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Analysis of Pharmaceutical Samples using Liquid Chromatography Techniques

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Abstract:

High-performance liquid chromatography technology (HPLC is one of the most important analytical methods used to analyze drugs and determine their components with high accuracy. This research focuses on using HPLC to estimate the amount of paracetamol in pharmaceutical tablets, which is one of the common medications used as a pain reliever and fever reducer. Verifying the accuracy and reliability of this analytical method is crucial to ensuring the quality and safety of drug products. The methodology involved preparing samples by crushing the tablets and dissolving them in a suitable solvent to obtain a homogeneous solution. Analysis was performed using a Supelco C18 25)cm x 4.6mm column)Determine the appropriate mobile phase, flow rate, and wavelength used for detection (243 nm). Standard solutions and actual samples were injected into the HPLC machine, where compounds were separated and the peak area and retention time for each sample was recorded. The resulting data was used to create a calibration curve and determine the paracetamol concentration in the unknown samples. The results showed a linear relationship between paracetamol concentration and peak area, which reflects the accuracy and reliability of the methodology used. The retention time was constant at approximately 5.2 min, indicating stable device performance and consistency of analysis conditions. The amount of paracetamol in the pharmaceutical tablets was estimated with high accuracy, and the results were found to be within acceptable limits according to pharmaceutical standards. The research concluded that HPLC technology is an effective and accurate tool to estimate the amount of paracetamol in pharmaceutical tablets. These results strengthen confidence in using HPLC as a standard for ensuring the quality and safety of pharmaceutical products. The study recommends that this technology should be widely adopted in quality control laboratories to ensure that products comply with international health standards.

Keywords: High performance liquid chromatography, paracetamol, pharmaceutical quality, quality control, quantitative analysis.

Introduction

In the testing and control of premarket and drug marketing procedures in the past 10 years, high-performance liquid chromatography has replaced many spectroscopic and gas chromatographic methods in quantitative and qualitative analysis. In the early period of application of high-performance liquid chromatography it was thought that it would become a complementary method to gas chromatography, however, today it has almost completely replaced gas chromatography in drug analysis where The application of a liquid mobile phase with the possibility of shifting the mobile polarity during chromatography and all other modifications of the mobile phase depending on the properties of the substance being tested, is a great advantage in the separation process compared to other methods.

Greater choice of stationary phase is the next factor that enables good separation to be achieved. The separation line is associated with specific and sensitive detection systems, such as fluorescence spectrometer, diode detector, electrochemical detector, and other interconnected systems such asHPLC-MS and HPLC-NMR, which are the basic elements on which this broad and effective application of the HPLC method is based.

The purpose of analyzing any drug using high-performance liquid chromatography (HPLC is to confirm the identity of the drug, provide quantitative results as well as monitor the progress of disease treatment.[i]

Review references

Chromatography

Nowadays, chromatography is known as the mainstay of separation science and is widely applied in various pharmaceutical industries and research organizations around the world. [ii]Chromatography is defined as the separation of a group of compounds into defined entities using two phases; One is mobile and the other is fixed, This technique was first invented by Mikhail Semyorevich Tsoit, a Russian botanist of Italian descent, later considered the "father of chromatography."[iii]Chromatography combines two Greek words, i.e., chromo means “colour” and graphene means “writing”. Generally, the separation method in chromatography involves the main steps which start from the retention or adsorption of a substance(s) in the stationary phase and then separation of the adsorbed substances with the help of the mobile phase. This is followed by recovery of the separated material by a continuous flow of the mobile phase called ilution; This is preceded by a quantitative and qualitative analysis of the washed materials.[iv]

Types of chromatography

There are different types of chromatography that can be based on the nature of both phases, methods of operation of chromatography, based on separation, based on ilution technique.

The nature of the mobile phase and the stationary phase.

Different types of chromatography techniques are available depending on the type of phase used such as gas–liquid chromatography, gas–solid chromatography, liquid–liquid chromatography: divided into column split chromatography and paper split chromatography, solid–liquid chromatography includes thin layer chromatography (TLC), high-performance liquid chromatography (HPLC) and column chromatography[v].

Chromatographic methods

There are two types of chromatography, which depend entirely on the polarity of the two phases used for separation, i.e. the interaction between the mobile phase of the solute and the stationary phase. When the two phases are polar, their affinity is greater; The same applies when the two

phases used are nonpolar; Their familiarity is greater; But when one phase of the solvent is polar and the other is nonpolar, the reaction is less.[vi]

Normal phase chromatography

This technique uses a polar solvent for the stationary phase and a nonpolar solvent for the mobile phase. In this, the polar compound is involved for a long time in the column compared to the non-polar compounds, which migrate faster due to their affinity towards the stationary phase. For pharmaceutical analysis where normal phase chromatography is directed, columns composed of silica gel are used[vii].

Reverse phase chromatography

It is just the opposite of normal phase chromatography, as the stationary phase used here is nonpolar and the mobile phase is polar in nature. Thus, the polar compounds, i.e. the mobile phase, are ejected first and the stationary compounds, which are nonpolar compounds, are retained for a long time. Hence, reversed phase chromatography is the most widely used technique in pharmaceutical industries as most of the components have polar nature which are not retained in the column for a long time. Bound hydrocarbons are used e.gC8 (carbon 8), C18, C4, or octadecylsilane (ODS) as a stationary phase in pharmaceutical analysis[viii].

High performance liquid chromatography

High-pressure liquid chromatography or high-performance liquid chromatography (HPLC) is known asHPLC is a specialized technology that uses columns as well as liquid chromatography to separate, characterize, quantify and examine the active molecules present in the mixture. Compared with other traditional techniques, high-performance liquid chromatography has greatly improved performance. The expansion of high-performance liquid chromatography has evolved from conventional column chromatography and its performance (efficiency and accuracy), which is enhanced by the use of a stationary phase consisting of spherical particles ranging from 2 µm to 5 µm in diameter. Thus, due to the small particles, the head pressure forces the moving solvent or liquid to pass through the column under high pressure. For this reason it is known as high pressure liquid chromatography.[ix]

Technology is appliedHPLC to separate not only volatile compounds but also ionic species, polymeric substances, unstable natural products, macromolecules as well as high molecular weight functional groups[x].

The pump is used inHPLC to allow a sample mixture dissolved in a liquid solvent to pass through a solid sorbent filled into the column. Individual components in the sample bind differently to the sorbent, allowing different flow rates for different components and most importantly separation of components as they flow out of the columns[xi].

It worksHPLC is based on the principle of absorption and separation. In an HPLC column, the compounds to be separated are introduced as a mixture, but the different components migrate at a different rate, according to their relative affinity toward the stationary phase[xii]. Elements with greater affinity move toward the stationary phase at a slower rate, while elements with less affinity move toward the stationary phase at an accelerated rate. Since no two compounds have the same affinity toward the stationary phase, the components are separated showing the principal components ofHPLC in Figure (1).[xiii]

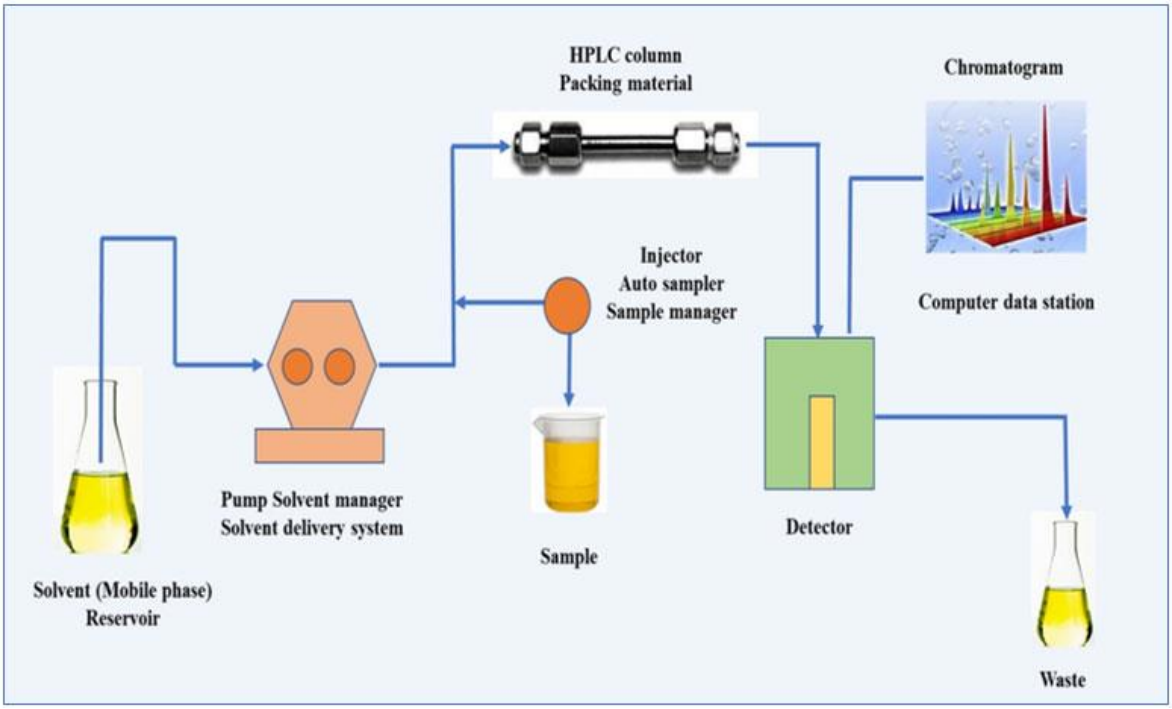

Figure (1): The main components of high-performance liquid chromatography

The concentration of the mobile phase varies and depends on the sample composition. Therefore, the mobile solvent is usually a mixture of polar and nonpolar liquid solvents. Now, there are generally four bottles used as moving tanks during a runHPLC, which contains different solvents. Each bottle of the glass tank has a capacity of 1 litre. The choice of mobile phase used for separation purposes depends on the type of separation required, i.e. isodensity and gradient separation.[xiv]

For isodensity separations, solvents with the same polarity numbers or eluent strength are used to prepare the mobile phase (if a fixed ratio is used) or, in general, this can be pure solvent but in gradient separation, the polarity of the solvent gradually improves. Next, the solvent composition must be changed[xv].

Separation principle

Adsorption chromatography

This technique, which relies on the principle of adsorption for separation, is known as absorption chromatography. Separation of a mixture of compounds occurs due to difference in affinity towards the stationary phase. Compounds with a high affinity toward the stationary phase move slowly as opposed to compounds with a lower affinity toward the stationary phase, which move at a faster rate. Examples include column chromatography, thin layer chromatography, and high-performance liquid chromatography[xvi].

Partition chromatography

When two immiscible liquids are present, the solute mixture must be circulated through the column according to their partition coefficient. The process involves passing the mixture dissolved in the mobile phase through a column of stationary phase. The less soluble component moves into the mobile phase faster and vice versa, resulting in separation of compounds based on their partition coefficients. Example: gas-liquid chromatography, column chromatography, etc.[xvii]

Elote technique

On the basis of the Eilot technique, two commonly used methods are Equal Eilot and Stepped Eilot. The method involving a constant and uniform rotation of the moving solvent throughout the separation process is called isoelectric elution. During the procedure, elotic strength or polarity is maintained; Whereas if a mobile phase of low polarity or low escape force is used, with a constant increase in polarity or escape force throughout the process, the technique is called gradient escape.[xviii].

Type of analysis

Qualitative analysis determines the nature of the compound, the impurities present and the presence of active ingredients in the compound mixture, which can be determined using retention time values. While quantitative analysis determines the number of different components present in the mixture and is determined by comparing the sample peak area to the standard peak area[xix].

Other chromatographic methods

Ion exchange chromatography

It is one of the most powerful techniques for separation of charged particles which is capable of separating almost all charged particles including nucleotides, small amino acids, large proteins, etc. Using ion exchange chromatography, inorganic ions can also be separated and the method can be used for preparative and analytical purposes. The main disadvantage associated with ion exchange chromatography is the buffer requirement. The cost of work is also high due to the insulator used to separate the components[xx].

Size exclusion chromatography or gel permeation chromatography

In this technique, separation of particles according to molecular size occurs using a gel. The method is suitable for determining the quaternary and tertiary structures of amino acids as well as determining the molecular weight of polysaccharides. For separation purposes, gels that are soft in nature like polyacrylic amide, agar gel and dextran are used for separation purpose. Apart from soft gels, some semi-rigid gels such as alkyl, dextran and polystyrene dispersed in non-aqueous medium are also used for analysis[xxi].

Chiral phase chromatography

Using a chiral stationary phase, separation of the optical isomers, i.e., dextro and lipo forms, is achieved. Silica gel is used as the most suitable static medium. The chiral stationary phase is much more expensive than the chiral stationary phase such as carbon[xxii].

Bioaffinity chromatography

This method is generally used in various fields such as microbiology, biochemistry, and biotechnology, where the reversible reactions of protein and ligand are completely separation dependent. Solid supports with a bioaffinity matrix are covalently attached to the ligand, which interacts with the ligands bound to the column to retain proteins.[xxiii].

High performance liquid chromatography applications inanalysis Pharmaceutical samples

High-performance liquid chromatography can be used to detect impurities in pharmaceutical products and ultimately to verify product quality. High-performance liquid chromatography can be used to determine the quantity of pharmaceutical products, to study the biopharmaceutical and pharmacokinetic properties of drug dosage forms, to study the stability of drug dosage forms, to determine the dissolution of tablets and to ensure proper production and purity of drugs.[xxiv]

The measurement shown in the figure2It is a chromatogram obtained from the plasma of depressed patients 12 hours before oral dexamethasone administration.

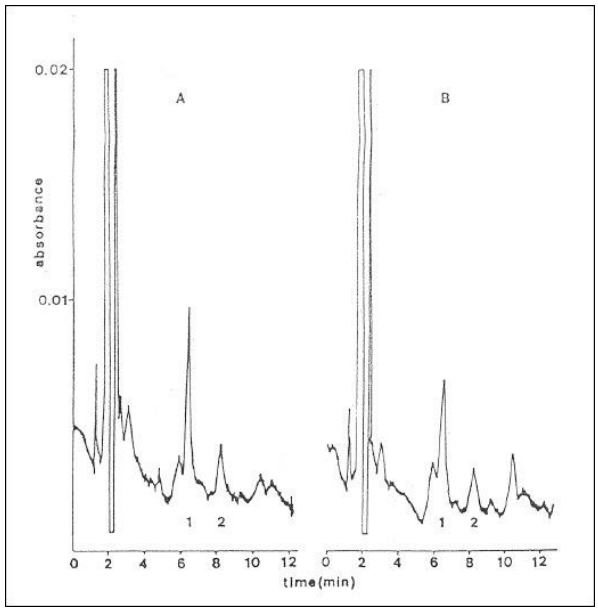

Figure (2): Plasma cortisol concentration was calculated to be 108 ng/mL (A). Plasma hormonogram of the same subject 12 hours after oral administration of 0.5 mg dexamethasone.[xxv]

1. Method development and validation:

According to the FDA, verification is defined as the establishment of documented evidence that provides a high degree of assurance that a given process will consistently produce a product that meets pre-specified quality specifications and attributes. Method development and validation is a time-consuming and complex process: laboratories need to evaluate combinations of mobile phase, pH, temperature, column chemistry, and gradient profiles to obtain robust and reliable separation of each activity. Using With UPLC, analysis times are as short as one minute, and methods can be optimized in just one or two hours, greatly reducing the time required for method development and validation..

2. Manufacturing and QA/QC:

Identity, purity, quality, safety and potency are the important factors to consider while manufacturing a drug product. Successful production of high-quality pharmaceutical products requires that raw materials and finished pharmaceutical products meet purity specifications, Continuous monitoring of material stability is also a component of quality assurance and control. Technology is usedUPLC is in highly regulated quantitative analyzes performed in quality assurance and quality control laboratories, Providing consistent, high-quality products plays an important role in the patented analytical method.

3. Identification of fixed-dose combination products (FDC):

Compound fixed dose product (FDC) is a formulation that combines two or more drugs in a single dosage form available at certain fixed doses. Method development for more than one drug becomes very complex if the solubility varies greatly. Therefore, these compounds need to be analyzed using more advanced techniques such as UPLC-PDA and UPLC-QTOF/MS. A large number of samples can be analyzed daily due to short analysis times.

4. Stability test/stress test:

According toICHQ1A (R2), Forced degradation studies or forced degradation studies of a drug substance are performed to identify degradation products, determine the intrinsic stability of the

molecule, and develop stability-indicating power for analytical methods. The analytical technique mostly used for forced degradation studies is HPLC-UV and/or HPLC-MS but the main drawback is that these techniques are time consuming and do not provide sufficient information regarding the resolution of all impurities and degradation products. The UHPLC/Q-TOF-MS system provides changeable collision energy values that allow mass information to be generated with acceptable accuracy and precision, which ultimately helps in structure elucidation, determining drug fragmentation patterns, identifying degradation products, and establishing degradation mechanismsto, UPLC technology has been successfully applied to determine acelofenac, paracetamol and their degradation products in tablets.

5. Detect and identify impurities:

Determination of the impurity profile is a mandatory requirement by various regulatory bodies and is directly related to the quality, safety and efficacy of a drug product. Impurities are drug-related substances that develop during formulation or when storing drugs. Mass spectrometry is one of the best techniques for detecting impurities. The impurities present can be detected by the additional peaks and the highest value of the mass peaks from the compound itself and from the fragmentation pattern. The rapid change of collision energy generates molecular fragments, allowing rapid identification of compounds along with their associated impurities. On the basis of the different fragments of the compounds, one can easily suggest their structure.

6. Dissolution test:

For quality and release control in pharmaceutical manufacturing, dissolution testing is essential in the formulation, development and production process. The dissolution profile is used to demonstrate the reliability and batch-to-batch uniformity of the active ingredient. For quality and release control in pharmaceutical manufacturing, dissolution testing is essential in the formulation, development and production process. In sustained-release dosage formulations, testing for higher potency drugs is particularly important as dissolution can be the speed-limiting step in drug delivery. The dissolution profile is used to demonstrate the reliability and batch-to-batch uniformity of the active ingredient. In addition, newer and more effective formulations require increased analytical sensitivity. He provides UPLC provides accurate and reliable online automated sample acquisition. It automates dissolution testing, from dropping the pill to initiating the test, through data acquisition and analysis of sample fractions, to managing the dissemination and distribution of test results.

7. The Pharmacokinetic and bioequivalence studies

For pharmacokinetic, toxicological, and bioequivalence studies, determining the amount of drug in biological samples is an important part of development programs. It is sensitive and selectiveUPLC-PDA at low detection levels generates accurate and reliable data that can be used for a variety of different purposes, including statistical pharmacokinetic (PK) analysis. Drugs are generally low molecular weight and are tested during clinical and preclinical studies.

8. Identification of metabolites:

After the development stage of any chemical compound, identification of its metabolite is a mandatory process. To obtain complete knowledge of the therapeutic efficacy of a compound, it is necessary to identify all relevant metabolites. It has been successfully used to study metabolites using transitionsMS/MS selected molecular ions and product ions for quantification in the sample matrix. Identification of human metabolites of paracetamol was performed by single column HPLC and UPLC columns for sub-2 μm particles combined with TOF-MS. The UHPLC system yielded three times more sensitivity and detection of metabolites than the HPLC system. The metabolite profile in different biological samples was investigated by applying UHPLC/Q-TOF coupled with Metabo Lynx™ software. Biotransformation of new chemical entities (NCE) is essential for drug

discovery. When a compound reaches the development stage, metabolite identification becomes a structured process.

9. Peptide Mapping:

Determining the chemical structure of a peptide bound to a protein is known as peptide mapping, This technique provides real-world results when used to analyze complex molecules such as proteins, Structural identification of proteins can become easier by applying temporal mass detection

10. Analysis of natural products and herbal medicines:

Complex samples of natural products and traditional herbal medicines were identified. For example, separating Asian ginseng and adulterated American ginseng preparations by qualitative and quantitative analysis through this technique. This method helps in identifying and elucidating the structure of many similar compounds such as ginsenoside and pseudo-ginsenoside. The chemical components of a popular Chinese herbal formula used to treat intestinal and stomach infections have been studied by fingerprinting. Technology availabilityUPLC provides high-quality separation and detection capabilities to identify active compounds in highly complex samples from natural products and traditional herbal medicines.

11. Screening for antibiotics in surface water and wastewater:

This technology has been applied in the detection of antibiotics from incoming and outgoing surface water and wastewater. These compounds are transported by washing machines and transferred to wastewater during the manufacturing process. Several antibiotics such as ofloxacin and ciprofloxacin along with other substances have been identified and the presence of residues of these substances in pharmaceutical industry wastewater has been confirmed. Identification and quantitation of different pharmaceutical compounds belonging to different therapeutic classes such as analgesics, anti-inflammatories, cholesterol-lowering agents, psychiatric drugs, anti-ulcer agents, antibiotics and beta-blockers were performed by a multi-residue method. The separation time for all compounds was 10 minutes. The mass measurement of the compounds was performed by the collision disintegration method (CID) by choosing different values of impact energy to fragment vehicles

12. Monitoring of therapeutic drugs:

Monitoring the concentration of beta-lactam antibiotics in the plasma of patients with different pharmacokinetics. method has been appliedUPLC-MS/MS for the simultaneous determination of two beta-lactamase inhibitors and seven beta-lactamase antibiotics in human plasma. The main benefit of this technique is the faster analysis speed compared to other methods used for this type of multianalyzer. This technique is now widely applied for toxicological analysis of biological samples. These toxins can be generated during the biotransformation of drugs, byproducts, intermediates, enantiomers, impurities, degradation products, and adjuvants to drug products. These substances are found in the blood; Plasma and urine at very low levels are easily identified and measured.

13. Screening of synthetic compounds:

This technology has become critical tools for use in high-throughput quality control screening of synthetic medical compounds. The use of more conventional techniques such as NMR cannot meet these high-throughput analytical needs due to relatively poor sensitivity, high sample purity requirements, the necessity of operator experience, and the use of expensive solvents. To simplify analytical procedures, automation is demonstrated along with software application for accurate mass measurement.

14. Identification of unknown pesticides:

This method is used to determine pesticides in vegetables and fruits. Insecticides with a complex chemical composition of organic and inorganic compounds. Identification of these toxicants from vegetables is essential for public health, which can be determined by this sensitive method. Pesticide residues present at a very low concentration level collected from the packaging materials used for packing the fruit were analyzed by the technology..

15. Analysis of doping materials:

The method has been applied to control doping and analyze stimulant substances from different classes such as beta blockers, stimulants, diuretics and narcotics. However, methods developed for screening doping materials were used and finally applied for quantification. The World Anti-Doping Agency found (WADA has determined that the method is reliable for identifying these restricted drugs. The method applied has been validated according to the guidelines set forth in the World Anti-Doping Code.

16. Screening for organic pollutants in water:

This technique is used to identify organic contaminants in natural water and wastewater. Samples were obtained from solid phase extraction procedures. The analysis was performed using the standard addition technique. Organic pollutants from different sources were mixed with water samples at different concentrations and analyzed using this technique. The developed method was applied to detect several organic pollutants, antibiotics, anti-inflammatory drugs and analgesics.

17. High-throughput screening(HTS):

The systematic approach to screening and analyzing organic, synthetic and pharmaceutical compounds is known as high-throughput screening(HTS). HTS technology has been widely used in drug design and discovery to identify, quantify, and characterize chemical compounds. By applying HTS methods, therapeutic, metabolic, pharmacokinetic, toxicological, analytical and degradation data can be obtained for new drugs. Reduces cost, time, and consumption of materials and solvents during drug development. The design and discovery of a new drug depends on complete information regarding its useful therapeutic derivatives. The therapeutic efficacy of a compound depends on the formation of all its derivatives during the biotransformation process and it is necessary to identify all of these derivatives. The metabolism of a drug and the formation of its derivatives during the process of drug metabolism is known as metabonomics. In the era of advanced and modified chromatography techniques, UHPLC/Q-TOF-MS is a relatively new technology and has been widely used in drug discovery and development.

18. Iodization byproducts in drinking water(IBP):

With helpUPLC connected to electron sputter ionization triple quadrupole mass spectrometry (ESI-tqMS), several chlorinated and chlorine-ammoniated water iodinated byproducts (IDBPs) were quantitatively analyzed and their structures proposed.

19. Toxicity studies:

During the drug development process, toxicity issues eliminate drug candidates, causing financial losses to the organization. Estimating drug candidates in terms of their potential to inhibit or induce metabolic enzymes, toxicity or drug interactions in the body is a complex task. He providesUPLC provides accurate detection thanks to its high resolution. In addition, its high sensitivity allows detection of peaks at low concentrations. These factors help reduce analysis time and reduce sample analysis failures.

Methodology:

Determination of the amount of paracetamol in tablets is a common application of high-performance liquid chromatography(HPLC) This technology is used to accurately separate and

analyze chemical compounds, allowing the amount of active ingredient in the tablets to be determined. The methodology described below addresses the detailed steps used in this analysis.

- **Technology selectionused**

Because paracetamol is a relatively nonpolar small molecule,Reverse-phase HPLC for its high sensitivity and resolution.

The chromatographic system consists of a model(Shimadzu HPLC model LC-20AT) with UV detector model (SPD-20A Model) And a columnC18 (250 mm x 4.6 mm, 5 µm particle size), which is ideal for the separation of non-polar compounds such as paracetamol. The column temperature was set at 30 °C to ensure consistent peak shapes and retention times.andThe mobile phase consists of a mixture of water (with 0.1% formic acid) and methanol(70:30). Formic acid improves the peak shape and enhances detection,A case was presentedHPLC with a particle size of 5 µm (Table 1).

Table (1) conditions HPLC(HPLC conditions.)

Column	Supelco C_{18} (25cm×4.6mm),5 µm
Wavelength	243 nm
Mobile phase	Acetonitrile – pH3 (40:60)
Retention time	2.2 minutes
Flow rate	1.5 ml/min
Temperature	Ambient
Injection volume	10 µL

- **Sample preparation**

Crushing tablets: A known number of paracetamol tablets (5 tablets) are weighed and finely crushed to obtain a homogeneous powder.

Dissolution: This solution was prepared by dissolving 5.75 g of monobasic ammonium phosphate in about 80 ml of water, adding sufficient acetic acid to adjust the pH to 3 and diluting it to 100 ml with distilled water in a volumetric flask. As A standard stock solution of paracetamol (1 mg/ml) was prepared in the mobile phase, and Standard solutions working in the range (10-100 µg/mL) were prepared by diluting the stock solution.

Sonication and filtration(Sonication and Filtration): The solution is sonicated for 10 minutes to ensure complete dissolution of The API is then filtered through a 0.45 µm membrane filter to remove any undissolved particles.

Dilution: The filter is further diluted with the solvent mixture to achieve the desired concentration (e.g., 50 µg/mL).

- **Road HPLC To analyze medicine (Paracetamol):**

A series of standard solutions containing 10–100 µg/mL paracetamol and sample solution of pharmaceutical preparations were applied respectively. 10 µl of each solution was injected into the column in duplicate and the chromatograms were recorded. The calibration graph was created by plotting the mean peak area against the paracetamol concentration, so that the curve should be linear, with a value ofr^2 is close to 1.0. The limit of detection (LOD) was determined to be 0.5 µg/ml, and the limit of quantification (LOQ) was 1.5 µg/ml. The concentration of the unknown was calculated from the regression equation derived from the concentration and peak area data, or it was read from the calibration graph, as was done Determine the amount of paracetamol by comparing the peak area of the test preparation with the standard preparation at the same time.

➢ Data analysis

Colorimetric interpretation: The paracetamol peak appears at a retention time of approximately 4.5 minutes, where The peak area is measured and compared to the calibration curve to determine the concentration in the tablet sample.

Quantification: Based on the calibration curve, the concentration of paracetamol in the sample is calculated. For example, if the peak area corresponds to a concentration of 48 µg/mL, the amount of paracetamol in the tablet will be calculated accordingly.

Results:

Methods have been developed HPLC for drug identification has gained great interest in recent years due to its importance in quality control of drugs and pharmaceutical products. The aim of this study is to develop an accurate, sensitive, rapid, selective and reproducible HPLC method for the determination of paracetamol in pure samples of its pharmaceutical formulation. Using the columnC18 is most commonly used with UV detection. The detection wavelength of 243 nm was chosen in order to achieve good sensitivity for the quantification of paracetamol in tablets, whileThe mobile phase provided good separation at room temperature under these conditions using a flow rate of 1.5 ml/min and a retention time of 2.2 min..

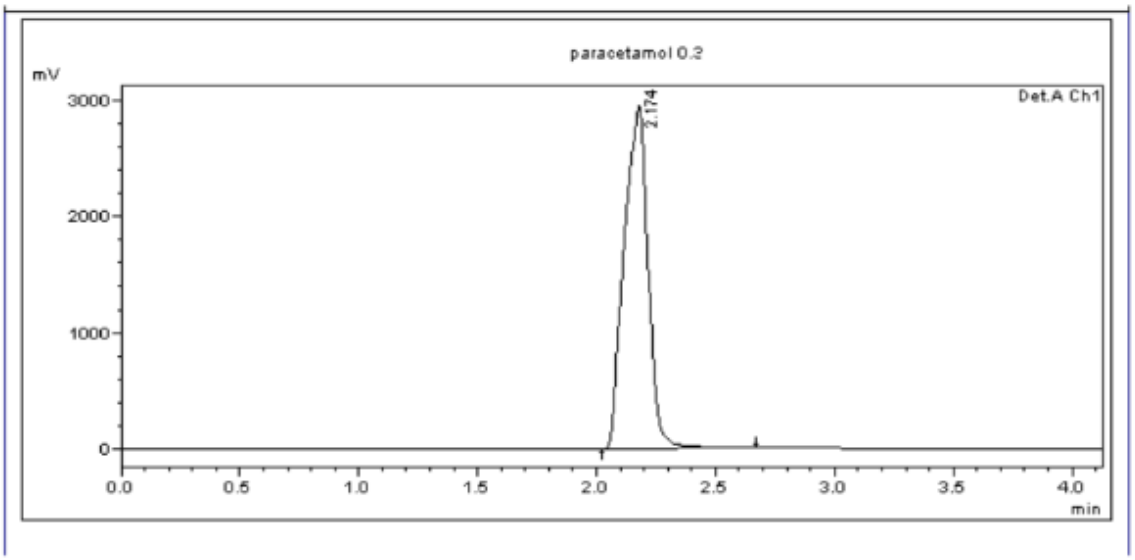

Figure (1) the a plan the Chromatogram the Typical (paracetamol 50 µg/ml).

Calibration curve (Calibration Curve)

Table (1) Calibration curve (Calibration Curve) for paracetamol

Peak area (units)((Peak Area (units)	Standard concentration (µg/ml)
90,000	10
225,000	25
450,000	50
675,000	75
900,000	100

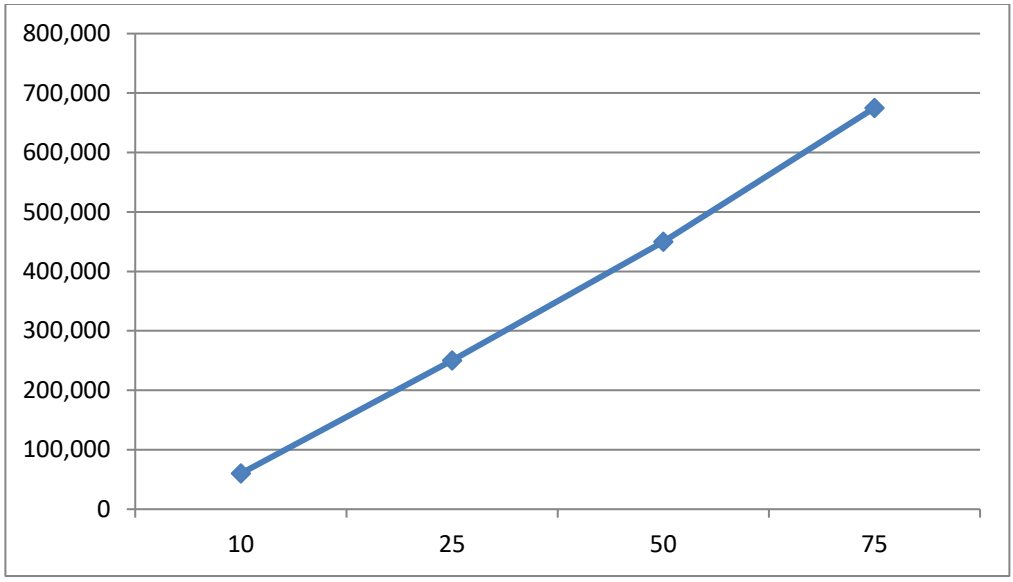

Figure (2) Calibration curve (Calibration Curve) for paracetamol

Calibration curve data show a strong linear relationship between the paracetamol concentration (range 10 to 100 µg/mL) and the corresponding peak area detected by the system. HPLC. As the paracetamol concentration increases, the peak area also increases proportionally, with the peak area being 90,000 units for the 10 µg/mL solution and 900,000 units for the 100 µg/mL solution. This linearity is critical because it indicates that the HPLC method is reliable for measuring unknown concentrations of paracetamol based on peak area. In sample analysis, a peak area of 450,000 units was recorded for the paracetamol sample, which corresponds to a concentration of 50 µg/mL based on the calibration curve. Considering the dilution factor applied during sample preparation, this concentration is equivalent to 500 µg/mL in the original solution

Method validation results (Method Validation Results)

value (Value)	standard unit (Parameter)
0.999	Sin((Linearity(r^2)
1.2	Accuracy((Precision (RSD %))
0.5 µg/mL	Detection limit(Limit of Detection (LOD
1.5 µg/mL	Limit of quantification(Limit of Quantification (LOQ))

Table (2) Method validation results(Method Validation Results)

The results of method validation confirm the robustness and reliability of the method HPLC used to measure the amount of paracetamol in pharmaceutical samples. Linearity, indicated by an r^2 value of 0.999, shows that the method shows a strong linear correlation between paracetamol concentration and peak area across the range tested, where This high linearity ensures that the method can accurately quantify the active ingredient over a wide concentration range. Accuracy is reflected in the relative standard deviation (RSD) of 1.2%, indicating that the method produces consistent and reproducible results when the same sample is analyzed multiple times, as Low refugee status determination (RSD) is essential to ensure the stability and reliability of the method's performance.

The standard deviation at zero concentration was calculated and this value was used to calculate the limit of detection and limit of quantification. Detection limits were calculated (LOD) and quantitative estimation (LOQ) using the following formulas: LOD= 3.3σ/s) and(LOQ=(10σ/s where

σ is the standard deviation of the response and s is the slope of the regression line. It is found Detection limit (The LOD and limit of quantification (LOQ) are 0.5 µg/mL and 1.5 µg/mL, respectively. These values demonstrate the sensitivity of the method, meaning that it can reliably detect and measure very low concentrations of paracetamol. This sensitivity is critical to ensure that trace amounts of the active ingredient can be measured accurately, making the method suitable for both quality control and regulatory compliance in pharmaceutical analysis.

Sample analysis results Paracetamol

Amount of paracetamol inDiscs(mg) Amount of Paracetamol in Tablet (mg)	Concentration in original solution (µg/mL) Concentration in Original Solution (µg/mL)	Calculated concentration (µg/mL) Concentration Calculated (µg/mL)	Peak area (units) Peak Area (units))	Retention time(min)(Retention Time(min))	Sample
500		50	450,000	4.5	1

Table (3) Sample analysis results Paracetamol

The results of the sample analysis provide a detailed overview of the evaluation HPLC to measure the amount of paracetamol in tablets, where the retention time refers to the time it takes for the paracetamol to move through the HPLC column and reach the detector after injection. In this analysis, paracetamol was detected at 4.5 minutes, which is consistent with the expected retention time and detected during the chromatography process, consistent with expectations based on the calibration standards., and This constant retention time helps confirm the identity of the compound, indicating that the peak detected corresponds to paracetamol and not another substance.

The peak area represents the amount of paracetamol present in the sample, and the area under the peak color curve is directly proportional to the concentration of the compound. In this case, the peak area of 450,000 units indicates the amount of paracetamol detected by the system HPLC which corresponds to a calculated concentration of 50 µg/mL in the analyzed solution. This value is used to calculate the paracetamol concentration in the sample based on the previously developed calibration curve. Given the dilution factor used in sample preparation, this concentration translates to 500 µg/mL in the original solution before dilution. When considering the total volume used in the preparation, this indicates that the tablet contains 500 mg of paracetamol, which matches the amount shown. These results confirm that the tablet dose is accurate and within expected specifications, confirming the effectiveness of the method in routine quality control of paracetamol tablets.

Conclusion

By observing the previous results, it is possible conclusion What follows:

Yes The analysis was demonstrated using high-performance liquid chromatography(HPLC) to determine the amount of paracetamol in standard solutions with high accuracy and reliability, which confirms the effectiveness of this method in the quantitative analysis of drugs.

The results obtained show a clear linear relationship between the paracetamol concentration and the measured peak area, indicating that increasing the paracetamol concentration leads to a proportional increase in the resulting signal., This linear pattern is essential to confirm the validity of the analytical methodology, as it allows the creation of a reliable calibration curve that can be used to accurately determine paracetamol concentrations in actual samples..

Shows stability of retention time(Retention Time) at about 5.2 minutes in all standard samples, providing stability in the performance of the chromatographic column and consistency in the analysis conditions. This stability is a vital element to ensure accurate quantification, as it ensures that the compound being analyzed exhibits the same behavior under the same conditions, reducing the possibility of analytical errors resulting from unexpected fluctuations in the instrument or environmental conditions.

The results obtained reinforce confidence in the technical ability HPLC can accurately separate and identify paracetamol over a wide range of concentrations, making it a powerful tool in monitoring pharmaceutical quality and ensuring that products conform to required pharmaceutical standards. This analysis also demonstrates the effectiveness of the preparatory procedures and selection of appropriate operating conditions, such as column type, flow rate, and wavelength, all of which contributed to achieving accurate and consistent results.

Conclusion

The application of the high-performance liquid chromatography method, as well as the selection of chromatographic analysis conditions, including a chromatographic column, a mobile phase, and the wavelength of the detector used, are of great importance in the analysis of such preparations. This leads to accurate and reliable results. The low economic cost of this method leads to the possibility of its widespread use in the analysis of pharmaceutical preparations and its application in drug control. In this study, a method was developed HPLC is accurate, simple, fast and has been validated for the determination of paracetamol in Pharmaceutical samples. The method was selective using an analytical columnC18 and applicable to pharmaceuticals. Hence, the developed method was recommended for controlling the entire drug manufacturing process as well as for quality control of the final product due to its high recovery and accuracy.

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