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CHROMATOGRAPHY TECHNIQUE USED FOR QUANTITATIVE AND QUALITATIVE ANALYSIS OF DIFFERENT TYPES OF SAMPLES

Seema

Research scholar S.P.C. Govt College, Ajmer (Rajasthan)

Dr. Seema Garg

Professor , S.P.C Govt College, Ajmer (Rajasthan)

ABSTRACT

Chromatography is based on the premise that molecules in a mixture can be separated from each other by applying them onto the surface or into the solid stationary phase (stable phase), and then moving with the assistance of a mobile phase. Molecular properties such as adsorption (liquid-solid), partition (liquid-solid), and affinity, as well as variances in their molecular weights, are among the parameters that have an impact on the effectiveness of this separation process. Because of these variances, certain components of the mixture spend more time in the stationary phase of the chromatographic system, and they travel through the system more slowly, while other components of the mixture pass quickly into the mobile phase, and they exit the system more quickly. Traditional Chinese herbal tea was created over a long period of time by Chinese people for the purpose of disease prevention and health care under the direction of traditional Chinese medicine. In 2006, the Chinese government recognised traditional Chinese herbal tea as an important part of China's intangible cultural heritage. WLJHT was the subject of our prior phytochemical investigation, during which numerous polyphenol constituents, such as phenolic acids and flavonoids, were isolated and their structures were deduced. Due to the chemical complexity of WLJHT, there is no reliable method for controlling the product's quality. WLJHT is known as the "King of herbal tea." For the purpose of evaluating the quality of WLJHT, it has been suggested that three different phenolic acids be used. However, as is well knowledge, the comprehensive efficacy of herbal tea can be attributed to the several components found in positive plants. Both a quantitative study and a qualitative study on service-learning were analysed, and references to these aspects were made throughout in order to make the distinctions between the two methods of instruction more clear. In conclusion, both the positive and negative aspects of each strategy were discussed.

Keywords*:Quantitative and Qualitative Analysis, Chromatography Technique*

INTRODUCTION

Chromatography is based on the premise that molecules in a mixture can be separated from each other by applying them onto the surface or into the solid stationary phase (stable phase), and then moving with the assistance of a mobile phase. Molecular properties such as adsorption (liquid-solid), partition (liquid-solid), and affinity, as well as variances in their molecular weights, are among the parameters that have an impact on the effectiveness of this separation process. Because of these variances, certain components of the mixture spend more time in the stationary phase of the chromatographic system, and they travel through the system more

slowly, while other components of the mixture pass quickly into the mobile phase, and they exit the system more quickly.

In accordance with this methodology, the chromatography method can be broken down into three distinct components. The stationary phase is always made up of a "solid" phase or "a layer of a liquid adsorbed on the surface of a solid support." This phase is known as the "stationary" phase.

Isolated individual molecules

The fundamental component that is responsible for the effective separation of molecules from one another is the type of interaction that occurs between the stationary phase, the mobile phase, and the substances that are present in the mixture. The separation and identification of tiny molecules such as amino acids, carbohydrates, and fatty acids can be accomplished relatively efficiently using chromatography techniques that are based on partitioning. On the other hand, affinity chromatographies, such as ion-exchange chromatography, are superior methods for the separation of macromolecules such as nucleic acids and proteins. Paper chromatography is utilised in the separation of proteins, as well as in studies related to protein synthesis; gas liquid chromatography is utilised in the separation of alcohol, esther, lipid, and amino groups, as well as the observation of enzymatic interactions; and molecular-sieve chromatography is utilised in particular for the determination of the molecular weights of proteins. The purification of RNA, DNA particles, and viruses can be accomplished through the use of agarose-gel chromatography [4]. In the field of chromatography, a stationary phase can be either a solid phase or a liquid phase that is coated on the surface of a solid phase. A gaseous or liquid mobile phase will flow across a stationary phase as the system operates. When the mobile phase is a liquid, the technique is referred to as liquid chromatography (LC), but when it is a gas, the technique is referred to as gas chromatography (GC). The analysis of gases, as well as combinations of volatile liquids and solid material, can be accomplished through the use of gas chromatography. Particularly useful for thermally unstable and non-volatile samples is the technique of liquid chromatography.

Types of chromatography

1. Chromatography using columns
2. Chromatography based on ion exchange
3. Chromatography using gel-permeation (also known as molecular sieve)
4. Chromatography based on affinity
5. Chromatography on paper 5.
6. Chromatography using a thin layer
7. A method called gas chromatography
8. Chromatography using dyes and ligands
9. Chromatography for the study of hydrophobic interactions
10. Chromatography using a pseudo-affinity gradient
11. High-pressure liquid chromatography (also known as HPLC) and column chromatography

The various characteristic components of proteins, such as their size, shape, net charge, stationary phase utilised, and binding capacity, can each be purified using chromatographic methods. Proteins have different characteristic features, such as these: size, shape, net charge, stationary phase used, and binding capacity. Column chromatography is the technique that is used the majority of the time among these methods. The

purification of biomolecules is accomplished with the help of this method. First, the sample that needs to be separated is applied to a column, which represents the stationary phase; subsequently, the wash buffer represents the mobile phase (Figure 1). It is ensured that their flow will take place through the interior column material that is supported by fibreglass. In a manner that is time- and volume-dependent, the samples are brought to the bottom of the apparatus and aggregated there.

Chromatography based on ion exchange

The separation technique known as ion-exchange chromatography relies on the electrostatic interactions that occur between charged protein groups and the solid support material, also known as the matrix. Ionic ties are responsible for the protein's affinity for the column, which is achieved by the matrix having an ion load that is diametrically opposed to that of the protein that needs to be separated. Altering the pH of the buffer solution, increasing the concentration of ion salts, or increasing the ionic strength of the buffer can be used to separate proteins from the column [8]. Anion-exchange matrices are the name given to positively charged ion exchange matrices, and they are able to adsorb proteins with a negative charge. However, matrices that are bound with negatively charged groups are referred to as cation-exchange matrices, and they are able to adsorb proteins that have a positive charge.

Chromatography using gel-permeation (also known as molecular sieve)

This technique separates macromolecules according to the differences in the sizes of their constituent components by employing materials that include dextran as the primary discriminating factor. This technique is utilised for the most part in the determination of the molecular weights of proteins as well as the reduction of the salt concentrations in protein solutions. The stationary phase of a gel-permeation column is made up of non-living molecules that have very small pores. The solution, which may contain molecules of varying size, is carried through the column in a continuous stream at a pace that is held constant. Molecules that are larger than the pores in the gel particles are unable to pass through the gel, and instead, they are trapped between the gel particles in a confined space. Larger molecules are able to flow more quickly through the column because they can fit through the pores between the porous particles. Diffusion into pores occurs for molecules that are smaller than the pores, and as molecules continue to get smaller, they exit the column with retention durations that are correspondingly longer. The most common type of Sephadeks used for column construction is the G type. In addition to these materials, dextran, agorose, and polyacrylamide are also utilised as column components.

chromatography based on affinity

Enzymes, hormones, antibodies, nucleic acids, and certain proteins can all be purified with the help of this chromatography method. The filling material of the column is held together by a ligand that is able to form a compound with a particular protein (such as dextran, polyacrylamide, or cellulose, for example). The particular protein that forms a compound with the ligand is the one that is bound to the solid support, also known as the matrix, and is the one that is kept in the column. Free proteins are the ones that are removed from the column. After that, the bound protein escapes the column by altering the ionic strength of the column's environment by adjusting the pH or adding a salt solution, whichever comes first.

The study of chromatography

The support material for paper chromatography consists of a layer of cellulose that is heavily saturated with water. This technique utilised a substantial piece of filter paper as the support, and the "liquid phase" was represented by water droplets that had been lodged in the paper's pores. The mobile phase is made up of the suitable liquid that is stored in the developing tank. Chromatography performed on paper is known as "liquid-liquid" chromatography.

Chromatography using a thin layer

Chromatography using a thin layer is referred described as "solid-liquid adsorption" chromatography. In this technique, the ary phase station utilises a solid adsorbent substance that is deposited on glass plates. It is possible to use as an adsorbent material any of the solid substances that are used in column chromatography, such as alumina, silica gel, or cellulose. Using this technique, the mobile phase moves higher while passing through the stationary phase. Because of capillary action, the solvent makes its way up the thin plate that has been saturated with the solvent. During the course of this process, it also propels the mixture that was previously dropped on the lower regions of the plate with a pipette upwards at varying flow rates. The separation of the analytes is completed as a result. This upward travelling velocity is influenced by the polarity of the substance, both in its solid phase and in the solvent. In situations in which the molecules of the sample are colourless, florescence, radioactivity, or a particular chemical substance can be utilised to generate a visible coloured reactive result in order to identify their positions on the chromatogram. This can be accomplished through the employment of a specific chemical substance. Under normal lighting or ultraviolet illumination, the formation of a colour that can be seen can be detected. Calculating the ratio between the lengths travelled by the molecule and the solvent is one way to determine where each molecule is located within the mixture. This ratio may be found by dividing the distance travelled by the molecule by the distance travelled by the solvent. This value of measurement is referred to as relative mobility, and it is represented by the sign Rf. Rf values are utilised for the purpose of qualitatively describing the compounds.

In gas chromatography,

The method known as stationary phase utilises a column that is positioned within the apparatus and contains a liquid stationary phase that is adsorbed onto the surface of an inert solid. The term "gas-liquid" chromatography refers to what is known as gas chromatography. The He or N2 gases that make up its carrier phase are the components of this phase. Under a significant amount of pressure, the mobile phase, which is an inert gas, is moved through a column. The sample that will be analysed is vaporised before being introduced into a mobile phase that is composed of gas. The components that make up the sample are spread out across the solid support in a manner that distributes them between the mobile phase and the stationary phase. Gas chromatography is a technology for the exceptionally effective separation of very minute molecules that is straightforward, versatile, incredibly sensitive, and can be put into practise very quickly. It is put to use in the process of separating extremely trace levels of analytes.

OBJECTIVE OF THE STUDY

1. To conduct research on the chromatography method, which can be applied to the quantitative and qualitative examination of a wide variety of sample types
2. To conduct research on the Qualitative and Quantitative Analysis of the Principal Components of WLJ Herbal Tea Using a Variety of Chromatographic Techniques

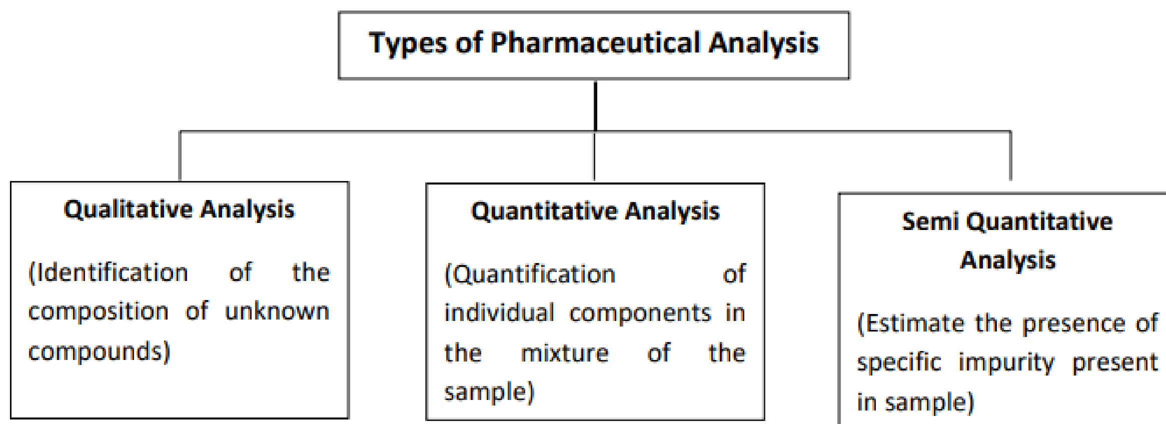
METHODOLOGY

The high-performance thin-layer chromatography (HPTLC) technique may be utilised for quantitative and qualitative analyses, the optimisation of analysis time, the separation of two constituents as well as multicomponent naturally occurring mixtures, and more. In order to analyse pharmaceuticals in multicomponent dosage forms using HPTLC, one must have a fundamental understanding of the sample's nature, namely its polarity, structure, stability, and volatility, as well as the solubility requirements. The creation of the approach includes a significant amount of time spent doing operations of trial and error. In multicomponent analysis, deciding which mobile phase to use and where to begin doing the analysis are two of the most challenging problems. Silica gel is the one that is most suited to and makes the most sense for every type of medicine. Therefore, selecting the stationary phase is a very straightforward alternative.In order to optimise the mobility phase, three different levels of approaches are utilised. In the initial stage, we make use of certain solvents, and then we look for other examples of these solvents that have a moderate segregation power for the pharmaceuticals that are being evaluated. The second level covers a variety of functions that include either raising or reducing the solvent strength by employing water or hexane. Even though plain solvents are tested at the third level, mixes of the selected solvents from the first and second levels are also tested, and these mixtures can be further optimised by the addition of modifiers such as acids or bases. Evaluating analytes can be done using either the absorbance method or the fluorescence mode. If the analytes are not entirely detected, however, it may be necessary to switch either the mobile phase or the stationary phase, or it may be necessary to seek assistance from pre or post chromatographic derivatization. A very minor alteration in the composition of the mobile phase leads to an acceptable chromatogram that has all of the desired peaks in symmetry and is properly separated, which assists in optimisation.When using two-dimensional chromatography, it is possible to increase the capacity of the spot, which is comparable to the peak capacity in HPLC. This is accomplished by constructing the plate with two unique solvents.When the sample is transferred to the TLC plate by hand, there is a degree of ambiguity about the droplet's size as well as its position. Automation is an excellent method for overcoming this uncertainty. Piezoelectric devices and inkjet printers have recently been utilised in the process of putting the sample method to automation into action.

Pharmaceutical Analysis: -

Analysis of substances on both the qualitative and quantitative levels is the focus of the field of chemistry known as pharmaceutical analysis. This subfield of chemistry is also known as pharmaceutical testing. It encompasses the analytical processes that are used to assess the purity, safety, and quality of the medicine, as well as the separation of the components of the drug and the determination of the structure of chemical compounds.

Table: 1



Analytical Techniques:-

Analytical procedures are processes that enable us to know subjectively and/or quantitatively the composition of any material and the chemical state in which it is situated, or to identify and qualify the substances. Analytical techniques may be broken down into two categories: qualitative techniques and quantitative techniques. For the purpose of determining the composition of medicines, a wide range of analytical techniques, including chromatographic, electrochemical, titrimetric, spectroscopic, and electrophoretic analysis, as well as their respective method applications, have been utilised.

In this article, we will discuss the spectroscopic and chromatographic methods of analysis, as well as their applications in the pharmaceutical industry.

Spectroscopy:

The study of the ways in which different types of materials interact with light or other forms of electromagnetic radiation is the focus of the scientific discipline known as spectroscopy. Electromagnetic waves of a certain wavelength or range of wavelengths are used in this technique to identify the qualitative and quantitative examination of materials.

Principle: The study of spectroscopy is predicated on the interaction of electromagnetic radiations with the subject matter being investigated. When a beam of electromagnetic radiation falls upon a sample (atoms or molecules), the radiation is either absorbed, reflected, transmitted, or scattered depending on how it interacts with the material. The spectrum that is obtained as a result of this is then analysed.

Spectrum: - a graph that shows the relationship between the frequency of light and the wavelength of electromagnetic radiation as a function of the response.

Spectrophotometer: - The measurement of the spectrum in spectroscopy is performed with the assistance of this apparatus.

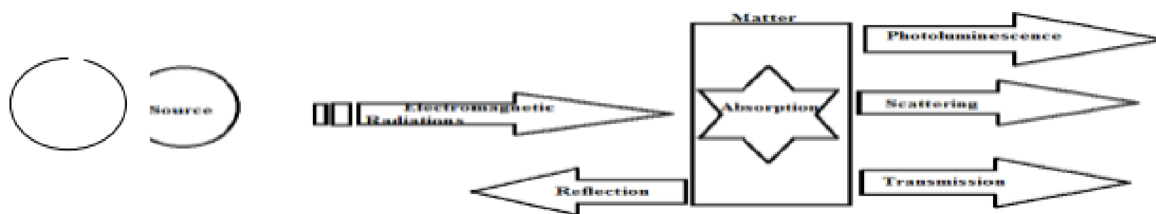


Figure: 1 Principle of Spectroscopy

The primary benefit of UV visible spectroscopy technologies is that they need less time to complete and require a less amount of manual labour. This technique has a very high degree of accuracy. Over the past several years, there has been a dramatic rise in the number of pharmaceutical analyses that make use of UV-Visible spectrophotometers, in particular those that focus on the examination of dosage forms.

UV-Visible spectroscopy:

Principle: A beam of light is reflected off of a sample surface, and the attenuation of the beam of light is measured once it has been done so. The movement of an electron within a molecule from a lower level to a higher level results in the formation of an ultraviolet absorption spectrum. Only in the presence of conjugated pie electrons does the portion of this area that is easily accessible (with a wavelength range of 200-800 nm) display absorption.

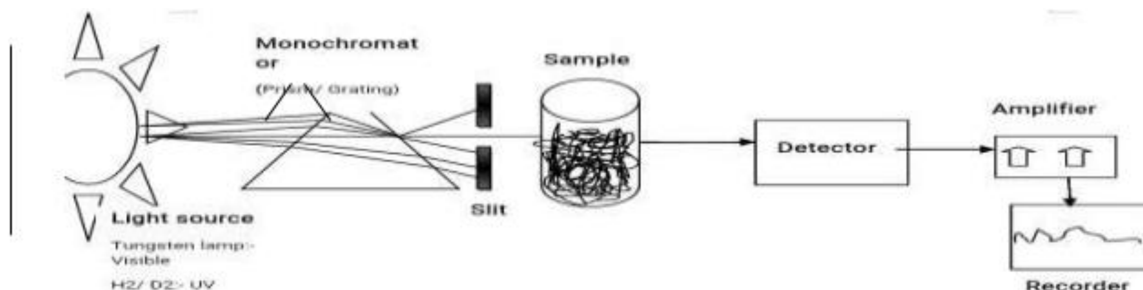


Figure: 2 Spectroscopic Equipment for the Ultraviolet and Visible Spectrum

Table 2 The Differences Between Quantitative and Qualitative Analytics

Quantitative Analytics	Qualitative Analytics
Close-ended questions with multiple-choice format, surveys, polls or questionnaires.	Open-ended questions with interviews and observations.
Mathematical and statistical analysis communicated with numbers, graphs and charts.	Verbal communication and analysis of summarizations, categorizations and interpretations.
Impartiality, fast and reliable data collection methodology, larger sample sizes.	More detailed insights, methodology encourages deeper discussion.

Unable to learn more context in answers, abnormal research environment, limited answers for data collection and insights.	Smaller sample sizes, more risk of biasness, requires highly skilled moderator.
Finance, accounting, consulting.	Healthcare, health sciences, social sciences, legal, e-commerce, marketing.

A quick overview of quantitative and qualitative analytics. Both have their uses, depending on the situation. Business students must understand the differences and when to use them.

CONCLUSION

Analyzing a quantitative and qualitative service-learning study with these components helped elucidate the differences. Finally, each approach's pros and cons were listed. This work developed UPLC/Q-TOF-MS/MS, HPLC-MS/MS, and HPLC-ELSD methodologies to identify and determine WLJHT's key ingredients. Based on their accurate molecular weights and fragmentation patterns, UPLC-MS immediately discovered or tentatively characterized 34 WLJHT molecules. HPLC-ELSD and HPLC-MS/MS accurately measured 17 main WLJHT components. Herbal pigments were initially separated by colour using chromatography. Its use expanded over time. Chromatography is a sensitive and effective separation process nowadays.

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