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REVIEW ON QUANTITATIVE DETERMINATION OF PRESERVATIVES - METHYL PARABEN AND PROPYL PARABEN BY THIN LAYER CHROMATOGRAPHY

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Abstract
In the field of analytical chemistry and various pharmaceutical fields the separation techniques are widely used to study specific compounds from a mixture or a complex material. One such separation technique is called Chromatography. A simple, rapid, and cost-effective TLC method was developed and validated for the quantitative determination of methyl paraben and propyl paraben in pharmaceutical and cosmetic products. The method employed a mobile phase consisting of ethyl acetate-methanol-water (80:10:10, v/v/v) and showed good linearity (R2 > 0.99), accuracy (recovery 95-105%), precision (RSD < 2%), and specificity. The limits of detection and quantitation were 0.1 µg and 0.5 µg, respectively. This TLC method can be used for quality control and regulatory purposes.

Keywords: Thin layer chromatography, Methyl paraben, propyl paraben ,quantitative determination, preservatives..

Introduction

Chromatography is a technique used for separating the solutes or components present in a mixture. This separation takes place on the basis of the relative volume of each solute present in the moving fluid stream, which is known as mobile phase, and also present in the stationary phase. The mobile phase can comprise of a liquid or a gas and the stationary phase comprises of a solid or a liquid. The discovery of chromatography was done by Mikhail S.Tsvet, who was a Russian botanist. In the year 1901, Tswett discovered the physicochemical basis of separation and applied it in a scientific way to separate plant pigments. He specifically focused on the carotenoids and the chlorophylls of the plants. Since he did his research majorly on the coloured components of the plants, hence he named this method as chromatography which also derives meaning from Greek words. Chromatography can be effectively used for the purpose of separation, analysis, and purification of various components which can include food, pesticides, pharmaceuticals, tissue extracts and also air and water samples [1].

This technique is widely used in many pharmaceutical and chemistry domains because of its precision and applicability in these areas. Some of the important uses of Chromatography are as follows:

Chromatography and manufacturing of drugs

In the pharmaceutical industry the drugs are produced primarily using high-performance liquid chromatography. This technique is considered apt for compound separation before characterization while making the drug. This technique defines the quantitative composition of various compounds in a drug. Hence, it is widely used in quality control testing of the drug. This type of chromatography is also used in drug characterization [2]. It is also used in purification of products during various stages of synthesis, using modern automated processes in a timely manner.

This exercise results in a more refined final product.

Chromatography and testing of drugs

Chromatography is widely used in drug testing by various law bodies like police and forensic. In the testing the bodily fluids such as blood or urine are tested using chromatography to separate the naturally occurring compounds that result from metabolic breakdown of ingested material. In drug testing urine samples are preferred over blood samples because most of the drug compounds have relatively shorter half life in blood rather than that in urine. In urine many drug compounds can be detected even after several months of consumption.

Thin layer chromatography (TLC) is one of the simplest chromatographic techniques used for the separation and identification of compounds. This is also used to monitor the progress of chemical reaction at every step. We can also check the purity of synthesized compounds at very short time span. Thin layer of some inert material such as; alumina (Al,O), silica gel (SiO2), magnesium oxide (MgO), etc. is uniformly spread on glass plate either manually or mechanically and the solution of mixture is applied on it. After the development of plate with suitable mobile phase, components of mixture get separated as spot at different places on the plate [3].

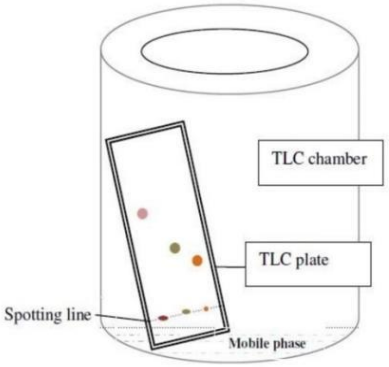

Fig.1: TLC chamber

Thin layer chromatography is similar to paper chromatography only instead of paper thin layer of stationary material is used. The advantage of using layer of stationary over paper is that, we can use very corrosive solvents such as hydrochloric acid and sulphuric acid as mobile phase without destroying the stationary phase. These solvents are very useful to separate and identify high molecular weight biological compounds. This technique is also useful to study variety of compounds such as natural extracts, sugars, amino acids, dyes, biological fluids, food colourings etc. Inorganic cations and anions also get separated by thin layer chromatography [2].

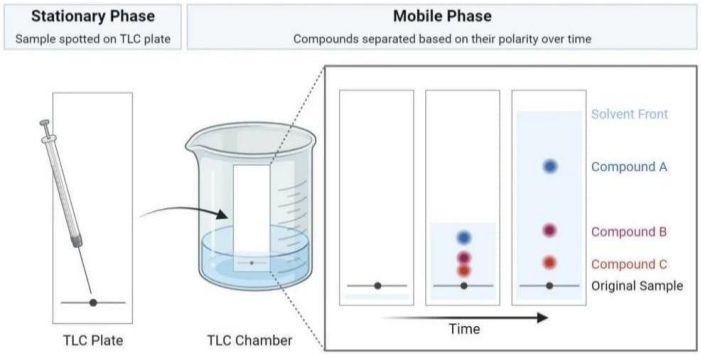

Fig.2: Thin layer chromatography

1.Separation
2.Idntification
3.Quantification

1. **To develop a TLC method:** Develop a TLC method for the separation and quantitation of methyl paraben and propyl paraben.
2. **To optimize the TLC conditions:** Optimize the TLC conditions, including the mobile phase, plate development, and detection, for the best separation and quantitation of methyl paraben and propyl paraben.
3. **To validate the TLC method:** Validate the TLC method for linearity, accuracy, precision, specificity, and limits of detection and quantitation.

4. **To determine the preservatives in pharmaceutical and cosmetic products:** Apply the developed and validated TLC method to determine the amounts of methyl paraben and propyl paraben in various pharmaceutical and cosmetic products.
5. **To compare the results with other analytical methods:** Compare the results obtained by the TLC method with those obtained by other analytical methods, such as HPLC or GC [4].

Principle of TLC

Thin layer chromatography uses a thin glass plate coated with either aluminum oxide or silica gel as the solid phase. The mobile phase is a solvent chosen according to the properties of the components in the mixture. The principle of TLC is the distribution of a compound between solid fixed phases (the thin layer) applied to a glass or plastic plate and a liquid mobile phase (eluting solvent) that is moving over the solid phase. A small amount of a compound or mixture is applied to a starting point just above the bottom of TLC plate [4]. The plate is then developed in the developing chamber that has a shallow pool of solvent just below the level at which the sample was applied.

The solvent is drawn up through the particles on the plate through the capillary action, and as the solvent moves over the mixture each compound will either remain with the solid phase or dissolve in the solvent and move up the plate. Whether the compound moves up the plate or stays behind depend on the physical properties of that individual compound and thus depend on its molecular structure, especially functional groups. The solubility rule "Like Dissolves Like" is followed. The more similar the physical properties of the compound to the mobile phase, the longer it will stay in the mobile phase. The mobile phase will carry the most soluble compounds the furthest up the TLC plate. The compounds that are less soluble in the mobile phase and have a higher affinity to the particles on the TLC plate will stay behind [4].

Rf values

The behavior of an individual compound in TLC is characterized by a quantity Known as Rf and is expressed as a decimal fraction. The Rf is calculated by dividing the distance the compound travelled from the original position by the distance the solvent travelled from the original position (the solvent front).

Rf = Distance of centre of spot from starting point /Distance of solvent front from starting point. The Rf value is a constant for each component only under identical experimental condition. It depends upon number of factors4.

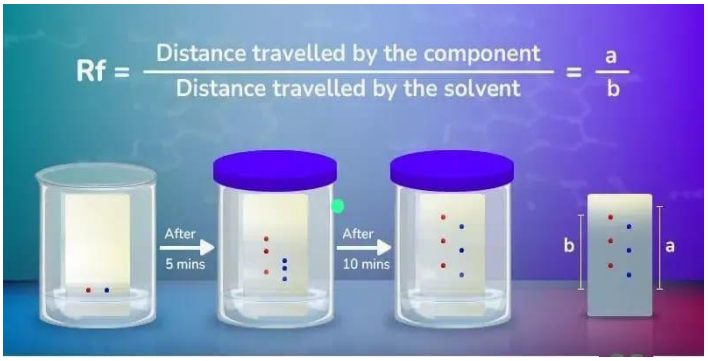

Fig.3: Steps of thin layer chromatography

The TLC Experiment

Visualization

When the solvent front has moved to within about 1 cm of the top end of the adsorbent (after 15 to 45 minutes), the plate should be removed from the developing chamber, the position of the solvent front marked, and the solvent allowed to evaporate. If the components of the sample are colored, they can be observed directly. If not, they can sometimes be visualized by shining ultraviolet light on the plate or by allowing the plate to stand for a few minutes in a closed container in which the atmosphere is saturated with iodine vapor. Sometimes the spots can be visualized by spraying the plate with a reagent that will react with one or more of the components of the sample.

Analysis

The components, visible as separated spots, are identified by comparing the distances they have traveled with those of the known reference materials. Measure the distance of the start line to the solvent front. Then measure the distance of center of the spot to the start line. Divide the distance the solvent moved by the distance the individual spot moved. The resulting ratio is called Rf-value. As the chemicals being separated may be colorless, several methods exist to visualize the spots. Often a small amount of a fluorescent compound, usually manganese

activated zinc silicate, is added to the adsorbent that allows the visualization of spots under a blacklight (UV254). The adsorbent layer will thus fluoresce light green by itself, but spots of analyte quench this fluorescence, Iodine vapors are a general unspecific color reagent, Specific color reagents exist into which the TLC plate is dipped or which are sprayed onto the plate. Once visible, the Rf value, or retention factor, of each spot can be determined by dividing the distance traveled by the product by the total distance traveled by the solvent (the solvent front). These values depend on the solvent used, and the type of TLC plate, and are not physical constants.

Preparative TLC

TLC can also be used on a small semi-preparative scale to separate mixtures of up to a few hundred milligrams. The mixture is not "spotted" on the TLC plate as dots, but rather is applied to the plate as a thin even layer horizontally to and just above the solvent level. When developed with solvent the compounds separate in horizontal bands rather than horizontally separated spots. Each band (or a desired band) is scraped off the backing material.

The backing material is then extracted with a suitable solvent (e.g. DCM) and filtered to give the isolated material upon removal of the solvent. For small-scale reactions with easily separated products, preparative TLC can be a far more efficient in terms of time and cost than doing chromatography. Obviously, the whole plate cannot be chemically developed or the product will be chemically destroyed. Thus this technique is best used with compounds that are colored, or visible under UV light. Alternatively, a small section of the plate can be chemically developed e.g. cutting a section out and chemically developing it, or masking most of the plate and exposing a small section to a chemical developer like iodine [4].

Applications of Thin Layer Chromatography

Testing, identification, stability testing, assay, and content uniformity testing of intermediates, raw materials, and drug products, with the analysis of sample analytes. Often degradation products, synthetic intermediates, and process related impurities do not have chromophores hence cannot be detected by the UV detector. Thus, these types of impurities are frequently specified by the TLC analysis.

Sometimes impurities are eluted at the solvent front in the HPLC, and that may be complicated to quantify and monitor. Modification in the mobile phase or HPLC column could not be sufficient to solve them adequately. On the other hand, occasionally the impurities take more time to elute from the column and they can't be detected, but TLC method is open in which whole samples are evaluated Thin layer chromatography is used in the early stage of drug development. Some of the applications of thin layer chromatography are described below:

1) **To Check Purity of Sample**
   - Purity of sample can be determined with TLC Direct comparison is done between the sample and the standard or authentic sample; if any impurity is detected, and then it shows extra spots and this can be detected easily.
2) **TLC in Compound Identification**
   - Thin layer chromatography can be employed in purification, isolation and identification of natural products like; volatile oll or essential oil, fixed oil, waxes, alkaloids, glycosides, steroids, etc.
3) **Monitoring of Chemical Reactions**
   - Reaction mixture can be examined by Thin layer chromatography to access whether the reaction is complete or not. This method is also used in checking other separation processes and purification processes like distillation, molecular distillation, etc.
4) **TLC in Biochemical Analysis**
   - Thin layer chromatography is extremely useful in Isolation or separation of biochemical metabolites or constituent from body fluids. Blood plasma, serum, urine, etc.
5) **In Chemistry**
   - TLC methodology is increasingly used in chemistry for the separation and identification of compounds which are closely related to each other. It is also used for identification of cations and anions in inorganic chemistry.
6) **In Pharmaceutical Industry**
   - Various pharmacopoeias have adopted TLC technique for detection of impurity in official monographs.
7) **Various medicines like**
   - Anti-histaminic, sedatives, anticonvulsant, tranquillizers antibiotics, analgesics, local anaesthetics, steroids have been tested qualitatively by TLC method.
8) **In Food and Cosmetic Industry**
   - TLC method is used for separation and identification of colours, preservatives, sweetening agent, and various cosmetic products5.

Preservatives

Preservatives are substance added to food to inhibit the microbial spoilage .chemicals food spoilage by enzymatic and nonenzymatic mechanism may be controlled by specific additives e.g.,antioxidents and antibrowning agents. Food preservation is used from the ancient times. Food preservatives becomes an essential thing nowadays, this plays an important role during food transportation. Preservatives are the substances, which are used to prevent food spoilage from microorganism. This will preserve the food for a long duration from the spoilage [7].

Dentification of the preservatives in pharmaceuticals by TLC.

- The most of used preservatives are-Methyl paraben
  -Propyl paraben
  -Germaben II
  -Imidazolidinyl urea

Materials and Methods

Stationary phase: silica gel 60, silica gel F254 pre-coated TLC plate on A1 support Mobile phase: mixture of solvents, Detection: UV lights, specific reagents Parabens**:**

Alkyl esters of p-hydroxybenzoic acid, also known as parabens, are widely used as antimicrobial agents in food products, pharmaceutical preparations,cosmetic and toiletries consumer products. This family of chemicals mainly includes methylparaben, ethyl paraben, npropylparaben, iso-propylparaben, n- butylparaben, iso-butylparaben and benzyl paraben, and their log Dow, pKa and aqueous solubility. It had been found that antimicrobial activity increased as the chain length of the ester group of paraben increased. However, esters of longer alkyl chains are of limited applications due to their lower solubility in water. To reach a satisfied activity, parabens are usually used as mixtures according to their antibacterial synergistic effect. Among all parabens, methylparaben and propylparaben are often used together. Parabens have been added to food for a very long time, and the use of parabens has steadily increased in many more food categories over the years. They are employed in several foods including processed vegetables, baked goods, fats and oils, seasonings, sugar substitutes, coffee extracts, fruit juices, pickles, sauces, soft drinks and frozen dairy products at concentrations between 450 and 2000 mg kg [17].

Methylparaben

One of the parabens compounds with the chemical formula C8H8O3 is the methyl ester of phydroxy benzoic acid as the IUPAC name is methyl 4-hydroxy benzoate. It is available in the form of white crystals, but industrial use grades may be light grey or light tan. Contacting with air and light causes oxidation and darkening of colour. Methyl paraben is soluble in water, methanol, and ether and slightly soluble in chloroform.

Experimental Procedure for the Determination of Preservatives by TLC

Principle

- The preservatives are extracted from the acidified sample with acetone.
- After filtration, the acetone solution is mixed with water, and in alkaline medium the fatty acids are precipitated as their Ca salts.
- The alkaline acetone/water mixture is extracted with diethyl ether to remove lipophilic substances.
- After acidification the preservatives are extracted with diethyl ether.
- An aliquot of the diethyl ether extract is spotted on a silica gel coated thin-layer plate.
- After development of the plate, the chromatogram obtained is observed under UV light and visualized using Million's reagent.

Procedure

- Weigh accurately about 1g of sample into a 125ml flask.
- Add 4 drops of HCL4M, add 40ml of acetone and mix.
- HEAT the mixture to about 6oc until complete extraction.
- Cool and shack for 1minute.
- Adjust the solution at <3 using 4M KOH.
- Add calcium chloride dihydrate and shack.
- Filtrate the solution into a 250ml separating funnel, containing 75ml diethyl ether, and shake for 5min.
- Allow the phases to separate.
- Discard the upper layer (diethyl ether phase).
- Collect the aqueous phase (lower layer) in a 100ml separating funnel [9].

Determination

- Active the plates at 100c for 10 minutes.
- Apply 10ML of each of the reference solution and 100ML of the sample solution on base line of TLC plate.

- A stream of air can be used to facilitate evaporation of the solvent.
- Transfer an adequate volume of the development solvent into a developing tank of suitable size.
- Place the TLC plate in the chamber and develop at room temperature.
- Remove the plate from development tank and dry it.
- Examine the plate under UV light.
- Visualize the preservatives in the chromatogram with millions reagent.
- Calculate the Rf value for each spot.
- Compare the spots obtained from the sample solution with those of standard solution with respect to their Rf values, their behavior under radiation and the colour, After visualization [10].

Conclusion

A simple, rapid, and cost-effective TLC method was developed and validated for the quantitative determination of methyl paraben and propyl paraben in pharmaceutical and cosmetic products. The method showed good linearity, accuracy, precision, and specificity. The limits of detection and quantitation were 0.1 µg and 0.5 µg, respectively. The developed TLC method can be used for routine quality control analysis of methyl paraben and propyl paraben in various products. The method's advantages, including simplicity, speed, and low cost, make it an attractive alternative to more complex and expensive chromatographic techniques. This study demonstrates the potential of TLC as a valuable analytical tool for the quantitative determination of preservatives in pharmaceutical and cosmetic products.

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Conflict of Interest

No

Informed Consent

Not Applicable.

Ethical Statement

Not Applicable.

Author Contribution

All authors are contributed equally.

Reference

1. Shashank Tiwari1and Shreya Talreja2. Thin Layer Chromatography (TLC) VS. Paper Chromatography: A Review. Acta Scientific Pharmaceutical Sciences (ISSN: 2581-5423). 2022;6(9): 05- 09.
2. Sanjeet Kumar1, K. Jyotirmayee2, Monalisa Sarangi2. Thin Layer Chromatography: A Tool of Biotechnology for Isolation of Bioactive Compounds from Medicinal Plants. 2012; 126-132.
3. Bansode Badal Mahadev, 2Garad R.S, 3Dr.Santosh Jain, 4Kamble Manoj, 5Shinde Vivek Thin Layer Chromatography. 2023;11(5).
4. Archana A. Bele and Anubha Khale H. K. College of Pharmacy, Jogeshwari Mumbai ,Maharashtra, India. Anoverview on thin layer chromatography.2010;2(2):256-267.
5. Bansode Badal Mahadev, 2Garad R.S, 3Dr.Santosh Jain, 4Kamble Manoj, 5Shinde Vivek. Thin Layer Chromatography. 2023;11(5).
6. Gedela Vamsi Krishna. Review on the role of food preservatives and its efficiancy. 2017;2(2).
7. Mariam Farag Ambarak. Determination of methylparaben in some cosmetics and pharmaceutics using liquid-liquid extraction and spectrophotometric technique.2019.
8. Syeda Rakhshinda Zareen*1,Dr. Osman Ahmed1 , Reshma1 , Mohammed Sayeed Uddin1 and Dr. Anas Rasheed2.Thin layer chromatography.2023;10(2).
9. RAJESH M. KAMBLE* , SANTOSH G. SINGH and SHRAWAN SINGH. Simultaneous Determination of Preservatives (Methyl Paraben and Propyl Paraben) in Sucralfate Suspension Using High Performance Liquid Chromatography. 2011, 8(1), 340-346.

10. Quach, H. T, Steeper, R L, Griffin, G. W, Separation of plant pigments by thin layer chromatography. Journal of chemical education. 2004, 81, 385-7.
11. Singhal S., Singhal N., Agarwal S., Pharmaceutical Analysis II, Thin layer chromatography, Pragati prakashan, First edition, 2009, 98-111.
12. Kasture A.V., Mahadik K.R, Wadodkar S.G, More H.N., A textbook of pharmaceutical analysis, Instrumental methods, Nirali Prakashan, 9th edition, 2005, vol II, 18-30.
13. Quach, H. T, Steeper, R L, Griffin, G. W, Separation of plant pigments by thin layer chromatography. Journal of chemical education. 2004, 81, 385-7.
14. Skoog D. A., Holler F.J. and Nieman T.A., "Principles of instrumental analysis, Saunders college publishing, 5th edition, 2006 ,761-766.
15. Chatwal G. R., Anand S.K, Instrumental methods of chemical analysis, Himalaya publishing house,5th edition,2008, 2.599-2.616.
16. Beckett A.H, Stenlake J.B., Practical pharmaceutical chemistry, Thin layer chromatography, CBS publishers, 4th edition, 2005, 115-128.
17. Yadav P, Garg N, Kumar S. Improved shelf stability of Mulberry juice by combination of preservatives. Indian J Natural Prod Resources. 2014;5(1):62-66.
18. Sarkar S, Saha S, Rai C, Bhattacharyya S. Effect of storage and preservatives on antioxidant status of some refrigerated fruit juices. Int J Curr Microbiol App Sci. 2014;3(7):1007-1013.
19. M Ali and V. Agrawal, Thin-layer chromatography of aromatic amines, Separation Science and Technology, 37, 2002, 363 - 377.
20. S Singhal, N singhal, S Agarwal, Pharmaceutical analysis-II, Thin Layer Chromatography, Pragati Prakashan, first edition, 2009, 98-111.
21. A. Mohammad, S.A. Bhawani and S. Sharma, Analysis of herbal products by thin-layer chromatography: a review, International Journal of Pharma and Biosciences, 1(2), 2010, 1-50.
22. A.H. Beckett, J.B.Stenlake, Practical pharmaceutical chemistry, thin layer chromatography, CBS publishers, 4th edition, 2005, 115-128.
23. Singhal S., Singhal N., Agarwal S., Pharmaceutical Analysis II, Thin layer chromatography, Pragati prakashan, First edition, 2009, 98-111.
24. Kasture A.V., Mahadik K.R, Wadodkar S.G, More H.N., A textbook of pharmaceutical analysis, Instrumental methods, Nirali Prakashan, 9th edition, 2005, vol II, 18-30.
25. Quach, H. T, Steeper, R L, Griffin, G. W, Separation of plant pigments by thin layer chromatography. Journal of chemical education. 2004, 81, 385-7.
26. Skoog D. A., Holler F.J. and Nieman T.A., "Principles of instrumental analysis, Saunders college publishing, 5th edition, 2006 ,761-766.
27. Chatwal G. R., Anand S.K, Instrumental methods of chemical analysis, Himalaya publishing house,5th edition,2008, 2.599-2.616.
28. Beckett A.H, Stenlake J.B., Practical pharmaceutical chemistry, Thin layer chromatography, CBS publishers, 4th edition, 2005, 115-128.
29. Ali M. and Agrawal V., Thin-layer chromatography of aromatic amines, Separation Science and Technology, 2002, 37, 363 - 377.