



International Journal of Science, Architecture, Technology and Environment

A Review on Paper Chromatography: Principle, Types, Procedure and Pharmaceutical Applications

Ferujul Hoque^{1*}, Kaja Reshma²

¹Department Of Pharmaceutical Analysis Nimra College of Pharmacy Vijayawada 521456 AP INDIA

²Entrepreneurship Cell Coordinator Department of Pharmaceutical Analysis Nimra College of Pharmacy Vijayawada 521456 AP INDIA

*Corresponding author, reshmakaaja@gmail.com

DOI: <https://doi.org/10.63680/ijstate092651.43>

Abstract

Paper chromatography is considered a simple, inexpensive, and effective chromatographic technique used for the separation, identification, and analysis of different components present in a mixture. It is a type of planar chromatography in which a sheet of specially prepared paper acts as the supporting medium. Separation occurs mainly because different components of a mixture have different affinities for the stationary and mobile phases. When a suitable solvent moves through the paper by capillary action, the components travel at different rates and form separate spots. The technique can be performed by different methods such as ascending, descending, circular, and two-dimensional paper chromatography. The separated components can be identified by comparing their migration distances and calculating the retention factor (Rf) value. Paper chromatography has applications in pharmaceutical analysis, the identification of drugs, the examination of plant pigments, the separation of amino acids, the analysis of dyes, and research laboratories. Although modern instrumental techniques provide greater sensitivity and accuracy, paper chromatography remains valuable due to its simplicity, low cost, and ease of operation. This article discusses the principle, types, procedure, Rf value, factors affecting separation, applications, advantages, limitations, and precautions associated with paper chromatography.

Keywords: Paper chromatography, chromatography, stationary phase, mobile phase, separation, retention factor, Rf value, pharmaceutical analysis.

1.INTRODUCTION

Chromatography plays a significant role in separating, identifying, and analyzing the components of a mixture. The word chromatography is derived from the Greek words “chroma” that means color and “graphein” that means writing. Different chromatographic techniques have been widely used in pharmaceutical analysis, chemistry, biochemical, food analysis, environmental analysis, and biological research.

The separation of a mixture's components using chromatography is based on their different distribution or interaction between the stationary and mobile phases. A chromatographic separation takes place when a mixture's components travel at different speeds because of the variations in the materials' properties. Their individual migration rates enable the separation of the mixture's components and their subsequent determination.

Multiple chromatographic techniques have been developed including paper chromatography, thin-layer chromatography, column chromatography, gas chromatography, and high-performance liquid chromatography. Paper chromatography technique is one of the simplest forms of planar chromatography. It is a sophisticated method that requires merely inexpensive equipment and does not require elaborate apparatus.

The paper chromatography is primarily used for the qualitative analysis of minute amounts of substances including amino acids, sugars, dyes, plant pigments, and some pharmaceutical substances. Hence, the method involves using the sample on a sheet of chromatographic paper and developing the chromatogram by using a suitable solvent system. Indeed, through the capillary action, the solvent will pass through the paper, allowing for the chromatographic separation of the mixture's components. Because of the different affinities of the sample's components for the stationary and mobile phases, they will travel at different speeds, ultimately forming spots on the paper.

The separated components can be visualized using a suitable visualization technique. Furthermore, a ratio of the distances can be expressed as a Retention factor (R_f) value. Since paper chromatography is an easy and inexpensive method, it remains an essential analytical technique for students, teaching laboratories, and some analytical industries.

2. HISTORICAL BACKGROUND

The development of chromatography resulted from the achievement of a Russian-Italian botanist Mikhail S. Tswett in 1903, who managed to separate plant pigments from a solution with the help of adsorbent. He succeeded by passing the solution through a column with an adsorbent, as a result of which the movement of various plant pigment groups caused the formation of different coloured bands on the adsorbent. After that, the term 'chromatography' was coined to refer to the technique.

Chromatography principles were further examined and developed by other scientists after realizing its great application potential. The new technique was initially used to separate various chemical compounds, and it later evolved to be applied to various substances from colourless plant extracts to drugs and proteins.

The chromatographic method of paper came into prominence in the 1940s as partition chromatography. It provided a simple way of analyzing and separating small amounts of various substances. The method was particularly useful in the analysis of amino acids, carbohydrates, peptides, dyes, and plant products.

The development of paper chromatography can be considered a significant breakthrough because of the relative simplicity of the method and the fact that simple and cheap equipment was enough to separate complex mixtures with a small amount of the substance. Due to the mentioned advantages, paper chromatography was widely used in teaching students, biochemical studies, pharmaceutical analysis, and routine laboratory work.

After that, chromatography has been further advanced, and now laboratories use more efficient methods such as thin-layer chromatography, high-performance liquid chromatography, and gas chromatography. However, paper chromatography is still used as an underlying knowledge in the study of chromatography principles.

3. DEFINITION

Paper chromatography is a type of planar chromatography which is primarily concerned with the separation, detection and qualitative analysis of the mixture's contents. When being used, a sheet of chromatographic paper was employed as the support medium and an appropriate solvent or mixture of solvents was used as the mobile phase.

As the solvent is sucked up the chromatographic paper, spots of a mixture content will appear on different positions due to the different attractions of the stationary phase and mobile one. The separated components can be visualized by the means of visualization techniques. Then they can be identified based on their R_f values or the ratio of the distances traveled by the solvent front and the component.

4. PRINCIPLE AND MECHANISM OF PAPER CHROMATOGRAPHY

4.1 PRINCIPLE

The paper chromatography is largely based on the principle of partition, wherein the ingredients of a mixture partition between stationary and mobile phase associated with chromatographic paper and solvent respectively.

The chromatographic paper acts as a stationary phase whereas suitable solvent is taken as a mobile phase. A small amount of sample mixture is applied on the chromatographic paper and is developed with the help of suitable solvent. During development process, the solvent rises through the chromatographic paper by capillary action and extracts the sample and moves it along with it.

At this point, the components of the mixture get partitioned between stationary and mobile phases. The components that have higher affinity with the mobile phase travel faster with the mobile phase. On the other hand, the components that have higher affinity with stationary phase are retained in the stationary phase for longer time and hence travel slow.

Therefore, different components of the mixture travel different distance and get separated into individual spots.

4.2 MECHANISM OF SEPARATION

A small amount of the mixture is applied on the marked origin line of a chromatographic paper. After drying the spot, the paper is kept in a chamber containing suitable solvent system. The spot should always stay above the level of solvent. The solvent then rises up the paper through capillary action and interacts with the spot.

The components get dissolved in the solvent and get separated during migration. The separation occurs due to continuous partitioning of the components between the stationary phase associated with the paper and the mobile phase. Since different components have different affinity towards the stationary and mobile phase, they get separated.

The components with higher affinity towards the mobile phase travel faster and farther than the ones with higher affinity towards stationary phase. After development, the chromatogram is taken out from the chamber and dried. The spots are visualized by suitable method.

The migration behavior of the component can be calculated in terms of R_f value. The R_f value is helpful to compare the spots and identify the components of the mixture.

4.3 SEPARATION SEQUENCE

Sample spot application – Solvent development – Capillary movement – Partition between two phase – Migration – Separation – Spot detection – R_f calculation

Therefore, the basic principle of paper chromatography is partition. According to this principle, the components of the mixture get partitioned between stationary and mobile phase and get separated due to their different affinities towards the two Phase.

5. COMPONENTS OF PAPER CHROMATOGRAPHY

The proper performance of paper chromatography requires several key components, each of which plays a vital role in the separation process. The basic components of paper chromatography are chromatographic paper, stationary phase, mobile phase, sample, developing chamber, sample applicator, solvent reservoir, and appropriate detection system.

5.1 Chromatographic Paper

Chromatographic paper is the medium, on which development of chromatogram takes place. It is mainly made up of cellulose and also has a porous structure, which allows for the capillary movement of the mobile phase. The paper should be of uniform thickness, appropriate porosity, mechanical strength, and low impurities to achieve good separation.

5.2 Stationary Phase

The stationary phase is the phase, which is stationary during the chromatographic development. In common paper chromatography, the water associated with the cellulose fibre of the paper is considered as the stationary phase. The paper acts as a support for this stationary phase. The different interactions between components of the mixture with the stationary phase lead to their differential migration.

5.3 Mobile Phase

The mobile phase consists of a solvent or mixture of solvents, which are moved by capillary forces through the chromatographic paper. It dissolves and transports the components of the sample mixture on the chromatographic paper. The choice of mobile phase depends on the chemical nature of the substances to be separated and significantly influences the separation quality and obtained R_f values. Water, alcohols, acetone, butanol, acetic acid, and their mixtures can be used as solvents, depending on the analytical requirements.

5.4 Sample

The sample is the substance or mixture of substances, which need to be separated and analyzed. A small amount of the sample is usually applied to the paper as a concentrated spot on the origin line. The sample spot must not be overloaded since it may cause spots to be too broad and overlapping, leading to poor resolution.

5.5 Developing Chamber

The developing chamber is a closed vessel, where the chromatogram is developed. It contains the mobile phase at the bottom and provides an enclosed environment for capillary action. Sealing of the chamber should be done to minimize solvent evaporation and allow the chamber atmosphere to become saturated with solvent vapour, which increases chromatographic consistency and reproducibility

5.6 Sample Applicator

A capillary tube or micropipette or some other suitable applicator is used to apply a small quantity of sample on the chromatographic paper. The sample should be applied carefully as a concentrated spot on the paper, in order to obtain well-defined chromatographic spots.

5.7 Solvent Reservoir

The solvent reservoir consists of the area in the developing chamber containing the mobile phase. The solvent level should be below the origin line so that the sample spot does not come directly into contact with the solvent.

5.8 Detection and Visualization System

After development, individual separated spots can be revealed if they are visible, or otherwise, appropriate detection methods need to be used. Depending on the nature of the separated substances, the detection can be carried out using ultraviolet light, iodine vapour, ninhydrin or other suitable chemical detecting reagents.

Thus, each component of the paper chromatographic system contributes to obtaining effective, interpretable, and reliable separation.

6. TYPES OF PAPER CHROMATOGRAPHY

There are various types of paper chromatography which can be classified according to the direction of solvent movement and method used for chromatographic development. The major types are: ascending, descending, ascending–descending, circular or radial and two-dimensional paper chromatography

6.1 Ascending Paper Chromatography

In ascending paper chromatography, the mobile phase moves upwards or against gravity through chromatographic paper mainly by capillary action. The sample is applied on a marked origin line near the lower end of paper. The paper lower end is placed in a developing chamber, which contains the mobile phase. However, the sample spot is kept above the solvent level. As the solvent rises through the paper, the different components of the sample migrate at different rates and become separated.

Ascending chromatography is a simple procedure, commonly used for qualitative separation and demonstration of chromatographic principles.

6.2 Descending Paper Chromatography

In descending paper chromatography, the mobile phase moves downward through chromatographic paper. The solvent is placed in a reservoir in the upper part of developing chamber and a sample is applied near the upper part of the paper. The solvent flows downward through the paper under the combined influence of capillary action and gravity.

Descending paper chromatography can provide a relatively long development path and may be useful when greater separation is required.

6.3 Ascending-Descending Paper Chromatography

Ascending-descending paper chromatography combines two types of solvent movements. During the first part of development, the solvent moves upward through the paper. During second part of solvent development, the solvent is directed downward. The method provides an extended path for solvent movement and may improve the separation of components in complex mixtures.

6.4 Circular or Radial Paper Chromatography

Circular paper chromatography, also called radial paper chromatography, uses a circular sheet of chromatographic paper. The sample is generally applied near the centre of the paper and the mobile phase moves radially outward. As the solvent spreads outward, the components of the mixture migrate at different rates and become separated in radial patterns. Circular paper chromatography can provide rapid development and is particularly useful when a circular chromatographic format is convenient.

6.5 Two-Dimensional Paper Chromatography

Two-dimensional paper chromatography is a specialized method of separation of complex mixtures, particularly when a one-dimensional separation does not provide adequate resolution. In two-dimensional paper chromatography, the sample is applied near one corner of the paper and developed first with one solvent system. The paper is then removed, dried and rotated, usually by 90 degrees, and developed in the second direction using second solvent system. The use of two different solvent systems provides separation based on two different sets of interactions. Consequently, components of the mixture that overlap after the first development can be further resolved with the second set of solvents.

7. EXPERIMENTAL PROCEDURE OF PAPER CHROMATOGRAPHY

The experimental procedure of paper chromatography involves the preparation of the sample and chromatographic paper, selection of the suitable mobile phase, the application of the sample, the development of a chromatogram, and the detection of the separated components.

7.1 Materials Required

The basic materials required are chromatographic paper, sample solution, suitable mobile phase, developing chamber, capillary tube or micropipette, pencil, ruler, forceps, and suitable visualization or detection reagents.

7.2 Preparation of Sample

A suitable solution of the material to be analysed is prepared using an appropriate solvent. The sample is sufficiently concentrated to provide detectable spots but should not be overloaded onto the chromatographic paper. If necessary, the solution may be filtered to remove insoluble particles.

7.3 Preparation of Chromatographic Paper

A suitable sheet of chromatographic paper is cut to the required size. A straight baseline or origin line is drawn about 1–2 cm from the bottom edge of the paper with a pencil. The positions on which the sample solution must be applied to are also marked on this line. A pencil is preferred because graphite does not normally dissolve and migrate with the developing solvent.

7.4 Preparation of Mobile Phase

A suitable solvent or solvent mixture that is appropriate to the chemical nature of the components to be separated is selected. The mobile phase should give satisfactory mobility and good separation of individual components.

7.5 Application of Sample

A small amount of the prepared sample solution is applied to the marked origin with a capillary tube or micropipette. The spot is allowed to dry. A higher sample concentration may be achieved by making several small applications at the same position, allowing the spot to dry between applications. A compact spot is preferred as an oversized spot can lead to poor separation of the components.

7.6 Chamber Saturation

The selected mobile phase is added into the developing chamber. The chamber is closed and allowed to equilibrate with the solvent vapour before any development. Chamber saturation helps reduce solvent evaporation and can improve the reproducibility of the chromatographic development.

7.7 Development of Chromatogram

The prepared chromatographic paper is placed in the developing chamber in such a way that the sample spot is above the level of the mobile phase. The chamber is closed and the solvent is allowed to move through the paper by capillary action.

As the solvent progresses through the paper, the components of the sample distribute themselves differently between the stationary and mobile phases. Therefore, they will move at different rates and separate into individual spots.

7.8 Marking the Solvent Front

The paper is removed from the chamber when the solvent has travelled a sufficient distance. The solvent front is immediately marked with a pencil because the solvent may evaporate rapidly. The chromatogram is then allowed to dry.

7.9 Detection of Separated Components

The separated components are detected using an appropriate visualization technique. Coloured substances are often directly detectable, whereas colourless substances require detection using ultraviolet light, iodine vapour, ninhydrin, or other suitable chemical reagents. The choice of detection method depends on the chemical nature of the substances being analysed.

8. PAPER DEVELOPMENT TECHNIQUES

8.1 Ascending Development

In ascending development, the mobile phase moves upwards through the chromatographic paper mainly by capillary action. The sample is applied near the bottom edge of the paper, and the paper is placed into the developing chamber so that the sample spot is above the level of the mobile phase.

8.2 Descending Development

In descending development, the mobile phase moves downwards through the chromatographic paper. The solvent is supplied from an upper reservoir. Gravity helps the movement of the mobile phase in addition to capillary forces.

8.3 Circular or Radial Development

In circular development, a circular sheet of chromatographic paper is used. The sample is generally applied near the centre. The mobile phase is supplied from an upper reservoir and moves radially outwards. Separation of the components occurs at different distances from the centre of the sheet.

8.4 Two-Dimensional Development

In two-dimensional paper chromatography, the sample is first developed in one direction using one solvent system. After the paper dries, it is rotated and developed in the second direction using another solvent system. Two-dimensional paper chromatography provides improved separation and is particularly suitable for separating complex mixtures with components having similar migration characteristics.

8.5 Ascending-Descending Development

In ascending-descending development, there is an initial movement of the solvent upwards followed by movement downwards. This method provides an extended development path and may be useful when additional separation is needed.

Overall, careful attention and control of sample application, solvent composition, chamber conditions, development distance, and visualization are essential for obtaining clear and reproducible chromatographic separation.

9. RETENTION FACTOR (R_F) VALUE

Retention factor is also commonly referred to as R_f value. In paper chromatography, R_f value is expressed mathematically as the ratio of the distance travelled by a component to the distance travelled by the solvent front from the origin.

Formula:

$$R_f = \text{Distance travelled by the component} / \text{Distance travelled by the solvent front}$$

Since R_f value is represented by the ratio of the two distances, its unit is not defined. For normal chromatographic conditions, R_f value generally lies in the range of 0 to 1.

For example:

If a component has travelled a distance of 4 cm from the origin and the solvent front has travelled a distance of 8 cm, the R_f value will be:

$$R_f = 4/8 = 0.50$$

Therefore, the R_f value of the component would be 0.50.

R_f value of the substances can be used for qualitative comparisons of various chromatographic components. An unknown substance can be compared with the known reference substance developed in the same

chromatographic system. Similar Rf values for an unknown substance and a known reference substance may confirm a component in a mixture, but an Rf value for a substance alone is not sufficient for the identification.

10. FACTORS AFFECTING RF VALUE

The Rf value of a substance is not a universal constant. The value of Rf always depends on the chromatographic conditions under which the experiment is carried out. The major factors affecting Rf value are nature and composition of mobile phase, properties of chromatographic paper, temperature, humidity, sample concentration, saturation of the developing chamber, pH, and nature of the sample.

10.1 Nature and composition of mobile phase

The solvent system has a major impact on migration of the various components. Alteration in the polarity of the solvent or changing the solvent composition or solvent ratio can cause changes in the Rf values and separation efficiency.

10.2 Properties of chromatographic paper

The composition of the paper, thickness, porosity, and moisture content can affect the solvent movement and subsequently influence the migration of the sample components.

10.3 Temperature

Temperature can influence the evaporation of solvents and the solubility of the various compounds. Therefore, it can have an impact on sample migration during the chromatographic process.

10.4 Humidity

Humidity can influence the moisture content of the chromatographic paper which may in turn influence the distribution of the substances present between the mobile and stationary phases.

10.5 Sample concentration

High concentrations of a sample can lead to broad or tailing spots thus making it difficult to measure accurate Rf values. A highly concentrated dot of a sample will provide good separation.

10.6 Chamber saturation

The degree of saturation of the developing chamber with solvent vapour can affect the development of a chromatographic pattern. Chamber saturation should be consistent to obtain good results.

10.7 pH

pH of mobile phase generally influences the ionization of substances capable of existing in different ionic form. This can influence the interactions of substances with stationary and mobile phases and consequently their Rf values.

10.8 Nature of the sample

Chemical properties of a substance can influence its interaction with the stationary and mobile phases and subsequently its migration

10.9 Development conditions

Various development conditions such as development distance, volume of solvent, chamber size, and development time can also influence chromatographic performance. Therefore, it is always critical to standardize development conditions while comparing Rf values.

11. APPLICATIONS OF PAPER CHROMATOGRAPHY

Paper chromatography has found applications in pharmaceutical analysis, biochemistry, food analysis, natural product research and educational laboratories. Despite the wide variety of more sophisticated chromatographic techniques, paper chromatography has proved to be useful for simple separations, qualitative analysis and demonstrating chromatographic principles.

11.1 Pharmaceutical Analysis

Paper chromatography can be used for preliminary separations and qualitative comparisons of particular pharmaceutical substances. An unknown sample can be compared to a reference substance with the same chromatographic conditions by examining the position of spots and their Rf values.

11.2 Separation of Amino Acids

The application of paper chromatography to the separation and identification of amino acids represents a valuable classical technique. The different amino acids exhibit different migration characteristics due to differences in their chemical properties and the interaction with the stationary and mobile phases. Ninhydrin is frequently used for visualization of amino acid spots.

11.3 Separation of Sugars and Carbohydrates

The technique can be used for the separation and qualitative examination of various sugars and carbohydrates. It can be applied in biochemical and related food studies.

11.4 Analysis of Plant Pigments

Plant pigments such as chlorophylls, carotenoids and xanthophylls can be separated through appropriate chromatographic conditions. This application demonstrates the differing migration behaviour of the pigments present in plant extracts.

11.5 Analysis of Dyes and Inks

Paper chromatography can be used to separate the individual components of mixtures of dyes. It is useful in the examination of writing inks, food colours, textile dyes and certain printing inks.

11.6 Natural Product Analysis

Paper chromatography may be used as a preliminary technique for examining extracts of natural materials and comparing their chromatographic patterns. It can provide valuable information during the initial investigation of constituents of plant origin.

11.7 Biochemical Applications

The technique has historically been used for the separation and examination of amino acids, sugars, peptides and other small biomolecules. It therefore has an important place in the development and teaching of basic biochemical analytical methods.

11.8 Food Analysis

Paper chromatography can be used for the qualitative examination of certain food colours, sugars, additives and plant-derived constituents.

11.9 Educational and Research Applications

Due to its simple procedure and relatively lowcost it is useful in teaching laboratories for demonstrating the separation, solvent migration, spot development and calculation of R_f values.

12. ADVANTAGES OF PAPER CHROMATOGRAPHY

The following advantages exist for paper chromatography:

1. Simple and relatively easy to perform.
2. Relatively inexpensive equipment and materials.
3. Only a small amount of sample is generally required.
4. Multiple samples can be explored on a single sheet when properly arranged.
5. Does not require sophisticated instrumentation for basic applications.
6. Valuable for qualitative separation and comparison.
7. Many separated substances can be detected using simple visualization methods.
8. It is particularly useful for teaching and illustrating fundamental chromatographic principles.

13. LIMITATIONS OF PAPER CHROMATOGRAPHY

Despite the usefulness of paper chromatography, it has certain limitations:

1. The sensitivity may be less than many of the modern instrumental techniques
2. It is primarily suitable for qualitative and, in some cases, semi-quantitative analysis, rather than highly precise quantitative analysis.
3. Complex mixtures may not always achieve adequate resolution.
4. Development may require considerable time especially if the method and solvent system is used.
5. Temperature and humidity can influence chromatographic development.
6. Reproducibility may be affected by the variation in paper characteristics, solvent composition, chamber conditions and the development conditions.
7. Traditional paper chromatography generally involves manual sample application, development and detection.
8. Not equally suitable for all classes of chemical substance.

14. PRECAUTIONS IN PAPER CHROMATOGRAPHY

Proper precautions should be taken to obtain reliable chromatographic results.

1. Clean and suitable chromatographic paper should be used.
2. The origin line should be drawn with a pencil rather than an ink pen.
3. The sample should be applied as a small, concentrated spot.
4. The sample spot must remain above the level of the mobile phase.
5. A suitable and clean solvent system should be used.
6. The developing chamber should remain closed during development.
7. Avoid unnecessary handling of the chromatographic paper.
8. Allow the sample spot to dry before development.
9. Handle the paper with care to prevent contamination.
10. The solvent front should be marked immediately after removing the chromatogram from the chamber.
11. Experimental conditions such as solvent composition, temperature, humidity and development distance should be kept as consistent as possible when comparing results.

By taking these precautions it can be ensured that clear spots, good separation and reproducible chromatographic results can be achieved.

15. CONCLUSION

Paper chromatography is a simple, inexpensive, and efficient chromatographic technique that allow a separation and preliminary identification of various chemical substances. This technique is based on the differential distribution of substances between a stationary phase incorporated into chromatographic paper and a moving solvent phase. Various components are migrated at different rates resulting in their separation.

A variety of methods such as ascending, descending, circular, ascending–descending, and two-dimensional paper chromatography can be chosen depending on the nature of the sample and the analysis purpose. The R_f value is helpful for comparison of migration of separated components under similar experimental conditions.

Paper chromatography has a wide range of applications from pharmaceutical analysis, pharmacognosy, and biochemistry to food analysis, natural-product research, and laboratory education. The main benefits of the method include its simplicity, cost-effectiveness, low requirement for samples, and equipment. However, its limitations include a relatively low resolution and sensitivity compared to several modern instrumental techniques.

Advanced chromatographic and spectroscopic techniques have replaced paper chromatography in many pharmaceutical analytical laboratories. However, this method still plays a critical role in separation of compounds and qualitative analysis, research,

and teaching. Thus, paper chromatography remains an essential and easy-to-implement chromatographic technique in pharmaceutical and scientific education.

ACKNOWLEDGEMENTS: The author is thankful to Guide Prof Kaja Reshma Professor Entrepreneurship Cell Coordinator Nimra College of Pharmacy for advice in writing this Article.

Declaration of Conflicting Interests:

The authors declare no potential conflicts of interest with respect to the research, authorship and publication of this article.

Funding:

The author received no financial support for the research, authorship and publication of this article.

16. REFERENCES

1. Skoog DA, Holler FJ, Crouch SR. Principles of Instrumental Analysis. Cengage Learning.
2. Willard HH, Merritt LL, Dean JA, Settle FA. Instrumental Methods of Analysis. CBS Publishers & Distributors.
3. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry. CBS Publishers & Distributors.
4. Vogel AI. Vogel's Textbook of Quantitative Chemical Analysis. Pearson Education.
5. Chatwal GR, Anand SK. Instrumental Methods of Chemical Analysis. Himalaya Publishing House.
6. Sharma BK. Instrumental Methods of Chemical Analysis. Goel Publishing House.
7. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman and Hall.
8. Stahl E. Thin-Layer Chromatography: A Laboratory Handbook. Springer.
9. United States Pharmacopeia–National Formulary (USP–NF). United States Pharmacopeial Convention.
10. International Pharmacopoeia. World Health Organization.