Active Pharmaceutical Ingredient (API) in a Drug Product by paper chromatography and determining pH of API samples

Project based lab report submitted for the partial fulfillment of requirement for the award of B. Tech in Biotechnology

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This is to certify that **2200010064–T.SANTHOSH,2200010078 – M.TRINADH,2200010094 – G.THRINADH,2200010133 – K.INDU PRIYA,2200010136 - CH.ANIRUDH** of Biotechnology Department, KL University has done this project based lab entitled **"Active Pharmaceutical Ingredient (API) in a Drug Product by paper chromatography and determining pH of API samples"** during their B.Tech second year in academic year 2023- 2024.

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DECLARATION

We, **2200010064–T.SANTHOSH,2200010078 – M.TRINADH,2200010094 – G.THRINADH,2200010133 – K.INDU PRIYA,2200010136 - CH.ANIRUDH**of second year Biotechnology Department, KL University hereby declare that the project-basedlab work entitled **"Active Pharmaceutical Ingredient (API) in a Drug Product by paper chromatography and determining pH of API samples"**was carried out by us under the guidance Bankuru Navyatha. This work has not been submitted in part or whole for award of any Degree or Diploma from any other University / Institute.

Place: Vaddeswaram

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ABSTRACT

The determination of the Active Pharmaceutical Ingredient (API) in a drug product using paper chromatography. Paper chromatography is a simple and cost-effective technique for separating and analyzing mixtures of compounds. In this experiment, chromatography paper strips were prepared and spotted with samples of the drug product containing the API. A suitable solvent system was used to develop the chromatogram, allowing the separation of the API from other components present in the drug formulation. Visualization of the chromatogram was achieved using UV light, enabling the detection of the API spot. The distance traveled by the API spot and the solvent front was measured, and the retention factor (Rf) value was calculated to assess the migration behavior of the API. The method provided a qualitative assessment of the presence of the API in the drug product. Further analysis, such as comparison with a reference standard, can confirm the identity of the API. Paper chromatography offers a rapid and accessible approach for preliminary screening of API content in pharmaceutical formulations, serving as a valuable tool for quality control and assurance in drug manufacturing and analysis.

The pH of Active Pharmaceutical Ingredient (API) samples serves as a critical parameter in pharmaceutical formulations, influencing stability, solubility, and efficacy. This study presents a systematic approach to determine the pH of API samples using precise laboratory techniques. Methodologies encompass pH meter calibration, sample preparation, and measurement protocols ensuring accuracy and reproducibility. Various factors affecting pH, such as temperature and ionic strength, are meticulously controlled to minimize errors. Statistical analysis is employed to validate results and ascertain confidence levels. The study aims to provide a robust framework for pH determination, essential for quality control in pharmaceutical manufacturing. Understanding the pH characteristics of API samples is fundamental for ensuring product integrity and patient safety.

CONTENTS

INTRODUCTION

Active Pharmaceutical Ingredients (APIs) are the biologically active components responsible for the therapeutic effects of a drug. Analyzing APIs within drug products is crucial to ensure their quality, efficacy, and safety. Among various analytical techniques, paper chromatography stands out as a versatile and cost-effective method for separating and identifying chemical compounds present in complex mixtures. Additionally, determining the pH of API samples provides valuable insights into their stability and behavior under different physiological conditions. This combined approach offers a comprehensive understanding of API characteristics essential for pharmaceutical development and quality control.

Paper chromatography is a type of partition chromatography where the stationary phase is a piece of paper, and the mobile phase is a solvent. The separation is based on differences in the affinity of the components for the stationary and mobile phases. It has been widely employed in pharmaceutical analysis due to its simplicity, rapidity, and effectiveness in separating closely related compounds.

The principle behind paper chromatography involves the migration of the components of a mixture through the paper at different rates. The separation occurs based on factors such as molecular size, polarity, and solubility. By comparing the migration distances of known standards with those of the API, one can identify and quantify the components present.

In pharmaceutical analysis, paper chromatography finds applications in determining the purity of APIs, identifying impurities, assessing the stability of formulations, and monitoring the progress of chemical reactions. Additionally, it is invaluable in quality control processes to ensure consistency and compliance with regulatory standards.

Complementing paper chromatography, pH determination of API samples provides critical information about their chemical properties and behavior in biological systems. pH, a measure of hydrogen ion concentration, profoundly influences drug solubility, dissolution rate, and stability. Variations in pH can alter the bioavailability and efficacy of the drug, making pH determination a vital aspect of pharmaceutical analysis.

The pH of API samples can be measured using various techniques, including pH meters, pH indicators, and titration methods. Understanding the pH profile of APIs helps in formulating dosage forms with optimal stability and bioavailability. Moreover, it aids in predicting potential interactions between the API and excipients, as well as assessing compatibility with physiological environments.

Key objectives of this project include:

To separate and identify the components of the API using paper chromatography.
To quantify the components present in the API sample.
To determine the pH of the API sample using appropriate methods.
To correlate the results obtained from paper chromatography and pH determination.
To assess the implications of the findings on the quality and stability of the drug product.

MATERIALS REQUIRED

Materials required for determining API by Paper Chromatography :

1. Drug product containing the API
2. Chromatography paper strips
3. Solvent system (e.g., mixture of water and alcohol or other suitable solvent)
4. Spotting capillary or pipette
5. Developing chamber (glass jar or beaker with a lid)
6. Ruler or measuring tape
7. Pencil or marker
8. UV lamp or other visualization technique (optional)
9. Reference standard of the API (if available)

Materials required for determining pH for the API samples :

1. Glass rod
2. Beaker
3. pH meter
4. Tissue paper
5. Distilled water
6. NaOH as basic solution
7. HCL as acidic solution

METHODOLOGY

Determining API by Paper Chromatography

1. Preparation of Sample:

If the drug product is in a solid form (e.g., tablet), crush or grind a small portion of the tablet into a fine powder.
If the drug product is in a liquid form (e.g., syrup), dilute a small volume of the product with a suitable solvent to obtain a clear solution.

2. Preparing the Chromatography Paper:

Cut a strip of chromatography paper to the desired size (usually 10-15 cm long and 1-2 cm wide).
Using a pencil or marker, draw a line near the bottom of the paper, approximately 1-2 cm above the edge.

3. Spotting the Sample:

Using a spotting capillary or pipette, apply a small volume (e.g., 1-2 µL) of the sample solution onto the marked line on the chromatography paper. Allow the spot to dry completely.

4. Developing the Chromatogram:

Pour the solvent system into the developing chamber to a depth of about 1 cm.
Place the chromatography paper strip into the chamber, ensuring that the spotted end is above the level of the solvent.
Seal the chamber with a lid to prevent solvent evaporation and allow the chromatogram to develop.
Remove the paper strip from the chamber when the solvent front is close to the top of the paper (but not touching the spot). Mark the solvent front with a pencil.

5. Visualization of Chromatogram:

If using a UV lamp, visualize the chromatogram under UV light. The API spot may fluoresce under UV light, aiding in its detection.
Alternatively, use a suitable visualization technique such as staining with a specific reagent or exposing the chromatogram to iodine vapor.

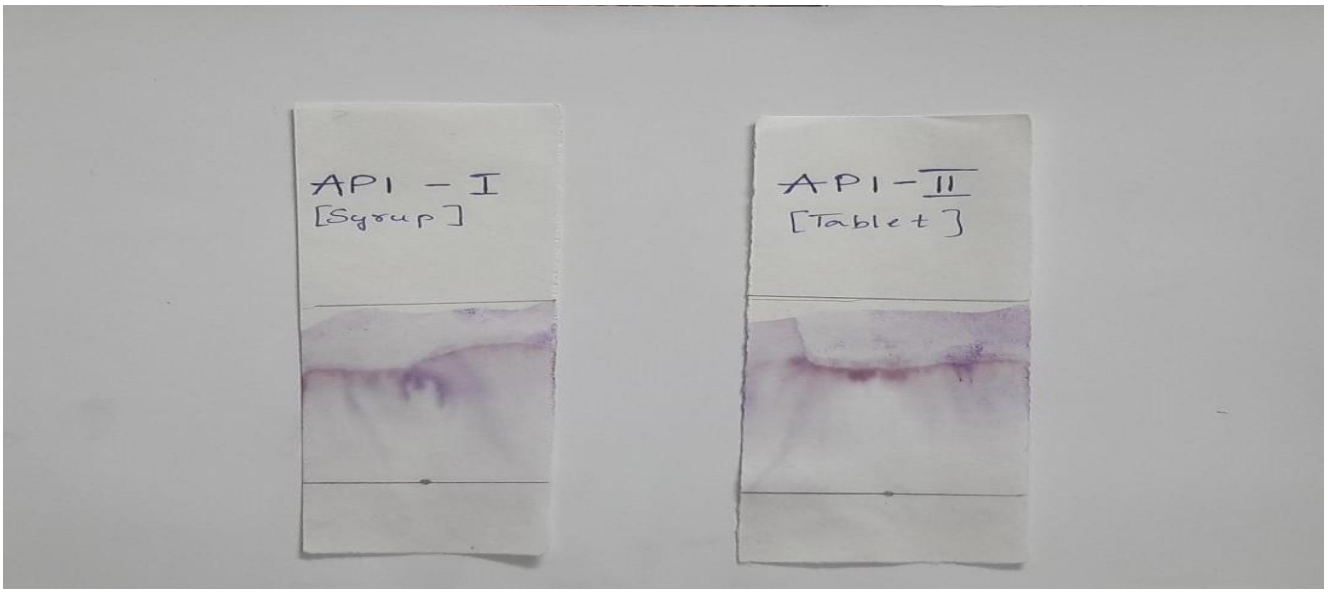
Determining pH of API samples :

1. Take 20ml of sample in a beaker and insert the pH probe to measure the pH
2. Measure the pH and make the solution either as acidic/basic accordingly, note down the volume of acid/base required.
3. After adding each drop of acid or base, the solution is mixed properly with a glass rod and noted the pH
4. After all the ten readings and pH is calculated by a calibration curve by plotting volume of acid/base on X-axis and pH on Y-axis.

S.NO	Volume of NaOH/HCL added to TABLET (API)	pH of sample
1	100 µl	5.20
2	200 µl	5.55
3	300 µl	6.14
4	400 µl	6.62
5	500 µl	8.22
6	600 µl	9.78
7	700 µl	10.1
8	800 µl	10.4
9	900 µl	10.7
10	1000 µl	11.0

3.RESULTS AND DISCUSSIONS

Results of paper chromatography



•Measure the distance traveled by the API spot and the solvent front from the origin line.
•Calculate the Rf (retention factor) value for the API spot using the formula:

RF = Distance travelled by solute
Distance travelled by solvent

RF(Syrup) = 3.5
6

Retention factor = 0.58

RF(Tablet) = 4
7

Retention factor = 0.57k

CONCLUSION

paper chromatography effectively separated the API from the drug product, allowing for its identification. The determined pH of the API samples provided additional quality control data. This combined approach offers a simple and reliable method for initial API analysis in drug products

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