IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
December 2022 Vol.:26, Issue:1

Method Development and Validation of an RP- HPLC Method for Determination of Levocetirizine

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Suyash Gupta* [1], Nainshi Omar [1], Riya Mathur [1], Archan Gupta [2], Abhinav Prasoon Mishra[3]

1-Research Scholar, Advance Institute of Biotech and Paramedical Sciences, Kanpur, India.

2-Research Scholar, Chhatrapati Shahu Ji Maharaj University, Kanpur, India.

3-Professor of Advance Institute of Biotech and Paramedical Sciences, Kanpur, India.

Submitted: 20 November 2022
Accepted: 26 November 2022
Published: 30 December 2022

HUMAN JOURNALS
www.ijppr.humanjournals.com

Keywords: Validation, Quantification, Reproducible, linearity, Reinjection stability, Chromatographic, hyphenated techniques, interferences)

ABSTRACT

Analytical method development and validation is a good research tool in the field of pharmaceutical chemistry which is utilized to determine the drug content from the mixture, and pharmaceuticals formulation because of the industrial scenario and literature, by using HPLC techniques, revealing the utility of analytical instruments like different chromatographic & hyphenated techniques for the quantification of API's at greater accuracy and precision in the quantification of drugs in formulation even at low concentration. Determination of Active ingredients in terms of purity is essential in the department of quality assurance and quality control for the pharmaceutical dosage form, prior to its entry into the market. Nowadays, efficacy of the drugs and potency are playing major role in patient compliance during therapy. However, in recent decades there are lots of strategies related to analytical were framed for the analysis of drugs in quality control & quality assurance aspects. There is a rapid advancement and developments in the field of pharmaceutical chemistry where sensitive chromatographic and spectral techniques could be evoked for quantification and analysis of active analytes in pharmaceuticals in Pharma industry, the analyst plays a vital role in FDA approval of newer potent drugs with respect to method development, validation, and determination of drugs. Analytical methods have to be simple, sensitive, selective, rugged and reproducible and the validation parameters like selectivity(matrix interference), sensitivity, linearity, precision and accuracy batches (minimum three), matrix effect, recovery, ruggedness, stock solution stability, reinjection stability, long-term stability, dilution integrity were proved for selected drugs. The main goal of this research work is selected based on the increasing needs of the pharmaceutical industry in developing suitable analytical methods.

INTRODUCTION

Allergies are chronic inflammatory illnesses characterized by abnormal immune responses to allergens, which are substances found in the environment. A variety of proteins with diverse origins can act as allergens in different situations, causing allergic reactions. **[1].** Symptoms of an allergy range from unpleasant to fatal outcomes among patients. Medical professionals, business leaders, and academics agree that an immune system exposure to a particularly dangerous antigen is what triggers an allergic reaction. **[2].**

Allergies can cause misfunctioning in the human body. Atopy allergy is a condition due to the exposure of the human body to environmental stimuli, thereby causing the production of IgE antibodies in cells. This condition has genetic susceptibility and is not commonly found in people; which is why every atopic reaction is an allergic reaction. **[3].** There are various allergens present in the environment such as chemical allergens, food allergens, and aeroallergens which are responsible for allergic symptoms, such as skin reactions, allergic rhinitis, anaphylaxis, and asthma. **[4,6].** Chemical allergens include dyes, hair fragrances, creams, and skin care products; food allergens include genetically modified foods, peanuts, tree nuts, and eggs; aeroallergens include spores, dust mites, and pollens.

In 1906, Dr. Clemens von Pirquet coined the word “allergy”, after examining his patients for hypersensitive to commonly non-toxic items such as dust, pollen, and specific meals. **[7]**

Earlier, allergies represented a very broad category of inappropriate inflammatory hyper-immune sensitive reactions. The majority of the time, it was thought that a certain group of immune system cells in the body were to blame for the inflating. Further, research proved disproportionate activation of particular immune cells IgE& its mechanism, which releases inflammatory mediators. In 1963, P. Gell and R. Coombs presented a new classification to distinguish different hypersensitive reactions based on immunological components and the process. **[8,9]**

Allergies are one of the most common chronic disorders in Europe today, impacting more than 60 million people according to conservative estimates. Allergies were once thought to be rare disorders at the turn of the twentieth century, but their prevalence has risen dramatically in recent decades. Environmental changes, such as climate channelization and microbial sterilization, have led to the industrial and technological revolution, as well as an urban, sedentary lifestyle. Contrarily, this has affected the type, intensity, and diversity of external stimuli, thereby altering the regular immune responses. Allergies affect nearly all body

organs in multiple forms. They have a broad spectrum of symptoms, which causes their presentations to varying among different phenotypes. European studies have highlighted the prevalence of allergic rhinoconjunctivitis, asthma, and allergic atopic dermatitis in 30%, 20% and 15% of the European population, respectively. **[10,11].** Moreover, similar prevalence has been found in other countries such as the United States and Australia. An allergy epidemic is increasing owing to the occurrence of medication allergies, occupational allergies, and insect sting allergies coupled with food allergies which are the most common and severe. Nevertheless, a significant and rising proportion of patients ~15% to ~20% have severe, disabling diseases and are constantly afraid of dying from an asthma attack or anaphylactic shock **[12].**

ALLERGY RESEARCH: INFORMATION GAPS:

- The incidence of allergic patients has increased, making it more challenging to pinpoint the precise origin or cause of allergic reactions.

- The method of the allergy's impulsive character, the source of the allergic phenomenon is unclear and yet unproven.

- With few viable therapy choices for a complete recovery from the illness, the relationships between microorganisms, the immune response, and allergic sickness are misunderstood and wrongly diagnosed.

It is crucial to recognize that hypersensitivity has a negative influence on the quality of patient's life as well as their families, further influencing their personal, professional, and lifestyle decisions, irrespective of age, incidence, or nationality. Allergies may affect various aspects of life, including social interactions, academic achievement, sports and work performance, and sleep. **[13]**

PATHOPHYSIOLOGY OF ALLERGY

Pathophysiology aspects of allergic rhinitis and associated hypersensitivity are primarily divided into two stages of retaliation, namely, the early phase and the late phase. Mast cell degranulation, which happens shortly after an allergen encounter, is linked to the early phase of retaliation. Patients with allergic rhinitis have a concentration of mast cells close to the nasal mucosal epithelial site. These cells are crucial in maintaining the late-phase response, which occurred hours before an allergen exposure**. [14]**

Generally, after 2 hours to 8 hours of exposure to allergy antigens, inflammatory mediators are generated, resulting in tissue degradation and severe loss of mucosal epithelial cells. These inflammatory mediators cause the lag in the reaction, thereby increasing the sensitivity to recurrent exposure to specific drugs. Mast cells are abundant in the human body and are secreted in response to chemical mediators. The conjunctiva and blood vessels of the respiratory tract have a disproportionately high number of mast cells. Histamine is produced as a result of mast cell degranulation and is the main factor in several allergy diseases. The histamine compound interacts with specific histamine receptors, leading to vasodilation along with unbroken brawniness exclusion. This causes a rapid fluid ejaculation that passes into the nasal tract**.[15]**

ALLERGY CAUSES AND RISK FACTORS

Allergens are substances that have been identified to cause allergy and hypersensitivity reactions in the human body. Evaluation of risk factors associated with various allergies is vital for diagnosis, treatment of patients, and modification to conduct preventive actions for future exposure. The risk factors are primary & secondary. The primary risk factor is the premature occurrence of atopic disease, while the secondary risk factor is from environmental stimuli, affecting allergic symptoms in patients with existing hypersensitivity. There are two types of sensitivity risk factors: the host and the environment.**[16].**

CLASSIFICATION OF ALLERGIC DRUGS

1. **Highly Sedative**:

Diphenhydramine, DimenhydrSedativeomethazine, Hydroxyzine

2. **Moderately sedative**:

Cyproheptadine, Pheniramine, Meclozine, Cinnarizine

3. **Mild Sedative:**

Clemastine, Dexchlorpheniramine, Chlopheniramine, Triprolidine,

4. **Second-Generation Antihistaminics:**

Loratadine, Fexofenadine, Rupatadine, Cetirizine, Desloratadine, Levocetirizine, Mizolastine, Azelastine, Ebastine.**[17]**

MECHANISM ACTION

It is an anti-histamine of the third generation that is non-sedative. The mechanism of the drug includes a block of histamine that is expelled from mast cell degranulation. The chemical released is bound to histaminic receptors present in the lymphatic system, thereby confining the release of other allergens coupled with the rising blood supply to the inflammation site.

CONTRAINDICATIONS AND SIDE EFFECTS

The most common adverse reactions include mild sleepiness, headaches, mouth dryness, dizziness, impaired vision, and weariness.

PHARMACOKINETICS

Absorption

This includes oral medication that is rapidly and thoroughly absorbed, reaching its peak plasma concentration in 0.9 hours.

Distribution

The milk is expected to receive the apparent distribution of 0.4 L/kg. 91–92% of plasma proteins, predominantly albumin, were bound.

Metabolism

The metabolic threshold was limited to (< 14 %).

Elimination

The elimination in urine is (85.4 %) and in feces is (12.9 %). **[18].**

Analytical methods have shown tremendous progress & positive results in the development and validation of pharmaceutical studies, which are used to extract drug content from large quantities of pharmaceutical dosage forms as well as from biological samples like blood serum and urine. By combining spectral and chromatographic techniques, researchers were able to demonstrate the value of analytical tools comparable to increased chromatographic and hyphenated methods of measurement of APIs with increased the speed and quality in the measurement for substances into the sometimes at minute quantities, natural substances concentrations.

Evaluation of pharmaceutical formulations should be carried out at least in duplicate but preferably in three copies once the optimal method free from interference is chosen as the most appropriate one. The gathering of measured results, that reflect an expected analysis test, decided, transforms a basic computation into information. The outcomes of practical experiments will almost always involve some degree of mistake and may be accurate in any physio-chemical divination. For this reason, it is always necessary to quantify the level of uncertainty to accurately and precisely interpret the data. Thus, this makes it imperative to validate the method that has been created and demonstrate the technique's suitability for the accurate analysis of the material that is the subject of the research.**[19].**

METHOD DEVELOPMENT

These processes play an essential function during the creation of a new medication, diverse pharmaceutical dosage forms, and analytical techniques. These methods are employed to examine the potency, openness, authenticity, and safety of pharmaceutical drugs. The HPLC technique aims to separate, calculate, and identify the primary active substance, any response impurities, all readily available synthetic intermediaries, and any degradants. **[20].**

At one extreme, it may include modifying an existing technique to make it appropriate for a new application by making minimal adjustments. **[21].**

For the evaluation of new or products where there are no established methodologies or processes, new ones are always being developed. Innovative methods are developed to reduce the value of time aside from accuracy and to investigate the presence of either pharmacopeia-compliant or no pharmacopeia-compliant products. Primary runs are used to optimize and validate these procedures. To replace the current approach within the comparative laboratory data with all available advantages and downsides, many alternative methods are devised and put into practice.

The scientific field of analytical chemistry uses cutting-edge technology to arrange things using analytical methods. The generation of thorough, high-quality, and reliable analytical information depends heavily on analytical instrument development. Chromatographic, electrochemical, spectroscopic, hyphenated, or different analytical methods are all possible.

Method development is the process of choosing the best analytical technique to regulate how preparation is set up. To measure the absorption of the following models, the research laboratory should use this procedure of supporting that can be an analytical technique. The

development of analytical procedures must follow the protocols and acceptance standards outlined in the ICH guidelines Q2(R1), and they must be employed in GMP and GLP environments. **[22]**

There are novel approaches that originated from an innovative product when the methodology is defined. Preliminary executions enhance and validate these approaches. Alternative methods of exchanging this approach are created and performed inside the laboratory comparison data with all of the benefits and drawbacks readily available. **[23]**

NEED FOR METHOD DEVELOPMENT

Drug evaluation shows how drugs are recognized and described in their deiform and organic fluid forms. The main purposes of analytical strategies are to collect data on efficacy (which may directly relate to the requirement of a specific quantity), contamination (associated with medication security), bioavailability (which includes significant drug characteristics including crystals type, drug homogeneity, or dissolution rate), stability (which reveals a degrading substance), or the impact of manufacturing limitations. These data are used to confirm that the medicinal product assembly is stable. **[24]**

MEASURES FOR NEW ANALYTICAL METHOD DEVELOPMENT

The finalization of the product is based on a pharmacological study. Most of the time, this is a pharmacopeia's temporary categorization. As a result, a new analytical approach to acquiring these medicines is required. **[21]**

STEPS INVOLVED IN THE METHOD'S DEVELOPMENT

The publication phase is when documentation begins. It is recommended to set up a system for thorough documentation. A lab notebook or an electronic database must be used to keep track of all study-related information. **[25]**

METHODOLOGICAL REQUIREMENTS

The analytical figures that are deemed to be the fundamental requirements of an analytical procedure are defined. The following requirements must be satisfied detection, selectivity, nonlinearity, range, accuracy, and precision. **[26]**

NEW METHOD DEVELOPMENT REQUIREMENTS

- It's conceivable that the drug or drug combination isn't included in any pharmacopeias.
- Because of patent laws, a reliable method of drug analysis might not be mentioned in these writings.
- There are no approved analysis methodologies for the drug in the form of preparation that are not presently available due to the interference that the excipients in the formulation generate.
- Analytical techniques for a drug that is taken in conjunction with another drug may not exist.

The present analytical methods may mandate the use of costly chemicals or solutions. It could also include laborious, unpredictable extraction and separation methods **[28]**.

HPLC METHOD DEVELOPMENT STEPS

Method development for High-performance liquid chromatography (HPLC) appears complicated due to the huge range of equipment, columns, eluant, and operating factors involved. The technique, which typically goes through the stages below, is influenced by the type of analytes:

Move 1 – Choosing the HPLC method and initial system

Move 2 - Choosing of required basic circumstances

Move 3 – Optimizing approach

Move 4 – Validation of Analytical Technique

Move 1 – Choosing of the HPLC method and initial system

A primary stage in developing an HPLC procedure is to do a literature search to see if the transfer has carried out and, if so, under what circumstances. Time will be saved by avoiding needless investigational effort. Make sure the HPLC system you choose has a strong likelihood of the substance can be analyzed, for instance, if polarized solutes are contained in the sample, reverse phase High-performance liquid chromatography would bargain sufficient preservation or determination, but normal phase HPLC would be noticeably more challenging **[29]**.

Sample collection and preparation

In an ideal situation, the sample would break down during the initial mobile phase. The sample may be combined with formic acid, acetic acid, or salt to boost solubility if issues with stability or solubility prevent it from doing so naturally. As long as the sample volume added is small compared to the column volume, these additions have no impact on the separation. The potential for one or more additional peaks to elute in the empty container following sample injection is the only disadvantage of employing large sample quantities. Sample preparation is a critical step in HPLC analysis because it ensures that the solution that will be injected into the column is uniform and homogeneous. The goal of sample preparation is to produce an aliquot of a sample that is basically interference-free and functional.

The sample solvent is appropriate for desired HPLC method and would disintegrate not impacting material persistence/solubility with in the solvent system. Sample collection is the first step in the preparation process, which is then followed by insertion of material onto an HPLC column. The effectiveness, precision, and ease of the approach are significantly impacted by each of these sample preparation tasks that are essential **[30]**.

Move 2 - Choosing of required basic circumstances

By verifying that none of the analytes have below 0.5 for the load demand (Peaks doubling could be caused by inadequate retention), or more than 10-15, this stage provides the optimal retention conditions for all analytes. (Excessive retention has the impacts of large peaks, lengthy processing times, and reduced detectability) **[29]**.

Move 3 - Optimization approach

Once there are enough separations to meet the necessary separations and sensitivity, the experimental parameters should be changed. Planned/systematic analysis of factors includes gradients, fluid velocity, temp, specimen, solvent system and ratios, pH (ionized), quantities, diluents, type of solvent, and feed flow rate will be used to develop the experimental setup for the stability-indicating test **[31]**.

Move 4 - Validation of Analytical Technique

Utilizing laboratory tests to evaluate a procedure's performance uniqueness to the specifications for its intended usage is the process of verifying an analytical technique. The

first step in validating analytical methods and procedures is the applicant's meticulous and well-planned collection of validation data to support analytical processes **[32].**

VALIDATION

Pharmaceutical analysis is a branch of research that examines the identification, quantity, and concentration of compounds in a given sample. To support biopharmaceutical and pharmacokinetic investigations, the pharmaceutical analysis includes dosage forms, bulk materials, and, more recently, biological samples.**[28]** Analysis can be classified as either qualitative or quantitative. Pharmaceutical substances were created and identified using instrumental methods. To manage the process and quality of chemicals during both research and development and manufacturing, analytical techniques must be developed in large part because they are being accepted for product development. Spectroscopic or chromatographic techniques can be used to build analytical methods, and each has a unique set of requirements that must be followed when carrying out these operations. Each step of the analytical process must be examined for any operational parameters that potentially have an impact as to how highly the medication's active content in the presence of the matrix affects the effective material of any pharmacological product in the existing ecological factors and a matrix **[33].**

In the United States, the concept of "VALIDATION" was first introduced in 1978. The concept of validation has evolved throughout time, with its applications spanning from computerized systems incorporating clinical trials, process control, and labelling to analytical processes used to evaluate the quality of medicinal Components and prescription on products. It is advisable to think of the validation process as a vital part of cGMP. The act of evaluating dependability or proving efficacy is referred to as validation. It involves teamwork amongst employees from various plant departments. To assure that the product (equipment) will meet the requirements of anticipated analytical applications, method validation is a "process of establishing recorded proof" **[34]**.

Chemical Evaluation is undertaken

- The discovery of improved drugs.
- Process Assessment by ICH Guidelines.
- The study of clinical medicine.
- Therapeutic Pharmacokinetics Research **[35].**

Importance Of Validation

1. Quality Assurance
2. Time-bound
3. Improvisation of process
4. Quality cost reduction
5. Nominal mix-ups, and bottlenecks
6. Minimizing production or sample batch failures, enhancing efficiency and productivity
7. Minimal rejections
8. Enhanced output
9. Skipping capital expenses
10. Few complaints regarding errors in the process
11. Not much testing of finished items and goods in the process
12. Improved fast and dependable startup of sophisticated equipment
13. Easy scaling up of development work
14. Equipment maintenance is simpler
15. More process awareness among employees
16. Quicker automation
17. Obtaining government permission for the development and launch of products requires government compliance with validation procedures **[36]**.

Process Validation:

"Process validation" is defined as "the establishment of written proof that certain procedures regularly generate a product that meets its planned requirements and quality features" **[37-38].**

Analytical Method Validation:

Analytical approaches need to be validated for a variety of reasons. Between them are legal specifications, appropriate scientific practices, and quality assurance standards. According to 311.165c of the Code of Federal Regulations, "the accuracy, sensitivity, specificity, and repeatability of test procedures applied by the company should be established and recorded." To demonstrate the precision, sensitivity, specificity, and reproducibility of the analytical method used, researchers aim to use outstanding science. Last but not least, the quality assurance department's administration like to make sure that the analytical procedures employed by the division it makes their goods available have been adequately approved for their intended purpose, ensuring the product is safe for human consumption **[39-40]**.

Steps in Method Validation:

1. Create an operation procedure or validation methodology for the Validation
2. Describe the method's use, objective, and range.
3. Establish the performance metrics and acceptance standards.
4. Describe validation studies
5. Check the equipment's pertinent performance characteristics.
6. Make items qualifiable, such as chemicals and benchmarks.
7. Carry out pre-validation tests
8. If necessary, modify the method's parameters acceptance criteria.
9. Carry out exhaustive (internal and external) validation studies.
10. Create Standard Operating Procedures for carrying out procedures in daily basis.
11. Specify the revalidation criterion.
12. Describe the types and timing of the routine's analytical quality control (AQC) checks or system suitability evaluations.
13. Describe the results of the validation experiments **[41]**.

Need for validation of the method

When it is critical to ensure that a technique of analysis' functioning features is sufficient to be employed for a certain analytical problem, for example, it should be examined for validation.

- When an established method needs to be perfected.
- Creation of a new technique for the given issue
- Application of an established approach in a different lab or with different analysis or technique
- To demonstrate the consistency of two distinct processes, such as a fresh method and a reference pharmaceutical technique.

All processes and tests are necessary to verify the dependability of a process for quantitative estimates in a sample. A sample consists of a component or a known sequence of components that are to be validated using an analytical technique.

The US Food and Drug Administration has published some instructions in the USP called "Eight phases of analytical technique validation"**[42]**.

Validation of method components:

Characteristics of analytical performance assessed during technique validation include:

1. Accuracy
2. Precision
3. Linearity
4. Limits of detection and quantitation
5. Specificity
6. Range
7. Robustness

Accuracy

Accuracy is the degree to which a quantified worth resembles real or recognized standard. The difference between the mean value that was discovered and actual value is usually used to define accuracy. By using the technique on samples with known analyte concentrations, Lt is computed. These have to be contrasted with baseline and blank solutions to make sure there isn't any interference. The accuracy is then determined as a percentage of the analyte recovered by the assay based on test findings. The test-based recovery of known added analyte levels is how it is frequently defined **[43].**

Precision

It shows the consistency (stretch) of a group of measurements that were gathered by repeatedly sampling the same homogeneous substance under the same circumstances. The reproducibility of an analytical process is gauged by precision. It has two parts: repeatability and intermediate precision. The fluctuation that a single analyst encounters on a single piece of equipment is known as repeatability. It makes no distinction between variation caused by the method used to prepare the samples and variation caused by the tool or system.

During validation, the analytical approach is used to assess several duplicates of an assay composite sample to determine repeatability. The recovery value is calculated. Intermediate precision is the phrase used to describe variation inside a laboratory, such as on different days, with various instruments, and by various analyzers **[44-45]**.

Linearity

The ability of an analytical process to produce a result according to the analyte concentration (amount) in the sample is known as linearity. If the procedure is linear, either directly or through a precise mathematical translation, the test results are proportional to the analyte concentration in samples lying within a certain range. Linearity is often indicated by the confidence interval around the slope of the regression line **[46]**.

Limits of detection and quantitation

The smallest percentage of either an analytical procedure that could be observed but not measured is known as the detection limit (LOD). Typically, 3:1, a LOD is a concentration at a specific signal-to-noise ratio. The limit of quantitation is the lowest concentration of an analyte in a sample that can be quantified with reasonable precision and accuracy under the

established operating conditions of the technique (LOQ). The signal-to-noise ratio for LOQ is 10:1, according to the ICH. The formulae given below can be used to calculate the response's standard deviation as well as the slope of the calibration curve(s) at values close to the LOD (SD) **[47-48].**

Specificity

Specificity is the ability to accurately assess the analyte in the presence of possibly present components. Common examples include impurities, degradants, matrices, and other compounds. Other supporting analytical methods may make up for the lack of specificity in a particular analytical methodology (s). This definition results in the identification of the analyte and the assurance of that identification. Testing for related compounds, heavy metals, residual solvents, and other contaminants are just a few examples of the purity tests used to make sure that all analytical methods accurately identify the presence of contaminants in an analyte. Obtaining an accurate result by assaying allows for the exact declaration of an analyte's presence or concentration in a sample (content or potency) **[49]**.

Range

The difference between the highest and lowest values of an analyte that can be identified with reasonable precision, accuracy, and linearity is the range of a technique. It is usually given in the same units as the test findings and calculated using a linear or nonlinear response curve (i.e., when many ranges are involved, as shown below) **[50]**.

Robustness

Robustness is the ability of an analytical technique to withstand minor but intentional modifications to the method parameters. The dependability of an analytical approach in real-world applications may be determined by how robust it is **[51]**.

HPLC

Another name for High-Performance Liquid Chromatography is High-Pressure Liquid Chromatography. It is a common analytical method for identifying, classifying, and figuring out how much of each element there is in a mixture. The HPLC procedure forces the solvent under high pressure, as opposed to how it ordinarily flows through the column owing to gravity (up to 400 atmospheres). This makes it possible to separate the sample into various components according to relative affinities **[52-57].**

An HPLC system consists of a mobile phase reservoir system, a pump, a column, and a detector that saves and displays the findings of the separation. The HPLC pump delivers the mobile phase and sample to the column. The most important part of the HPLC system is the column. The HPLC column's most popular adsorbent substance is silica gel. How the sample elutes is affected by the mobile phase as well as how the sample interacts with the material of the column. Water and organic solvents like acetonitrile and methanol make up the mobile phase. Buffers may occasionally be present in the mobility phase. Elution is available in two forms:

(1) Gradient elution happens when the mobile phase's composition changes while the sample is analyzed or separated.

(2) When the mobile phase's composition is consistent throughout the analysis, HPLC isocratic elution takes place **[58-64].**

In High-performance liquid chromatography, pumps are utilized to move pressurized liquid solvent carrying the combination of materials into a column that is stuffed with strong adsorbents. Because each trial element interacts with the other differently, distinct flow rates will be produced for each component, leading to the separation of column components. By pumping a pressurized fluid and a sample blend through a portion that is dense with adsorbent, HPLC forces the specimen segments to separate. The adsorbent, the section's dynamic component, is typically a granular substance made up of solid particles with diameters ranging from 2 to 50 micrometers (e.g., silica, polymers, etc.) **[65-75].**

HPLC employs greater operational pressures than traditional ("low weight") liquid chromatography and moves the portable stage across the segment using pressure rather than gravity (50 bar to 350 bar). Because scientific HPLC can only isolate a very small amount of material, column section measurements range from 30 mm to 250 mm in distance and 2.1 mm to 4.6 mm in width. Smaller sorbent particles are also used to create HPLC segments (often 2 to 50 m in size). HPLC is a well-liked chromatographic technology since it has a high defining or resolution control (the volume to distinguish mechanisms) through separating combinations **[76-85].**

Principle of HPLC

A porous material column is first filled with the stationary phase (the sample solution), and then the liquid phase (the mobile phase) is forced over the column at an increasing gravity.

The separation principle is the adsorption of a solute on a stationary phase in line with the solute's affinity for the stationary phase **[86].**

Fast insertion of the mobile phase into the column is a feature of high-performance liquid chromatography (HPLC). Column efficiency is dramatically improved because much smaller adsorbent or support particles may be employed. As an effect, as compared to conventional column chromatography, the analysis period is reduced by one to two instructions of scale **[87].**

Types of HPLC

Normal Phase HPLC-

In this technique, the division is created by polarization. Silica predominates in the polar stationary phase, whereas hexane, chloroform, and diethyl ether make up the non-polar phase. The polar trials are kept in the column.

Reverse Phase HPLC-

This HPLC phase is exactly contrary to the regular phase. While the stationary phase is nonpolar or hydrophobic, the mobile phase is polar. Non-polar nature will be preserved more the more of it there is.

Size-exclusion HPLC-

In the column, the substrate molecules will be correctly integrated and regulated. Different molecular sizes will cause components to separate.

Ion-exchange HPLC-

The ionically charged surface of the stationary phase is different from that of the sample. In this approach, polarity serves as the basis for the division. The polar stationary phase is dominated by silica, whereas the non-polar phase is composed of hexane, chloroform, and diethyl ether. The column is used to hold the polar samples **[88].**

Mobile phase/solvent reservoir.

Glass is frequently used as the mobile phase's reservoir. Our HPLC solvent reagent container is often used in combination with a reservoir. Teflon tubing sometimes referred to as the pump's "inlet line," is used to convey the solvent from the reservoir to the pump. The Agilent

1100 is one HPLC system that contains specialized chambers for one or more mobile phase reservoirs. The degassing and isolation of the mobile phase from air contact may be made possible by additional properties of the reservoirs in these systems **[89].** Glass is a typical reservoir for the mobile phase. Our HPLC solvent reagent container is usually used in conjunction with a reservoir. Teflon tubing also referred to as the pump's.

"Inlet line," carries solvent from the reservoir to the pump **[90].**

Solvent delivery system

A pulse-free mobile phase is delivered reliably and independently to the HPLC by a solvent delivery system **[89]**.

INTRODUCTION TO SPECTROSCOPY

The most common and commonly used quantitative analytical methods in routine laboratory work are those based on UV-visible light measurements. Currently available commercial tools are practical and simple to use. The steps involved in spectrophotometric procedures don't take much time or effort. These methods are very economically valuable.

Every laboratory must employ this method since it is one of the most affordable for regular analysis with equivalent accuracy and precision. However, the fundamental zero-order technique has a minimal selectivity restriction. Since the absorbance values of the analyte and matrix are added to create the recorded UV-Visible spectrum, interference from other sample components will change the absorbance result. Although the indicated absorbance bands are frequently clearly defined, background interferences make them hard to see. Solvent extraction could be used to separate the analytes from one matrix and transfer them to another matrix to address this problem, but this method carries a higher risk of analyte loss, contamination, and delay. One of the most effective and straightforward methods to improve sensitivity and selectivity is to derivatize spectra. By making this change, matrix interferences and spectral background can be eliminated. Derivative spectroscopy maintains the key tenets of conventional spectroscopy, including Beer's Law and the dependence of derivatization form on analyte concentration (relative to absorbance value in zero-order).**[91]**

Basic Principle Of UV-VIS Spectrometry

The foundation of UV-VIS spectroscopy is the assumption that certain wavelengths of UV-VIS light may be absorbed by liquid pollutant molecules. The amount of light transmitted is

limited by electron migration from the ground state to an excited state, that absorbs light of a particular wavelength. UV-VIS spectroscopy is used to identify water pollutants because of the Lambert-Beer equation, which shows that there is a high link between the material's concentration and its absorption spectra.

Absorption spectrum

The spectrum of a substance's absorption reveals how much light it absorbs at various wavelengths. An absorption curve is produced by plotting the wavelength values along the ordinate's axis and the absorbance values along the abscissa's axis. The maxima or the places of the peaks, of light absorption as determined by absorptivity at specific wavelengths are a property of an absorption spectrum.

Lambert's Law

According to Lambert's Law, the momentum of monochromatic light diminishes as it enters a homogeneous absorbing channel, and the width of the channel is proportional to the strength of the incident radiation.

Beer's law plot

The analyte is collected as a reference sample, and solutions with specified concentrations are produced. A calibration curve is created by aligning the known concentrations along the axis of abscissas and the matching absorbances along the axis ordinates. All of the solutions' absorbances are measured at a certain wavelength. Unknown analyte intensity in the sample under investigation is determined using the calibration curve **[92].**

Applications of UV- Vis Spectroscopy

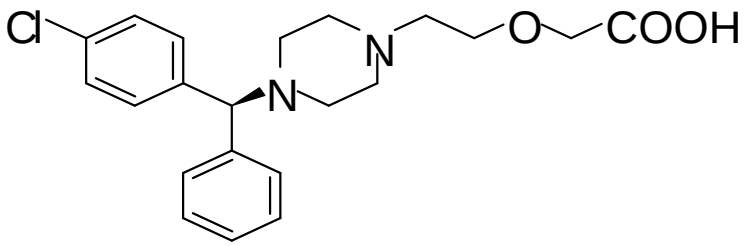
UV –vis spectroscopy has much different application.

1. Clarification of the structures of organic compounds
2. Finding impurities
3. Qualitative research
4. Quantitative research.
5. Chemical analysis

6. As an HPLC detector
7. Acid and base dissociation constants
8. Quantitative evaluation of a drug substance
9. Calculating molecular weight
10. Variances of the Beer-Lambert law **[93].**

DRUG PROFILE

Molecular structure



Chemical name

➢ (R)-2-[2-[4-[(4-chlorophenyl)phenylmethyl]piperazin-1yl]ethoxy]aceticacid dihydrochloride

Molecular formula

➢ C21H25N2O3Cl.2HCl

Molecular weight

➢ 461.8

Category

➢ Anti-histamine agent

Description

➢ White or almost white powder.

Solubility

➢ It is freely soluble in water, and insoluble in acetone and dichloromethane.

Melting Point

Standard value	Observed average value
228 ºC - 229ºC	228.5 ºC

Storage

- Store protected from moisture

MATERIALS AND METHODS

MATERIAL USED

Drug

Levocetirizine (Lcz) Active Pharmaceutical Ingredient was obtained as a bounty analyte from Simson Pharma Limited, B-307, Sarita Building, Prabhat Industrial Estate, Western Express Hwy, Dahisar East, Mumbai, Maharashtra 400068.

Fig: 1 Standard sample of levocetirizine

Formulation

LECET (Skymap Pharmaceuticals Pvt. Ltd, Roorkee) Tablet formulation containing Levocetirizine Dihydrochloride 5 mg was purchased from a local Pharmacy in Kanpur.

Reagent and Chemicals

All of the compounds were HPLC and analytical grade. Triethylamine, purified water, and Methanol (AR grade) were the substances employed in the investigation.

INSTRUMENT

The present work was carried out using a variety of tools.

a) Analytical Balance (SF-400D, Sartorius, Germany)

b) Centrifuge Apparatus

c) pH meter

d) Sonicator Apparatus.

e) HPLC, Shimadzu, Japan, LC-20AP auto sampling device.

f) The visible spectroscope, Shimadzu UV-1800, Serial No. A114548, produces a twofold beam of light from the ultraviolet chamber's dual wavelength UV lamp.

Fig: 2 Sonicator Apparatus

Fig: 3 A HPLC system for levocetirizine

Fig: 4 Shimadzu, UV-spectroscope

SELECTION OF DETECTION WAVELENGTH

According to the analyte being researched, the wavelength chosen by the HPLC technique enables sufficient analysis for the procedure chosen, making it simple to recognize and address the active component under investigation. The following steps were used to prepare an equivalent volume of pure and standard medication solution in methanol: Triethylamine (69+19+12) is added to the mixture to cleanse the water. The overlying graph is then presented after scanning this mixture over a 231 nm UV region.

CHROMATOGRAPHIC STIPULATIONS

- The organic phase ratio of the mobile phase is to be changed by ± 2 % absolute. (i.e. Mobile phase A as such and Triethylamine: Methanol: Purified water (69:19:12) for 2%.
- Colum Temperature is to be changed to ± 5˚c (i.e.,35˚c and 45˚c).
- Column: 4.6-mm × 25-cm; 5-µm packing L3.
- Flow rate to be changed by ± 10% (i.e., 0.9 ml /min and 1.1 ml/min).
- Mobile phase a pH to be changed by ± 0.2 units (i.e., pH 2.3 and pH 2.7)
- Wavelength: 231nm.
- Injection volume: 20 µl.

APPARATUS

a) Volumetric flasks (10, 25, 50, 100, 250, 500 mL, Borosilicate Glass Type I).

b) Whatman filter paper no. 41.

c) Pipettes (1, 2, 5, 10mL, Borosilicate Glass Type I)

d) Measuring cylinders.

e) Beakers.

f) Funnel.

g) Glass road.

h) Spatula.

i) Butter paper.

j) Microscope filter (0.45 ,25mm).

PREPARATIONS OF STANDARD SOLUTION

Composing of solvent part

Prepare a mixture of triethylamine, methanol, and purified water in the ratio (69+19+12) following the testing process.

Preparation of standard stock solution

A typical drug called levocetirizine weighs exactly 50 mg (0.05g). A 250 ml volumetric flask was filled with the sample, and 10 ml of distilled water was added. The volumetric flask was filled with 190ml of filtered water, and then the contents were shaken before being added to a sonicator for a further 5 minutes of sonication. The Levocetirizine Standard Stock Solution was created in an amount of 200 ml.

Preparation of Standard Solution

The process of creating a standard solution using a diluent. The 50 ml volumetric flask is filled with 5 ml of the standard stock solution, creating 45 ml of the diluent and 50 ml of the levocetirizine standard solution.

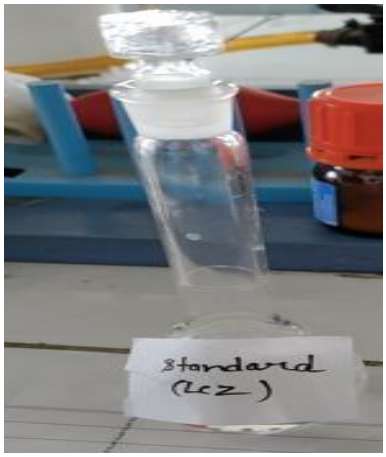


Fig: 5 Standard solution levocetirizine.

Preparation of Sample Solution:

Levocetirizine was applied in the form of 20 properly weighed pills that were then ground into a fine powder. After precisely measuring the levocetirizine powder and adding it to a 250 ml volumetric flask along with 150 ml of diluents, the volumetric flask was put into a sonicator equipment to sonicate the made-up solution for around 5 minutes. The sonication technique results in the production of 200 ml of diluent. Now, my sample was centrifuged for 5 minutes at 500 rpm, filtered through Microsep filter paper, and the filtrate was then collected. Collecting 5 ml from the filter and diluting it to a volume of 45 ml using a diluent will be how we create our sample solution.

Total weight of 20 tablets = 3.75gm

Weight of tablets after crushing =3.54

The average weight of 20 tablets= 3.75\20 =0.1875

VALIDATION OF DEVELOPED METHOD

Linearity

According to the concentration of analyte in a sample within a series, linearity is the ability of an analytical procedure to translate experimental data into a direct or distinct mathematical form. Within the confines of the analytical technique, it must be standard procedure.

In many cases, the regression line's slope and range are expressed in the same units as the result trials (for instance, g/ml, percent, parts per million, parts per billion, etc.). A pendant corollary is a frequent way to define linearity.

The linearity of the suggested method was investigated by creating a solution of the widely used drug Levocetirizine for range selection. Levocetirizine standard solution was put as a band onto the HPLC in the suggested study project, generated under the aforementioned chromatographic circumstances, using 231 nm as the detection wavelength in the absorbance approach, and creating the standardization curvature based on the findings.

Linearity Table

Linearity level	Linearity stack solution to be taken in ml	Dilute to ml with diluent
50%	2.0	50
80%	4.0	50
100%	6.0	50
120%	8.0	50
150%	1.0	50

Fig: 6 Linearity level

RESULT AND DISCUSSION

HPLC Technique for Measurement of Several Anti-Allergic Prescription medications: Development and Validation

Sneezing, coughing, eye discomfort, and nasal congestion are all signs of allergic rhinitis, a common inflammatory illness. Several factors might induce allergic rhinitis, including a

person's diet and any substances to which they may be allergic. They are recognized as allergen-causing compounds. There is a substantial influence on the patient's life level of effectiveness and existence It frequently coexists with several other respiratory conditions. Asthma is one such ailment. Even though this relationship is not fully understood, scientists believe it exists. That description covers a single illness characterized by inflammation and allergies. In both circumstances, riposte could be necessary. The use of oral second-generation antihistamines in conjunction with Leukotriene for just the management of chronic rhinitis, receptor antagonists, as well as the proper prevention and care for allergic rhinitis, has been investigated in literature.

Selection & optimization of mobile phase:

As per test procedure, a 90:10:0.1 % combination of methanol, filtered water, and triethylamine was utilised as the mobile phase.

VALIDATION OF HPLC METHOD

Linearity

By creating a solution of the reference material levocetirizine for the selection of the range, the linearity method could be carried out. Levocetirizine standard solution used in this suggested study endeavor has a strength of 200 to 400 ng/spot.

The linear regression equation was **Y= 0.002x + 0.161**with a correlation coefficient of $\mathbf{R^2}$**=1** for levocetirizine. Respectively depicted in Fig: 7.

The linear regression equation was **Y= 0.0023x + 0.1626** with correlation coefficient of $\mathbf{R^2}$**=0.9962**for levocetirizine. Respectively depicted in Fig: 6.8.

The linear regression equation was **Y= 0.002x + 0.165** with a correlation coefficient of $\mathbf{R^2}$**=1** for levocetirizine. Respectively depicted in Fig: 9.

The linear regression equation was **Y= 0.018x + 0.1685** with correlation coefficient of $\mathbf{R^2}$**=0.9924** for levocetirizine. Respectively depicted in Fig: 10.

The linear regression equation was **Y= 0.002x + 0.169** with correlation coefficient of $\mathbf{R^2}$**=1** for levocetirizine. Respectively depicted in Fig: 11.

The linear regression equation was **Y= 0.003x + 0.17** with correlation coefficient of **R^2=1** for levocetirizine. Respectively depicted in Fig: 12.

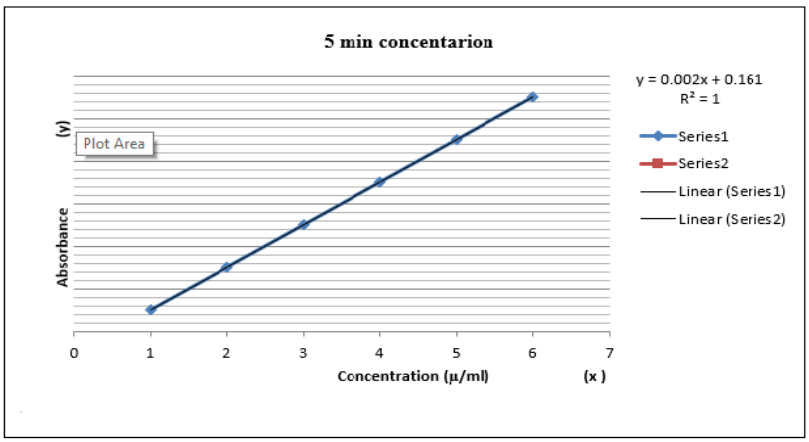


Fig: 7 Calibration curve for levocetirizine (5 min concentration)

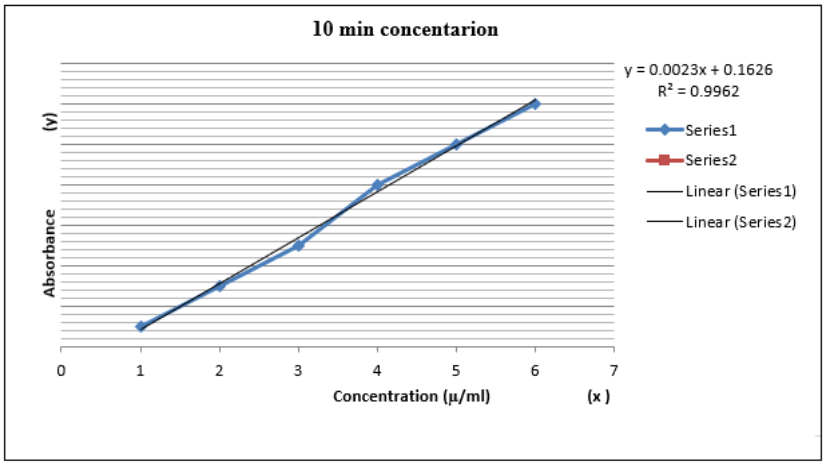


Fig: 8 Calibration curve for levocetirizine (10 min concentration)

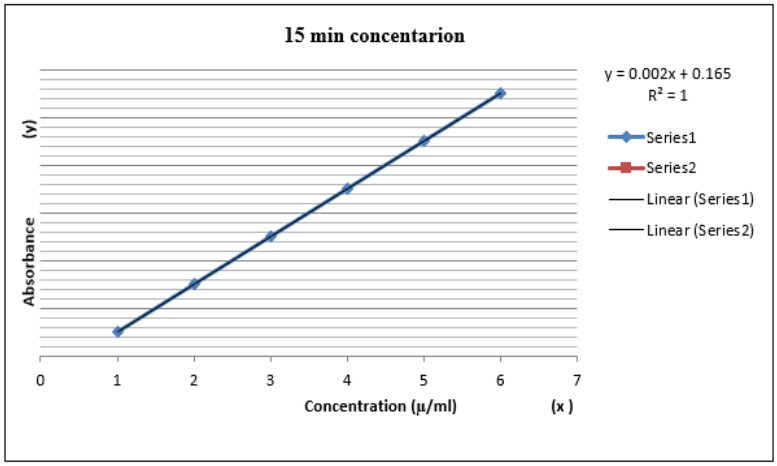


Fig: 9 Calibration curve for levocetirizine (15 min concentration)

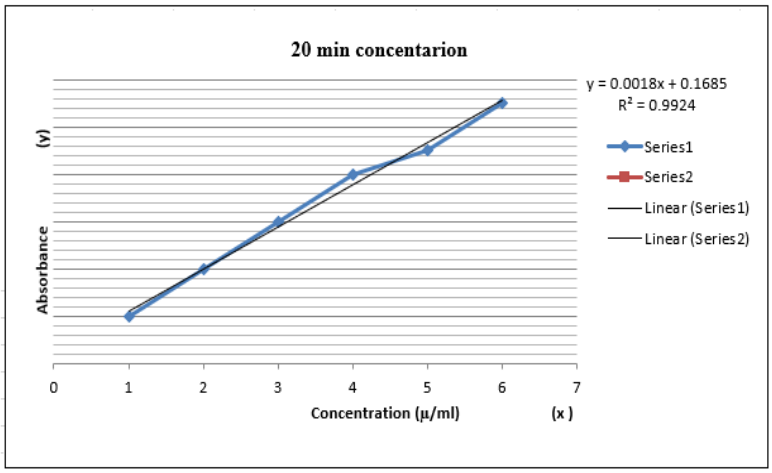


Fig: 10 Calibration curve for levocetirizine (20 min concentration)

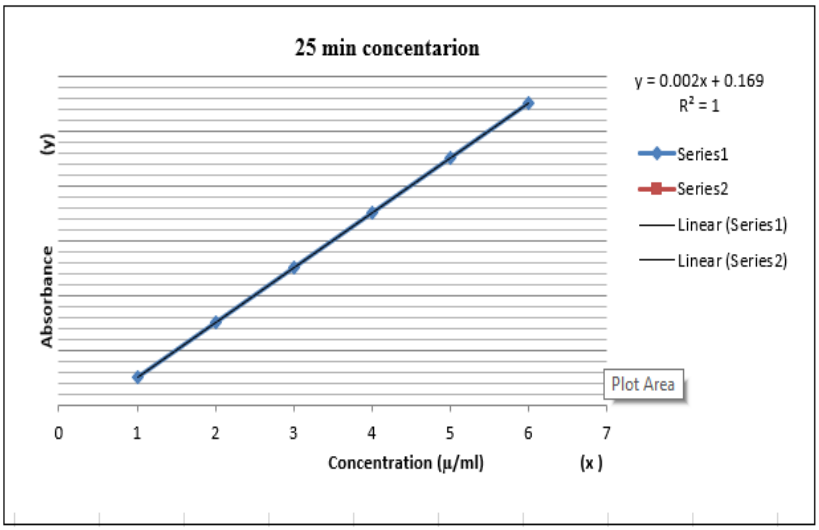


Fig: 11 Calibration curve for levocetirizine (25 min concentration)

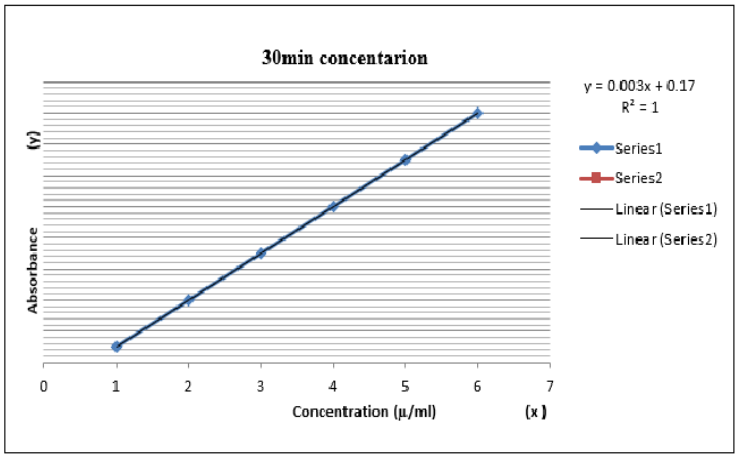
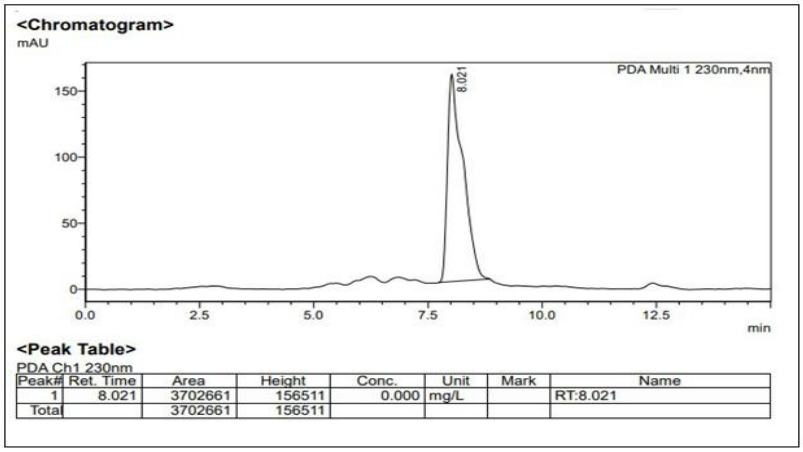


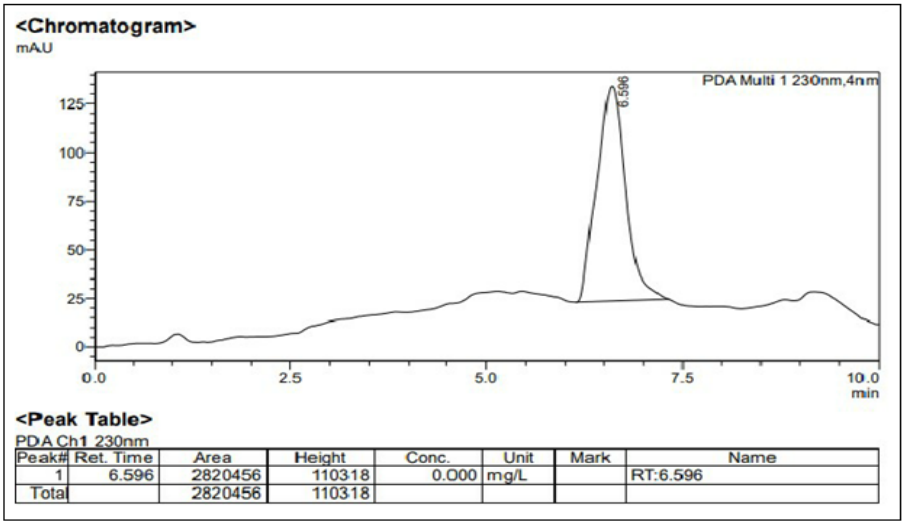
Fig: 12 Calibration curve for levocetirizine (30 min concentration)



<Peak Table>
PDA Ch1 230nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.021	3702661	156511	0.000	mg/L		RT:8.021
Total		3702661	156511				

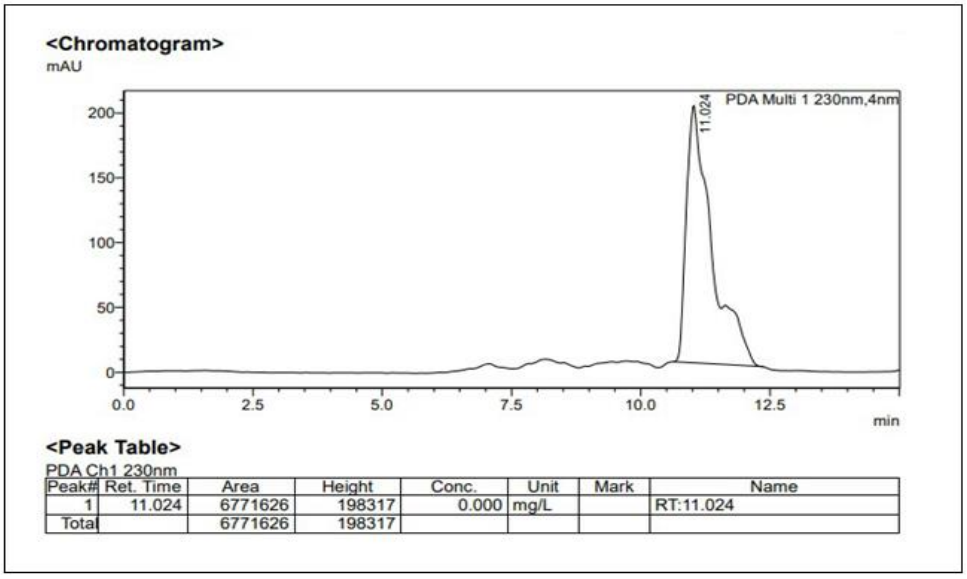
Fig: 13 Chromatogram of levocetirizine standard solution (LCZSTD, 90:10)



<Peak Table>
PDA Ch1 230nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	6.596	2820456	110318	0.000	mg/L		RT:6.596
Total		2820456	110318				

Fig:14 Chromatogram of levocetirizine standard solution (LCZSTD,70:10)



<Peak Table>
PDA Ch1 230nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	11.024	6771626	198317	0.000	mg/L		RT:11.024
Total		6771626	198317				

Fig:15 Chromatogram of levocetirizine sample solution (LCZSAMPLE)

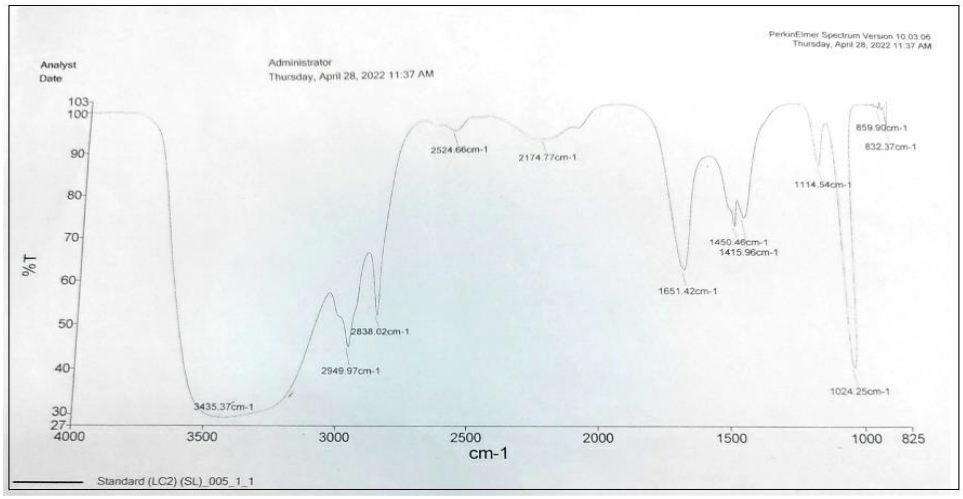


Fig: 16 IR Spectra of standard levocetirizine solution (LC2SAMPLE)

CONCLUSION

The results of the study concluded that investigation and examination led to a conclusion about a method of development and validation for the thesis. For the simultaneous estimation of Levocetirizine, which exhibited several characteristics, the proposed RP-HPLC method was developed. The outcomes were satisfactory by ICH recommendations. Therefore, it can be said that this RP-HPLC method is appropriate for dissolution studies as well as quality control of raw materials and formulations. A series of easy steps are involved in method development. The method is validated using ICH guidelines, and all conditions have been optimized to meet the separation's requirements. The purpose of this thesis is to offer a straightforward method for using strategies with the appropriate scientific foundation in order to enhance the caliber of the development and validation of bioanalytical methods of levocetirizine by RP-HPLC method.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENT:

We sincerely acknowledge the honorable Director of AIBPS, Kanpur for the resources and substantial support. We also extend our thanks to HOD, Advance Institute of Biotech and Paramedical Sciences, AIBPS, Kanpur for facilitating us. We acknowledge Dr. Abhinav Prasoon Mishra Professor AIBPS Kanpur for his valuable support and suggestions throughout the process.

REFERENCES

1. Woodfolk, J.A.; Commins, S.P.; Schuyler, A.J.; Erwin, E.A.; Platts-Mills, T.A. Allergens, sources, particles, and molecules: Why do we make IgE responses? Allergol. Int. 2015, 64, 295–303.
2. Genuneit, J.; Seibold, A.M.; Apfelbacher, C.J.; Konstantinou, G.N.; Koplin, J.J.; La Grutta, S.; Logan, K.; Perkin, M.R.; Flohr, C. The Task Force 'Overview of Systematic Reviews in Allergy Epidemiology (OSRAE)' of the EAACI Interest Group on Epidemiology. Overview of systematic reviews in allergy epidemiology. Allergy 2017, 72, 849–856.
3. Morgenstern, V.; Zutavern, A.; Cyrys, J.; Brockow, I.; Koletzko, S.; Kramer, U.; Behrendt, H.; Herbarth, O.; von Berg, A.; Bauer, C.P. Atopic diseases, allergic sensitization, and exposure to traffic-related air pollution in children. Am. J. Respir. Crit. Care Med. 2008, 177, 1331–133
4. Sharma, N.; Patiyal, S.; Dhall, A.; Pande, A.; Arora, C.; Raghava, G.P. AlgPred 2.0: An improved method for predicting allergenic proteins and mapping of IgE epitopes. Brief. Bioinform. 2021, 22, 294.
5. Isaacs, K.K.; Goldsmith, M.-R.; Egeghy, P.; Phillips, K.; Brooks, R.; Hong, T.; Wambaugh, J.F. Characterization and prediction of chemical functions and weight fractions in consumer products. Toxicol. Rep. 2016, 3, 723–732.
6. Kimber, I.; Basketter, D.A.; Gerberick, G.F.; Ryan, C.A.; Dearman, R.J. Chemical allergy: Translating biology into hazard characterization. Toxicol. Sci. 2011, 120 (Suppl. 1), S238–S268.
7. Basu, S.; Banik, B. Hypersensitivity: An overview. Immunol. Curr. Res. 2018, 2, 1000105.
8. Wani, N.A.; Mir, M.A.; Qayoom, H.; Mehraj, U.; Nisar, S.; Sheikh, B.A.; Suhail, S. Gell and coomb's classification of hypersensitivity. In The Fundamentals of Hypersensitivities and Allergies; Nova Science Publishers Inc.: New York, NY, USA, 2020; pp. 35–74.
9. Mir, M.A.; Mehraj, U.; Nisar, S.; Sheikh, B.A.; Suhail, S.; Qayoom, H. Hypersensitivity reaction. In The Fundamentals of Hypersensitivities and Allergies; Nova Science Publishers Inc.: New York, NY, USA, 2020; pp. 1–34.
10. World Health Organization FSn307. http://www.who.int/mediacentre/ factsheets/fs307/en/index.html.
11. Asher MI, Montefort S, Bjorksten B, Lai CK, Strachan DP, Weiland SK, Williams H: Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. Lancet 2006, 368(9537):733–743.
12. .Kauppi P, Linna M, Martikainen J, Makela MJ, Haahtela T: Follow-up of the Finnish Asthma Programme 2000–2010: reduction of hospital burden needs risk group rethinking. Thorax 2012, [Epub ahead of print] PMID 22504963.
13. Wertz DA, Pollack M, Rodgers K, Bohn RL, Sacco P, Sullivan SD: Impact of asthma control on sleep, attendance at work, normal activities, and disease burden. Ann Allergy Asthma Immunol 2010, 105(2):118–123.
14. Robert A. Nathan, (2003), Critical review on pharmacotherapy for allergic rhinitis with leukotriene receptor antagonists & compared with other available diagnosis: Annals of Allergy, Asthma, & Immunology, 90(1), 182-191.
15. Evans R., (1993), Pathophysiology & natural history of asthma, allergic rhinitis & atopic dermatitis: Allergy Principles and Practice. 4th ed. St. Louis, MO: Mosby; 1993, 1109-1136.
16. Pawankar, R.; Canonica, G.W.; Holgate, S.T.; Lockey, R.F. Allergic diseases and asthma: A major global health concern. Curr. Opin. Allergy Clin. Immunol. 2012, 12, 39–41.

17. Tripathi KD,Essentials Of Medical Pharmacology,Jaypee Brothers Medical Publisers,Eighth Edition,2019.
18. IP 2007; Martindale, The extra Pharmacopoeia, 2005; Goodman and Gillman's 2001; The Merck Index 2006; Clarke's Analysis of Drugs and Poisons 2004; http://www.en.wikipedia.org/wiki/Levocetirizine.html.)
19. Jeffery G. H., Bassett J., Mendham J. and Denney R. C., (1994a), Vogel's Textbook of Quantitative Chemical Analysis: Introduction, Harlow, Essex, England: Longman group UK Ltd. 5 th edition. 3-2
20. Chatwal.RGAnand.K (2007). Textbook of instrumental methods of chemical analysis. 2.567
21. Breaux J, Jones K, Boulas P., Understanding and implementing efficient analytical methods develop- ment and validation. Pharm Technol Anal Chem Test. 2003; 5:6-13.
22. Sharma S, Goyal S, Chauhan K, A Review on Analytical method development and Validation, International Journal of Applied Pharmaceutics, 2018; 10(6):8-15.
23. Chauhan Ashish, Mittu1 Bharti and Chauhan Priyanka (2015). Analytical Method Development and Validation. A Concise Review. l. *J Anal Bioanal Tech* 6(1).
24. Shah D, Nandakumar S, Jaishankar GB, Chilakala S, Wang K, Kumaraguru U. Pre-Term Exposure Patterns in Neonatal Intensive Care Unit Alters Immunological Outcome in Neonates. J Aller Ther. 2011; 2(7)
25. Swartz ME, Krull IS, editors. Analytical method development and validation. CRC Press; 2018 Oct 3
26. Grubbs FE. Errors of measurement, precision, accuracy and the statistical comparison of measuring instruments. Technometrics. 1973; 15(1):53-66.
27. Karnes HT, March C. Precision, accuracy, and data acceptance criteria in biopharmaceutical analysis. Pharmaceutical research. 1993; 10(10):1420-6
28. Sethi PD, HPLC Quantitative Anal-ysis of Pharmaceutical Formulations. 1st Edn., New Delhi: CBS Publishers & Distributors (2001)
29. Mohammad Tet al., HPLC Method Development and Validation for Pharmaceutical Analysis- A Review. Internationale Pharmaceutica Sciencia. 2012; 2(3): 14
30. Snyder LR, Kirkland JJ and GlajchJL.In:PracticalHPLC Method Development. 2nd ed, 2001
31. Vibha G et al., Development and validation of HPLC method - a review. International Research Journal of Pharmaceutical and Applied Sciences. 2012; 2(4): 22-23
32. Bliesner DM. In: Validating Chromatographic Methods. John Wliey& sons Inc. 2006, 88-92.
33. F. Bressolleetal. , Validation of liquid chromatographic and gas chromatographic methods Applications to pharmacokinetics, JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS, B 686 (1996) 3 10.
34. Ramamurthy, M.andSarvanakumar.K; pharmaceutical validation. The Eastern Pharmacist. 1997, XL, 476, 45-47.
35. Glajch JL, Kirkland JJ. In:PracticalHPLC Method Development. 2nd ed, 2001.
36. Available form: http://www.transfusionguidelines.org.uk/docs/pdfs/oig_ method_validation_procedure_01.pdf [Last accessed on 2011 March 21].
37. Tamaoka J and Komagata K. Determination of DNA base composition by reversed-phase high-performance liquid chromatography. FEMS Microb let. 1984.
38. Svec F and Frechet MJJ. Continuous rods of macroporous polymer as high-performance liquid chromatography separation media. Anal Chem. 1992;64:820-822.
39. Shintani H. Validation Study in membrane chromatography adsorber and phenyl hydrophobic membrane chromatography adsorber for virus clearance and removal of many other components. Pharm Anal Acta. 2013;S2:005.
40. Badgujar DC, et al. Pathogenicity of mutations discovered in BRCA1 BRCT domains is characterized by destabilizing the hydrophobic interactions. J Cancer SciTher. 2012;4:386-393. 55. Ukuku DO, et al. Effect of thermal and radio frequency electric fields treatments on Escherichia coli bacteria in apple juice. J MicrobBiochem Technol. 2012;4:76-81.
41. Available from: http://www.reagecon.com/TechPapers/ guidemethodvalidation.pdf [Last accessed on 2011 March 21].
42. Sethi PD, HPLC Quantitative Anal-ysis of Pharmaceutical Formulations. 1st Edn., New Delhi: CBS Publishers & Distributors,1996; 113203.
43. Agalloco J. Validation: an unconventional review and reinvention, PDA J. Pharm Sci Tech 1995; 49, 175–179.

44. Aleem H, Zhao Y, Lord S, McCarthy T and Sharratt P; Pharmaceutical process validation: an overview, J Proc Mech Eng 2003; 217, 141-151.
45. Lambert J. Validation Guidelines for Pharmaceutical Dosage Forms. Health Canada / Health Products and Food Branch Inspectorate 2004; 7-15.
46. Prabh Simran Singh, Gagan Shah. Analytical Method Development and Validation. Journal of Pharmacy Research 2011; 4(7): 2330-2332.
47. TangriPranshu, Rawat Prakash Singh, Jakhmola Vikash: Validation: A Critical Parameter for Quality Control of Pharmaceuticals. Journal of Drug Delivery & Therapeutics 2012; 2(3): 34-40.
48. Webster P. Analytical procedures and method validation. Enviormental protection agency; 2001
49. Mohamad T, Mohamad MA, Chattopadhyay M. Particle size role, Importance and Strategy of HPLC Analysis An update. International Archives of BioMedical and Clinical Research. 2016; 2(2): 5-11.
50. Weston A, Brown PR. HPLC and CE Principles and Practice. Academic press California; 1997.
51. Ngwa G. Forced Degradation Studies. Forced Degradation as an Integral part of HPLC Stability Indicating Method Development. Drug Delivery Technology. 2010; 10(5).
52. Analytical chemistry- R. M. Verma-2016.
53. Lin G, et al. Determination of sodium tanshinoneiia sulfonate in rat plasma by high performance liquid chromatography and its application to pharmacokinetics studies. Pharm Anal Acta. 2015;6:383.
54. Analytical chemistry-Christian-2009:347.
55. High performance liquid chromatography- P. D. Sethi.
56. Thammana M. A review on high-performance liquid chromatography (HPLC). Res Rev: J Pharm Anal 2016;5:22-8.
57. Malviya R, Bansal V, Pal OP, Sharma PK. High-performance liquid chromatography: a short review. JGPT 2010;2:22-6.
58. Kazakevich YV. HPLC method development for pharmaceuticals. Sep Sci Technol2007;8:13–44.
59. Bhardwaj SK, Dwivedi K, Agarwal DD, Analytical S. A review on analytical method development and validation. Int J Appl Pharm 2018;10:8-15.
60. Azim Md Sabir, Mitra Moloy, Bhasin Parminder S. HPLC method development and validation. Int Res J Pharm 2015;5:76–81.
61. Jimidar MI, De Smet M. HPLC method development for pharmaceuticals; 2007.
62. Snyder LR, Glajch JL, Kirkland JJ. Practical HPLC method development. Vol. 2nd ed.; 1997
63. Elshanawane AA, et al. Development and validation of HPLC method for simultaneous estimation of brimonidine tartrate and timolol maleate in bulk and pharmaceutical dosage form. J Chromatograph SeparatTechniq. 2014;5:230.
64. Mustafa S, et al. An improved high performance liquid chromatographic method for tryptophan analysis in rat brain administrated by seaweed. J Anal Bioanal Tech. 2014;5:188.
65. Caglar S and Alp AR. A validated high performance liquid chromatography method for the determination of saxagliptin and metformin in bulk, a stability indicating study. J Anal Bioanal Tech. 2014;S12:010
66. Abdallah MA. Validated stability-indicating hplc and thin layer densitometric methods for the determination of pazufloxacin: application to pharmaceutical formulation and degradation kinetics. J Chromatograph SeparatTechniq. 2014;5:218.
67. deFigueiredo NB, et al. Determination of 3,4-methylenedioxymethamphetamine (mdma) in confiscated tablets by high-performance liquid chromatography (HPLC) with diode array detector. J Forensic Res. 2010;1:106.
68. Shah I, et al. A novel method for determination of fenofibric acid in human plasma using HPLC-UV: application to a pharmacokinetic study of new formulations. J Anal Bioanal Tech. 2014;S12:009.
69. Gurupadayya BM and Disha NS. Stability indicating hplc method for the simultaneous determination of ceftriaxone and vancomycin in pharmaceutical formulation. J Chromatograph SeparatTechniq. 2013;4:207.
70. Shintani H. HPLC separation of amino acids is appropriate? Pharmaceut Anal Acta. 2013;4:e158.
71. Akan JC, et al. Determination of organochlorine, organophosphorus and pyrethroid pesticide residues in water and sediment samples by high performance liquid chromatography (HPLC) with UV/visible detector. J Anal Bioanal Tech. 2014;5:226

72. Parbhunath OL, et al. Optimization and validation of a reverse-phase high performance liquid chromatography assay with ultra-violet detection for measuring total l-ascorbic acid in food and beverage products. J Anal Bioanal Tech. 2014;5:201
73. Szterk A, et al. Comparison of various detection systems coupled to high performance liquid chromatography for determination of tocopherols in meat. The influence and comparison of the most popular sample preparation method. J Anal Bioanal Tech. 2013;S2:005.
74. Lories IB, et al. High performance liquid chromatography, TLC densitometry, first-derivative and first-derivative ratio spectrophotometry for de-termination of rivaroxaban and its alkaline degradates in bulk powder and its tablets. J Chromatograph SeparatTechniq. 2013;4:202.
75. Chierentin L and Nunes Salgado HR. Development and validation of a simple, rapid and stability-indicating high performance liquid chromatography method for quantification of norfloxacin in a pharmaceutical product. J Chromat Separation Techniq. 2013;4:171.
76. Srinivasarao K, et al. Validated method development for estimation of formoterol fumarate and mometasone furoate in metered dose inhalation form by high performance liquid chromatography. J Anal Bioanal Tech. 2012;3:153.
77. Sun H, et al. A Rapid and effective method for simultaneous determination of residual sulfonamides and sarafloxacin in pork and chicken muscle by high performance liquid chromatography with accelerated solvent extraction–solid phase extraction cleanup. J Chromat Separation Techniq. 2012;3:154.
78. Virkar PS, et al. Development and validation of a high performance liquid chromatography method for determination of telmisartan in rabbit plasma and its application to a pharmacokinetic study. J Anal Bioanal Tech. 2012;3:133.
79. Gugulothu DB, et al. A versatile high performance liquid chromatography method for simultaneous determination of three curcuminoids in pharmaceutical dosage forms. Pharmaceut Anal Acta. 2012;3:156.
80. Devika GS, et al. Simultaneous determination of eprosartan mesylate and hydrochlorthiazide in pharmaceutical dosage form by reverse phase high performance liquid chromatography. Pharm Anal Acta. 2011;2:122
81. Dwivedia, S.K.; Agarwala, D.D. A Review: HPLC Method Development and Validation. Int J Analytical Bioanalytical Chem 2015;5(4):76-81.
82. Sultana, N.; Arayne, S.; Shah, S.N. Development and validation for the simultaneous quantification of prazosin, amlodipine, diltiazem and verapamil in API, dosage formulation and human serum by RP-HPLC: application to in vitro interaction studies. Med Chem 2014;4:770-7.
83. Lu M, et al. Hydrophobic fractionation enhances novel protein detection by mass spectrometry in triple negative breast cancer. J Proteomics Bioinform. 2010;3:029-038.
84. Qiao G, et al. Modified a colony forming unit microbial adherence to hydrocarbons assay and evaluated cell surface hydrophobicity and biofilm production of vibrio scophthalmi. J BacteriolParasitol. 2012;3:130
85. Wiese S., Teutenberg T. and Schmidt T. C. (2011), A general strategy for performing temperature-programming in high performance liquid chro-matography—Prediction of segmented temperature gradients, Journal of Chromatography A, 10. (39), 6898-6906. https://doi.org/10.1016/j.chroma.2011.08.022
86. Devika GS, et al. Simultaneous determination of eprosartan mesylate and hydrochlorthiazide in pharmaceutical dosage form by reverse phase high performance liquid chromatography. Pharm Anal Acta. 2011;2:122
87. Dwivedia, S.K.; Agarwala, D.D. A Review: HPLC Method Development and Validation. Int J Analytical Bioanalytical Chem 2015;5(4):76-81.
88. Lu M, et al. Hydrophobic fractionation enhances novel protein detection by mass spectrometry in triple negative breast cancer. J Proteomics Bioinform. 2010;3:029-038.
89. Qiao G, et al. Modified a colony forming unit microbial adherence to hydrocarbons assay and evaluated cell surface hydrophobicity and biofilm production of vibrio scophthalmi. J BacteriolParasitol. 2012;3:130
90. Wiese S., Teutenberg T. and Schmidt T. C. (2011), A general strategy for performing temperature-programming in high performance liquid chro-matography—Prediction of segmented temperature gradients, Journal of Chromatography A, 10. (39), 6898-6906.
91. Owen A.J. (1996). Fundamentals of UV-Visible spectroscopy: In: Principles and applications of UV-Visible spectroscopy, Germany: Hewlett-Packard. 10- 18.

92. Kirk G. F., (1966), Development & Publication of new spectrophotometric methods of analysis: Talanta, 13(1), 1-14.
93. L.D.S. YADAV,OraganicSpectroscopy,originally published by Kluwer academic publishers in 2005, 43-44.