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Development of Identification Irbesartan Drug Irovel 150 Mg by Utilising HPLC

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Abstract

Analytical chemistry uses high-performance liquid Chromatography (HPLC), originally known as high pressure liquid Chromatography, to separate, identify, and quantify components in mixtures. The mixtures may come from liquid solutions containing food, chemicals, medicines biological, environmental, and agricultural materials, among other sources. In addition to a range of other human and animal studies, the development and validation of HPLC techniques play crucial roles in the discovery, development, and production of innovative drugs. This article covers the use of HPLC to separate the medication Irbesartan. Evaluating the quantity of drug is one way to perform drug quality assurance, which is necessary to ensure the safety and quality of pharmaceutical products. In this work, a stability indicating RP-HPLC technique for estimating Irbesartan degradation and process-related contaminants is developed and validated. An addition to pharmaceutical preparations it can also be used for biological matrices samples that require a small amount of sample analysis. The assay and impurity levels in bulk active and prepared medicinal products are frequently determined using high performance liquid Chromatography (HPLC). To demonstrate the capacity to identify a broad range of impurities that may arise in pharmaceuticals, impurity profile analyses are necessary. HPLC instrumentation consists of a solvent reservoir, pump, injector, column, detector, integrator, or acquisition and display system. The brain of the system is the column where separation occurs. Among other things, HPLC can be used to identify, quantify, and resolve a compound. For the purpose of quality control of medication components, it is the most adaptable, secure, trustworthy, and rapid chromatographic technology available.

Keywords: HPLC; Irbesartan; Chromatography; Hypertension; Sonicator

Abbreviations

HPLC: High-Performance Liquid Chromatography; IEC: Ion-Exchange Chromatography; TLC: Thin-Layer Chromatography; CC: Column Chromatography; GC: Gas Chromatography; HIC: Hydrophobic Interaction Chromatography; IMAC: Immobilized Metal Affinity Chromatography; SEC: Size Exclusion Chromatography; NYHA: New York Heart Association; AUC: Area Under Curve; GSC: Gas-Solid Chromatography; GLC: Gas-Liquid

Chromatography; ODS: Octadecyl-Silica; TMS: Trimethyl Silane.

Introduction

Analytical chemistry is used to determine the qualitative and quantitative composition of material under study. Both these aspects are necessary to understand the sample material. Analytical chemistry is divided into two branches, quantitative and qualitative. A qualitative analysis gives us information about the nature of samples by knowing about the presence or absence of certain components. A quantitative analysis provides numerical information as to the relative amount of one or more of this component [1].

Qualitative analysis has been related with response of binary nature, today, thanks to the development of chemometrics, qualitative analysis can be considerate a classificatory analysis [2]. HPLC is a form of column Chromatography that pumps at high pressure a sample (analyte) dissolved in a solvent (mobile phase) through a column with an immobilized Chromatographic packing material (stationary phase). The properties of the sample and the solvent, as well as the nature of the stationary phase, determine the retention time of the analytes, or how fast they pass through the column. As the sample passes through the column, analytes having the strongest interactions with the stationary phase exit the column the slowest, meaning they exhibit the longest retention time [3].

Chromatography

Chromatography is a physical method of separation of the mixture into its individual Components. It is used as an analytical technique to get information about what is present in the mixture, how much the individual compound is in mixture. It is also used as a purification method to separate and collect the components of mixture. Chromatography is Greek word where chromates mean color and graphs means writing. So, basically Chromatography is colour writing process. The main advantage that differentiates Chromatography from most other chemical and physical separation methods is that two mutually immiscible phases are brought into contact one is stationary phase and the other mobile [4]. The main advantage that differentiates Chromatography from most other chemical and physical separation methods is that two mutually immiscible phases brought into contact one is stationary phase and other mobile [5].

Types of Chromatography

- Column Chromatography
- Ion-exchange chromatography
- Gel-permeation (molecular sieve) Chromatography
- Affinity Chromatography
- Paper Chromatography
- Thin-layer Chromatography
- Gas Chromatography
- Dye-ligand chromatography
- Hydrophobic interaction Chromatography
- Pseudo affinity Chromatography
- High-pressure liquid Chromatography (HPLC) [6].

Column Chromatography: Column Chromatography is a technique which is used to separate a single chemical compound from a mixture dissolved in a fluid. It separates substances based on differential adsorption of compounds to the adsorbent as the compounds move through the column at different rates which allow them to get separated infractions. This technique can be used on small scale as well as large scale to purify materials that can be used in future experiments. This method is a type of adsorption Chromatography techniques [5].

Figure 1: Column Chromatography.

Ion-Exchange Chromatography: Ion-exchange Chromatography (IEC) is part of ion Chromatography which is an important analytical technique for the separation and determination of ionic compounds, together with ion-partition/interaction and ion-exclusion Chromatography. Ion Chromatography separation is based on ionic (or electrostatic) interactions between ionic and polar analytes, ions present in the eluent and ionic functional groups fixed to the Chromatographic support. Two distinct mechanisms as follows; ion exchange due to competitive ionic binding (attraction) and ion exclusion due to repulsion between similarly charged analyte ions and the ions fixed on the Chromatographic support, play a role in the separation in ion Chromatography [7].

Figure 2: Ion-exchange chromatography.

Gel-Permeation (Molecular Sieve) Chromatography: Gel permeation Chromatography basically belongs to a category of size exclusion Chromatography. The basic mechanism is the separation based on the size of the particles .The nature of solvent in usually organic. Polymers are generally analyzed by this technique. This technique was first investigated by J. C. Moore in 1964 and was also commercialized in the same year by Waters Corporation. The separation process in Gel permeation Chromatography is built on the radius of gyration of the particles that are to be separated. Porous beads packed in column are used for separation [8].

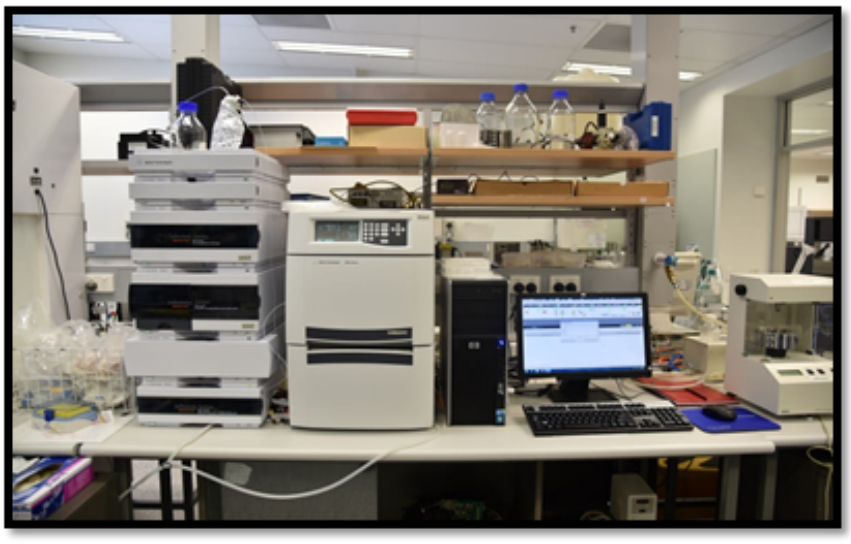


Figure 3: Gel-permeation (molecular sieve) chromatography.

Affinity Chromatography: The combination of bio affinity and Chromatography gave birth to affinity Chromatography. Affinity Chromatography is a type of liquid Chromatography that makes use of biological like interactions for the separation and specific analysis of sample components. The technique offers high selectivity, hence high resolution, and usually high capacity for the protein of interest. Purification can be in the order of several thousand-fold and recoveries of active material are generally very high. Affinity Chromatography (also called affinity purification) makes use of specific binding interactions between molecules. A particular ligand is chemically immobilized or "coupled" to a solid support so that when a complex mixture is passed over the column, those molecules having specific binding affinity to the ligand become bound [9].

Paper Chromatography: The stationary phase and the mobile phase are both liquids (partition Chromatography), the polar adsorbed water in the paper acts as the stationary phase in a 2D plate. The dissolving sample is placed as a small spot one half inch from the edge of filter paper and left to dry. The dry spot will be held at the front end in a closed chamber saturated with atmosphere, and the end closer to the sample contacts the solvent, which moves up or down by the capillary action (depending on the mode of action whether ascending means moves up along the paper or descending that moves down due to high viscosity of thus mobile phase) [10].

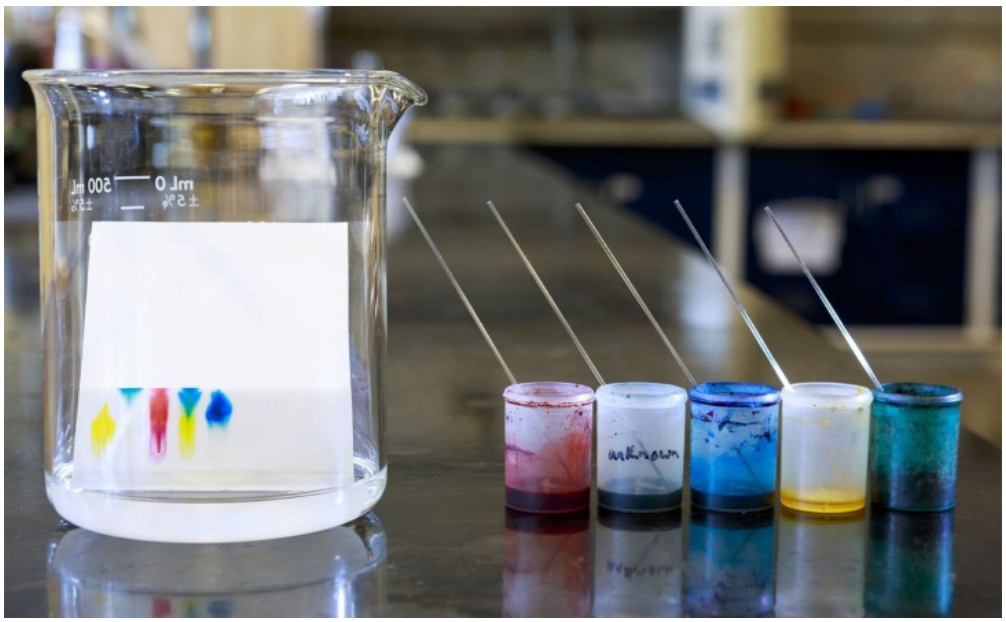


Figure 4: Paper chromatography.

Thin-Layer Chromatography: The attractive features of TLC include parallel sample processing for high sample throughput; accessibility of the sample for post Chromatographic evaluation free of time constraints; detection in the presence of the stationary phase (is somewhat) independent of mobile phase properties; and the stationary phase is normally used once only. It is generally agreed that thin layer Chromatography is most effective for the low-cost analysis of samples requiring minimal sample clean up or were. Thin-layer and high-pressure liquid Chromatography should be viewed as complementary techniques that can be distinguished by their different attributes, resulting in a preference for one approach over the other on a problem-by-problem basis [11].

Gas Chromatography: Modern Gas Chromatography was invented by James & Martin. Since the early 1950's this technique was first used for the separation of amino acids. Now GC has a large number of applications as this technique is rapid & has a great sensitivity. Both qualitative & quantitative analysis can be done through GC .a stationary phase and a mobile phase. The mobile phase is a chemically inert gas such as helium, nitrogen etc. Gas Chromatography is one of the unique forms of Chromatography that does not need the mobile phase for interacting with the analyte. The stationary phase is either solid adsorbent, termed gas-solid Chromatography (GSC), or a liquid on an inert support, termed gas-liquid Chromatography (GLC). The Criteria for the compounds to be analyzed in GC is volatility & thermos stability [12].

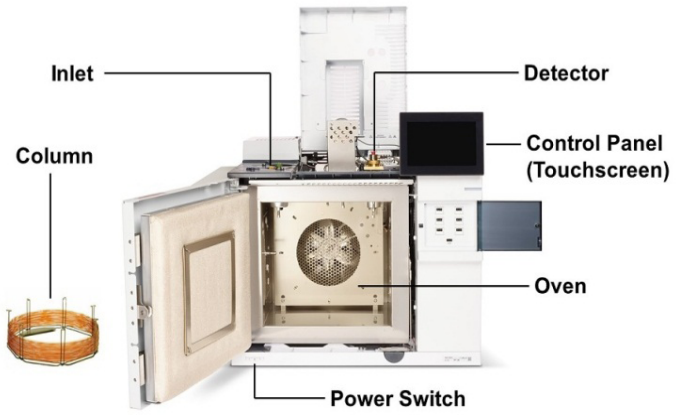


Figure 5: Gas chromatography.

Dye-Ligand Chromatography: Recent progress in the field of dye-ligand affinity Chromatography has improved our understanding of protein-dye interactions and enabled the design and synthesis of novel synthetic dye-ligands with improved selectivity, the biomimetic dye-ligands (3–14). According to the biomimetic dye-ligand concept, new dyes that mimic natural ligands of the targeted proteins are designed, after substitution of the terminal 2-aminobenzene sulfonate moiety of Cibacron Blue 3GA for a substrate-mimetic moiety [13].

Hydrophobic Interaction Chromatography: The proteins can be retained when eluent has relatively high salt concentration and eluted when concentration of salts drops. This method of protein separation, which is a variant of reversed phase Chromatography, is known as hydrophobic interaction Chromatography (HIC). Due to hydrophobic interactions, hydrophobic molecules undergo self-association in aqueous solvent. Hydrophobic interactions are of prime importance in biological molecules and are the driving force behind folding of globular protein, in association of protein subunits and in the binding of many small molecules to protein [14].

Pseudoaffinity Chromatography: Some compounds as anthraquinone dyes, and azo dyes can be used as ligands because of their affinity especially for dehydrogenases, kinases, transferases, and reductases the mostly known type of this kind of Chromatography is immobilized metal affinity Chromatography (IMAC) [6].

High-Pressure Liquid Chromatography (HPLC): High-Performance Liquid Chromatography, also known as High Pressure Liquid Chromatography, is a type of column Chromatography that is commonly used in biochemistry and analysis to separate, identify, and quantify active chemicals. It is a popular analytical technique for separating, identifying, and quantifying each element of a mixture. HPLC is a sophisticated column liquid Chromatography technology. The solvent normally flows through the column due to gravity, but in the HPLC process, the solvent is pushed under high pressures of up to 400 atmospheres so that the sample can be separated into different constituents based on differences in relative affinities [15].

Figure 6: High-pressure liquid chromatography (HPLC).

Principle of HPLC

HPLC principle is that solution of sample is injected into a column of porous material (stationary phase) and liquid phase (mobile phase) is pumped at higher pressure through the column. The principle of separation followed is the absorption of solute on stationary phase based on its affinity towards stationary phase HPLC is a special branch of column Chromatography in which the mobile phase is forced through the column at high speed. As a result, the analysis time is reduced by 1-2 orders of magnitude relative to classical column Chromatography and the use of much smaller particles of the adsorbent or support becomes possible increasing the column efficiency substantially [16].

History of HPLC

Preceding HPLC researchers utilized normal liquid natural action ways. Liquid natural action systems were to Associate in Nursing inefficient thanks to the rate of solvents being dependent on gravity Separations took numerous hours, and a few of the time days to complete. Gas natural process (GC) Fat the time was simpler than liquid natural process (LU), in any case, it had been trusty that gas stage partition and investigation in of extraordinarily polar high mass biopolymers was not possible. GC was ineffectual for a few organic chemists because of the thermal instability of the solutes, consequently, various techniques were hypothesized which might shortly bring about the advancement of HPLC [17].

Types of HPLC

Normal Phase Chromatography: In normal phase Chromatography, mobile phase is non-polar and stationary phase is Polar. Hence, the station phase retains the polar analyte. An increase in polarity of solute molecules increases the adsorption capacity leading to an increased elution time. Chemically modified silica (cyanopropyl, aminopropyl and diol) is used as a stationary phase in this Chromatography. For example A typical column has an internal diameter of around 4.6 mm, and a length in the range of 150 to 250 mm. Polar compounds in the mixture that are passed through the column will stick longer to the polar silica than the non-polar compounds [18].

Reverse Phase HPLC: Reversed phase chromatography has found both analytical and preparative applications in the area of biochemical separation and purification. Molecules that possess some degree of hydrophobic character can be separated by reversed phase Chromatography with excellent recovery and resolution Uses water organic as mobile phase, columns may be C (ODS), C phenyl, Trimethyl Silane (TMS), cyano as a stationary phase. It is the first choice for most samples, especially neutral or non-ionized Compound, that dissolve in water organic mixtures [19].

Size-exclusion HPLC: Size exclusion Chromatography (SEC), also called gel permeation Chromatography or gel filtration Chromatography mainly separates particles on the basis of size. It is also useful for determining the tertiary structure and quaternary structure of proteins and amino acids. This technique is widely used for the molecular weight determination of polysaccharides.

Ion-exchange HPLC: In Ion-exchange Chromatography, retention is based on the attraction between solute ions and charged sites bound to the stationary phase. Ions of the same charge are excluded. This form of Chromatography is widely used in purifying water, Ligand-exchange Chromatography, Ion-exchange Chromatography of proteins, High-pH anion-exchange Chromatography of carbohydrates and oligosaccharides, etc [20].

Bio-affinity Chromatography: Separation is based on specific reversible interaction of proteins with ligands. Ligands are covalently attached to solid support on a bio-affinity matrix, retaining proteins with interaction to the column-bound ligands. Proteins bound to a bio-affinity column can be eluted in two ways:

- Bio specific elution: inclusion of free ligand in elution buffer which competes with column bound ligand.
- A specific elution: change in pH, salt, etc. which weakens interaction protein with column-bound substrate.

Because of specificity of the interaction, bio-affinity Chromatography can result in very high purification in a single step (10- 1000fold) [21].

Instrumentation of HPLC

- Solvent Reservoir
- Pump
  - High pressure-1000 to 5000 psi
- Injector
  - Low pressure stops the flow
  - High pressure value
- Column
  - Normal Phase-organic (water-free) mobile phase
  - Silica gel-non-aqueous
  - Adsorption
  - Reverse phase (CR. C18) aqueous mobile phase
  - Partitioning
  - Ion-exchange-aqueous mobile phase
  - Molecular sieve aqueous mobile phase
  - Size
- Detector
  - Specific
  - Absorbance
  - Fluorescence
  - Electrochemical
  - Non-specific
  - Refractive Index
  - Radioactivity

- Conductivity [22].

Solvent Reservoir: Mobile stage substances are contained in a glass reservoir. The versatile stage, or dissolvable, in HPLC is typically a blend of polar and non-polar liquid segments whose particular fixations are changed relying upon the arrangement of the specimen [23].

Figure 7: High-performance liquid chromatography.

Injection of the Samples: A device such as a rotary valve that allows the introduction of a sample solution in the HPLC column under high pressure and flow conditions. An injection valve can be a standalone manual valve, or part of an auto sampler. It typically consists of a needle port, a rotor and stator combination, and an exchangeable sample loop [24].

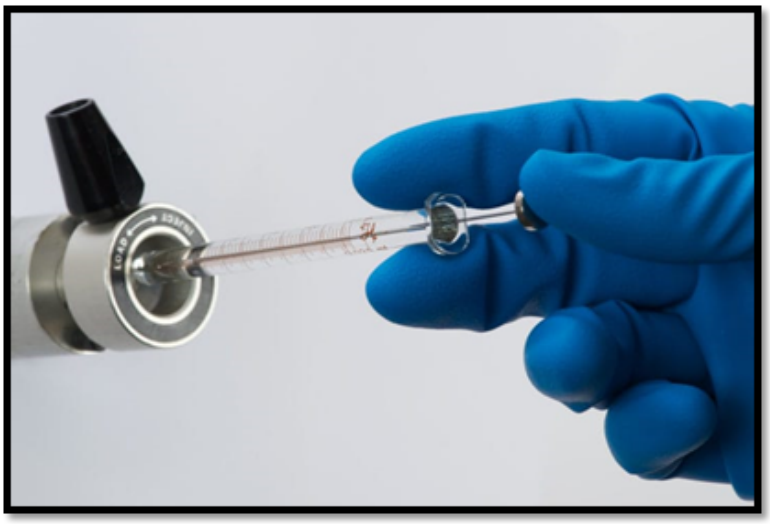


Figure 8: Injection of the samples.

Pump: The most important component of HPLC in solvent delivery system is the pump, because its performance directly affects the retention time, reproducibility and detector sensitivity. The particle size of the stationary phase is 5-10 µm. So, resistance to flow is observed. This is the reason that high pressure is required, and this is provided by using pumps the different types of pumps include: Constant pressure pump which uses a constant pressure to the mobile phase, the flow rate through the column is determined by the flow resistance of the column and any other restrictions between the pump and the detector outlet. Constant flow pump generates a given flow of liquid, so that the pressure

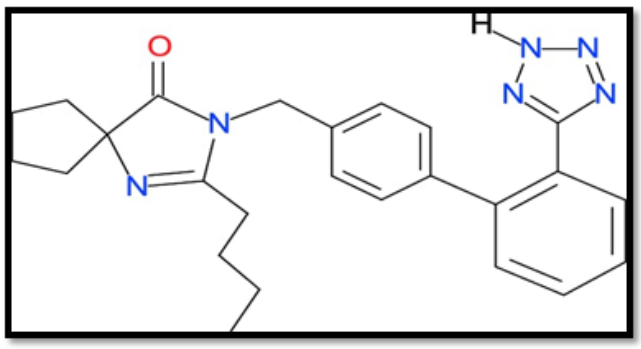
developed depends on the flow resistance [25].

Column: HPLC system consists of a simple flow through sequence of one or a few columns. If this sequence is branched and switching devices are employed to interface individual columns, it then becomes possible to exert powerful control on the quality and the basis for the separation and LC becomes a versatile and powerful separation system [26].

Detector: The detector has the ability to identify (detect) every molecule that elutes (comes out) from the column. In order for the chemist to quantitatively examine the sample components, a detector measures the quantity of those molecules. The liquid chromatogram, or graph of the detector response, is produced by the detector as an output to a recorder or computer. When a substance has travelled through the column, it can be determined in a number of different ways. UV spectroscopy is typically used to detect particular chemicals. Numerous organic substances absorb UV light of different wavelengths [27].

Drug Profile

Drug name: Irovel 150 Tablet
Active ingredient: Irbesartan
Category: Angiotensin II receptor antagonist
Structure:



IUPAC Name: 2-butyl-3-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl] methyl]-1, 3-diazaspiro [4.4] non-1-en-4one.

Irbesartan: Irbesartan (Irb) is an angiotensin II (AT1 receptor) inhibitor which is used to treat hypertension alone or in combination with other drugs. The molecular weight of Irbesartan is 428.5 g/mol. The log P value of this drug is 10.1 and its quantitative solubility in water is 5.9 × 10−2 mg/ L at 25°C. This low solubility is a challenge in the drug development process as it leads to a poor drug release profile and low therapeutic effect [28].

Pharmacodynamic properties: A single dose of Irbesartan 100 or 200mg reduced pulmonary capillary wedge pressure and systemic vascular resistance in 20 patients with heart failure [New York Heart Association (NYHA) Class II to IV]. Aortic wall and cardiac hypertrophy were reduced in spontaneously hypertensive rats treated with Irbesartan.

Pharmacokinetics properties: The bioavailability of Irbesartan is 60 to 80% after oral administration and is not affected by food. After administration of Irbesartan 50 to 300mg, maximum plasma drug concentrations (Cmax) are achieved within 1 to 2 hours (Tmax). The pharmacokinetic profile of Irbesartan is not markedly influenced by gender [29].

Use of Irbesartan: Irbesartan belongs to this group of drugs and is approved for the treatment of hypertension, and is indicated for lowering BP either alone or in combination with other antihypertensive agents. The antihypertensive efficacy of angiotensin II receptor antagonists in patients with mild to moderate hypertension has been evaluated and compared with ACE inhibitors, calcium antagonists, beta-blockers, and diuretics in several studies [30].

Irbesartan Safety and Tolerability: Irbesartan demonstrated a placebo like tolerability profile, even at the highest doses administered. An integrated analysis combined all safety data from nine placebo controlled, 4 to 12-week Irbesartan mono therapy studies [31].

Dosage and Administration: Irbesartan is available as tablets (75, 150 and 300mg). Irbesartan may be administered with other antihypertensive agents and with or without food. For treatment of hypertension, the recommended initial dose of Irbesartan is 150mg once daily. The dosage can be increased to a maximum dose of 300mg once daily as needed to control blood pressure. For treatment of nephropathy in type 2 diabetic patients, the recommended dose is 300mg once daily. In dose adjustment in volume and salt depleted [32].

Material of Method

Figure 9: Ultrasonic cleaner (sonicator).

Sr. No.	Equipment's
1	HPLC
2	Micro pipette
3	UltraSonic cleaner
4	Proline plus pipette
5	Weighing machine (mettler Toledo)
6	Column zodiac C8 analytical (5× 150mm×4.6mm)

Table 1: Equipments.

Sr. No.	Chemicals
1	HPLC grade ammonium format
2	HPLC grade acetone nitrate
3	HPLC grade methanol
4	HPLC grade deionised water
5	Ammonium format

Table 2: Chemicals.

Drug Name: Irovel 150 mg Tablet

Procedure

Figure 10: Irovel 150 mg Tablet.

Preparation of sample: Irbesartan formulation tablets (Irovel 150mg) were crushed to produce a finely powdered. 5 mg of Irbesartan powder, 1 ml of acetone nitrate is added to make it soluble in acetonitrile it gets partially soluble and to make it completely soluble 1 ml of methanol is added in it. Methanol becomes fully soluble then again 2 ml of methanol is added and 1 ml of HPLC grade deionized water is added in it. The flask was shaken to dissolve the drug, and UltiPro N66 Nylon 6, 6 membrane sample filter paper was used to filter the mixture after the preparation of sample sonication is done for one minute.

Blank sample preparation: Acetonitrile 1ml is added with 3 ml of methanol and deionized water 1ml in it. Sonication is done for one minute.

Preparation of mobile phase: Solvent A; 270 ml deionised water is added with 30ml methanol is added with 283 mg ammonium format Solvent B: 210 ml of acetonitrile is added with 90 ml of methanol. Sonicate both the solvent A and solvent B with the help of sonicator.

Working of HPLC

- Switch on a machine and connect tube A and B with solvent A and solvent-B.
- Then open the purging wall for washing of pump when purging wall is open 25% of B solvent and 75% of a solvent is used for removing water soluble impurities. 80% of B solvent and 20% of A.
- Solvent is used to remove organic components.
- Then blank sample is loaded in syringe for washing.
- After washing is done then Irbesartan sample is loaded with syringe.
- After the loading of sample knob was closed.
- Before closing the knob, the machine is set at auto zero to obtain the graph.

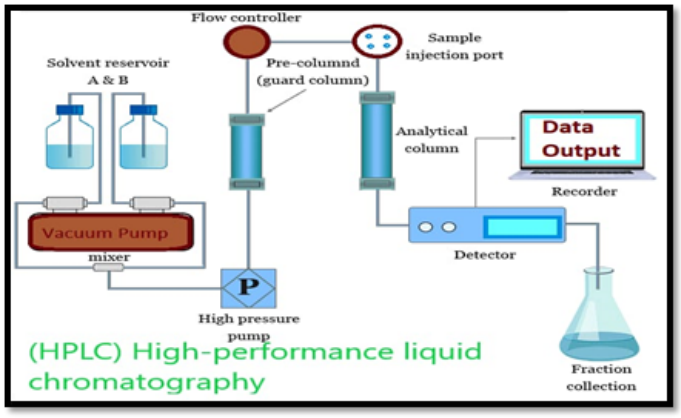


Figure 11: HPLC working.

Result and Discussion

Result

Sample Information		
Sr. No.	**Acquired by**	**Admin**
1	Sample Name	IRBESARTAN_MIBP83_08062023
2	Sample ID	IROVET150_VAISHNAVI_BAWANE
3	Vail#	**1**
4	Injection Volume	20 uL
5	Data Filename	IRBESARTAN_MIBP8_3_08062023.lcd
6	Method Filename	CILNIRBENEBI_RPHPLC.lcm
7	Batch Filename	-
8	Report Filename	Default.lcr
9	Date Acquired	06-08-2023 19:46
10	Data Processed	06-08-2023 20:06

Table 3: Sample Formulation.

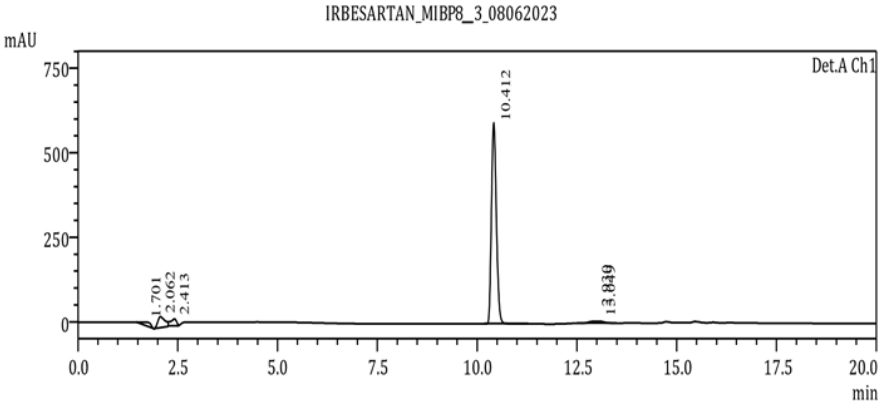


Figure 12: Det.A Ch1 / 230nm.

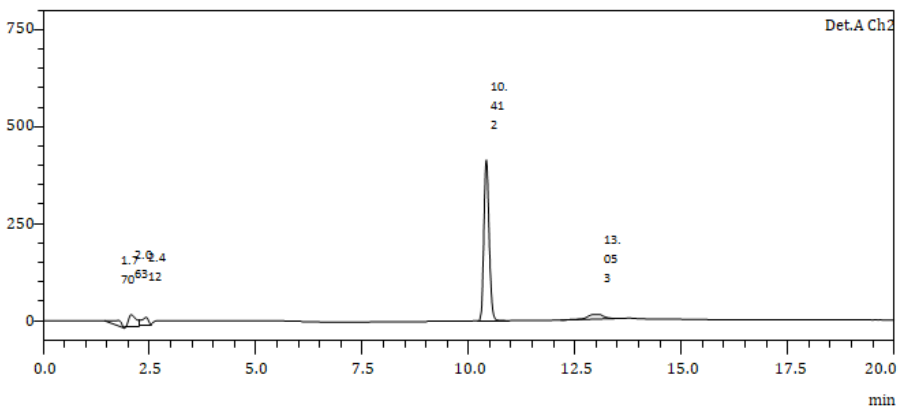


Figure 13: Det.A Ch2 / 250nm.

Peak Tables

Peak#	Ret. Time	Area	Theoretical Plate#	Area %	Tailing Factor	k'
1	1.701	183114	259.447	2.999	0.904	0
2	2.062	421304	467.034	6.9	0	0.212
3	2.413	225113	604.219	3.687	0	0.419
4	10.412	5122032	31132.277	83.889	1.231	5.123
5	12.93	71768	206.801	1.175	0	6.603
6	13.049	82404	1336.375	1.35	0	6.673
Total	-----	6105735	-----	100	-----	-----

Table 4: Detector A Ch1 230nm.

Peak#	Ret. Time	Area	Theoretical Plate#	Area %	Tailing Factor	k'
1	1.77	203001	249.719	4.28	0.693	0
2	2.063	405125	473.936	8.542	0	0.166
3	2.412	216089	571.112	4.556	0	0.363
4	10.412	3580212	31055.487	75.484	1.233	4.884
5	13.053	338568	7150.693	7.138	0.784	6.376
Total	-----	4742995	-----	100	-----	-----

Table 5: Detector A Ch2 250nm.

Discussion

HPLC is used in quality control to ensure the purity of raw materials, to control and improve process yields, to quantify assays of final products, or to evaluate products stability and monitor degradation. It is used for analyzing air and water pollutants. Federal and state regulatory agencies use HPLC to survey food and drug products. And Irbesartan drug we take it because Irbesartan is a helpful therapeutic option for treating patients with hypertension and diabetic nephropathy. It will be especially helpful for people who are intolerant to or do not respond well to anti-hypertensive medications from other pharmacological groups. For patients with mild-to-moderate hypertension, heart failure, myocardial infarction, and diabetic nephropathy, Irbesartan may be a suitable option for first-line treatment. The absorption spectrum was recorded over the range of 200–400 nm. The spectrum shows that Irbesartan exhibited two maxima at 257.4 and 202.6 nm. Irbesartan shows maximum absorbance at 224 nm. For the AUC method, the wavelength ranges between 225-230 nm and 258-265 nm respectively were selected with reference to the absorbance curves plotted between the wavelengths of 200-400 nm.

Conclusion

It was concluded that given work is effective with precised given procedure as well ensured the purity of ingredients, handling and improving process yields, quantifying tests of finished goods, assessing product stability, and keeping an eye on deterioration are all done with HPLC in quality control. It is employed to analyse contaminants in the air and water. HPLC is used by federal and state regulatory bodies to inspect food and pharmaceutical items. Additionally, we take the medication irbesartan because it is a beneficial therapeutic alternative for the treatment of diabetic nephropathy and hypertension in patients. It will be especially beneficial for those who do not respond well to anti-hypertensive drugs from other pharmacological categories or who are intolerant to them. Irbesartan may be a good choice for first-line therapy in individuals with mild-to-moderate hypertension, heart failure, myocardial infarction, and diabetic nephropathy.

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