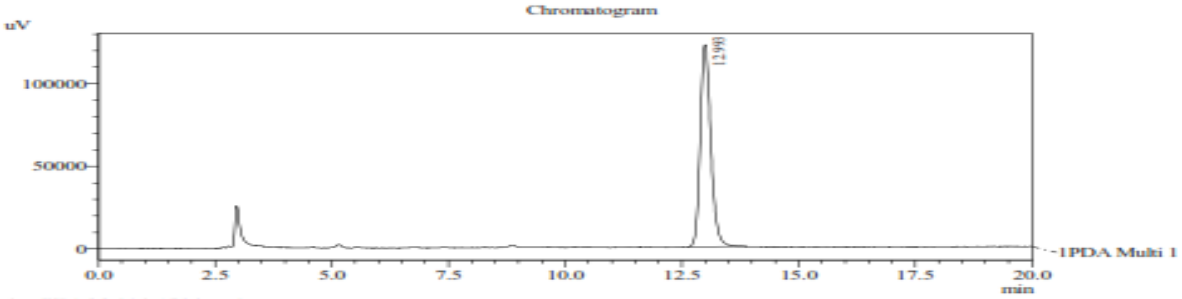
1 PDA Multi 1 / 214nm 4nm

Quantitative Results

PDA

ID#	Name	Ret. Time	Area
1	Acetylcysteine	12.949	1927206

Sample Name : Nefrosave Tablets
Sample ID : Solution Stability_36 Hr
Method Filename : Nefrosave_Acetyl cysteine.lcm
Vial : 3
Injection Volume : 10 uL



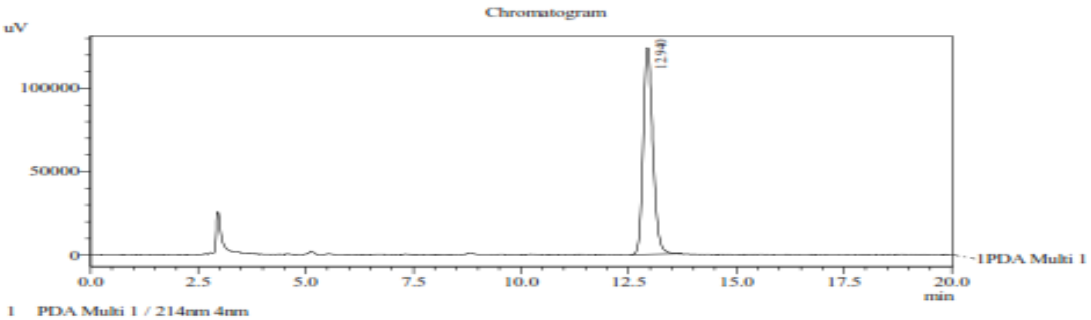
1 PDA Multi 1 / 214nm 4nm

Quantitative Results

PDA

ID#	Name	Ret. Time	Area
1	Acetylcysteine	12.993	1935742

Sample Name : Nefrosave Tablets
Sample ID : Solution Stability_48 Hr
Method Filename : Nefrosave_Acetyl cysteine.lcm
Vial : 3
Injection Volume : 10 uL



1 PDA Multi 1 / 214nm 4nm

Quantitative Results

PDA

ID#	Name	Ret. Time	Area
1	Acetylcysteine	12.940	1931686

SUMMARY REPORT OF ACETYLCYSTEINE

S.No	Parameters	Observation	Acceptance criteria
1	**Specificity** *Placebo* Interference	No peak was observed at the retention time of acetylcysteine in the chromatogram of *placebo*	The *placebo* chromatogram should not show any peak at the retention time of acetylcysteine.
2	**System precision**	1.29	Tailing factor: NMT 2.0
		10788	Theoretical plates :NLT 2000
		0.27	% RSD: NMT 2.0
3	**Linearity & range**	0.9998	Correlation coefficient: NLT 0.995
4	**Accuracy**	98.89 – 101.46 %	98.0 – 102.0%
5	**Method precision**	0.67	% RSD : NMT 2.0 (Assay)
6	**Ruggedness**	0.68	Overall % RSD NMT 2.0 (Assay)
7	**Robustness**	1.06 – 1.29	Tailing factor: NMT 2.0
		10788 – 16721	Theoretical plates: NLT 2000
		0.09 – 0.57	% RSD: NMT 2.0
8	**Solution stability**	0.35	%RSD NMT : 2.0 (Assay)

The results obtained in this study demonstrate that the estimation of acetylcysteine in tablet dosage form by HPLC method described is specific, linear, accurate, precise, ruggedness and robust. Therefore, the method is suitable for its intended use.

Specificity of taurine

Placebo solution was prepared separately at a concentration of 0.33 mg/mL of excipients blend. A solution of *placebo* was spiked with the taurine at its working concentration. The solution was analyzed as per the RP-HPLC method described.

Table 1 summarizes the retention time (RT), relative retention time (RRT) values obtained for *placebo* and taurine.

Acceptance criteria

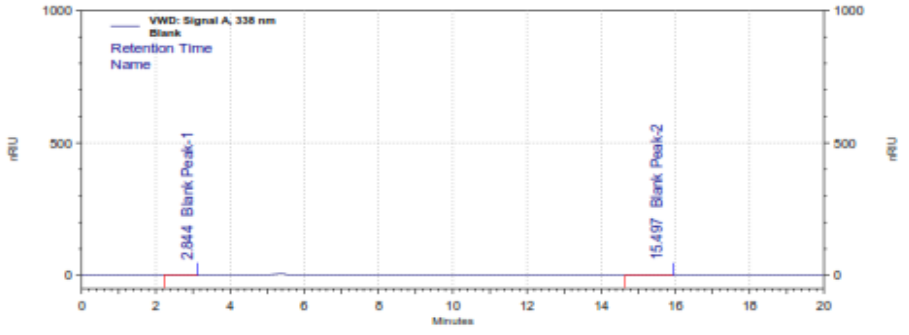
The *placebo* chromatogram should not show any peak at the retention time of taurine.

Table 1: Summary of retention time and relative retention time values for *placebo* peak of taurine

Peak name	Retention time (minutes)	Relative retention time
Blank peak-1	2.85	0.50
Blank peak-2	15.41	2.70
Taurine	5.70	1.00

No peak was observed at the retention time of taurine in the chromatogram of *placebo*.

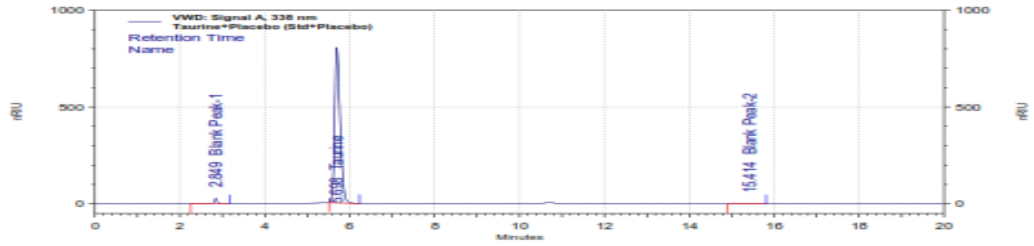
Method Name : NefroSave_TAURINE.met
Sample Name : Blank
Injection Volume : 10 µL
Vial No : 4



VWD: Signal A, 338 nm Results

Peak Number	Name	Retention Time	Area
1	Blank Peak-1	2.84	516224
2	Blank Peak-2	15.50	493507

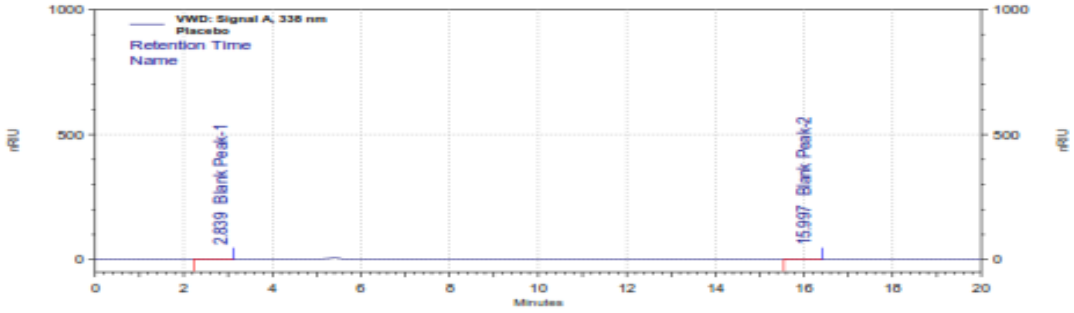
Method Name : NefroSave_TAURINE.met
Sample Name : Taurine+Placebo (Std+Placebo)
Injection Volume : 10 µL
Vial No : 7



VWD: Signal A, 338 nm Results

Peak Number	Name	Retention Time	Area
1	Blank Peak-1	2.85	2445395
2	Taurine	5.70	119583945
3	Blank Peak-2	15.41	417366

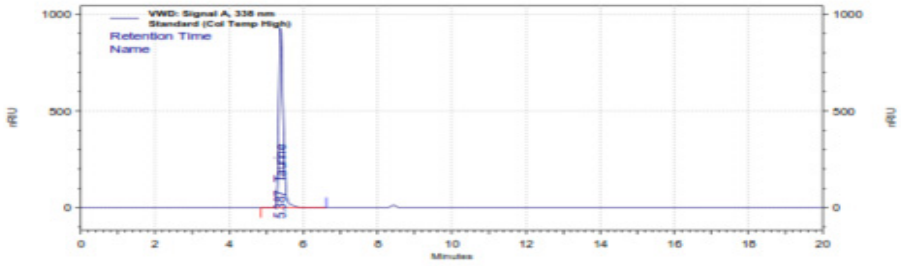
Method Name : NefroSave_TAURINE.met
Sample Name : Placebo
Injection Volume : 10 µL
Vial No : 5



VWD: Signal A, 338 nm Results

Peak Number	Name	Retention Time	Area
1	Blank Peak-1	2.84	528544
2	Blank Peak-2	16.00	513262

Method Name : NefroSave_TAURINE.met
Sample Name : Standard
Injection Volume : 10 µL
Vial No : 5



VWD: Signal A, 338 nm Results

Peak Number	Name	Retention Time	Area	Asymmetry	Theoretical plates (USP
1	Taurine	5.39	127959978	1.31	10701

System precision

A standard solution of 0.50 mg/mL of taurine was prepared and analyzed as per the method. Table 2 summarizes system suitability results.

Acceptance criteria

(i) The tailing factor for the taurine peak should not more than 2.0 in standard solution.

(ii) The number of theoretical plates for the taurine peak should not less than 2000 in standard solution.

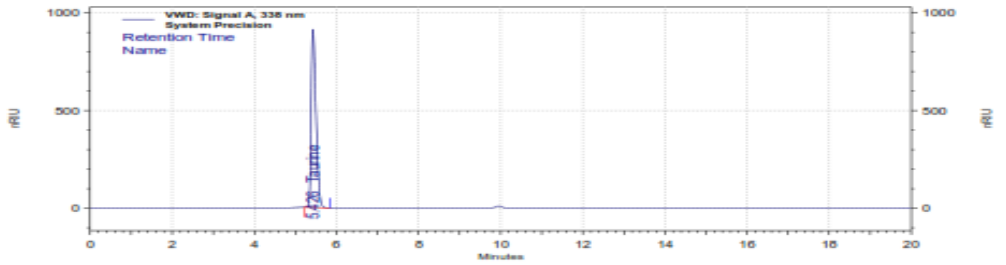
(ii) The relative standard deviation for the peak area of taurine should not be more than 2.0% in standard solution.

Table 2: Summary of retention time, % RSD of peak area, tailing factor and theoretical plates of the taurine peak

S.No	Retention time (Minutes)	Area	Tailing factor	Theoretical plates
1	5.43	122758536	1.56	10120
2	5.44	124554851		
3	5.42	123150389		
4	5.41	123847876		
5	5.40	123295736		
6	5.42	123710604		
	Average	**123552999**		
	% RSD	**0.51**		

The percentage relative standard deviation of peak area of taurine was 0.51 with the tailing factor and theoretical plates of 1.56 and 10120 respectively.

Method Name : NefroSave_TAURINE.met
Sample Name : System Precision
Injection Volume : 10 µL
Vial No : 5



VWD:
Signal A,
338 nm
Results

Peak Number	Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
1	Taurine	5.43	122758536	1.56	10120

Linearity and range

The linearity of the HPLC method was demonstrated for taurine ranging from 0.2500 mg/mL to 0.7500 mg/mL, which is equivalent to 50% to 150% of the taurine working strength. Five standard solutions at the concentrations within the mentioned range were prepared and analyzed as per the method. The linearity results obtained are shown in Table 3. Figure 1 shows the line of best fit for concentration versus peak area of taurine.

Acceptance criteria

The plot of concentration versus peak area should be linear with a correlation coefficient (R^2) not less than 0.995.

Table 3: Linearity of taurine

Level	% of taurine	Concentration (mg/mL)	Peak area
50%	50.12	0.25060	65341127
80%	80.19	0.40096	10372708
100%	100.24	0.50120	12412401
120%	120.29	0.60144	15106250
150%	150.36	0.75180	19086289
		correlation coefficient	0.9982

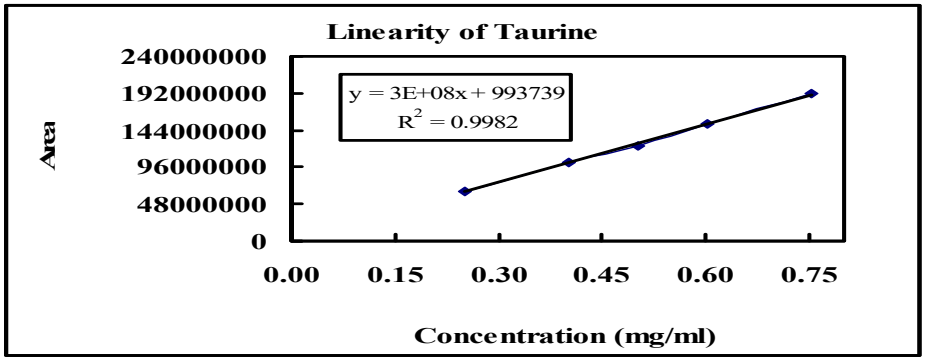
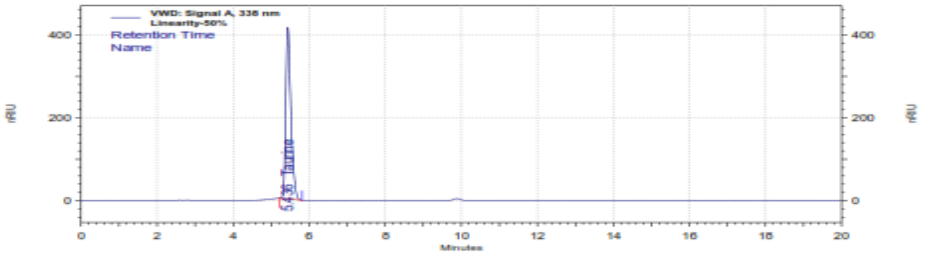


Figure 2: Linearity graph for taurine

Thus, the HPLC method for the estimation of taurine in tablet dosage form was shown to be linear in the range of 50% to 150% of the working concentration with a correlation coefficient of 0.9982. The range of the HPLC method for determining the assay of taurine in tablet dosage form is 50% to 150% of the working strength.

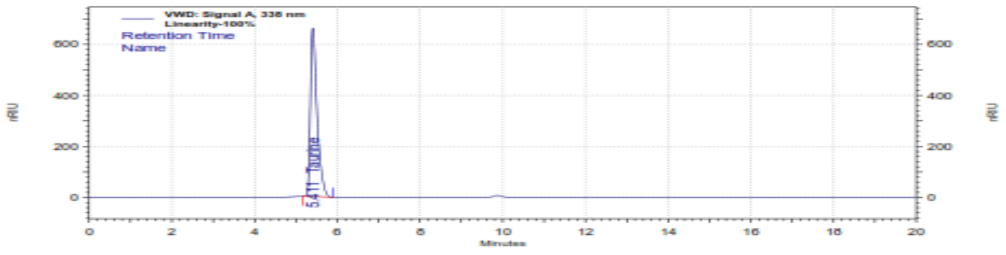
Method Name : NefroSave_TAURINE.met
Sample Name : Linearity-50%
Injection Volume : 10 µL
Vial No : 6



VWD: Signal A, 338 nm Results

Peak Number	Name	Retention Time	Area
1	Taurine	5.44	65341127

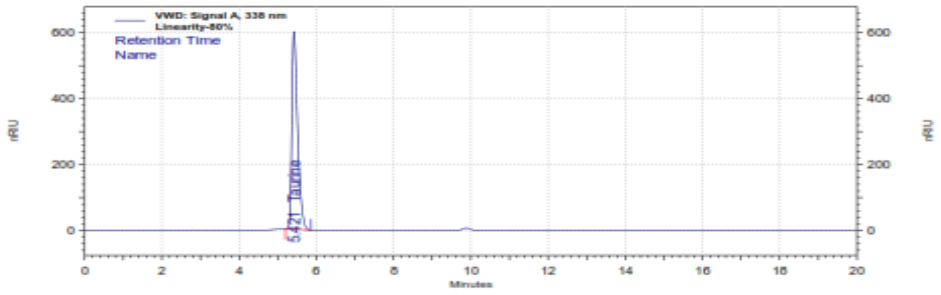
Method Name : NefroSave_TAURINE.met
Sample Name : Linearity-100%
Injection Volume : 10 µL
Vial No : 8



VWD: Signal A, 338 nm Results

Peak Number	Name	Retention Time	Area
1	Taurine	5.41	124124017

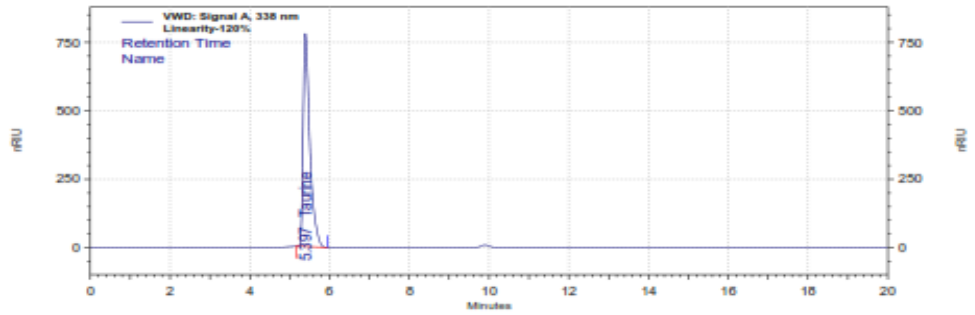
Method Name : NefroSave_TAURINE.met
Sample Name : Linearity-80%
Injection Volume : 10 µL
Vial No : 7



VWD: Signal A, 338 nm Results

Peak Number	Name	Retention Time	Area
1	Taurine	5.42	103727083

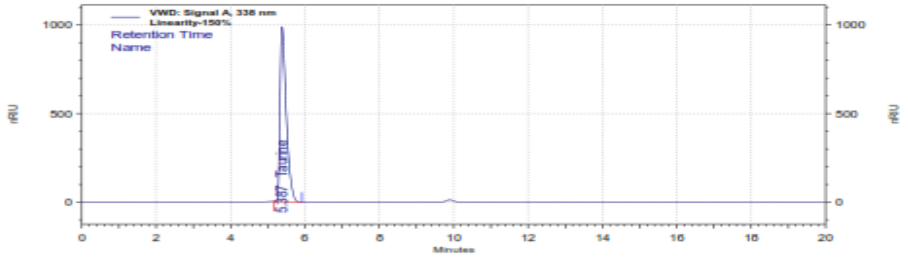
Method Name : NefroSave_TAURINE.met
Sample Name : Linearity-120%
Injection Volume : 10 µL
Vial No : 9



VWD: Signal A, 338 nm Results

Peak Number	Name	Retention Time	Area
1	Taurine	5.40	151062506

Method Name : NefroSave_TAURINE.met
Sample Name : Linearity-150%
Injection Volume : 10 µL
Vial No : 10



VWD: Signal A, 338 nm Results

Peak Number	Name	Retention Time	Area
1	Taurine	5.39	190862892

Accuracy

The accuracy of the method was determined by using three solutions containing *placebo* spiked with taurine at approximately 50%, 100% and 150% of its working strength. Each level was analyzed. The percentage recovery results obtained are listed in Table 4.

Acceptance criteria

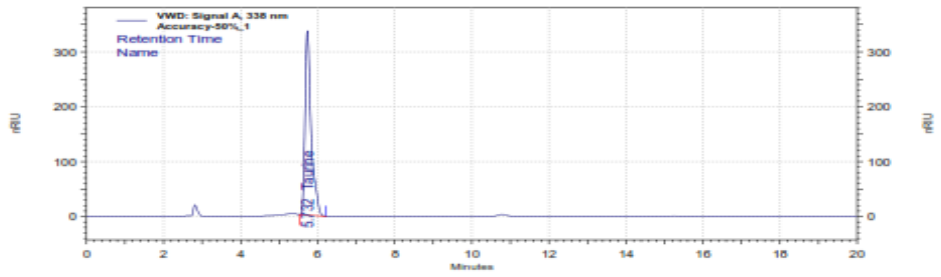
The recovery should be in the range of 98.0% – 102.0%.

Table 4: Accuracy of taurine

Level	% Taurine working strength	Theoretical conc.(mg/mL)	Measured conc.(mg/mL)	% recovery
50%	50.04	0.25020	0.25045	100.10
	50.24	0.25120	0.25414	101.17
	50.08	0.25040	0.25204	100.65
100%	100.04	0.50020	0.50262	100.48
	99.96	0.49980	0.49656	99.35
	100.08	0.50040	0.49598	99.12
150%	150.48	0.75240	0.76501	101.68
	150.20	0.75100	0.75375	100.37
	150.08	0.75040	0.75919	101.17

The percentage recovery values were in the range of 99.12%- 101.68% which is within the acceptance criteria.

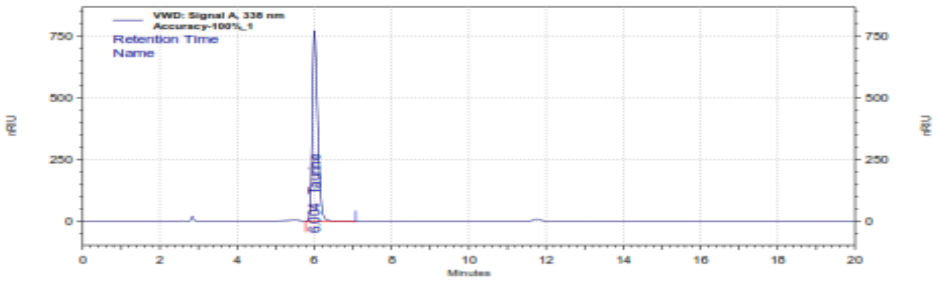
Method Name : NefroSave_TAURINE.met
Sample Name : Accuracy-50%_1
Injection Volume : 10 µL
Vial No : 6



VWD: Signal A,
338 nm Results

Peak Number	Name	Retention Time	Area
1	Taurine	5.73	62494101

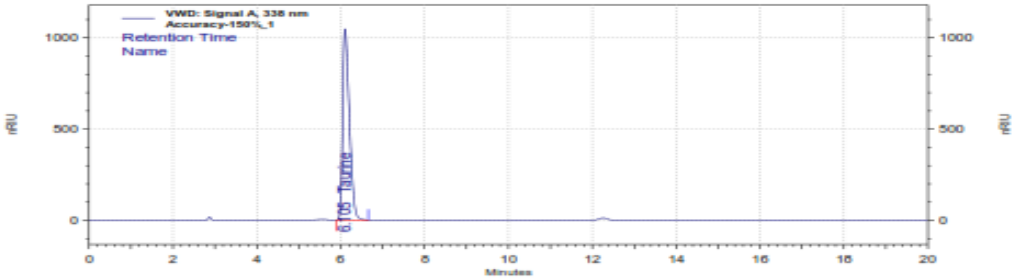
Method Name : NefroSave_TAURINE.met
Sample Name : Accuracy-100%_1
Injection Volume : 10 µL
Vial No : 9



VWD: Signal A,
338 nm Results

Peak Number	Name	Retention Time	Area
1	Taurine	6.00	126055593

Method Name : NefroSave_TAURINE.met
Sample Name : Accuracy-150%_1
Injection Volume : 10 µL
Vial No : 12



VWD: Signal A,
338 nm Results

Peak Number	Name	Retention Time	Area
1	Taurine	6.11	192715210

Method precision

The method precision was performed by analyzing a sample solution of tablet dosage form (B.No: D0349) as per the test method (six replicate sample preparation). Table 5 summarizes the results of precision for the method.

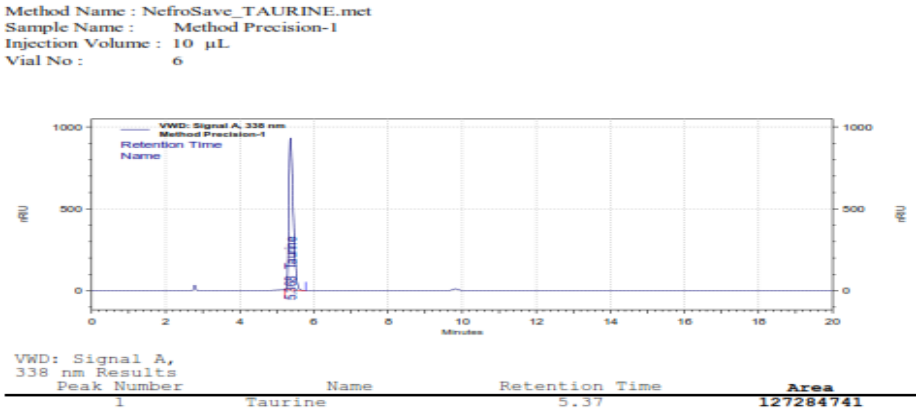
Acceptance criteria

The percentage relative standard deviation for the assay values should be less than 2.0.

Table 5: Summary of results for precision of the method

Sample	Level	% Assay (taurine)
1	100%	100.48
2	100%	101.63
3	100%	101.42
4	100%	101.09
5	100%	101.52
6	100%	101.75
	Average	**101.32**
	% RSD	**0.46**

The %RSD for the assay values of taurine in tablet dosage form was 0.46.



Ruggedness (Intermediate precision)

The ruggedness of the method was performed by analyzing a sample solution of tablet dosage form as per the test method (six replicate sample preparation) and injected each solution in duplicate using different instrument, column, reagent, and analyst on different days. The results of set I were compared with the results of set II. Table 6 shows the ruggedness of the method.

Acceptance criteria

The overall % RSD should not be more than 2.0%.

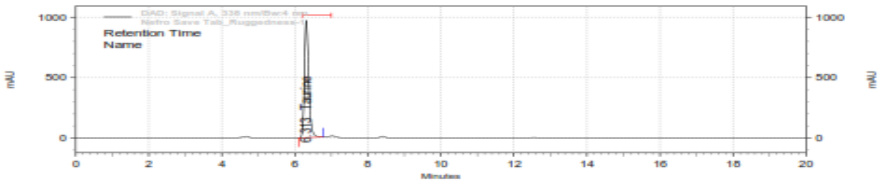
Table 6: Summary of results for ruggedness

Sample	% Assay (taurine)	
	Set - I	**Set - II**
1	100.48	100.33
2	101.63	101.40
3	101.42	99.31
4	101.09	99.18
5	101.52	100.28

6	101.75	99.77
Average	**101.32**	**100.05**
% RSD	**0.46**	**0.82**
Overall average	**100.68**	
Overall % RSD	**0.91**	
Set	**Set I**	**Set II**
Instrument	I/RD/HPC/01	I/RD/HPC/02
Column	Cosmosil,5C_{18}-MS-II (250 X 4.6 mm, 5µm), RD/COL/79	Phenomenex, Hyperclone-ODS 250 X 4.6mm, 5µm Column ID : RD/COL/58
Reagent	Sodium acetate anhydrous (Fisher) Methanol (Fisher) Acetonitrile (Finar)	Sodium acetate anhydrous (Rankem) Methanol (Finar) Acetonitrile (Advent)
Analyst	A.Baskar Palraj	R.Chitra
Day	01/03/2017	07/03/2017

The above result indicates that the test method is rugged for instrument to instrument, column to column, reagent to reagent, analyst to analyst and day to day variation. The overall % RSD for the assay value of taurine in tablet dosage form was 0.91.

Method: Nefrosave_Taurine.met
Sample Name : Nefro Save Tab_Ruggedness-1
Injection Volume : 10 µL
Vial: 6



DAD: Signal A, 338 nm/Bw:4 nm
Results

Name	Retention Time	Area
Taurine	6.313	16446118

Robustness

The following table (Table 7) shows the parameters of the method that were altered to test the robustness of the method. System suitability test was carried out to asses if these changes had a significant effect on the chromatography.

Table 7: Parameters altered for robustness test

Parameter / Condition	Actual	Low	High
Flow rate	1.00 mL/min	0.90 mL/min	1.10 mL/min
Mobile phase	Mobile phase A : Mobile phase B 50 : 50	Mobile phase A : Mobile phase B 52 : 48	Mobile phase A : Mobile phase B 48 : 52
Buffer pH	7.20	7.10	7.30
Column oven temperature	40°C	38°C	42°C

Acceptance criteria

(i) The tailing factor for the taurine peaks should not more than 2.0 in standard solution.

(ii) The number of theoretical plates for the taurine peaks should not less than 2000 in standard solution.

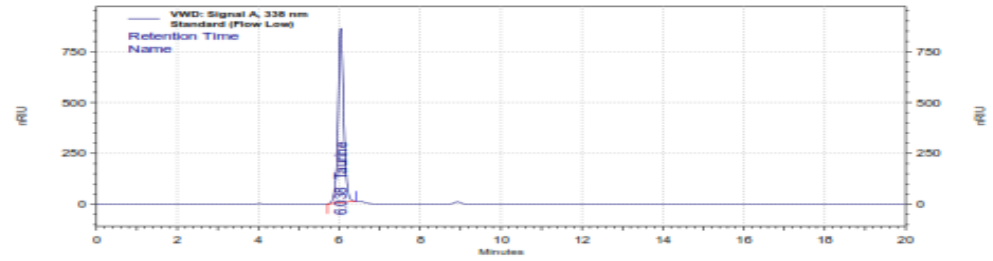
(iii) The relative standard deviation for the peak area of taurine should not be more than 2.0% in standard solution.

The taurine results obtained for robustness tests are summarized in table 8

Table 8: Summary of robustness results of taurine (Flow)

Flow	Actual	Low	High
Retention time (min)	13.18-13.24	14.14-14.18	11.55-11.65
Area	1888358-1900436	2145603-2153669	1733324-1754218
Tailing factor	1.29	1.21	1.20
Theoretical plates	10788	16721	15359
Average (Area)	1891268	2152542	1740558
%RSD (Area)	0.27	0.24	0.41

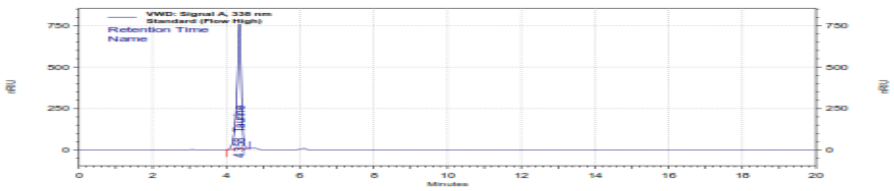
Method Name : NefroSave_TAURINE.met
Sample Name : Standard (Flow Low)
Injection Volume : 10 µL
Vial No : 5



VWD:
Signal A,
338 nm
Results

Peak Number	Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
1	Taurine	6.04	139324072	1.06	9529

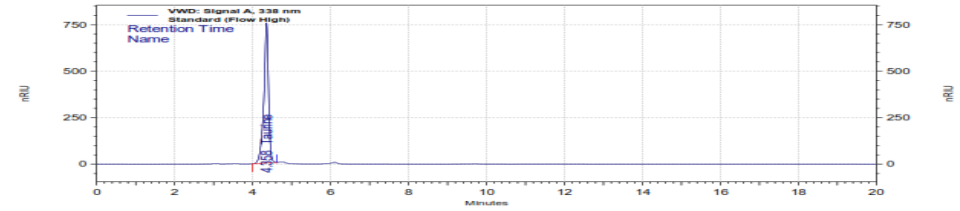
Method Name : NefroSave_TAURINE.met
Sample Name : Standard (Flow High)
Injection Volume : 10 µL
Vial No : 5



VWD:
Signal A,
338 nm
Results

Peak Number	Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
1	Taurine	4.36	104547629	0.81	7530

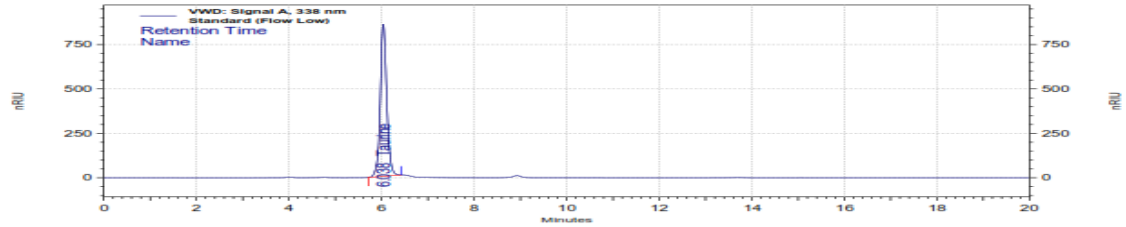
Method Name : NefroSave_TAURINE.met
Sample Name : NefroSave Flow High)
Injection Volume : 10 µL
Vial No : 5



VWD:
Signal A,
338 nm
Results

Peak Number	Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
1	Taurine	4.36	104547629	0.81	7530

Method Name : NefroSave_TAURINE.met
Sample Name : NefroSave (Flow Low)
Injection Volume : 10 µL
Vial No : 5



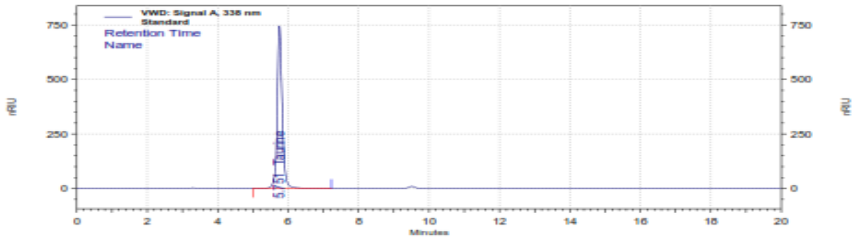
VWD:
Signal A,
338 nm
Results

Peak Number	Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
1	Taurine	6.04	139324072	1.06	9529

Table 9: Summary of robustness results of taurine (Mobile phase)

Mobile phase	Actual	Low	High
Retention time (min)	13.18-13.24	11.99-12.05	13.25-13.30
Area	1888358-1900436	1948798-1975414	1852245-1863360
Tailing factor	1.29	1.131	1.20
Theoretical plates	10788	15064	15359
Average (Area)	1891268	1962783	1858564
%RSD (Area)	0.27	0.57	0.24

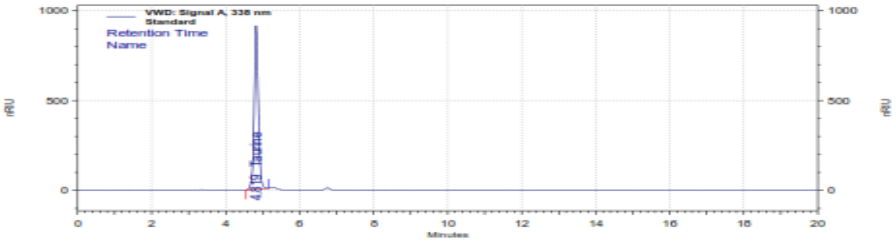
Method Name : NefroSave_TAURINE.met
Sample Name : Standard (Mobile Phase Low)
Injection Volume : 10 µL
Vial No : 5



VWD:
Signal A,
338 nm
Results

Peak Number	Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
1	Taurine	5.75	123446511	1.14	8883

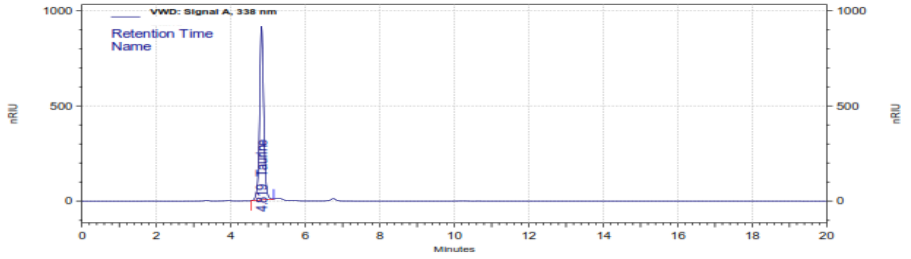
Method Name : NefroSave_TAURINE.met
Sample Name : Standard (Mobile Phase High)
Injection Volume : 10 µL
Vial No : 5



VWD:
Signal A,
338 nm
Results

Peak Number	Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
1	Taurine	4.82	118422942	1.07	9599

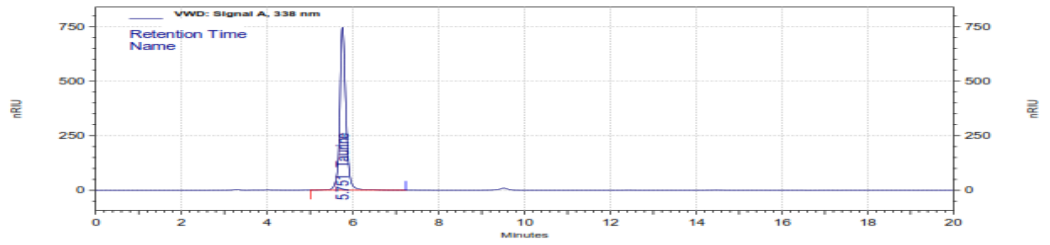
Method Name : NefroSave_TAURINE.met
Sample Name : NefroSave (Mobile Phase High)
Injection Volume : 10 µL
Vial No : 5



VWD:
Signal A,
338 nm
Results

Peak Number	Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
1	Taurine	4.82	118422942	1.07	9599

Method Name : NefroSave TAURINE.met
Sample Name : NefroSave (Mobile Phase Low)
Injection Volume : 10 µL
Vial No : 5



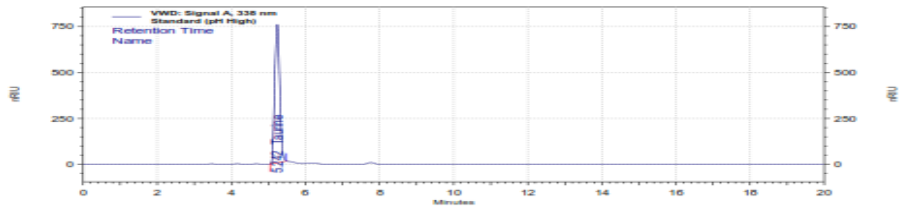
VWD:
Signal A,
338 nm
Results

Peak Number	Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
1	Taurine	5.75	123446511	1.14	8883

Table10: Summary of robustness results of taurine (Buffer pH)

Buffer pH	Actual	Low	High
Retention time (min)	13.18-13.24	12.97-13.05	11.66-11.75
Area	1892288-1900436	1879427-1885303	2018685-2034826
Tailing factor	1.29	1.06	1.09
Theoretical plates	10788	11501	13554
Average (Area)	**1891268**	**1890268**	**2024317**
%RSD (Area)	**0.27**	**0.41**	**0.30**

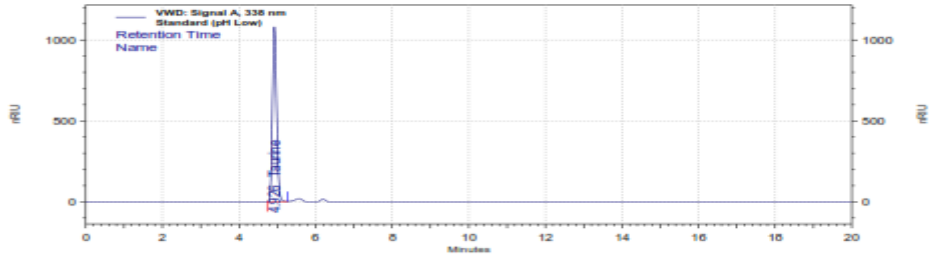
Method Name : NefroSave_TAURINE.met
Sample Name : Standard (pH High)
Injection Volume : 10 µL
Vial No : 5



VWD:
Signal A,
338 nm
Results

Peak Number	Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
1	Taurine	5.24	119343667	1.07	6914

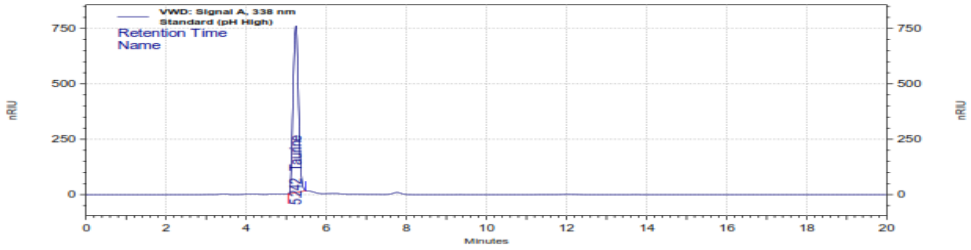
Method Name : NefroSave_TAURINE.met
Sample Name : Standard (pH Low)
Injection Volume : 10 µL
Vial No : 5



VWD:
Signal A,
338 nm
Results

Peak Number	Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
1	Taurine	4.93	138242323	1.12	9545

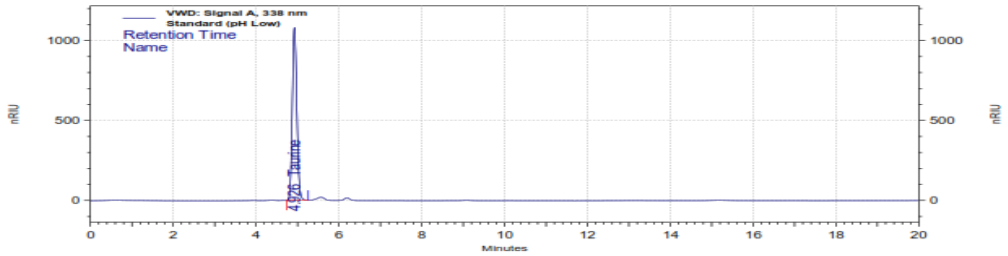
Method Name : NefroSave_TAURINE.met
Sample Name : NefroSave_ (pH High)
Injection Volume : 10 µL
Vial No : 5



VWD:
Signal A,
338 nm
Results

Peak Number	Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
1	Taurine	5.24	119343667	1.07	6914

Method Name : NefroSave_TAURINE.met
Sample Name : NefroSave_ (pH Low)
Injection Volume : 10 µL
Vial No : 5



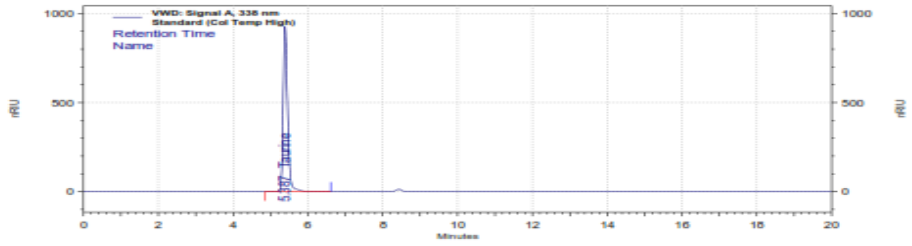
VWD:
Signal A,
338 nm
Results

Peak Number	Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
1	Taurine	4.93	138242323	1.12	9545

Table 11: Summary of robustness results of taurine (Column temperature)

Column temperature	Actual	Low	High
Retention time (min)	13.18-13.24	13.20-13.22	12.48-12.49
Area	1888358-1900436	1893207-1900998	1896528-1900962
Tailing factor	1.29	1.16	1.12
Theoretical plates	10788	15532	1 4663
Average (Area)	**1891268**	**1895568**	**1899345**
%RSD (Area)	**0.27**	**0.18**	**0.09**

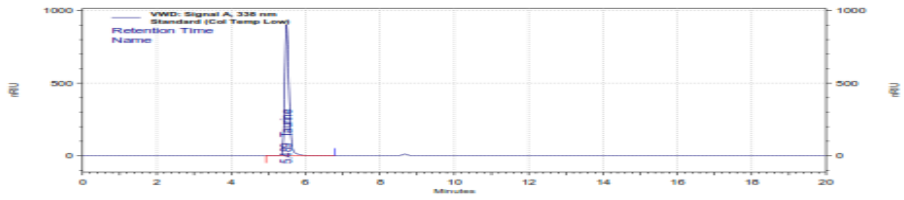
Method Name : NefroSave_TAURINE.met
Sample Name : Standard (Col Temp High)
Injection Volume : 10 µL
Vial No : 5



VWD:
Signal A,
338 nm
Results

Peak Number	Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
1	Taurine	5.39	127959978	1.31	10701

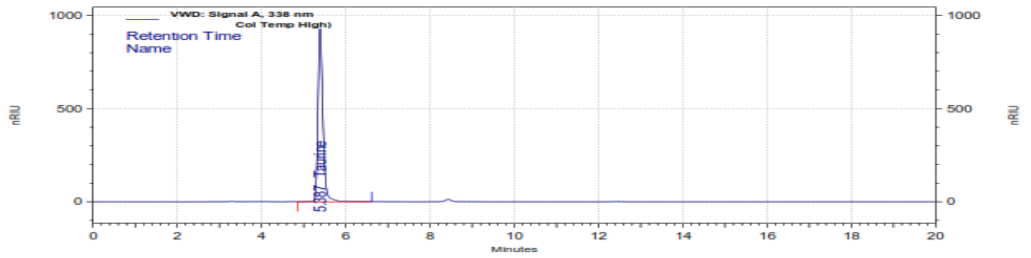
Method Name : NefroSave_TAURINE.met
Sample Name : Standard (Col Temp Low)
Injection Volume : 10 µL
Vial No : 5



VWD:
Signal A,
338 nm
Results

Peak Number	Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
1	Taurine	5.49	124309655	1.28	11024

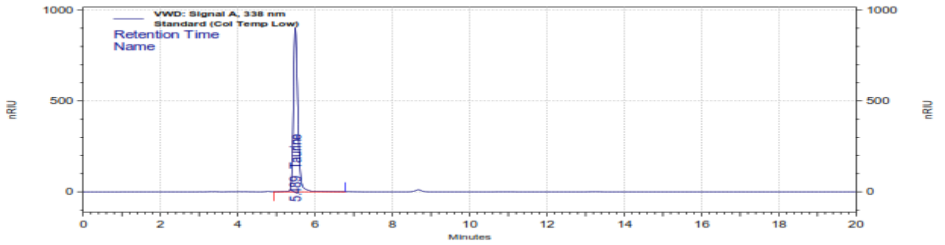
Method Name : NefroSave_TAURINE.met
Sample Name : NefroSave (Col Temp High)
Injection Volume : 10 µL
Vial No : 5



VWD:
Signal A,
338 nm
Results

Peak Number	Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
1	Taurine	5.39	127959978	1.31	10701

Method Name : NefroSave_TAURINE.met
Sample Name : NefroSave (Col Temp Low)
Injection Volume : 10 µL
Vial No : 5



VWD:
Signal A,
338 nm
Results

Peak Number	Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
1	Taurine	5.49	124309655	1.28	11024

Solution stability

A solution of tablet dosage form at 100% of working concentration was kept at 15°C. The solution stability was monitored at different time interval (Initial, 6 hours, 12 hours, and 24 hours). The results are summarized in Table 12.

Acceptance criteria

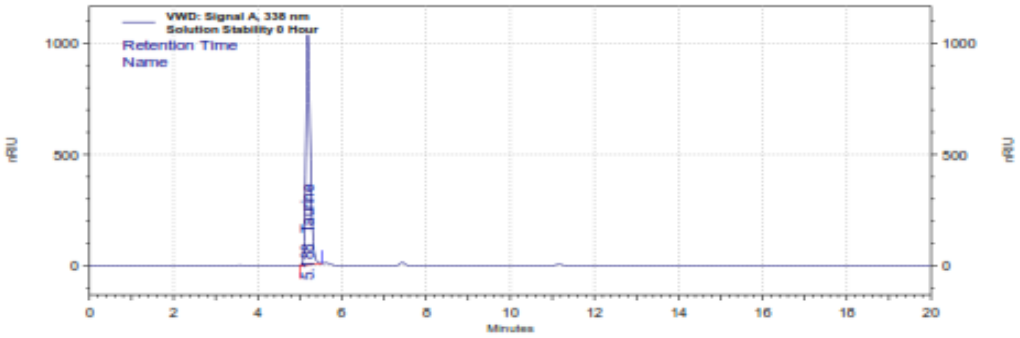
Record the results and assign the stability of solutions based on the experimental data. The relative standard deviation of assay results should not more than 2.0% for a stable solution.

Table 12: Summary of solution stability results

Time Intervals (hours)	Initial	6	12	18	24	% RSD
% taurine	100.17	100.49	101.18	103.06	102.56	**1.25**

The assay value of taurine in tablet dosage form was in the range of 100.17% to 103.06%. The % RSD between the assay results from the initial to 24 hours was 1.25, which is within the acceptance limit of 2.0%. Therefore, the tablet dosage form sample solutions are stable up to 24 hours at 15°C.

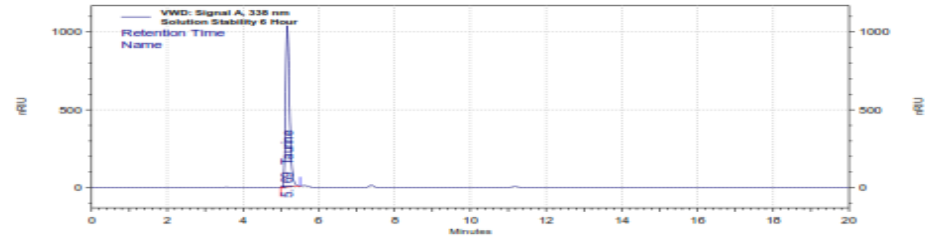
Method Name : NefroSave_TAURINE.met
Sample Name : Solution Stability 0 Hour
Injection Volume : 10 µL
Vial No : 6



VWD: Signal A, 338 nm Results

Peak Number	Name	Retention Time	Area
1	Taurine	5.19	**126075640**

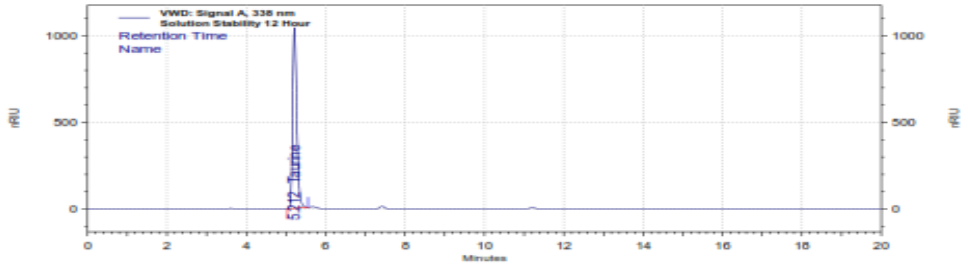
Method Name : NefroSave_TAURINE.met
Sample Name : Solution Stability 6 Hour
Injection Volume : 10 µL
Vial No : 6



VWD: Signal A, 338 nm Results

Peak Number	Name	Retention Time	Area
1	Taurine	5.17	**128015316**

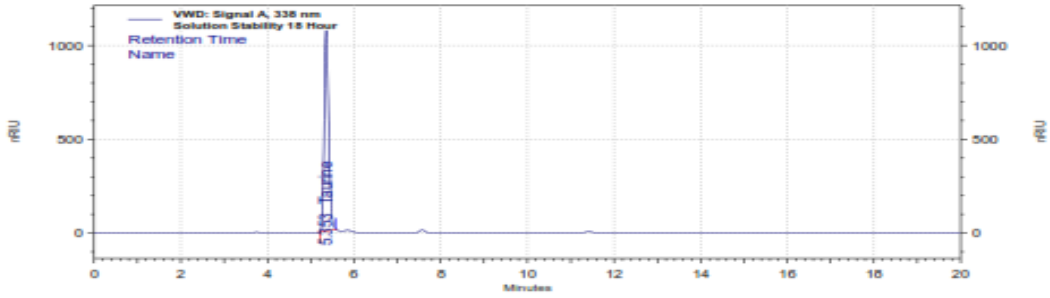
Method Name : NefroSave_TAURINE.met
Sample Name : Solution Stability 12 Hour
Injection Volume : 10 µL
Vial No : 6



VWD: Signal A, 338 nm Results

Peak Number	Name	Retention Time	Area
1	Taurine	5.21	128164437

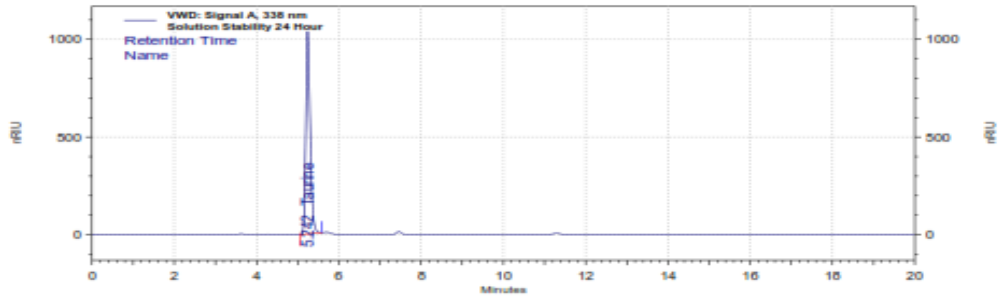
Method Name : NefroSave_TAURINE.met
Sample Name : Solution Stability 18 Hour
Injection Volume : 10 µL
Vial No : 6



VWD: Signal A, 338 nm Results

Peak Number	Name	Retention Time	Area
1	Taurine	5.35	130092526

Method Name : NefroSave_TAURINE.met
Sample Name : Solution Stability 24 Hour
Injection Volume : 10 µL
Vial No : 6



VWD: Signal A, 338 nm Results

Peak Number	Name	Retention Time	Area
1	Taurine	5.24	128857250

SUMMARY REPORT OF TAURINE

S. No	Parameters	Observation	Acceptance criteria
1	**Specificity** *Placebo* interference	No peak was observed at the retention time of taurine in the chromatogram of *Placebo*	The *placebo* chromatogram should not show any peak at the retention time of taurine.
2	**System precision**	1.56	Tailing factor: NMT 2.0
		10120	Theoretical plates: NLT 2000
		0.51	% RSD: NMT 2.0
3	**Linearity & range**	0.9982	correlation coefficient: NLT 0.995
4	**Accuracy**	99.12 – 101.68 %	98.0 – 102.0%
5	**Method precision**	0.46	% RSD : NMT: 2.0 (Assay)
6	**Ruggedness**	0.91	Overall % RSD NMT 2.0
7	**Robustness**	0.81 – 1.56	Tailing factor: NMT 2.0
		6914 – 11024	Theoretical plates: NLT 2000
		0.51 – 1.89	% RSD: NMT 2.0
8	**Solution stability**	1.25	%RSD NMT : 2.0 (Assay)

The results obtained in this study demonstrate that the estimation of taurine in tablet dosage form by RP-HPLC method described is specific, linear, accurate, precise, rugged and robust. Therefore, the method is suitable for its intended use.


CONCLUSIONS

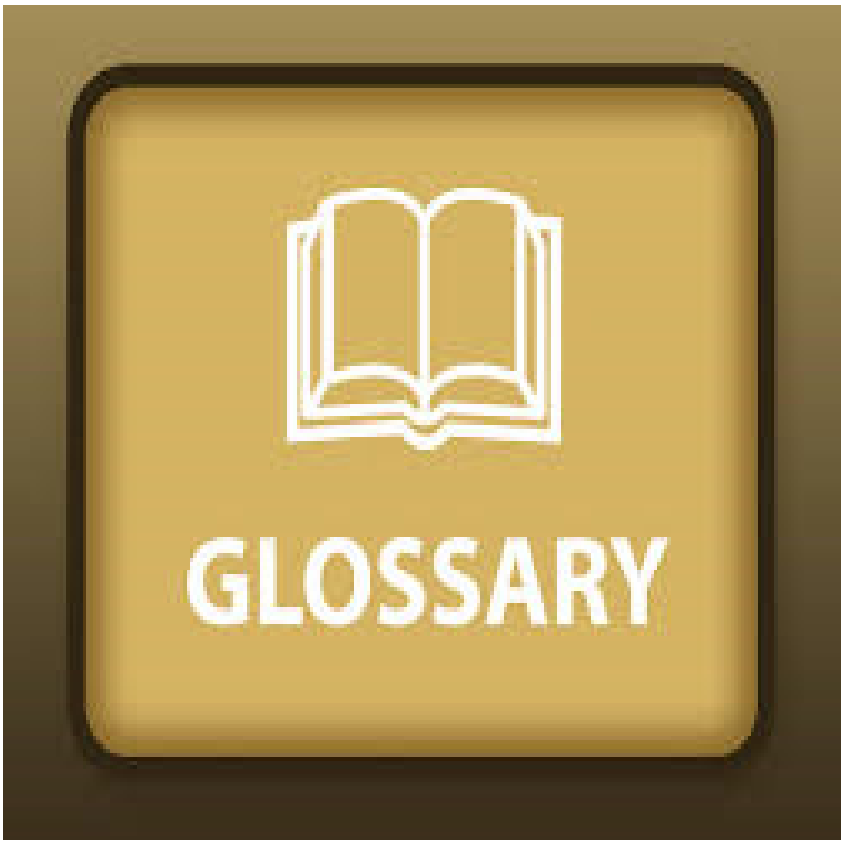
CONCLUSION OF ACETYLCYSTEINE

- Cosmosil, C_{18}, 250×4.6mm 5µm or equivalent as the stationary phase. Mobile phase is sodium metabisulphite solution adjusts the pH 3 using ortho phosphoric acid and flow rate 1 mL/ mins.
- Specificity no peak was observed at the retention time of acetylcysteine in the chromatogram of *placebo.*
- System precision shows %RSD value obtained was below 1.
- Retention time of acetylcysteine is 13.01.
- Correlation of coefficient for acetylcysteine is tailing factor of acetylcysteine is 0.9998.
- Quantitative estimation of acetylcysteine gives accuracy lies between which 98.89 – 101.46%
- By using to system to system suitability and analyst to analyst variability all the parameters met the system suitability.
- Percentage purity of acetylcysteine is 99.67%.

CONCLUSION OF TAURINE

- Cosmosil, C_{18}, 250×4.6mm5µm or equivalent as the stationary phase. Mobile phase is equally mix the Mobile phase A and B. pH 7.2 and flow rate 1 mL/ mins.
- Specificity no peak was observed at the retention time of taurine in the chromatogram of *placebo.*
- System precision shows %RSD value obtained was below 1.
- Retention time of taurine is 5.41.
- Correlation of coefficient for taurine is tailing factor of taurine is 1.56.
- Quantitative estimation of taurine gives accuracy lies between which 99.12 – 101.68 %
- By using to system to system suitability and analyst to analyst variability all the parameters met the system suitability.
- Percentage purity of taurine is 99.80%.
- **The results obtained in this study demonstrate that the estimation of taurine in tablet dosage form by RP-HPLC method described is specific, linear, accurate, precise, rugged and robust.**

The method is suitable for its intended use.


GLOSSARY

GLOSSARY

USP – United States Pharmacopeia

NMT – Not More Than

NLT- Not Less Than

RRT- Relative Retention Time

RSD- Relative Standard Deviation

RT- Retention Time

HPLC- High Performance Liquid Chromatography

RP-HPLC- Reverse Phase –High performance Liquid Chromatography

%- Percentage

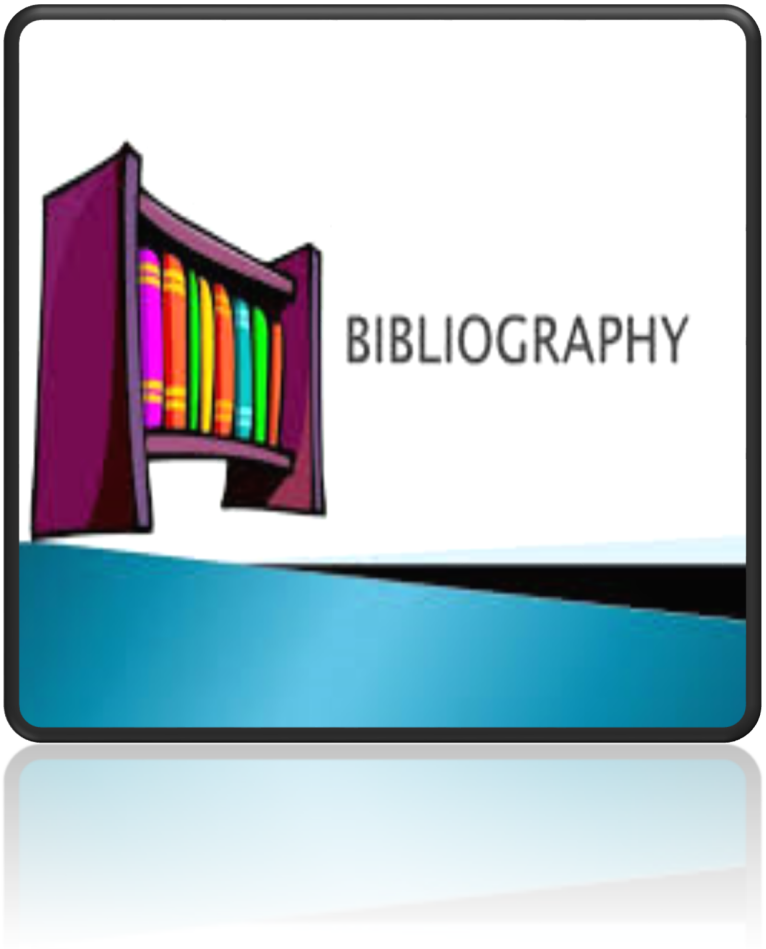
Mg – Millie gram

mL- Mille litter

pH- Potential hydrogen

Mins- minutes

$(R)^2$ - correlation coefficient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