DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-UPLC METHOD FOR DETERMINATION OF RELATED SUBSTANCES OF CINACALCET TABLETS

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

MASTER OF PHARMACY
IN
PHARMACEUTICAL ANALYSIS

Submitted by
Reg.No.:261331102

Under the Guidance of
Prof.K.K.NARASIMHULU,M.Pharm.,
Professor
Department of Pharmaceutical Chemistry
Jaya college of paramedical sciences
College of Pharmacy
Thiruninravur-602024.

DEPARTMENT OF PHARMACEUTICAL ANALYSIS
JAYA COLLEGE OF PARAMEDICAL SCIENCES
COLLEGE OF PHARMACY
THIRUNINRAVUR

APRIL 2015

CERTIFICATE

This is to certify that the dissertation entitled **" DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-UPLC METHOD FOR DETERMINATION OF RELATED SUBSTANCES OF CINACALCET TABLETS"** submitted by ***Reg No.:261331102*** partial fulfillment for the award of the degree of **MASTER OF PHARMACY** in **PHARMACEUTICAL ANALYSIS** by The Tamil Nadu Dr.M.G.R.Medical University is a work done by him during the academic year 2014-2015 at the Department of Pharmaceutical Analysis, Jaya College of Paramedical sciences, College of Pharmacy, Thiruninravur.

Prof.A.MAHESWARAN ,M.Pharm., PGDBM.,MBA.,(Ph.D.,)
Principal,
Jaya College of Paramedical sciences,
College of Pharmacy,
Thiruninravur-602024

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Prof. K.K.Narashimhulu, M.Pharm.,
Project Advisor
Dept.of Pharmaceutical Chemistry
Jaya College of Paramedical Sciences
College of Pharmacy
Thiruninravur-602024

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Analytical Chemistry is the oldest branch of Chemistry and is the foundation block on which other branches, namely, inorganic, organic, physical and biochemistry have grown to their present level. Understanding of these branches would not have been possible without an understanding and application of principles of analytical chemistry.

It has provided us a glimpse of matter from simple atomic structures to complex molecules to comprehend properties based on structural arrangements.

We have gained insight into the origin and evolution of the universe and life on our own planet through application of analytical techniques.

An understanding of composition has contributed to improvement of material characteristics of natural resources and industrial materials to the benefit of mankind.

Today we cannot think of even a single product of commercial use which has not been tested using analytical chemistry techniques before clearance for consumption

Earliest studies were mainly concerned with understanding the composition of environment and natural resources based on classical methods of analysis.

Classical Analysis also known as wet chemistry introduced quantitative studies and to this day forms the backbone of most university and industrial laboratories.

Earliest techniques were by and large gravimetric in nature with the objective of determination of elemental composition.

Titration methods evolved subsequently for acid-base and metals analysis of solutions.

Analytical Chemistry is poised to make even greater contributions to betterment of life and understanding of new materials.

Pharmaceutical analysis may be defined as a process or sequences of processes to identify and /or quantify a substance or drug, the components of a pharmaceutical solution or mixture or the determination of the structures of chemical compounds used in the formulation of pharmaceutical product. (Willard, *et al*.1986; Douglas, A.Skoog, *et al*, 2004; P.D.Sethi, 2001)

Various analytical techniques:

Electrochemical techniques involve the measurement of such electrical properties as voltage, current, resistance, and quantity of electrical charge. The various electrochemical technique includes

Amperometric technique
Voltammetric techniques
Potentiometric techniques
Stripping techniques
Coulometry
Electrogravimetry
Conductiometric techniques

Separation Techniques Chromatography is the collective term for a set of laboratory techniques for the separation of mixtures. The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase. The various constituents of the mixture travel at different speeds, causing them to separate. The various electrochemical technique includes

Gas chromatography
High performance liquid chromatography
Ion chromatography
Super critical fluid chromatography
Capillary electrophoresis
Planar chromatography
Displacement chromatography

Paper chromatography
Thin layer chromatography
Size exclusion chromatography
Chiral chromagraphy

Spectroscopic techniques are based on the measurement of the interaction between electromagnetic radiation and analyte atoms or molecules, or the production of such radiation by analytes. The most important consequence of such interaction is that energy is absorbed or emitted by the matter in discrete amounts called quanta. The absorption or emissions processed are known throughout the electromagnetic spectrum ranging from the gamma region to the radio wave region. Some of the important spectroscopical technique includes

Infrared spectrometry (dispersive and fourier transform)
Raman spectrometry
Nuclear magnetic resonance
Atomic absorption spectrometry
Inductively coupled plasma MS
Atomic fluorescence spectrometry
Ultraviolet/visible spectrometry
Molecular Fluorescence spectrometry
X-Ray Fluorescence spectrometry

Mass Spectrometry is an analytical chemistry technique that helps identify the amount and type of chemicals present in a sample by measuring the mass-to-charge ratio and abundance of gas phase ions.

Electron ionization MS
Chemical ionization MS
High resolution MS
Gas chromatography MS
Fast atom bombardment MS

Hyphenated techniques

GC – MS

ICP – MS

GC – IR

MS – MS

Microscopic and surface techniques

Atomic force microscopy

Scanning tunneling microscopy

Auger electron spectrometry

X-Ray photon electron spectrometry

Secondary ion MS (Gurdeep chatwal.R)

ANALYTICAL METHODOLOGY

Understanding and defining the problem

↓

History of the sample and background of the problem

↓

Plan of action and execution

↓

Analysis and reporting of results(Sanjay.B, 2007)

HPLC METHODS OF ANALYSIS FOR DRUGS

Most of the drugs in single/multi component dosage forms can be analyzed by HPLC method because of several advantages like rapidity, specificity, accuracy, precision and ease of automation in this method. HPLC method eliminates tedious extraction and isolation procedures. Some of the advantages are:

- ✓ Speed/ rapidity (analysis can be accomplished in 20 minutes or less).
- ✓ Greater sensitivity (various detectors can be employed).
- ✓ Improved resolution (wide variety of stationary phases).
- ✓ Reusable columns (expensive columns but can be used for many analysis).
- ✓ Ideal for substances of low volatility.
- ✓ Easy sample recovery, handling and maintenance.
- ✓ Instrumentation tends itself to automation and quantitation (less time and less labour).
- ✓ Precise and reproducible.
- ✓ Calculations are done by integrator itself.
- ✓ Suitable for preparative liquid chromatography on a much larger scale.

Significance of HPLC:

HPLC play an important and critical role in the field of pharmaceutical industries and analysis, since it is used to test the products and to detect the raw ingredient used to make them i.e., qualitative and quantitative analysis.

High-performance liquid chromatography (HPLC) is a chromatographic technique used to:

Separate and purify a mixture of compounds in the fields of analytical chemistry, biochemistry and industrial chemistry.

The main purposes for using HPLC are for identifying, quantifying and purifying the individual components of the mixture.

Moreover, the importance of HPLC uses in these fields falls under the stringent regulations established by the U.S. Food and Drug Administration (FDA). This obligates all pharmaceutical companies to test the quality of their products by using the HPLC before allowing them to sell it in the global market.

Ultra Performance Liquid Chromatography (UPLC):

A combination of pressurized chromatographic technology and below 2µ (two) micron particle size of stationary phase technology leads to advance Ultra Performance Liquid Chromatography (UPLC) and Rapid Resolution Liquid Chromatography (RRLC) technology. Technology of below 2µ (two) micron particle size leads many modifications in hardware part of the system like reduction of system volume, higher pump pressure capacity, injector and needle part, cell volume of detector as well as in software area and data acquisition rate.

In brief detail, small particle size columns leads to increase in pump pressure and increase surface area; for accurate and precise injection volume needle in needle technology with teflon material has come into the picture. Detector cell volume was reduced for better signals and resolution. Smaller particle size of 2 micron technology altered the machine and its application for faster way of analysis in current scenario of separation science. Requirement of this technology can be explained by van deemter equation and plot as shown. From this plot it reveals that there is minimum HETP against the linear velocity with the almost constant relation or maximum the theoretical plates can be achieved with particle size less than 2 micron.

ANALYTICAL METHOD VALIDATION

Method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use. For pharmaceutical analytical methods guidelines from the United States Pharmacopeia (USP), International Conference on Harmonization (ICH), and Food and Drug Administration (FDA) are adhered to in performing such validation.

Validation (Sethi 2008)

It is a process involving confirmation or establishing by laboratory studies that a method/procedure/system/analyst give accurate and reproducible result for intended analytical application in a proven and established range.

Types of Validation

- Prospective validation
- Retrospective
- Concurrent

Prospective Validation

This is employed when historical data of the product is not available or is not sufficient and in-process and finished product testing are not adequate to ensure reproducibility or high degree of compliance; such validation is conducted prior to release of either new product or product made under revised /new manufacturing process where revision may effect the product characters.

Retrospective Validation

This provides trend of comparative result i.e. review and evaluation of existing information for comparison when historical data is sufficient and readily available. Retrospective validation is acceptable provided specific test results generated by reliable analytical method on number of samples are available to allow statistical analysis. Simply pass / fail test results would not be accepted as part of retrospective validation - useful for trend setting.

Concurrent Validation

Based on information generated during implementation of a system. For this extensive testing and monitoring are performed as a part of initial run of the method. Concurrent validation verifies the quality characteristics of a particular batch and provides assurance that the same quality would be attained again when subsequent batches are manufactured and analyzed under similar conditions.

Purpose/ Reasons for validation

- Enables scientists to communicate scientifically and effectively on technical matters
- Setting standards of evaluation procedures for checking complaints and taking remedial measures.
- The consistency and reliability of validated analytical procedure is to produce a quality with all desired attributes, thus providing indirect cost saving from reduced testing or re testing and elimination of product rejection.
- As quality control process is not static, some form of validation / verification should continue till the validated process is in use.
- Retrospective validation is useful for comparison of results compliance to cGMP/GLP.
- Closer interaction with Pharmacopoeia forum to address analytical problems.
- International Pharmacopoeial harmonization particularly in respect of impurities determination and their limits.
- To provide high degree of confidence that the same level of quality is consistently built into each unit of finished product from batch to batch.

- For taking appropriate action, in case of non-compliance.

- It is a basic requirement for the product quality system.

- It assures that every lot of each product that is released to the market will consistently meet all the quality requirements

- It is capable of achieving the intended results.

Analytical parameters to be validated

- Accuracy
- Precision
- Selectivity (specificity)
- Linearity
- Range
- Sensitivity
- Limit of detection(LOD)
- Limit of quantification (LOQ)
- Ruggedness
- Robustness

Accuracy

It relates to the closeness of test results to true value i.e. measure of exactness of analytical method. It is expressed as % recovery by the assay of known/added amount of analyte in the linearity range. One can design experiments for recovery of known or spiked samples (usually 10% of the claim) in presence of expected matrix, keeping the matrix constant. Accuracy can also be determined by comparing the results with those obtained using an alternative method which has already been validated.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

Accuracy (Trueness)

The accuracy of an analytical procedure expresses the closeness of the test results obtained by that method to the true value (a conventional true value or an accepted reference).Accuracy is calculated as the percentage of recovery by the assay of the known added amount of analyte in the sample, or as the difference between mean and the accepted true value, together with confidence intervals. It is otherwise called trueness.

The ICH documents recommend that accuracy be assessed using a minimum of nine determinations over a minimum of three concentration level covering the specified range (i.e., three concentrations and three replicates of each concentration)

$$\text{It is expressed as \% bias} = \frac{\text{Observed amount} - \text{Original amount}}{\text{Original amount}} \times 100$$

Precision

The precision of an analytical procedure is the degree of agreement among the individual test result when the method is applied repeatedly the multiple samplings of a homogenous sample under the prescribed conditions. Precision of an analytical procedure is usually expressed as the standard deviation or relative standard deviation (Coefficient of variation) of a series of measurements. Precision may be a measure of either the degree of reproducibility or repeatability of the analytical method under operating conditions.

Repeatability

It refers to the use of the analytical procedure within the laboratory over a short period of time using the same analyst with same equipment.

Reproducibility

It refers to the use of analytical procedure in different laboratories as in a collaborative study.

Intermediate precision

It expresses within laboratory variation, on the different days or different analysts or equipment within the same laboratory. ICH documents recommend that repeatability should be assessed using a minimum of nine determinations covering the specified range for the procedure (i.e., three concentrations) or a minimum of six determinations at 100% of the test concentration.)

Detection limit (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value. Based on the standard deviation of the response and the slope, the detection limit of detection (LOD) may be expressed as

$$\mathbf{DL = 3.3\ \sigma / S}$$

Where,

σ is the standard deviation of the response.

S is the slope of the calibration curve (of the analyte).

Quantitation (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. It is a parameter used particularly for the determination of impurities and / or degradation products .The quantization limit expressed as the concentration of analyte (e.g., percentage parts per million) in the sample.

$$\mathbf{LOQ = 10\ \sigma / S}$$

Where,

σ = residual standard deviation of the response;

S = slope of the calibration curve (of the analyte)

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration of analyte in the sample.

Range

The range of an analytical procedure is the interval between the upper and lower concentration (amount) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision and linearity.

Ruggedness

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same sample under variety of conditions, such as different laboratories, analysts, instruments, lots of reagents, elapsed times, assay temperatures or days. Intermediate precision can be considered as ruggedness.

Robustness

The robustness of an analytical is a measure of its capacity to remain unaffected by small deliberate variations in methods parameters and provides an indication of its reliability during normal usage.

Type of validation	**Test for**
Specificity	Interference
Accuracy	Recovery; Linearity
Sensitivity	Limit of detection; Limit of Quantification
Precision	Repeatability ; Reproducibility ; Ruggedness
Personnel	Qualifications; Experience; Responsibility; Proficiency
Equipment	Specifications; Vendor Calibration; maintenance
Service	Sanitation; Water, waste disposal

Statistics in analytical chemistry

Statistics (Skoog, 2004) consist of a set of methods and rules for organizing and interpreting observations. It is the science of making effective use of numerical data relating to groups of individuals or experiments. Modern analytical chemistry is concerned with the detection, identification and measurement of the chemical, composition of unknown substances using existing instrumental techniques .It is a quantitative science, meaning that the desired result is almost always numeric. Quantitative results are obtained using devices or instruments that allow us to determine the concentration of a chemical in a sample from an observable signal. There is always some variation in that signal over time due to noise and/or drift within the instrument. We also need to calibrate the response as a function of analyte concentration in order to obtain meaningful quantitative data. As a result, there is always an error, a deviation from the true value, inherent in that measurement. One of the uses of statistics in analytical chemistry is therefore to provide an estimate of the likely value of that error, in other words, to establish the uncertainty associated with the measurement.

Statistical parameters

The precision or reproducibility of the analytical method is determined by repeating the analysis and various parameters.

Mean:

The mean of the any distribution is a measure of centrality, but in case of the normal distribution, it is equal to the mode and median of the distribution. The mean, or average, is obtained by dividing the sum of observed values by the number of observations, n.

$$\bar{X} = \frac{\sum X}{n}$$

Standard Deviation:

The standard deviation (SD) is a measure of data dispersion or variability. The standard deviation gives an idea of how close the entire set of data is to the average value. Data sets with a small standard deviation have tightly grouped; precise data.SD **is** also called the root mean square deviation as it is the square of the mean of the sum of the squares of the differences between the values and the mean of those values.

$$\mathbf{SD} = \sum(\mathbf{X} - \bar{\mathbf{X}})^{\mathbf{2}} / \sqrt{\mathbf{n} - \mathbf{1}}$$

Relative Standard Deviation:

The relative standard deviation (RSD) is also called coefficient of variation. This is useful when the SD is proportional to the magnitude of the measurement. It is defined as

$$\mathbf{RSD = SD / X}$$

$$\mathbf{\% \ RSD = SD / X \ x 100}$$

Regression equation

A regression is a statistical analysis assessing the association between two variables. It is used to find the relationship between two variables.

Regression equation (y) = mx+c

Where,

m = the slope of the regression line

c = the intercept point of the regression line and the y axis

Standard Error:

The standard error (SE) is

$$\mathbf{SE = SD / \sqrt{n}}$$

An example of the equation for the standard error of the mean reveals that means constructed from very large sample sizes will be very stable, i.e. nonvariable. New drugs and drug combination introduction into market is increasing every year. No proper analytical procedures are available because of the patent regulations. It is therefore necessary to develop newer analytical methods for such drugs.

Confidence Interval/Limits (CI):

The confidence interval for the mean is the range of values within which the population mean is expected to lie with a certain probability. The confidence level is the probability that true mean lies within a certain interval. It is often expressed as a percentage. The confidence limits describe the range within which we expect with given confidence the true value to lie.

$$\mathbf{CI = \bar{X} \pm 1.96\ \sigma / \sqrt{n}}$$

Where,

Σ	=	Sum of observations
$\bar{X}$	=	Mean or arithmetic average ($\Sigma X/n$)
X	=	Individual observed value
$X - \bar{X}$	=	Deviation of a value from the mean
n	=	Number of observations
S.D	=	Standard deviation

New drugs and drug combinations introduced into market is increasing every year. No proper analytical procedures are available because of the patent regulations. It is therefore necessary to develop newer analytical methods for such drugs. In the present investigation, an attempt has been made to develop simple, economical, accurate and reproducible spectrophotometric and UPLC methods for the estimation of fenofibric acid by formulations.

Cinacalcet: Cinacalcet is [(1R)-1-(naphthalen-1-yl)ethyl]({3-[3-(trifluoromethyl)phenyl]propyl}) amine. It is not an official drug in IP, BP and USP.

Empirical formula : $C_{22}H_{23}F_3N$

Molecular weight **:** 357.412 g/mol

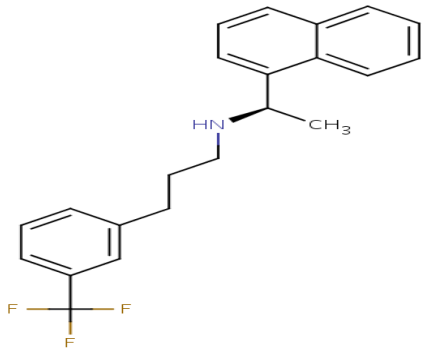

Fig 1: Structure of Cinacalcet

Description **:** Crystalline powder

Physical State : Solid

Solubility **:** Soluble in water (1 mg/ml at 25 °C), DMSO (79 mg/ml at 25 °C), ethanol (33 mg/ml at 25 °C), and methanol..

Storage **:** Store at room temperature

Boiling Point **:** 440.9 °C at 760 mmHg

Melting Point **:** 175-177 °C (dec.)

Mechanism of action:

Cinacalcet is a drug that acts as a calcimimetic (i.e. it mimics the action of calcium on tissues) by allosteric activation of the calcium-sensing receptor that is expressed in various human organ tissues.

- The calcium-sensing receptor on the surface of the chief cell of the parathyroid gland is the principal regulator of parathyroid hormone secretion (PTH).

- Cinacalcet increases the sensitivity of calcium receptors on parathyroid cells to reduce parathyroid hormone (PTH) levels and thus decrease serum calcium levels.

- As receptors are already active from the calcimimetic (Cinacalcet) the native rise and fall of Ca levels now interact with the remaining receptors, effectively lowering the threshold for activation of feedback on the parathyroid chief cells.

Adverse drug reaction:

- Diarrhoea,

- Lack of strength, Loss of appetite,

- Nausea, vomiting,

- Dizziness

- Weakness, stomach upset.

Contraindications:

- Hypocalcemia
- parathesias, myalgias
- muscle cramping, tetany
- convulsions

Drug interactions:

Cinacalcet is a strong CYP2D6 inhibitor and is partially metabolized by CYP3A4 and CYP1A2. Dose adjustments may be necessary if patients are on CYP3A4 and CYP1A2 inhibitors and medications that are metabolized by CYP2D

Brand names:

- Sensipar®, Mimpara®

Dosage and administration:

- Secondary hypertension- 30 mg once daily
- Parathyroid carcinoma and primary hyperparathyroidism-90 mg twice daily
- Hepatic impaired patients: 30 mg once daily
- Maximum dose: 300 mg once daily

For oral use. It is recommended that Cinacalcet be taken with food or shortly after a meal, as studies have shown that bioavailability of cinacalcet is increased when taken with food . Tablets should be taken whole and not divided.

Use:

- In the treatment of secondary hyperparathyroidism due to chronic kidney disease on dialysis.
- hypercalcemia in people with parathyroid carcinoma.
- To treat severe hypercalcemia in patients with primary hyperparathyroidism who are unable to undergo parathyroidectomy.

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An extensive literature review was done in cinacalcet tablet for estimation of related substances. It view of the literature cited for the estimation of cinacalcet tablet related substance

1. **AMRUTA B. LONI *et al.*, 2012** suggested the two simple, precise and economical UV methods have been developed for the estimation of cinacalcet Hcl in bulk and pharmaceutical dosage form. Method A is absorbance maxima method which is based on measurement of absorption at maximum wavelength, 281 nm. Method B is area under curve, in the wavelength range 249 – 299 nm.

2. **Siva Ganesh.N *et al.*, 2015** to develop a simple, rapid, efficient, cost effective and reproducible, stability indicating reverse phase high performance liquid chromatography method (RP-HPLC) for the quantification of cinacalcet in bulk and pharmaceutical dosage form. The RP-HPLC analysis was carried out on Inertsil ODS C18 with a mobile phase of methanol, acetonitrile and water in the ratio of 70:15:15 v/v/v. Detection was carried out at 280 nm using a PDA detector

3. **Manikandan K *et al.*, 2013** reported the stability-indicating liquid chromatography method has been developed and validated for the determination of Cinacalcet hydrochloride in a laboratory mixture as well as in a tablet formulation developed in-house. Efficient chromatographic separation was achieved on phenomenex C18 column (150 mm×4.6 mm, 5.0 µm) with mobile phase containing Methanol: Water (70:30v/v) pH adjusted to 3.6 with dilute Orthophosphoric acid at a flow rate of 1.3 mL/min and the eluent was monitored at 271 nm using Shimadzu LC-10AT-VP & LC-20 AD with Spinotech (Winchrome) software.

4. **I.A. DARWISH, 2013** performed a highly sensitive HPLC method with non-extractive sample preparation and UV detection has been developed and validated for the trace determination of cinacalcet (CIN) in human plasma. Paracetamol (PCM) was used as the internal standard. CIN and PCM were isolated from plasma by protein precipitation with acetonitrile. Chromatographic separation was achieved in isocratic mode on a C18 column (150 mm × 4.6 mm, i.d. 5µm particle size) by a mobile phase consisted of acetonitrile and 50 mM phosphate buffer (50:50 v/v) adjusted to pH of 7.4, at a flow rate of 1.0 mL/min. The eluted compoundswere monitored by UV detector at 235 nm.

5. **Jill S. Lindberg *et al.*, 2005** suggested that Management of secondary hyperparathyroidism is challenging with traditional therapy. The calcimimetic cinacalcet HCl acts on the calcium-sensing receptor to increase its sensitivity to calcium, thereby reducing parathyroid hormone (PTH) secretion. This phase 3, multicenter, randomized, placebo-controlled, double-blind study evaluated the efficacy and safety of cinacalcet in hemodialysis (HD) and peritoneal dialysis (PD) patients with PTH >300 pg/ml despite traditional therapy. A total of 395 patients received once-daily oral cinacalcet (260 HD, 34 PD) or placebo (89 HD, 12 PD) titrated from 30 to 180 mg to achieve a target intact PTH (iPTH) level of <250 pg/ml.

6. **S. HAYASHI1 *et al.* 2013** performed the effect of cinacalcet, a drug that acts as a calcimimetic through the allosteric activation of CaSR, on the loxoprofen-induced small intestinal lesions and investigated the mechanisms involved in the protective action. Male Sprague-Dawley rats were used without fasting

7. **Ibrahim A Darwish *et al,* 2012** reported the first report on the development of a novel spectrophotometric method for determination of cinacalcet hydrochloride (CIN) in its tablet dosage forms. Studies were carried out to investigate the reaction between CIN and 1,2-naphthoquinone-4-sulphonate (NQS) reagent. In alkaline medium (pH 8.5), an orange redcolored product exhibiting maximum absorption peak (lmax) at 490 nm was produced. The stoichiometry and kinetic of the reaction were investigated and the reaction mechanism was postulated. This color-developing reaction was employed in the development of a simple and rapid visible-spectrophotometric method for determination of CIN in its tablets.

8. **Mario Meola1 *et al,* 2009** Cinacalcet, in combination with conventional treatments, led to an improvement in biochemical and clinical parameters of sHPT and reduced glandular volume in patients with severe sHPT. Volume reduction was more evident in smaller glands. Longer term, larger, randomized clinical trials are needed to confirm these preliminary findings and to further define a more systematic approach in the treatment of sHPT.

9. **Amol K Choulwar *et al.*, 2011** suggested the safety and pharmacokinetics parameters of Cinacalcet Hydrochloride Tablets 90mg with the innovator (reference) product, on the basis that if two formulations exhibit similar drug concentration-time profiles in the blood/plasma, they should exhibit similar therapeutic effects. An open label, balanced, analyst blind, randomized, two-treatment, two-period, two sequence, single dose, crossover oral bioequivalence study of Cinacalcet Hydrochloride Tablets 90mg manufactured by Macleods Pharmaceuticals Ltd., India comparing with Sensipar (Cinacalcet Hydrochloride) Tablets 90mg manufactured by Amgen USA Inc. on 24 + 4 (standby) healthy, adult, human subjects under fed conditions

10. **Velasco N *et al.*, 2005 r**epoted that Rapid biochemical and clinical improvement ensued, followed by healing of the skin ulcers.

11. **B.D. Reams *et al.*, 2015** identified 17,791 eligible cinacalcet initiators who contributed 101,147 30-day follow-up intervals. Over half of all patients discontinued cinacalcet by month 4. Proximal PTH levels <150 pg/mL were associated with discontinuation: HR = 1.23 (95% CI: 1.11-1.36), whereas low Ca (<7.5 mg/dL) was suggestive of an association, HR = 1.10 (95% CI 0.92-1.32). Entering the Medicare Part D gap period increased discontinuation risk: HR = 1.19 (95% CI: 1.00-1.42), and low-income subsidy status decreased the risk of discontinuation: HR = 0.77 (95% CI 0.69-0.86). Increasing PTH, HR = 1.15 (95% CI: 1.08-1.23), and Ca levels, HR = 1.23 (95% CI: 1.15-1.31), may be early markers of discontinuation.

12. **Cunningham J *et al.*, 2005** randomization to cinacalcet led to significant reductions in the risk of parathyroidectomy, fracture, and cardiovascular hospitalization, along with improvements in self-reported physical function and diminished pain.

13. **R Garside *et al.*, 2007** reported a Seven trials comparing cinacalcet plusstandard treatment with placebo plus standard treatment were included in the systematic review. A total of 846 people were randomised to receive cinacalcet. Cinacalcet was more effective at meeting parathyroid hormone (PTH) target levels (40% vs 5% in placebo, $p < 0.001$). In those patients meeting PTH targets, 90% also experienced a reduction in calcium–phosphate product levels, compared with 1% in placebo.

14. **Block GA *et al.*, 2004** reported a Forty-three percent of the cinacalcet group reached the primary end point, as compared with 5 percent of the placebo group ($p<0.001$). Overall, mean parathyroid hormone values decreased 43 percent in those receiving cinacalcet but increase 9 percent in the placebo group ($p<0.001$). The serum calcium-phosphorus product declined by 15 percent in the cinacalcet group and remained unchanged in the placebo group ($p<0.001$). Cinacalcet effectively reduced parathyroid hormone levels independently of disease severity or changes in
vitamin D sterol dose.

15. **Kazunori Nakayama *et al.*, 2014** concluded Long-term administration of cinacalcet was associated with reduced progression of abdominal aortic calcification, and achieving appropriate calcium and phosphorus levels may reduce the rates of cardiovascular events and mortality in patients on hemodialysis.

16. **Ravinder *et al.*, 2009** author reported a rapid isocratic chiral LC method for the separation of (S)-cinacalcet from (R)-cinacalcet. Good resolution with RS > 3 was obtained using a Chiralpak- IA column (250 x 4.6 mm, particle size 5 µm) and n-hexane, ethanol and trifluoroacetic acid as the mobile phase (95:5:0.1v/v). This method was further used to determine the presence of (S)- cinacalcet in enantiopure pharmaceutical formulations containing (R)-cinacalcet. The method was validated following ICH guidelines.

17. **Eswara Rao Bammidi *et al.*, 2014** to develop and validate a simple, rapid, sensitive, and precise, degradation studies for Cinacalcet Hcl drug by RP-HPLC method as per ICH guidelines. The HPLC analysis used a reversed phase Agilent Zorbax C_{18} (250X4.6,5µm) column, a mobile phase constituted of buffer solution and methanol (30:70). The buffer was composed of 1ml orftho phosphoric acid in 1000 ml of water and adjusts P^H 2.1 with ortho phosphoric acid. Column temperature is 30^oc. This method in wavelength is detecting used for PDA detector and 10ml was injected. The retention time for cinacalet was 3.7min.

18. **Yueh-Ting Lee *et al.*, 2013** conclude that combination therapy of low-dose cinacalcet and calcitriol is more effective than calcitriol alone as a treatment for moderate and severe UHPT in chronic dialysis patients. Furthermore, this therapy is associated with improvement in hyperphosphatemia and hypercalcemia.

19. **Masafumi Fukagawa *et al.*, 2013** suggest that lower dose levels of cinacalcet, ascompared to those in US/EU studies, may be sufficient effectively suppress the serum iPTH levels and allow favourable management of the serum calcium and phosphorus levels in Japanese patients, having a longer average dialysis vintage.

20. **Iannazzo s *et al.*, 2010** concluded that Cinacalcet treatment could be considered a cost-effective treatment of SHPT in all reported countries. Results appear more homogeneous in the three southern countries. In Switzerland the cost of dialysis is very high (more than double than other countries).

21. **Gillespie Iain A *et al.*, 2015** observed results in the "real life" setting of the AROii observational cohort that closely mirrored the results of the EVOLVE RCT. Persistence-corrected analyses revealed a trend towards reduced all-cause mortality in haemodialysis patients receiving cinacalcet therapy.

22. **Waldemar Misiorowski *et al.*, 2007** proved that Cinacalcet appears to have been highly effective at controlling hypercalcemia in patients with parathyroid carcinoma.

23. **Pablo Ure˜na *et al.*, 2009** concluded This analysis of current European clinical practice shows that—consistent with findings from randomized controlled trials and retrospective observational studies—cinacalcet improves attainment of KDOQITM bone metabolism targets in dialysis patients with various stages of SHPT.

24. **Rita Guerra *et al.*, 2011** Calcimimetic agents represent a therapeutic alternative in transplant patients with persistent hyperparathyroidism, as they correct hypercalcemia and reduce PTH levels with no adverse effects on kidney function. Prospective, controlled studies should be designed to evaluate the long-term effects and evolution after suspension of the treatment

The aim of the study is to develop simple, novel methods for the Determination of related substances of Cinacalet in bulk and pharmaceutical dosage forms. The estimation of Cinacalcet impurities in degraded product have been reported and review of literature indicated that no validated analytical method have been reported for pharmaceutical formulation till date.

The objective of the present work is to develop analytical methods for the estimation of related substances in Cinacalcet tablets which comprises of the following.

- ✓ **Reverse Phase -Ultra Performance Liquid Chromatographic Method (RP-UPLC)**

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-UPLC METHOD FOR DETERMINATION OF RELATED SUBSTANCES OF CINACALCET TABLETS

UPLC refers to Ultra Performance Liquid Chromatography. It is improved in three areas: namely chromatographic resolution, speed and sensitivity of analysis. It uses fine particles and saves time and reduces solvent consumption. UPLC is derived from HPLC. An underlying principle of HPLC dictates that as column packing particle size decreases, efficiency and thus resolution also increases. As particle size decreases to less than 2.5µm, there is a significant gain in efficiency and it's doesn't diminish at increased linear velocities or flow rates according to the common Van Deemter equation. By using smaller particles, speed and peak capacity (number of peaks resolved per unit time) can be extended to new limits which is known as Ultra Performance. The classic separation method HPLC (High Performance Liquid Chromatography) has many advantages like robustness, ease of use, good selectivity and adjustable sensitivity. Its main limitation is the lack of efficiency compared to gas chromatography or the capillary electrophoresis due to low diffusion coefficients in liquid phase, involving slow diffusion of analyte in the stationary phase. The Van Deemter equation shows that efficiency increases with the use of smaller size particles but this leads to a rapid increase in back pressure, while most of the HPLC system can operate only up to 400 bar. That is why short columns filled with particles of about 2µm are used with these systems, to accelerate the analysis without loss of efficiency, while maintaining an acceptable loss of load to improve the efficiency of HPLC separations, the following can be done. (Madhava Prathap G and Amreen Nishat, (2013))

A. **Work at higher temperatures**- allows high flow rates by reducing the viscosity of mobile phase which significantly reduces back pressure.

B. **Use of monolithic columns**- contains polymerized porous support structure that provides lower flow resistances than conventional particle-packed columns.

Advantages of UPLC

- ✓ Decreases run time and increases sensitivity.
- ✓ Provides the selectivity, sensitivity, and dynamic range of LC analysis
- ✓ Maintaining resolution performance.
- ✓ Expands scope of Multi residue Methods
- ✓ UPLC's fast resolving power quickly quantifies related and unrelated compounds
- ✓ Faster analysis through the use of a novel separation material of very fine particle size
- ✓ Operation cost is reduced
- ✓ Less solvent consumption
- ✓ Reduces process cycle times, so that more product can be produced with existing resources
- ✓ Delivers real-time analysis in step with manufacturing processes

Disadvantages of UPLC

- ✓ Due to increased pressure requires more maintenance and reduces the life of the columns of this type. So far performance similar or even higher has been demonstrated by using stationary phases of size around 2 μm without the adverse effects of high pressure.

- ✓ In addition, the phases of less than 2 μm are generally non-regenerable and thus have limited use.

System suitability (IP, 1996):

System suitability test (SST) is commonly used to verify resolution, column efficiency and repeatability of a chromatographic system to ensure its adequacy far a particular analysis. According to USP and ICH, SST is an integral part of many analytical procedures. SST is based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as a whole.Once a method or system has been validated the task becomes one of routinely checking the suitability of the system to perform within the validated limits.

The simplest form of an HPLC system suitability test involves a comparison of the chromatogram trace with a standard trace (as shown below). This allows a comparison of the peak shape, peak width, and baseline resolution.

Alternatively these parameters can be calculated experimentally to provide a quantitative system suitability test report:

- ✓ Number of theoretical plates (efficiency)
- ✓ Capacity factor,
- ✓ Separation (relative retention)
- ✓ Resolution,
- ✓ Tailing factor
- ✓ Relative Standard Deviation (Precision)

These are measured on a peak or peaks of known retention time and peak width.

a) **Retention time (R_t)** is the time of emergence of the maximum of a component after injection.

b) **Symmetry factor or tailing factor (T)** is a measure of peak symmetry; it is unity for perfectly symmetrical peaks. Its value increases as tailing become more pronounced. As peak asymmetry increases, integration and hence precision, become less reliable.

$$T = \frac{W_{0.05}}{2f}$$

The assessment of peak shape is in terms of symmetry factor.

Where, $W_{0.05}$ = width of the peak at 5% height

f = distance from the peak maximum to the leading edge of the peak, when measured at 5% peak height (from the base line)

Number of theoretical plates (N) is measure of column efficiency. If the number of theoretical plate is high, the column is said to be highly efficient and vice versa. It is a measure of sharpness, which is important for the detection of trace elements.

$$N = 5.54 \left\{ \frac{t}{W_{1/2}} \right\}^2$$

The assessment of performance of efficient of a column is in terms of the number of theoretical plates.

a) Resolution is a measure of relative separation of two peaks. Resolution is defined as the distance between the two bands centers divided by the average width of the peaks determined at the bases of peaks.

$$R = \frac{2\,(t_2 - t_1)}{W_2 + W_1}$$

Where, t_2 and t_1 are retention times of first and second component and

W_2, W_1 are width of peak of first and second component eluted.

REVERSE PHASE- ULTRA PERFORMANCE LIQUIDCHROMATOGRAPHY

Instrument

Ultra performance chromatography

1. UPLC system (Water Aquity H class, USA)
2. PDA – detector (2996)
3. UV - detector
4. Auto sampler
5. Isocratic
6. Rheodyne valve injector with 10 µL fixed loop
7. Chromatographic column- CORTECS ^{TM} UPLC C18 (1.6 µ): 100 mm x 2.1 mm, Part No.186007116

Preparation of mobile phase

Preparation of Mobile Phase A

Mix 1 ml of ortho phosphoric acid with 1000mL of water. Filter through 0.2µ membrane filter and degas.

Preparation of Mobile Phase B

Mix methanol and acetonitrile in the ratio of 60:40 (%v/v). Filter through 0.2µ membrane filter and degas.

Chemical details:

CH0	:	3-(Trifluoromethyl) cinnamaldehyde
CH1	:	R(+)-Naphthylethylamine
CH2	:	(1-naphthalen-1-yl-ethyl)-[3-(3-trifluoromethylphenyl)-allyl]-amine hydrochloride (Olefin amine hydrochloride)
CH3	:	(1-Naphthalen-1-yl-ethyl)-bis-[3-(3-trifluoromethyl-phenyl)-propyl]-amine (Dimer Impurity)
CH4	:	3-(3-Trifluoromethyl-phenyl)-propylamine oxalate (Propylamine Impurity)
CH5	:	[3-(3-Difluoromethyl-phenyl)-propyl]-(1-naphthalen-1-yl-ethyl)-amine hydrochloride (Mono-Desfluoromethyl cinacalcet hydrochloride)
CH6	:	(1-Naphthalen-1-yl-ethyl)-(3-m-tolyl-propyl)-amine hydrochloride (3-Methyl Cinacalcet Hydrochloride)
CH7	:	N-[1-(R)-(1-naphthyl)ethyl]-3-(phenyl)-1-aminopropane hydrochloride (Des-trifluoromethyl cinacalcet hydrochloride)
Cinacalcet Standard		(1R)-1-(naphthalen-1-yl)ethyl]({3-[3 (trifluoromethyl)phenyl]propyl})amine

Preparation of 0.5N Hydrochloric acid

Dilute 42.5 mL of hydrochloric acid in to 1000mL with water.

Preparation of diluent

Mix methanol and 0.5N hydrochloric acid in the ratio of 50:50 (%v/v). Filter through 0.2µ membrane filter and degas.

Optimized Chromatographic Condition

Following parameters were used for RP-UPLC analysis of CINACALCET TABLETS

Pump Mode : Gradient

Stationary phase : CORTECS ^{TM} UPLC C18 (1.6 µ): 100 mm x 2.1 mm, Part No.186007116

Gradient program :

Time in minutes	Pump A (%)	Pump B (%)
0	75	25
9	50	50
15	40	60
20	40	60
22	75	25
27	75	25

Detection wavelength : 210 nm

Flow rate : 0.4 mL/min

Temperature : 35 ° C

Sample volume : 1 µl

Quantification method: External standard method

RELATED SUBSTANCES PROCEDURE

Preparation of Blank: Diluent

Preparation of Standard Solution:

Weight accurately 22mg of cinacalcet hydrochloride working standard and transfer into a 100ml volumetric flask, add about 70mL of diluent and sonicate to dissolve. Dilute to the volume with diluent and mix well. Dilute 2mL of this solution to 100mL with diluent (4ppm of cinacalcet)

Preparation of placebo Solution:

Weigh accurately and transfer placebo equivalent to 200mg of cinacalcet (subtract the 200mg from obtained equivalent weight) into a 100ml volumetric flask, add about 70mL of diluent and sonicate for 30minutes with intermittent shaking . Cool the solution to room temperature, dilute to the volume with diluent and mix well.

Preparation of Unspiked sample Solution:

Weigh not less than 20tablets and calculate the average weight. Crush the tablets in to fine powder. Accurately weigh the powdered sample equivalent to 200mg of cinacalcet and transfer into a 100ml volumetric flask, add about 70mL of diluent and sonicate for 30minutes with intermittent shaking . Cool the solution to room temperature, dilute to the volume with diluent and mix well. Filter through 0.45µ filter.

Preparation of Spiked sample Solution:

Weigh not less than 20tablets and calculate the average weight. Crush the tablets in to fine powder. Accurately weigh the powdered sample equivalent to 200mg of Cinacalcet and transfer into a 100ml volumetric flask, spiked known impurity at specification level and add about 70mL of diluent and sonicate for 30minutes with intermittent shaking . Cool the solution to room temperature, dilute to the volume with diluent and mix well. Filter through 0.45µ filter.

Preparation of Individual known impurities:

Prepare degradable impurities CH1, CH4, CH7 and process related impurities at specification level and inject into chromatograph and recorded chromatogram.

SYSTEM SUITABILITY

The system is suitable for analysis if and if only

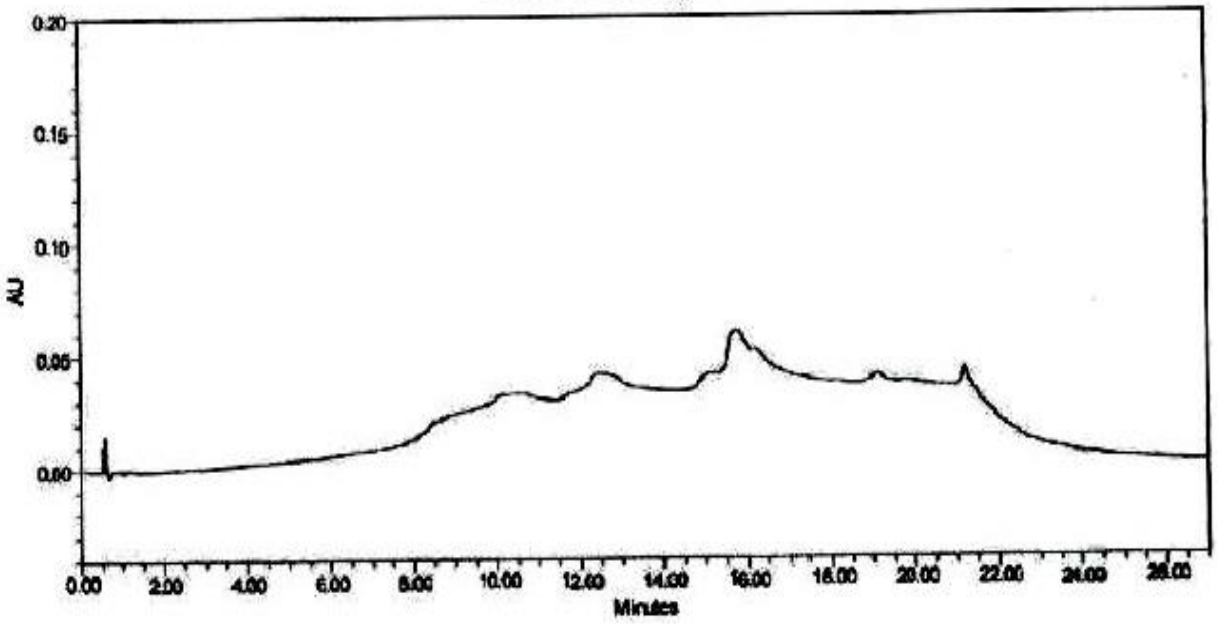
1.Tailing factor for Cinacalcet peak in standard solution is not more than 2.0

2.The number of the theorectical plates for Cinacalcet peak in standard solution is not less than 3000

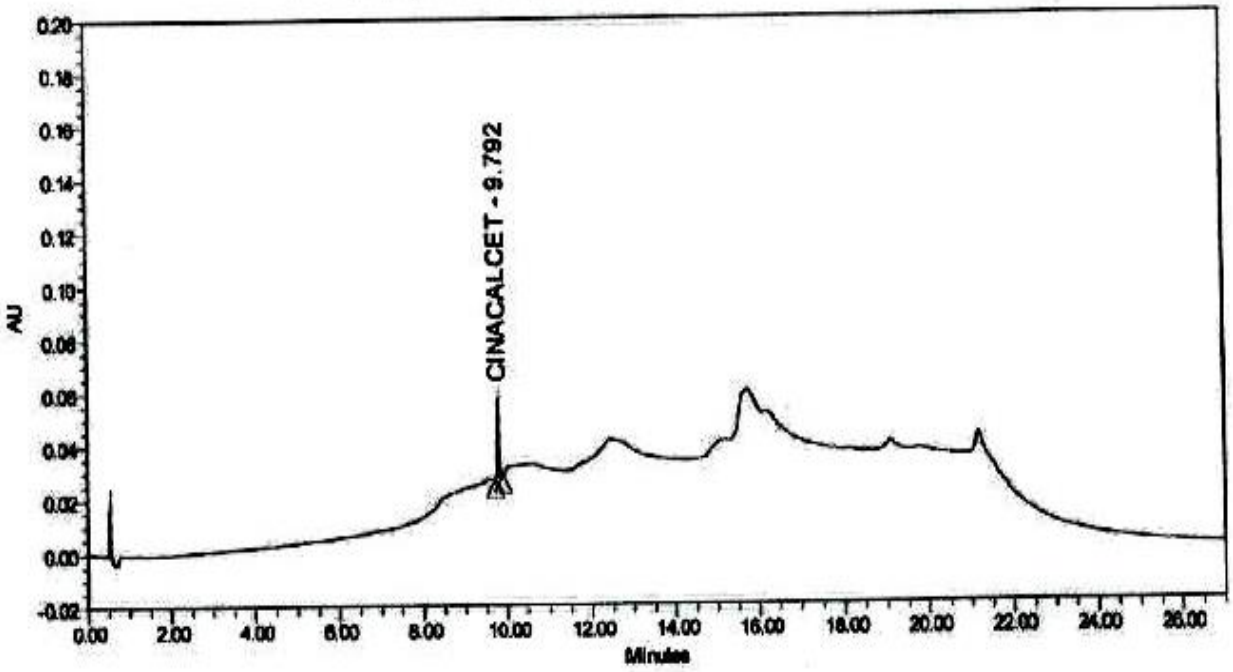
3.The percentage relative standard deviation for Cincalcet peak obtained from six replicate injection of standard solution is not more than 5.0

TYPICAL CHROMATOGRAMS

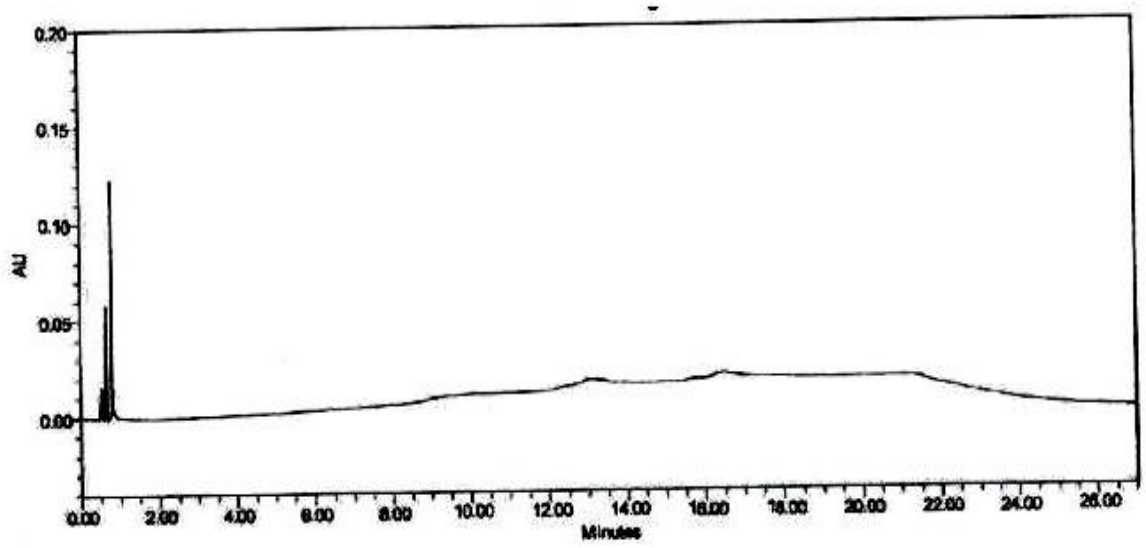
Chromatogram of blank



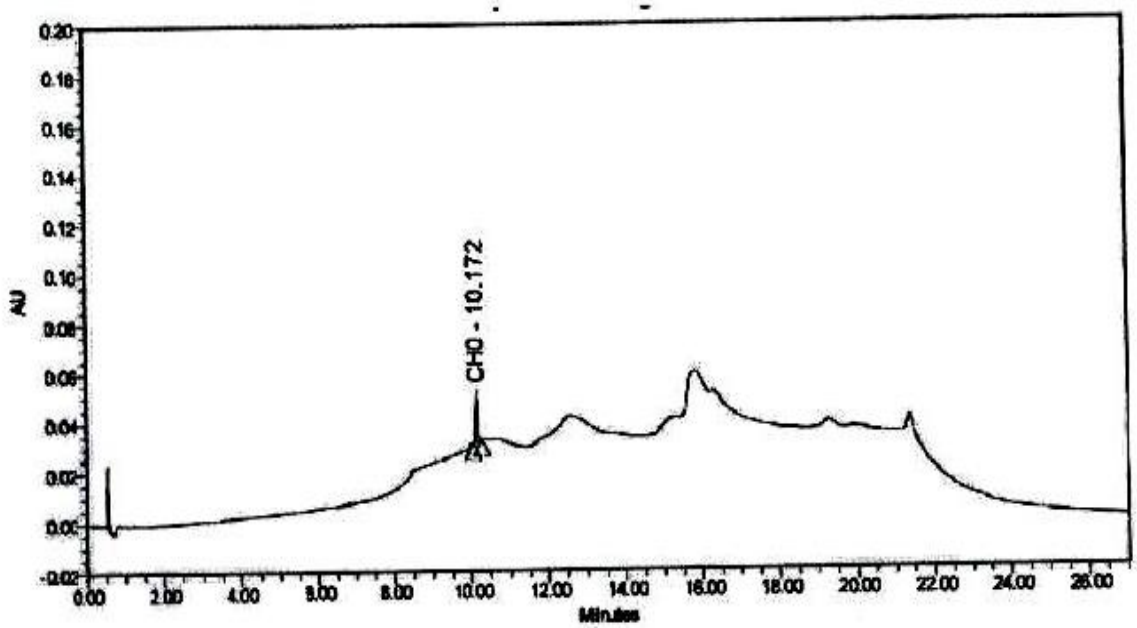
Chromatogram of Standard



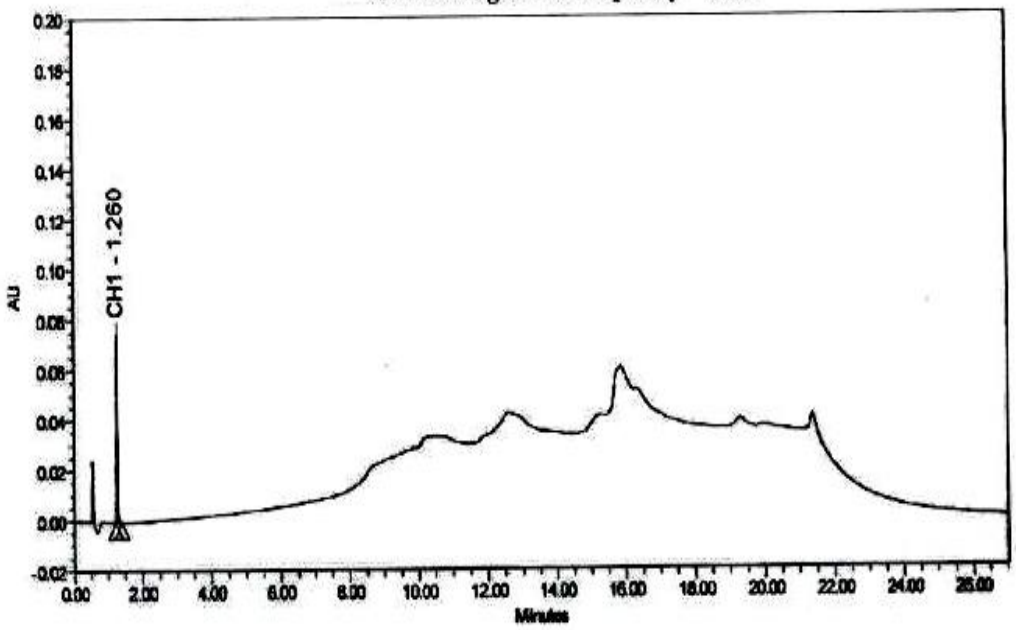
Chromatogram of Placebo



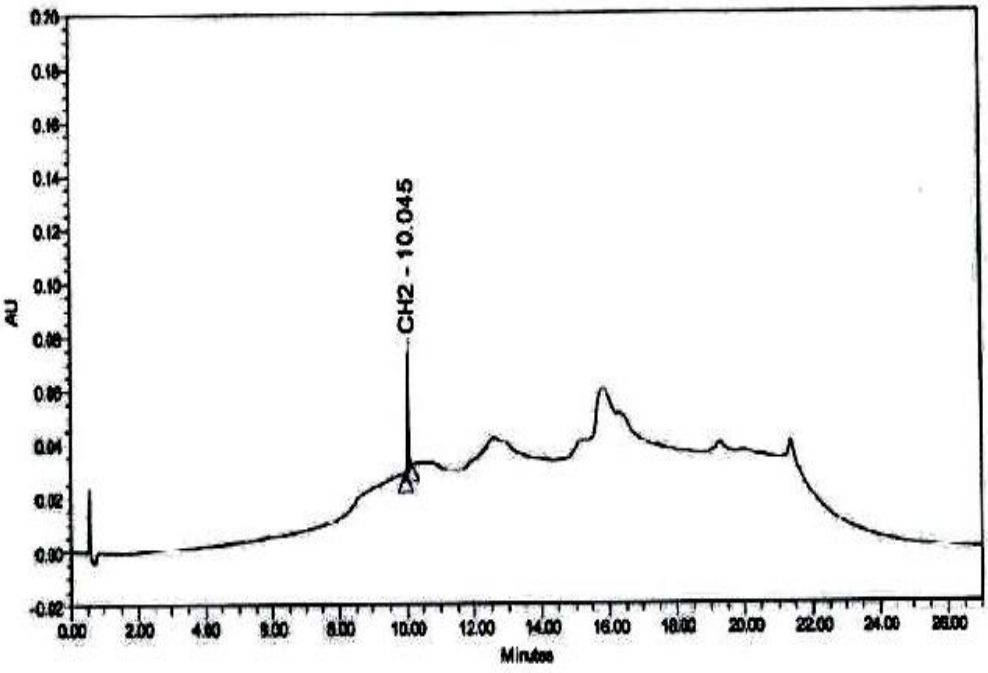
Chromatogram of Impurity-CH0



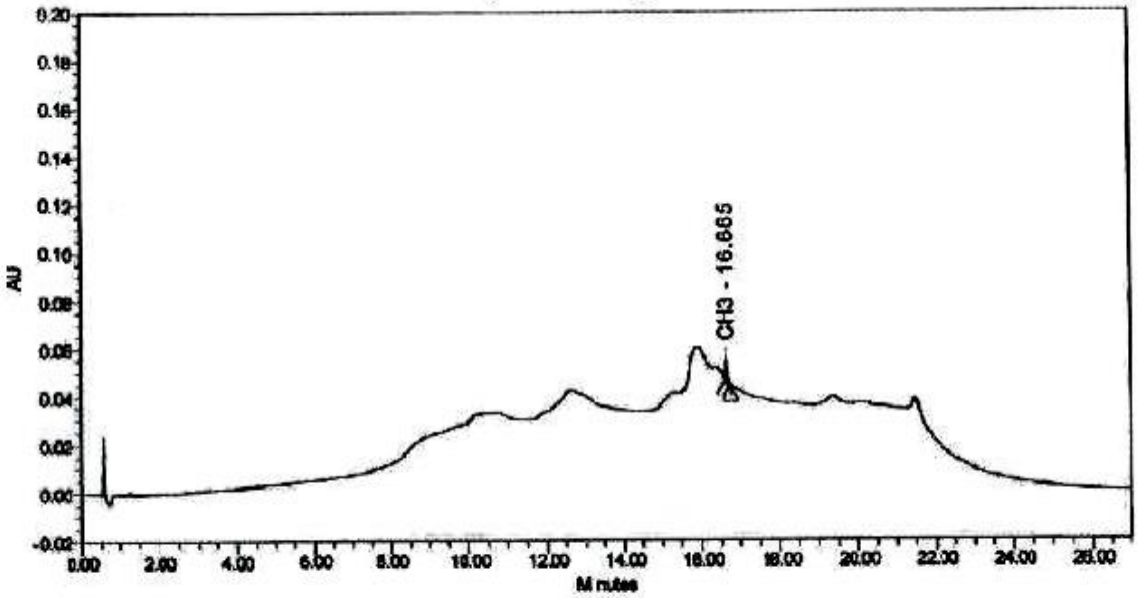
Chromatogram of Impurity – CH1



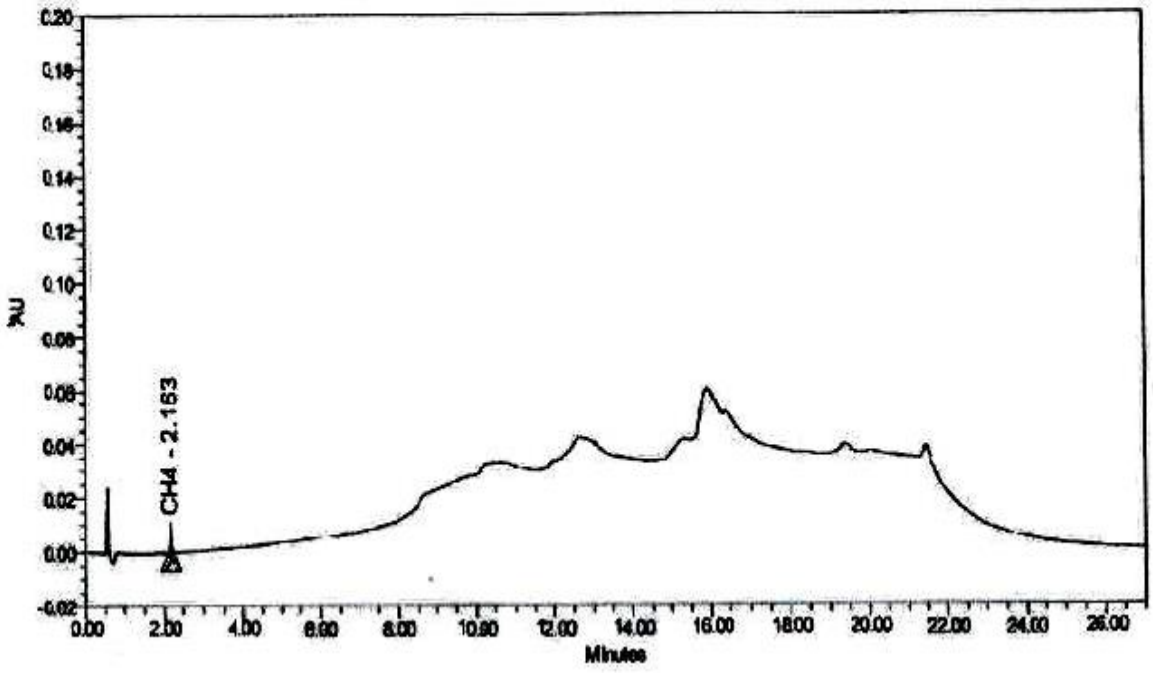
Chromatogram of Impurity – CH2



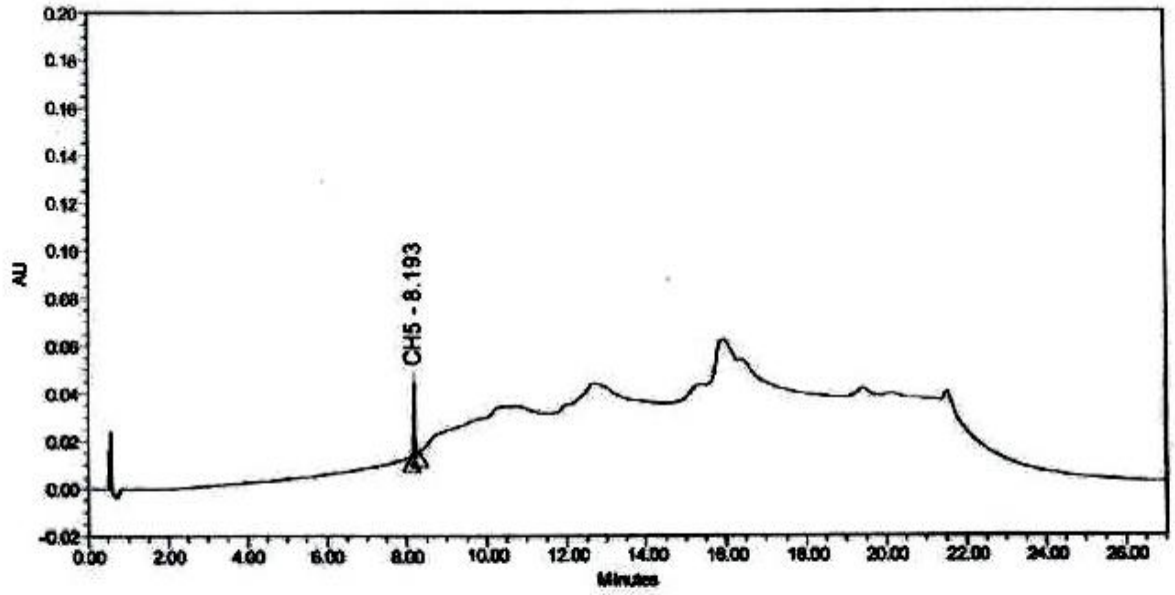
Chromatogram of Impurity – CH3



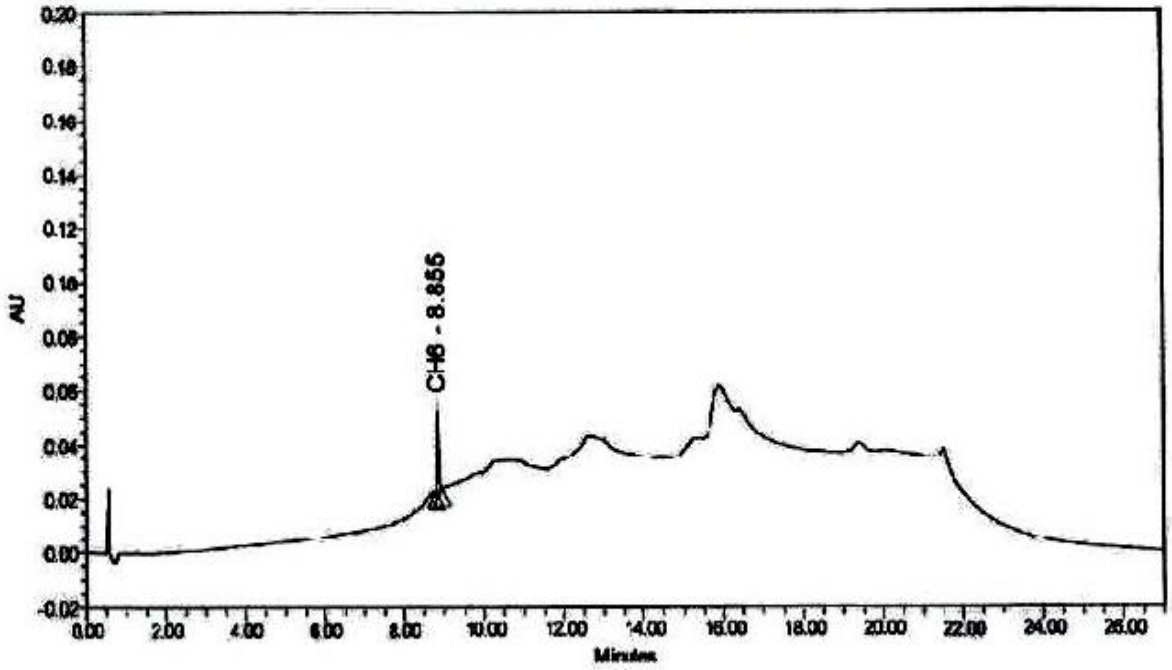
Chromatogram of Impurity – CH4



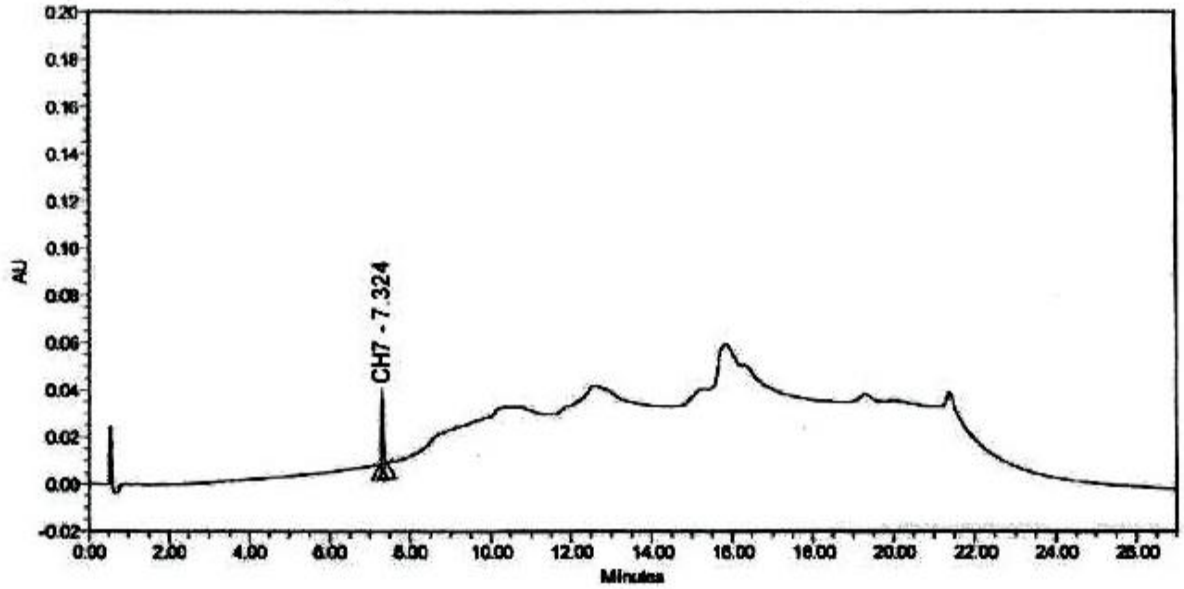
Chromatogram of Impurity – CH5



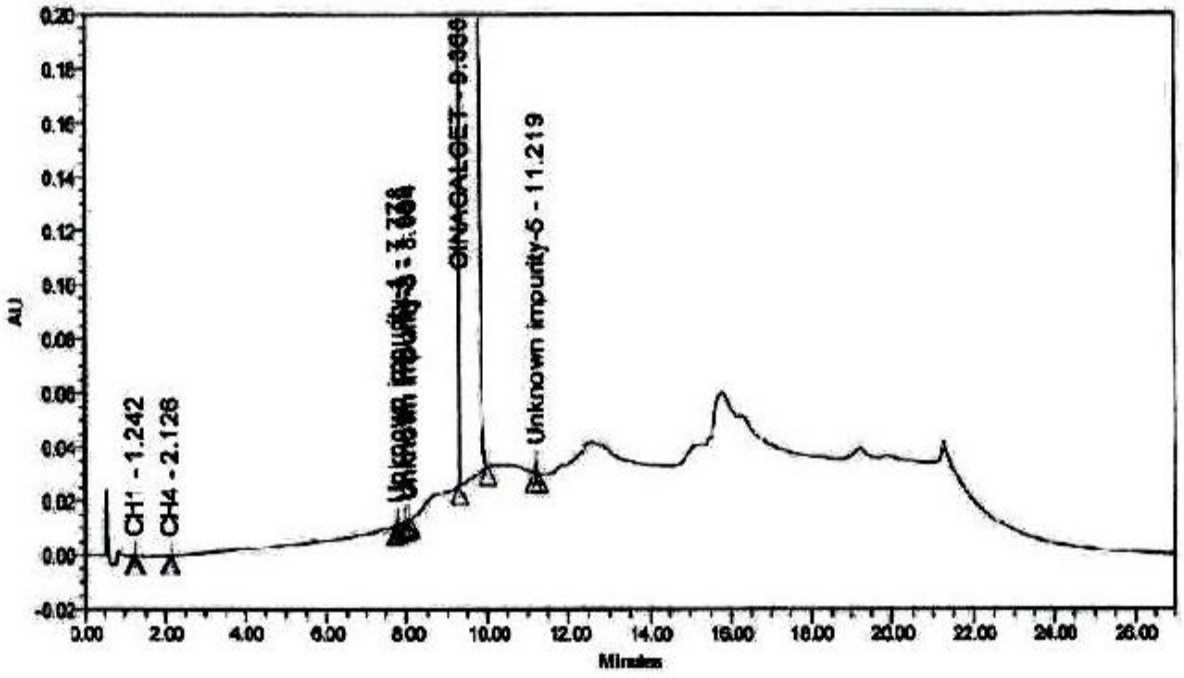
Chromatogram of Impurity-CH6



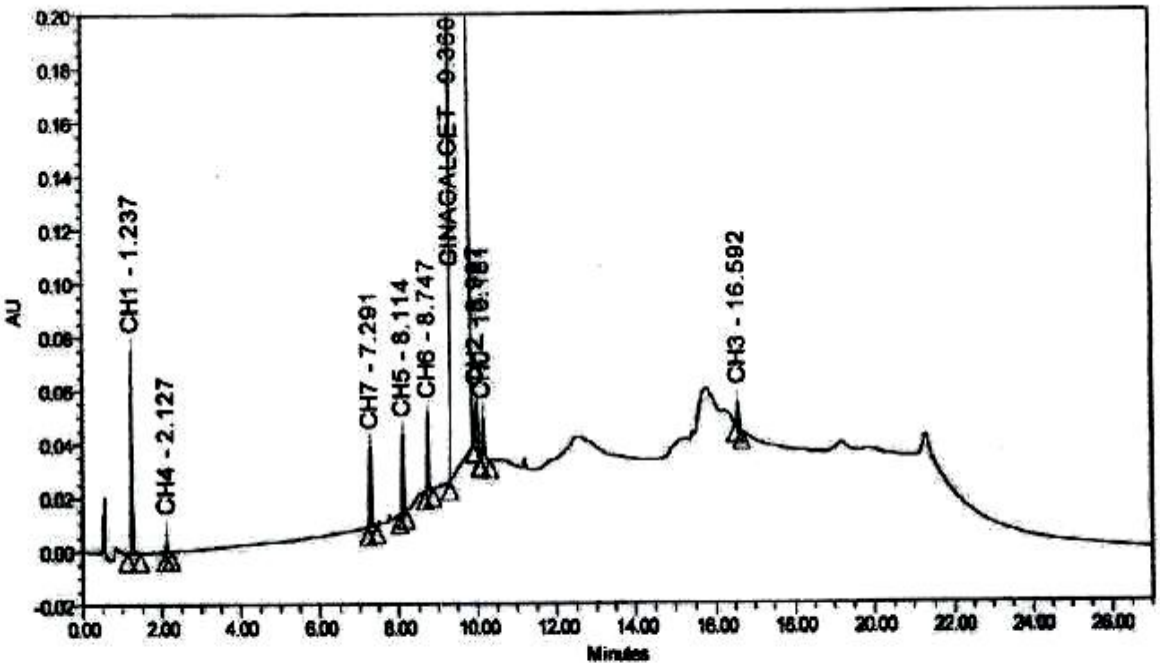
Chromatogram of Impurity-CH7



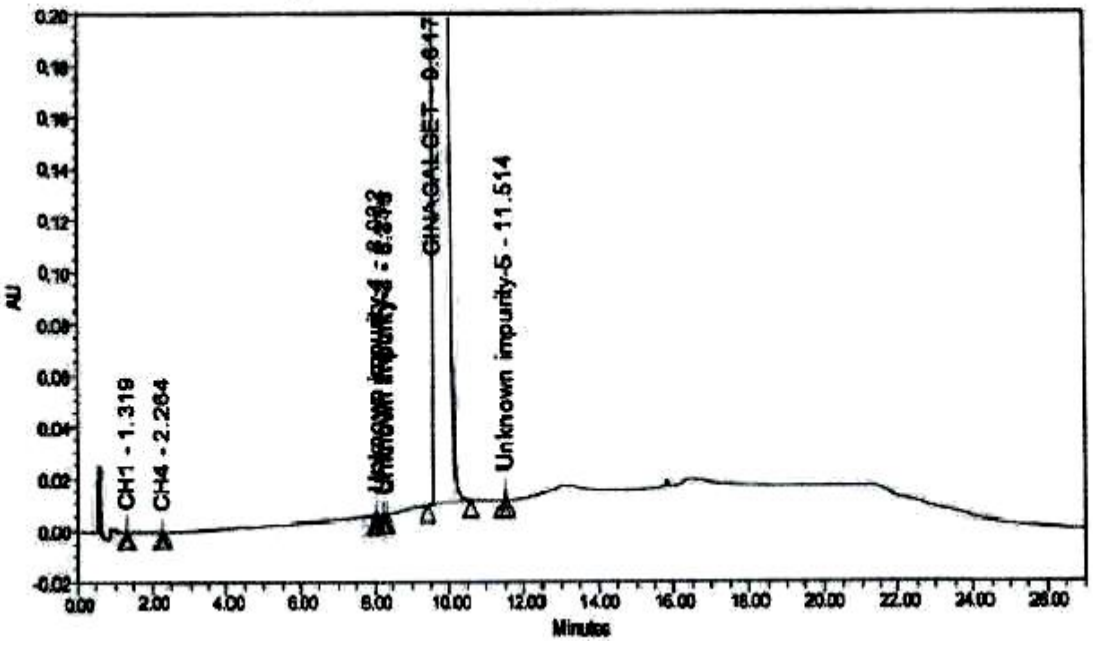
Chromatogram of unspiked sample



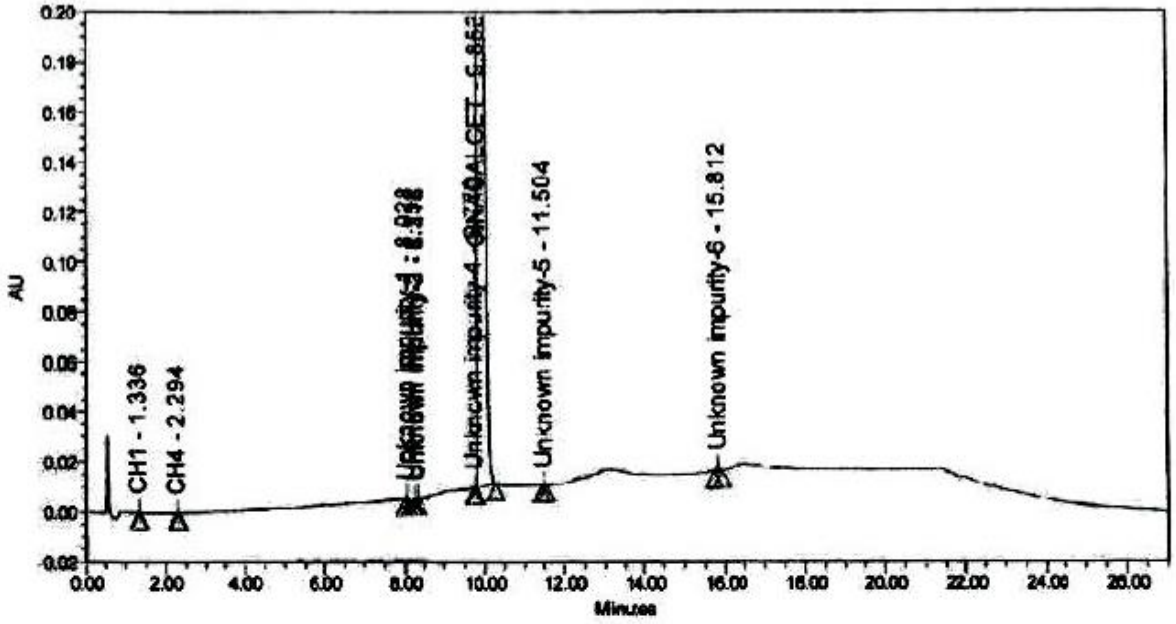
Chromatogram of impurities spiked sample



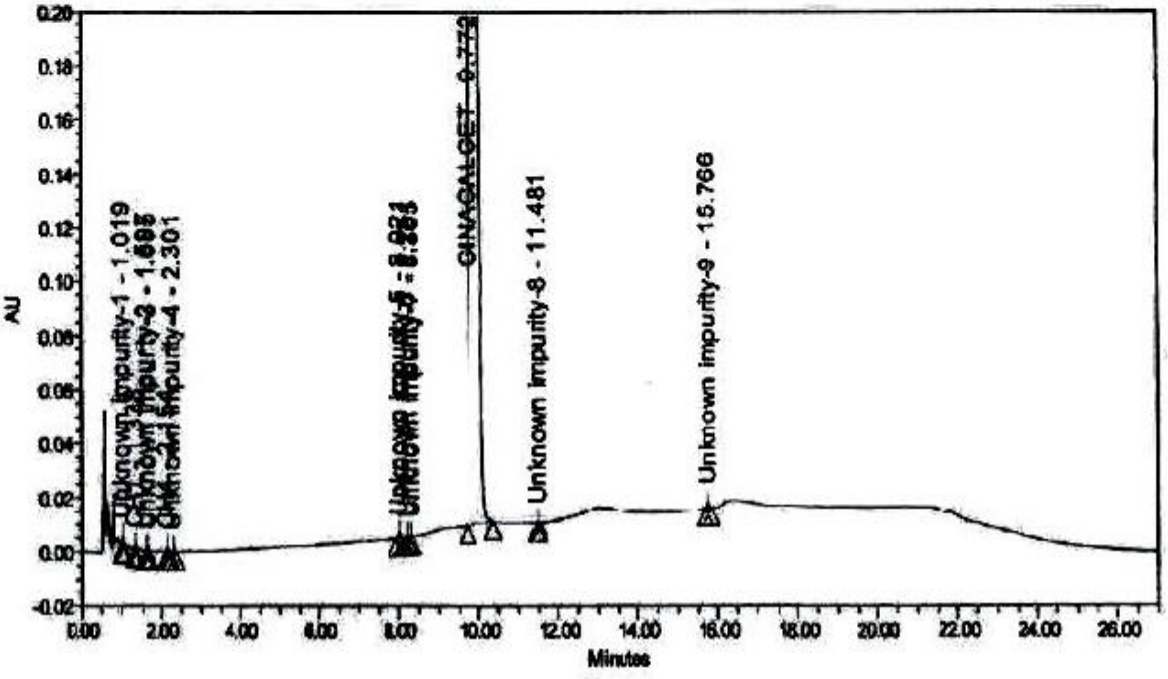
Chromatogram of unstressed sample



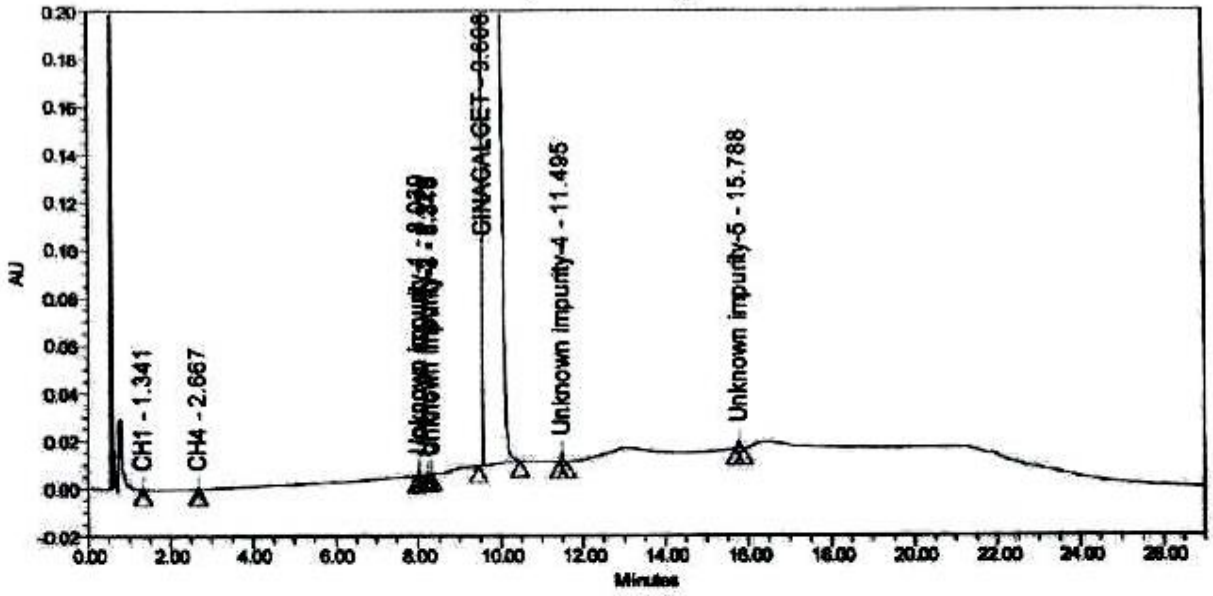
Chromatogram of acid stressed sample



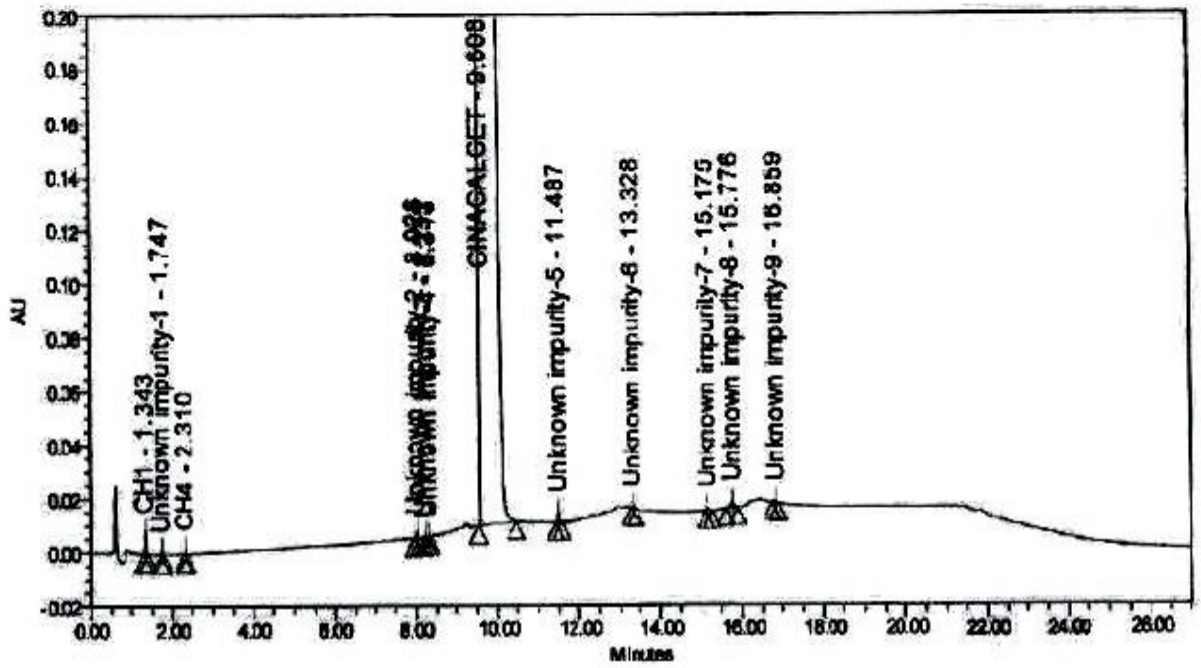
Chromatogram of alkali stressed sample



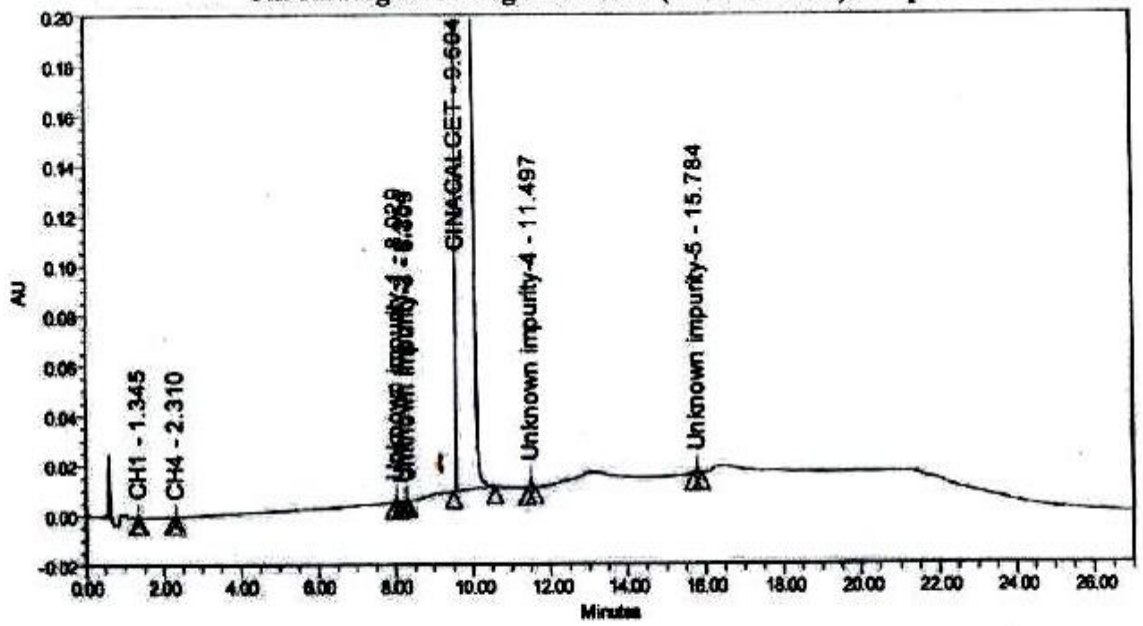
Chromatogram of peroxide stressed sample



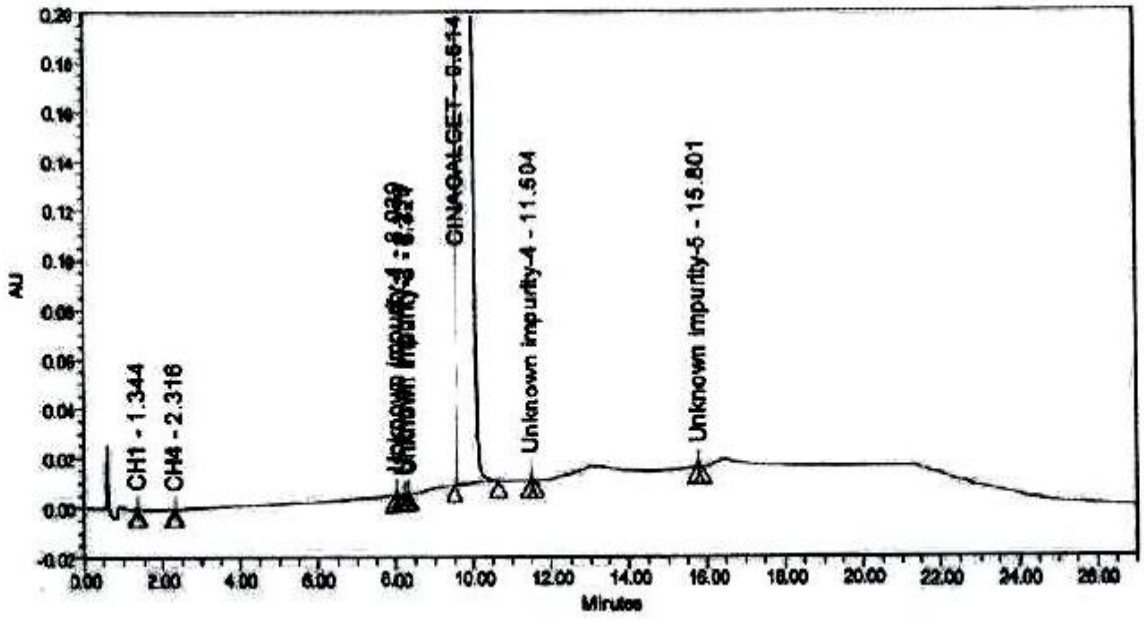
Chromatogram of thermal stressed sample



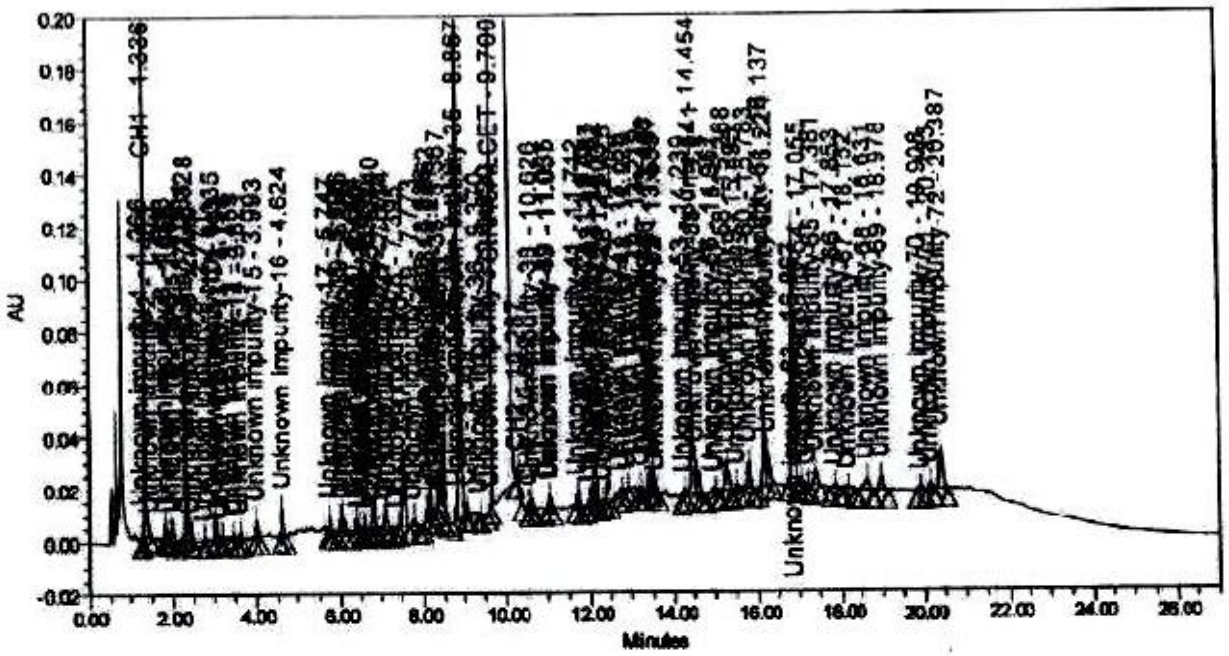
Chromatogram of light stressed (Uncontrolled) sample



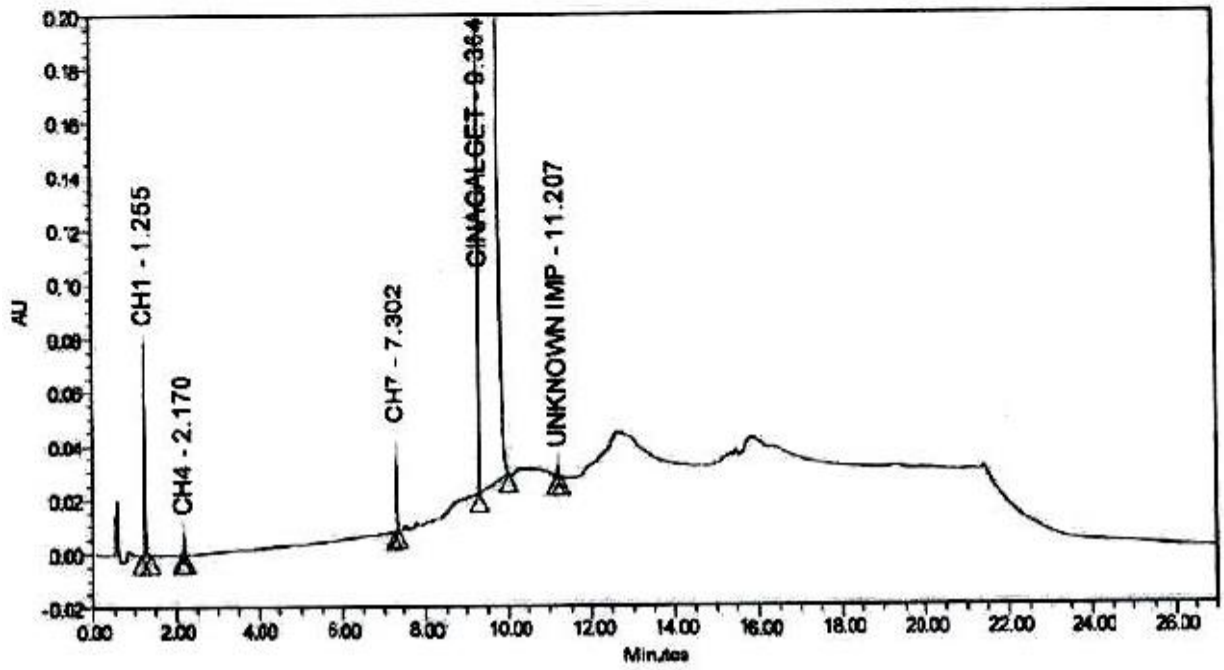
Chromatogram of light stressed (Controlled) sample



Chromatogram of $KMnO_4$ stressed sample



Chromatogram of Robustness Sample at Low wavelength (208 nm)



RP-ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY

An effort has been made to develop simple, precise, cost-effective and accurate method for the estimation of Cinacalcet related substances in pharmaceutical dosage form. A solution of 4 µg/mL of Cinacalcet and its impurities in diluent was scanned in the UV region to determine the detector wavelength. It was observed that Cinacalcet showed λ max at 210nm. Hence this was selected as the detecting wavelength for the estimation of cinacalcet and its impurities by UPLC.

Optimization of the chromatogram

The spiked solution of related compounds (CH1, CH4, CH7) and Cinacalcet were subjected to separation by RP-UPLC. The mobile phase was containing a isocratic mixture Methanol: Water (70:30%v/v) pH adjusted to 3.6 with dilute Orthophosphoric acid (Manikandan Krishnan) . The separation of all compounds form Cinacalcet was studied using this composition on UPLC column (CORTECS™ C18 50 x 2.1 mm, 1.7µm) and Waters (UPLC) system with the linear gradient program. The flow rate of 0.6 mL/min was selected with regards to the backpressure and analysis time as well. When studied was performed with above condition we observed that Impurity CH1 and Impurity CH4 was co-eluting. Impurity CH7 is well separated from Cinacalcet main peak and cinacalcet peak was relatively retained for longer time. Based on this experiment Impurity CH1 and Impurity CH4 was selected as a critical pair for separation. During this study column oven temperature was capped 40°C. Various types of solvents A and B were studied to optimize the method, which were summarized in below table with the observation. Based on above solvent selection experimental study optimized UPLC parameters were; flow rate 0.4mL/min; column oven temperature 35°C; gradient solvent program as per methodology(CORTECS™ C18 100 x 2.1 mm, 1.6µm); as a Solvent-A 0.1 % orthophosphoric acid and as Solvent-B mixture of acetonitrile and methanol in the ratio of 40:60 (v/v) respectively.

Summary of solvent used to optimize the method.

Solvent-A (Sol-A)	Solvent-B (Sol-B)	Observation/Remarks
0.1 % aqueous orthophoshoric acid	Mixture of methanol and water in the ratio of 70:30 (v/v)	Co-eluting peak of Impurity CH1 and Impurity CH4 was observed
0.1 % aqueous orthophoshoric acid	Mixture of methanol and water in the ratio of 50:50 (v/v)	Well separated peak of Impurity CH1 and Impurity CH4 was observed, peak split for Impurity CH7 and Cinacalcet
0.1 % aqueous orthophoshoric acid	Mixture of acetonitrile and methanol in the ratio of 90:10 (v/v)	Poor resolution between Impurity CH1 and Impurity CH4, and higher peak tailing for the peak of Cinacalcet main peak were observed.
1 % aqueous orthophoshoric acid	Mixture of acetonitrile and methanol in the ratio of 40:60 (v/v)	3.0 USP resolution was observed between Impurity CH1 and Impurity CH4.

This study covers the following parameters

1. Precision
    - 1a. System precision
    - 1b. Method precision
    - 1c. Intermediate precision (Ruggedness)
2. Specificity
    - 2a.Blank and placebo interference
    - 2b. Forced degradation
3. Limit of detection and limit of quantitation (LOD and LOQ)
4. Linearity
    - 4.1. Linearity for Cinacalcet and its impurities
5. Accuracy (Recovery)
6. Range
7. Solution stability
8. Filter interference study
9. Robustness.

PRECISION

SYSTEM PRECISION

Six replicate injections of standard solution were injected. The mean and percentage relative standard deviation (% RSD) for peak areas of Cinacalcet were calculated. The results are tabulated in table - 1.

Acceptance criteria

Percentage relative standard deviation (% RSD) is not more than 10.0

SYSTEM PRECISION

TABLE - 1

Injection No.	Peak area
1	65926
2	65610
3	65852
4	65910
5	65153
6	65438
Average	65648
% RSD	0.5

Conclusion

Percentage relative standard deviation (% RSD) value indicates an acceptable level of precision of the analytical system for the determination of related substances of Cinacalcet tablets 90mg.

METHOD PRECISION

Prepared six samples solution of 90mg tablets spiked with known impurities at specification and analyzed as per testing procedure. The percentage relative standard deviation (% RSD) for percentage of known impurities and total impurities were calculated and the results are tabulated in table - 2.

Acceptance criteria

Percentage relative standard deviation (% RSD) is not more than 10.0

METHOD PRECISION

TABLE – 2

Sample No.	Percentage of impurities (w/w)				
	CH 1	CH 4	CH 7	Highest Unknown impurity	Total Impurities
1	0.1908	0.2205	0.2120	0.0251	0.6484
2	0.1909	0.2220	0.2126	0.0245	0.6500
3	0.1908	0.2271	0.2115	0.0248	0.6542
4	0.1908	0.2257	0.2107	0.0246	0.6518
5	0.1888	0.2215	0.2124	0.0251	0.6478
6	0.1884	0.2198	0.2135	0.0251	0.6468
Avg	0.1901	0.2228	0.2121	0.0249	0.6498
% RSD	0.6	1.3	0.5	1.1	0.4

Conclusion

Percentage relative standard deviation (% RSD) value indicates an acceptable level of precision of the analytical method for the determination of related substances of Cinacalcet tablets 90mg.

INTERMEDIATE PRECISION (RUGGEDNESS)

Ruggedness of the method was verified by analyzing the six samples solution of Cinacalcet tablets 90mg. same batch which was used for method precision. The study was performed by different analyst using different instrument and different lot number of column on different day.

The percentage of known impurities and total impurities was determined. Calculated percentage relative standard deviation (% RSD) for percentage of known impurities, Highest individual impurity and total impurities in six samples and also calculated overall percentage relative standard deviation (% RSD) for percentage of known impurities, Highest individual impurity and total impurities of ruggedness results and method precision results. The results are tabulated in table-3.

Acceptance criteria

Percentage relative standard deviation (% RSD) is not more than 10.0

Overall percentage relative standard deviation (% RSD) is not more than 10.0 for known impurities, Highest individual Unknown impurity and total impurities.

TABLE – 3

INTERMEDIATE PRECISION

Sample No.	Percentage of impurities (w/w)									
	CH 1		CH 4		CH 7		Highest Unknown		Total Impurities	
	I	II	I	II	I	II	I	II	I	II
1	0.1908	0.1984	0.2205	0.1874	0.2120	0.2052	0.0251	0.0223	0.6484	0.6133
2	0.1909	0.1976	0.2220	0.1867	0.2126	0.2045	0.0245	0.0223	0.6500	0.6111
3	0.1908	0.1982	0.2271	0.1876	0.2115	02050	0.0248	0.0223	0.6542	0.6131
4	0.1908	0.2001	0.2257	0.1909	0.2107	0.2061	0.0246	0.0229	0.6518	0.6200
5	0.1888	0.1980	0.2215	0.1887	0.2124	0.2036	0.0251	0.0227	0.6478	0.6130
6	0.1884	0.1991	0.2198	0.1900	0.2135	0.2052	0.0251	0.0228	0.6468	0.6171
Avg	0.1901	0.1986	0.2228	0.1886	0.2121	0.2049	0.0249	0.0226	0.6498	0.6146
% RSD	0.6	0.5	1.3	0.9	0.5	0.4	1.1	1.2	0.4	0.5
Overall mean	0.1943		0.2057		0.2085		0.0237		0.6322	
Overall % RSD	2.3		8.8		1.8		5.2		2.9	

Where,

I - Analyst-1

II - Analyst-2

Conclusion

Percentage relative standard deviation (% RSD) value indicates an acceptable level of ruggedness of the analytical method for the determination of related substances of Cinacalcet tablets 90mg..

SPECIFICITY

BLANK AND PLACEBO INTERFERENCE

Blank, triplicate preparation of placebo, sample solution (unspiked) and sample solution spiked with known impurities, process related impurities of 90mg tablets at specification level were injected into the UPLC system. There was no interference from the blank and placebo at the retention time of known impurities, Process related impurities and main peak. Peak purity data reveals that Cinacalcet peaks was homogeneous and there were no co-eluting peaks at the retention time of Cinacalcet peaks.

The peak purity data of known impurities, process related impurities and Cinacalcet peaks in spiked sample are summarized in table-4a. The retention time of Cinacalcet and relative retention time of known impurities, process related impurities from spiked sample are compiled in table-4b. Interference of placebo is tabulated in table-4c.

Refer page no 27 to 32 for the chromatograms of blank, placebo, standard, individual impurity at specification level, sample (unspiked) and known impurities Process related impurities spiked sample.

Acceptance criteria

1) No peak elutes at the retention time of main peak and known impurity(s) in the blank and placebo.

2) Peak purity of main peak and known impurities peak should pass.

[Waters Empower software: Purity angle should be less than purity threshold and purity flag should be No]

SPECIFICITY

TABLE - 4a

	Peak Name	Purity Angle	Purity threshold	Purity flag
Impurity spiked sample	Impurity – CH 1	0.211	29.204	No
	Impurity – CH 4	2.001	90.0	No
	Impurity – CH 7	3.892	53.624	No
	Cinacalcet	0.591	1.336	No

TABLE - 4b

	Peak Name	RT	RRT
Impurity spiked sample	Impurity – CH 1	1.237	0.13
	Impurity – CH 4	2.127	0.23
	Impurity – CH 7	7.291	0.78
	Cinacalcet	9.36	1.00

RT - Retention time in minutes

RRT - Relative Retention Time

TABLE – 4c

Name	Interference
Blank	No interference
Placebo 1	No interference
Placebo 2	No interference
Placebo 3	No interference

Conclusion

The method is specific for determination of related substances of Cinacalcet tablet 90mg.

FORCED DEGRADATION

Forced degradation study was carried out by treating the sample under the following conditions.

a) Degradation by hydrochloric acid (Acid stressed sample)

Sample was dissolved in 40mL of methanol, treated with 10mL of 5N Hydrochloric acid and kept on 80°C incubator for 2 hour. Treated sample solution was analyzed.

b) Degradation by sodium hydroxide (Alkali stressed sample)

Sample was dissolved in 40mL of methanol, treated with 10 mL of 1N Sodium hydroxide and kept 80°C incubator for 2 hours. Treated sample solution was analyzed.

c) Degradation by hydrogen peroxide (Peroxide stressed sample)

Sample was dissolved in 40mL of methanol treated with 20 mL of 30 % solution of Hydrogen peroxide and kept on 80°C incubator for 2 hours. Treated sample solution was analyzed.

Degradation by oxidation (KMno4 stressed sample)

Sample was dissolved in 40mL of methanol treated with 20 mL of 20 % solution of potassium permanganate and kept on 80°C incubator for 2 hours. Treated sample solution was analyzed.

e) Degradation by thermal (Heat stressed sample)

Sample was kept in an oven at 105°C for about 48 hours. Treated sample solution was analysed.

f) Degradation by photo light [Controlled condition (wrapped in aluminum foil)]

Sample was exposed to visible light for 1.2 million Lux hours and UV light for 200 Watt hours/square meter in protected condition. Treated sample solution was analysed.

g) Degradation by photo light [Uncontrolled condition]

Sample was exposed to visible light for 1.2 million Lux hours and UV light for 200 Watt hours/square meter in protected condition. Treated sample solution was analysed.

The results of forced degradation studies are summarized in table - 5. Refer page no 32 to 37 for the chromatograms of unstressed and stressed samples.

Acceptance criteria

1. Peak purity for main peak should pass.[Waters Empower software: Purity angle should be less than purity threshold and purity flag should be No.]

2. Degradation is not more than 30 % in each condition.

3. Report the degradation data.

FORCED DEGRADATION

TABLE – 5

S. No	Condition	% Cinacalcet	% Net Degradation	Purity Angle	Purity Threshold	Purity flag
1	Unstressed Sample	99.95	-	0.675	1.752	No
2	Peroxide stressed sample	99.90	0.05	0.735	12.818	No
3	Oxidation stressed sample (KMno4)	85.90	14.05	0.329	1.757	No
4	Base stressed sample	99.75	0.20	0.108	2.584	No
5	Acid stressed sample	99.81	0.14	0.069	2.093	No
6	Photo light stressed sample (Controlled)	99.92	0.03	0.643	1.796	No
7	Photo light stressed sample (Uncontrolled)	99.91	0.04	0.660	1.773	No
8	Thermal stressed sample	99.83	0.12	0.616	1.708	No

Conclusion

The method is stability indicating for determination of related substances of Cinacalcet tablets 90mg.

LIMIT OF DETECTION AND LIMIT OF QUANTITATION (LOD and LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) is determined by signal to noise ratio method by using the formula.

Signal to noise ratio (S/N) = 2H/h

H - Height of the analyte peak

h - Height of the noise.

LOD and LOQ value was verified by giving six replicate injections of Mesalamine at this level. LOD and LOQ values are summarized in table 6a. The percentage relative standard deviation (% RSD) calculated for the peak areas and tabulated in table 6b and 6c.

Acceptance criteria

Signal-to-noise ratio 10:1 at the level of LOQ and 2:1 or 3:1 at the level of LOD.

Percentage relative standard deviation (% RSD) for peak areas at LOQ level is not more than 10.0

Report the percentage relative standard deviation (% RSD) for peak areas at LOD level.

TABLE – 6a

SUMMARY OF LOD AND LOQ VALUES

Name	LOD			LOQ		
	Con. in µg/mL	%	S/N	Con. in µg/mL	%	S/N
Impurity – CH 1	0.0240	0.001	3	0.0728	0.004	10
Impurity – CH 4	0.2943	0.015	4	0.8918	0.045	11
Impurity – CH 7	0.0669	0.003	4	0.2027	0.010	13
Cinacalcet	0.0664	0.003	5	0.2013	0.010	15

S/N: Signal to noise ratio.

% : Percentage calculated with respect to test concentration.

Precision at LOQ

TABLE – 6b

Sample No.	Peak area			
	CH 1	**CH 4**	**CH 7**	**Cinacalcet**
1	1872	2364	3522	3820
2	1821	2458	3465	3715
3	1808	2474	3474	3850
4	1829	2572	3479	3727
5	1813	2408	3408	3841
6	1873	2323	3588	3729
Avg	1836	2433	3489	3780
% RSD	1.6	3.6	1.7	1.7

Precision at LOD

TABLE – 6c

Sample No.	Peak area			
	CH 1	**CH 4**	**CH 7**	**Cinacalcet**
1	525	787	950	1213
2	512	808	1079	1135
3	558	811	1051	1217
4	557	826	1046	1154
5	587	817	1022	1188
6	579	803	1000	1124
Avg	553	809	1025	1172
% RSD	5.3	1.6	4.4	3.4

Conclusion

The method is Precise for determination of related substances at LOD and LOQ level of Cinacalcet tablets 30mg, 60mg, and 90mg.

LINEARITY

The linearity for Cinacalcet and known impurities in the range of about LOQ, 50%, 100%, 125% and 150. A graph was plotted with concentration (in µg/mL) on x-axis and peak areas on y-axis. Slope, y-intercept, correlation coefficient (r-value) and residual sum of squares (RSS) were determined. The results are tabulated in table – 7 graphically represented in figure 1.

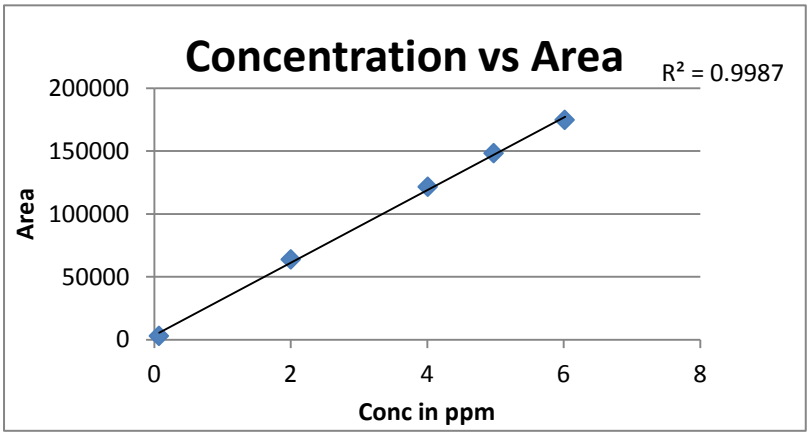
Acceptance criteria

Visual inspection of plot of the signal as a function of analyte concentration is linear and the value of correlation coefficient (CC) 'r' is not less than 0.97

LINEARITY
Impurity – CH1
TABLE – 7a

Level (%)	Concentration in µg/mL	Peak area
LOQ	0.0722	2996
50%	2.0069	63750
100%	4.0134	121516
125%	4.9772	148087
150%	6.0208	174613
Correlation coefficient	0.99935	
Slope	28915.3514	
Y-intercept	3354.3402	
Residual sum of squares	24689209.3476	

Linearity plot for Impurity – CH1

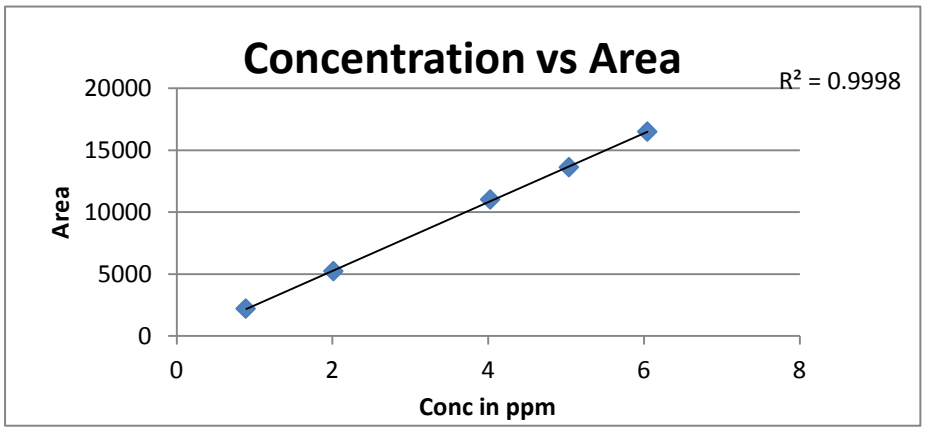


Impurity – CH4

TABLE – 7b

Level (%)	Concentration in µg/mL	Peak area
LOQ	0.8927	2196
50%	2.0158	5227
100%	4.0316	11002
125%	5.0394	13611
150%	6.0473	16487
Correlation coefficient	0.99992	
Slope	2775.6891	
Y-intercept	-302.7747	
Residual sum of squares	23325.6822	

Linearity plot for Impurity – CH4

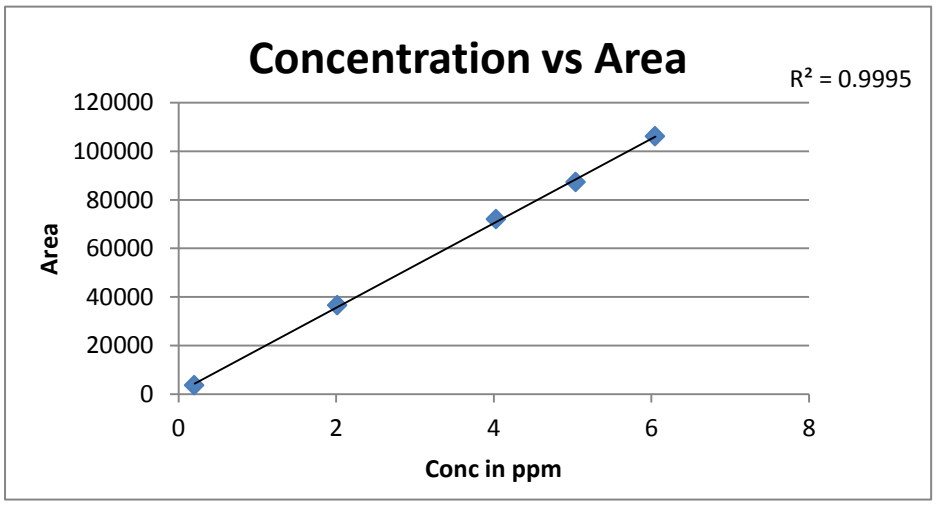


Impurity – CH7

TABLE – 7c

Level (%)	Concentration in µg/mL	Peak area
LOQ	0.2016	3629
50%	2.0163	36481
100%	4.0326	72026
125%	5.0408	87211
150%	6.0490	106095
Correlation coefficient	0.99973	
Slope	17400.8470	
Y-intercept	741.1368	
Residual sum of squares	3610868.2107	

Linearity plot for Impurity – CH7

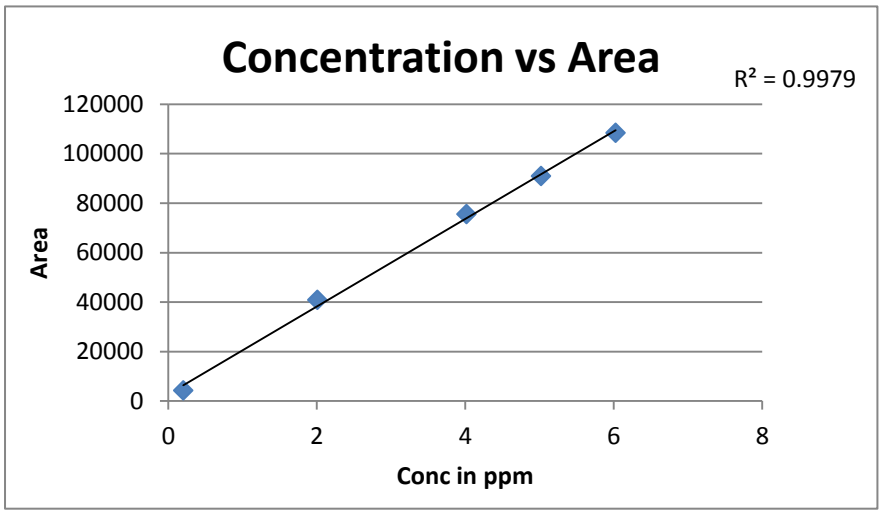


Cinacalcet

TABLE – 7d

Level (%)	Concentration in µg/mL	Peak area
LOQ	0.2009	4309
50%	2.0086	40834
100%	4.0173	75527
125%	5.0216	90912
150%	6.0259	108417
Correlation coefficient	0.99894	
Slope	17704.3119	
Y-intercept	2833.5991	
Residual sum of squares	14637532.5556	

Linearity plot for Impurity – Cinacalcet



RELATIVE RESPONSE FACTOR (RRF)

TABLE – 7e

Name of impurity	Relative response factor (RRF)
CH1	1.63
CH2	0.16
CH3	0.98

Conclusion

The detector response is directly proportional to concentration ranging from LOQ to 150% of specification limit for known impurities and Cinacalcet.

ACCURACY (RECOVERY)

Known amount of degradable impurities spiked in sample solution at about LOQ, 100% and 150% of specification limit in triplicate. The percentage recovery was calculated from the amount found and actual amount added and analyzed as per testing procedure. The percentage recovery was calculated from the amount found and actual amount added.
The results are tabulated in table – 8.

Acceptance criteria

Percentage recovery at each level is between 90.0 and 110.0
Percentage relative standard deviation (% RSD) is not more than 10.0 at each level.

Impurity – CH1

TABLE – 8

Level	Corrected Area	Amount found in µg	Amount added in µg	% Recovery	Mean	% RSD
Level - 1 (LOQ)	2147	0.0705	0.0722	97.6	98.3	0.6
	2174	0.0713	0.0722	98.7		
	2169	0.0712	0.0722	98.5		
Level - 2 (100%)	119098	3.9081	4.0138	97.4	98.1	0.6
	120456	3.9527	4.0138	98.5		
	120288	3.9471	4.0138	98.3		
Level - 3 (150)	180758	5.9314	6.0208	98.5	98.1	0.7
	180979	5.9387	6.0208	98.6		
	178596	5.8605	6.0208	97.3		

Impurity – CH4

TABLE – 8

Level	Corrected Area	Amount found in µg	Amount added in µg	% Recovery	Mean	% RSD
Level - 1 (LOQ)	2432	0.8130	0.8639	94.1	94.7	0.9
	2436	0.8143	0.8639	94.3		
	2472	0.8264	0.8639	95.7		
Level - 2 (100%)	12243	4.0928	4.0316	101.5	101.9	0.4
	12335	4.1235	4.0316	102.3		
	12302	4.1125	4.0316	102.0		
Level - 3 (150)	16778	5.6088	5.7594	97.4	96.6	1.5
	16794	5.6141	5.7594	97.5		
	16366	5.4710	5.7594	95.0		

Impurity – CH7

TABLE – 8

Level	Corrected Area	Amount found in µg	Amount added in µg	% Recovery	Mean	% RSD
Level - 1 (LOQ)	3536	0.1930	0.2016	95.7	95.4	1.0
	3555	0.1940	0.2016	96.2		
	3489	0.1904	0.2016	94.4		
Level - 2 (100%)	78093	4.2622	4.1767	102.0	102.7	0.6
	78912	4.3069	4.1767	103.1		
	78725	4.2967	4.1767	102.9		
Level - 3 (150)	107787	5.8829	6.0490	97.3	96.7	1.3
	108074	5.8985	6.0490	97.5		
	105517	5.7590	6.0490	95.2		

Cinacalcet

TABLE – 8

Level	Corrected Area	Amount found in µg	Amount added in µg	% Recovery	Mean	% RSD
Level - 1 (LOQ)	3794	0.2029	0.1992	101.8	101.8	0.7
	3766	0.2014	0.1992	101.1		
	3820	0.2043	0.1992	102.5		
Level - 2 (100%)	72916	3.9001	3.9850	97.9	97.7	0.2
	72611	3.8837	3.9850	97.5		
	72705	3.8888	3.9850	97.6		
Level - 3 (150)	104323	5.5799	5.7561	96.9	97.2	0.3
	104645	5.5971	5.7561	97.2		
	104969	5.6145	5.7561	97.5		

Conclusion

The analytical method meets the pre-established acceptance criteria for accuracy study as per ICH guidelines. Hence the method is accurate for the determination of related substances of Cinacalcet tablets 90mg.

RANGE

Range inferred from the data of linearity, accuracy and precision experiments.

Conclusion

The method was found to be linear, accurate and precise in the range of LOQ to 150% of specification limit.

SOLUTION STABILITY

Stability of analytical solution was verified by analyzing the standard and sample solution at initially and also at different time intervals as mentioned below by storing at ambient temperature. Calculated the cumulative percentage relative standard deviation (% RSD) for peak areas of Cinacalcet in standard solution and percentage of known impurities,highest individual impurity, total impurities in sample solution. The results are tabulated in table – 9a to 9b.

Acceptance criteria

Cumulative percentage relative standard deviation (% RSD) is not more than 10.0

STANDARD SOLUTION

TABLE-9a

Time in Hrs.	Peak area	Cum. % RSD
Initial	75213	-
8^{th} Hour	75803	0.6
12^{th} Hour	75752	0.4
21^{th} Hour	76073	0.5
24^{th} Hour	75341	0.5

SAMPLE SOLUTION

TABLE - 9b

Time in Hours	Impurity – CH1		Impurity – CH4		Impurity – CH7		Highest Unknown		Total impurities	
	%	Cum. % RSD	%	Cum. % RSD	%	Cum. % RSD	%	Cum. % RSD	%	Cum. % RSD
Initial	0.1908	-	0.2205	-	0.2120	-	0.0251	-	0.6484	-
6th Hour	0.1938	1.1	0.2220	0.5	0.2157	1.2	0.0245	1.7	0.6560	0.8
11th Hour	0.1941	0.9	0.2236	0.7	0.2193	1.7	0.0255	2.0	0.6625	1.1
19th Hour	0.1925	0.8	0.2259	1.0	0.2190	1.6	0.0245	2.0	0.6619	1.0
24th Hour	0.1922	0.7	0.2295	1.6	0.2250	2.2	0.0253	1.8	0.6720	1.3

Conclusion

Standard solution and filtered sample solution stable for 24 hours at ambient temperature.

7.5 FILTER INTERFERENCE STUDY

Filter interference study was performed on sample solution of cinacalcet tablets 90mg spiked with known impurities at specification level, centrifuged one portion of the solution and another portion was filtered through 0.45µm nylon filter and analyzed. The filtered sample results were compared to the results of centrifuged sample. Calculated the percentage difference of known impurity, highest unknown impurity and total impurities between the results of centrifuged and filtered sample with respect to centrifuged sample. The results are tabulated in table -10.

Acceptance criteria

The percentage difference between centrifuged and filtered sample results is not more than 10.0

FILTER INTERFERENCE STUDY

TABLE – 10

Name	Percentage of impurity (w/w)		% Difference
	Centrifuged sample	Filtered sample	
Impurity – CH1	0.1896	0.1884	0.6329
Impurity – CH4	0.2202	0.2198	0.1820
Impurity – CH7	0.2122	0.2135	-0.6126
Highest individual s	0.0247	0.0251	-1.6194

FILTER DETAILS

Filter Name	Pore size	Manufacturer	Batch No.
Nylon filter	0.45µm	Axiva	SFNY25RB

Conclusion

The percentage difference between the results of centrifuged and filtered sample is within the acceptance criteria. Hence 0.45µm nylon filter suitable for filtering the sample solution.

ROBUSTNESS

Robustness of the method was verified by deliberately varying the following conditions.

a. By changing the flow rate by ± 10%.

b. By changing the wavelength by ± 2 nm.

c. By changing the column oven temperature + 5°C.

System suitability was evaluated in each condition and sample solution of cinacalcet tablets 90mg was analyzed in triplicate. Calculated overall percentage relative standard deviation (% RSD) for percentage of Unknown impurity, Highest individual impurity and total impurities of each condition and method precision data.

The results are tabulated in table-11a, 11b, 11c, 11d, 11e 11f, 11g.

The system suitability parameters are tabulated in table-11h.

Acceptance criteria

System suitability should pass for each condition.

Overall percentage relative standard deviation (% RSD) is not more than 10.0 for known and total impurities.

ROBUSTNESS

LOW FLOW RATE

TABLE - 11a

Sample No.	Percentage of impurities (w/w)				
	CH 1	CH 4	CH 7	Highest Unknown	Total Impurities
Sample-1	0.1908	0.2205	0.2120	0.0251	0.6484
Sample-2	0.1909	0.2220	0.2126	0.0245	0.6500
Sample-3	0.1908	0.2271	0.2115	0.0248	0.6542
Sample-4	0.1908	0.2257	0.2107	0.0246	0.6518
Sample-5	0.1888	0.2215	0.2124	0.0251	0.6478
Sample-6	0.1884	0.2198	0.2135	0.0251	0.6468
Robustness-1	0.2058	0.2154	0.2261	0.0233	0.6706
Robustness-1	0.2062	0.2150	0.2265	0.0235	0.6712
Robustness-1	0.2050	0.2166	0.2253	0.0234	0.6703
Avg	0.1953	0.2204	0.2167	0.0244	0.6568
% RSD	4.0	1.9	3.2	3.1	1.6

ROBUSTNESS

HIGH FLOW RATE

TABLE - 11b

Sample No.	Percentage of impurities (w/w)				
	CH 1	CH 4	CH 7	Highest Unknown	Total Impurities
Sample-1	0.1908	0.2205	0.2120	0.0251	0.6484
Sample-2	0.1909	0.2220	0.2126	0.0245	0.6500
Sample-3	0.1908	0.2271	0.2115	0.0248	0.6542
Sample-4	0.1908	0.2257	0.2107	0.0246	0.6518
Sample-5	0.1888	0.2215	0.2124	0.0251	0.6478
Sample-6	0.1884	0.2198	0.2135	0.0251	0.6468
Robustness-1	0.1937	0.2081	0.2140	0.0216	0.6374
Robustness-1	0.1885	0.2025	0.2067	0.0209	0.6186
Robustness-1	0.1910	0.2007	0.2062	0.0207	0.6186
Avg	0.1904	0.2164	0.2111	0.0236	0.6415
% RSD	0.9	4.6	1.3	8.2	2.1

ROBUSTNESS

LOW WAVELENGTH

TABLE - 11c

Sample No.	Percentage of impurities (w/w)				
	CH 1	CH 4	CH 7	Highest Unknown	Total Impurities
Sample-1	0.1908	0.2205	0.2120	0.0251	0.6484
Sample-2	0.1909	0.2220	0.2126	0.0245	0.6500
Sample-3	0.1908	0.2271	0.2115	0.0248	0.6542
Sample-4	0.1908	0.2257	0.2107	0.0246	0.6518
Sample-5	0.1888	0.2215	0.2124	0.0251	0.6478
Sample-6	0.1884	0.2198	0.2135	0.0251	0.6468
Robustness-1	0.1779	0.2482	0.2164	0.0268	0.6693
Robustness-1	0.1744	0.2459	0.2116	0.0251	0.6570
Robustness-1	0.1750	0.2461	0.2107	0.0256	0.6574
Avg	0.1853	0.2308	0.2124	0.0252	0.6536
% RSD	3.9	5.3	0.8	2.7	1.1

ROBUSTNESS

HIGH WAVELENGTH

TABLE - 11d

Sample No.	Percentage of impurities (w/w)				
	CH 1	CH 4	CH 7	Highest Unknown	Total Impurities
Sample-1	0.1908	0.2205	0.2120	0.0251	0.6484
Sample-2	0.1909	0.2220	0.2126	0.0245	0.6500
Sample-3	0.1908	0.2271	0.2115	0.0248	0.6542
Sample-4	0.1908	0.2257	0.2107	0.0246	0.6518
Sample-5	0.1888	0.2215	0.2124	0.0251	0.6478
Sample-6	0.1884	0.2198	0.2135	0.0251	0.6468
Robustness-1	0.1974	0.1818	0.2112	0.0210	0.6114
Robustness-1	0.1929	0.1888	0.2037	0.0201	0.6055
Robustness-1	0.1936	0.1884	0.2046	0.0208	0.6074
Avg	0.1916	0.2106	0.2102	0.0235	0.6359
% RSD	1.4	8.8	1.7	9.1	3.3

ROBUSTNESS

LOW COLUMN OVEN TEMPERATURE

TABLE - 11e

Sample No.	Percentage of impurities (w/w)				
	CH 1	CH 4	CH 7	Highest Unknown	Total Impurities
Sample-1	0.1908	0.2205	0.2120	0.0251	0.6484
Sample-2	0.1909	0.2220	0.2126	0.0245	0.6500
Sample-3	0.1908	0.2271	0.2115	0.0248	0.6542
Sample-4	0.1908	0.2257	0.2107	0.0246	0.6518
Sample-5	0.1888	0.2215	0.2124	0.0251	0.6478
Sample-6	0.1884	0.2198	0.2135	0.0251	0.6468
Robustness-1	0.2074	0.2229	0.2283	0.0229	0.6815
Robustness-1	0.2027	0.2174	0.2223	0.0223	0.6647
Robustness-1	0.2040	0.2166	0.2240	0.0224	0.6670
Avg	0.1950	0.2215	0.2164	0.0241	0.6569
% RSD	3.8	1.6	3.1	5.0	1.8

ROBUSTNESS

HIGH COLUMN OVEN TEMPERATURE

TABLE – 11f

Sample No.	Percentage of impurities (w/w)				
	CH 1	CH 4	CH 7	Highest Unknown	Total Impurities
Sample-1	0.1908	0.2205	0.2120	0.0251	0.6484
Sample-2	0.1909	0.2220	0.2126	0.0245	0.6500
Sample-3	0.1908	0.2271	0.2115	0.0248	0.6542
Sample-4	0.1908	0.2257	0.2107	0.0246	0.6518
Sample-5	0.1888	0.2215	0.2124	0.0251	0.6478
Sample-6	0.1884	0.2198	0.2135	0.0251	0.6468
Robustness-1	0.2069	0.2228	0.2295	0.0239	0.6831
Robustness-1	0.2072	0.2239	0.2297	0.0238	0.6846
Robustness-1	0.2065	0.2215	0.2266	0.0235	0.6781
Avg	0.1957	0.2228	0.2176	0.0245	0.6605
% RSD	4.3	1.1	3.8	2.5	2.5

TABLE – 11g

SYSTEM SUITABILITY

S.No	Name of Experiment	Tailing factor	Theoretical plates	% RSD
1	Robustness (High wavelength-212 nm)	1.2	355259	0.2
2	Robustness (Low wavelength-208 nm)	1.2	355556	0.2
3	Robustness (Low flow rate-0.36 mL/min)	1.3	390550	0.3
4	Robustness (High flow rate-0.44 mL/min)	1.2	325950	0.6
5	Robustness (Low column oven temperature - 30°C)	1.3	369889	0.3
5	Robustness (High column oven temperature - 40°C)	1.3	334012	0.6

Conclusion

The overall percentage relative standard deviation (% RSD) was meeting for the conditions change in Low flow rate, High flow rate, Low wave length, High wave length, Low column oven temperature, High column oven temperature. Hence the method is robust .

The present work entitled **"Development and validation of stability indicating RP-UPLC method for determination of Related substances of Cinacalcet tablets"** comprises of the following novel methods which have not been reported till date.

Reverse phase-Ultra Performance Liquid Chromatography (RP-UPLC)

The ultra violet method involves the determination of Related substances of Cinacalcet tablets 90mg by External standard method. The drug obeyed Beer's Law at the concentration of 5-30 µg/mL. The correlation co-efficient was found to be 0.99 for the methods. The low percentage RSD value shows that the methods developed are not affected by the presence of sample matrix or devoid of interference by the excipients.

In RP-UPLC method, C_{18}column was used for estimation of impurities of Cinacalcet tablets. By trial and error method mobile phase chosen was Mobile phase A-0.1%v/v orthophosphoric acid and Mobile phase-B-Acetonitrile: Methanol (40.:60%v/v) and the effluents were monitored at 210nm for cinacalcet and its related compounds. The retention time was about 9.3. The chromatograms were then subjected to system suitability studies. The tailing factor and asymmetry factor were close to 1.2 which showed ascertained of the peak. The number of theoretical plate was found to be 355529 which proved the efficiency of the column. The correlation coefficient indicated linearity of the method. The %RSD values were < 10 which showed the reproducibility and specificity of the method and it can be used for routine analysis.

The RP-UPLC method developed for determination of related substance of cinacalcet tablets 90mg is simple, accurate, precise rapid economical and stability indicating. The RP-UPLC methods though utilizes costly equipment is more accurate and highly specific and well suitable for more number of sample analysis and simultaneous estimation of drugs. The run time (27 min) enables for rapid determination of impurities .

The method was validated for accuracy, precision, specificity, robustness, and detection and quantification limits, in accordance with ICH guidelines. Statistical analysis proved the method was precise, reproducible, selective, specific, and accurate for analysis of Cinacalcet and its impurities. The wide linearity range, sensitivity, accuracy, short retention time, and simple mobile phase showed that the method is suitable for routine quantification of impurities in Cinacalcet in pharmaceutical dosage forms with high precision and accuracy. Moreover, it may be applied for determination of Cinacalcet in the study of blend uniformity, tablet content uniformity and in-vitro dissolution profiling of Cinacalcet dosage forms, where sample load is higher and high throughput is essential for faster delivery of results

Therefore all the proposed validation methods could be used for routine analysis and are devoid of interference by sample excipients.

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