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QUALITATIVE ANALYSIS OF ANTIDIABETIC DRUGS BY THIN LAYER CHROMATOGRAPHY

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ABSTRACT

TLC has recently gained popularity as an analytical method it may be used in a variety of contexts, particularly for the purpose of determining the presence of contaminants in a chemical. Prior to high-performance liquid chromatography (HPLC), it may be employed as a preliminary analytical procedure. TLC is easy to understand conceptually, and it requires little preparation of materials in most cases. TLC is the gold standard in many fields, including industrial chemistry, environmental toxicity, food chemistry, water, inorganic and pesticide analysis, dye purity, cosmetics, plant materials, and herbal analysis. In this study, an attempt was made to review the basic principles and the importance of Thin Layer Chromatography (TLC) in research in general. Thin layer chromatography is a simple, cost-effective, and easy-to-operate planar chromatographic technique which has been used in general chemistry laboratories for several decades to routinely separate chemical and biochemical compounds. Traditionally, chemical and optical methods are employed to visualize the analyte spots on the TLC plate. Also it has a wide application in identifying impurities in a compound. Study highlights the review on TLC and its application of qualitative and quantitative estimation of bio-active compounds from medicinal plants.

Keywords: Thin Layer Chromatography, Mobile phase , Stationary phase, UV cabinet.

I. INTRODUCTION

Chromatography is the collective term for a set of laboratory techniques for the separation of mixtures into their components. All chromatographies have a stationary phase (a solid or a liquid supported on a solid) and a mobile phase (a liquid or a gas). The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material [1] called the stationary phase. The mobile phase flows through the stationary phase and carries the components of the mixture with it. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. There are different types of chromatography such as Column chromatography, Paper chromatography etc. among them Thin layer chromatography (TLC) is a widely employed laboratory technique and is similar to paper chromatography. However, instead of using a stationary phase of paper, it involves a stationary phase of a thin layer of adsorbent like silica gel, alumina, or cellulose on a flat, inert substrate. Compared to paper, it has the advantage of faster runs, better separations, and the choice between different adsorbents. .Thin layer chromatography (TLC) is among the most useful tools for following the progress of organic chemical reactions and for assaying the purity of organic compounds in phytochemistry and Biotechnology. Like all chromatographic methods, TLC takes advantage of the different affinity of the analyte[2] with the mobile and stationary phases to achieve the separation of complex mixtures of organic

molecules. A TLC plate is a sheet of glass, metal or plastic which is coated with a thin layer of a solid adsorbent. A small amount of the mixture to be analyzed[3] is spotted near the bottom of this plate. The TLC plate is then placed in a shallow pool of a solvent in a developing chamber so that only the very bottom of the plate is in the liquid [4]. A TLC plate is a sheet of glass, metal, or plastic which is coated with a thin layer of a solid adsorbent (usually silica or alumina). A small amount of the mixture to be analyzed is spotted near the bottom of this plate. The TLC plate is then placed in a shallow pool of a solvent in a developing chamber so that only the very bottom of the plate is in the liquid. This liquid, or the eluent, is the mobile phase, and it slowly rises up the TLC plate by capillary action. To determine the best solvent or mixture of solvents (a "solvent system") to develop a TLC plate or chromatography column loaded with an unknown mixture, vary the polarity of the solvent in several trial runs: a process of trial and error. Carefully observe and record the results of the chromatography in each solvent system. Hence increase the polarity of the solvent system, all the components of the mixture move faster (and vice versa with lowering the polarity). The ideal solvent system is simply the system that gives the best separation. TLC elution patterns usually carry over to column chromatography elution patterns. Since TLC is a much faster procedure than column chromatography, TLC is often used to determine the best solvent system for column chromatography. For instance, in determining the solvent system for a flash.

Separation of compounds is based on the competition of the solute and the mobile phase for binding places on the stationary phase. For instance, if normal phase silica gel is used as the stationary phase it can be considered polar. Given two compounds which differ in polarity, the more polar compound has a stronger interaction with the silica and is therefore more capable to dispel the mobile phase from the binding places. Consequently, the less polar compound moves higher up the plate. If the mobile phase is changed to a more polar solvent or mixture of solvents, it is more capable of dispelling solutes from the silica binding places and all compounds on the TLC plate will move higher up the plate. Practically this means that if you use a mixture of ethyl acetate and heptane as the mobile phase, adding more ethyl acetate results in higher Rf values for all compounds on the TLC plate. Changing the polarity of the mobile phase will normally not result in reversed order of running of the compounds on the TLC plate [5,6,7,8]. As the solvent slowly travels up the plate, the different components of the dye mixture travel at different rates[9] and the mixture is separated into different coloured spots[10]. The solvent is allowed to rise until it almost reaches the top of the plate. That will give the maximum separation of the dye components for this particular combination of solvent[11,12] and stationary phase.

S. No	Equipment	Make/Model
1	Electronic weighing balance	Contech, Model-125
2	UV-Visible transilluminator	Shimadzu, Model UV-1800
3	Hot air oven	Bio-Technique
4	Air Dryer	Philips

II. RESEARCH METHODOLOGY

2.1 APPARATUS: Volumetric flask, graduated pipette, Beakers, TLC Plates, TLC Chamber, Test tubes, Measuring cylinder, Capillary tubes.

2.2 CHEMICALS: Silica gel-G, Chloroform formic acid, Methanol, Saxagliptin, Metformin, Alogliptin, Distilled water.

2.3 METHOD: Thin layer chromatography (TLC)

2.4 PREPARTION OF TLC PLATES

1. Thin layer chromatography uses a thin glass plate coated with silica gel as the solid phase.
2. This mixture is spread as thick slurry on an unreactive carrier sheet, usually glass, the resultant plate is dried and activated by heating in an oven for thirty minutes at 110 °C.
3. To Prepared a silica gel TLC plates [13], draw a baseline 1cm from the bottom without disturbing silica gel. Use a small capillary

tube for spotting of the sample

4. Leave the space for unknown sample.
5. Spot your TLC plates with a mixture of crushed tablet and methanol.
6. After spotting the sample with standard and unknown sample allow it to dry.
7. Place a small amount of ethyl acetate as developing solvent in a chamber.
8. The liquid should cover the bottom of the breaker to a depth of 0.5cm.
9. Line the beaker within the piece of filter paper to saturate the atmosphere within it.
10. After drying place the plates in the development chamber.
11. Cover the beaker with an aluminium foil[14] and allow the solvent front to move up the plate.
12. The solvent will travel up the silica plate very quickly and it will reach the top within two minutes.
13. Allow the plate to dry for few minutes and place the plate in an iodine chamber in such a way the surface is completely exposed to iodine vapours.
14. Observe the colour of the spots that are appeared as a result of expousre to Iodine vapours.
15. Circle the spots and record chromatogram[10], measure the distance travelled from the baseline to the centre of each spot and distance travelled by solvent front.
16. R_f values are measured the distance travelled by the solvent, and the distance travelled by individual spots. When the solvent front gets close to the top of the plate, the plate is removed from the beaker and the position of the solvent is marked with another line before it has a chance to evaporate.
17. Calculate the R_f values for the samples and identify the unknown sample

Figure 1.0: Steps of Preparative TLC

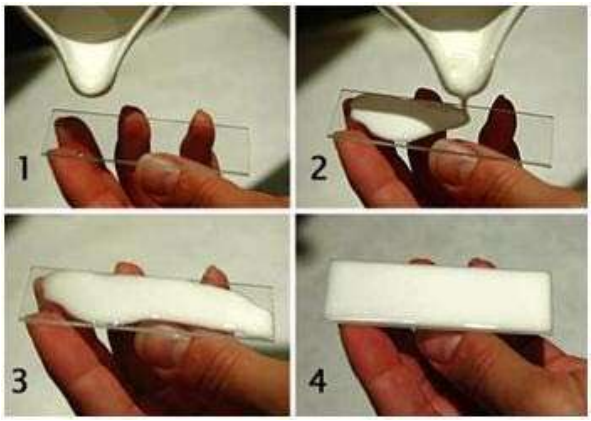

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Figure 2.0: TLC plate in chromatographic chamber

Sno	Sample	Distance travelled by solute	Distance travelled by solvent	Rf value
1	Saxagliptin	11.6	13	0.14
2	Metformin	3.6	13	0.4
3	Alogliptin	0.9	13	0.6
4	Mixture	11.3 8.7 4.1	0.86 0.66 0.31	0.86 0.66 0.31

Table 1.0 Analysis of Antidiadbetic Drugs by Thin Layer Chromatography (TLC)

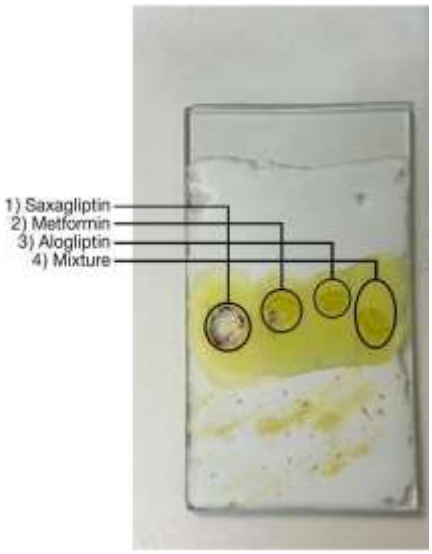

Fig . 3.0 TLC Plates after detection of spots

Fig. 4.0 TLC Under UV

III. RESULTS AND DISCUSSION

The separation and identification of given unknown mixture of antidiabetic Drugs by Thin Layer Chromatography was performed. The Rf value of the unknown sample was found to be 0.86 for spot 1 which corresponds to Saxagliptin. The Rf value of the unknown sample was found to be 0.66 for spot 2 which corresponds to Metformin. The Rf value of the unknown sample was found to be 0.31 for spot 3 which corresponds to Alogliptin. In Thin Layer Chromatography (TLC), Rf values indicate the relative distances travelled by solutes compared to the solvent front. The Rf value of the unknown sample was found to be 0.86 for spot 1 which corresponds to Saxagliptin. The Rf value of the unknown sample was found to be 0.66 for spot 2 which corresponds to Metformin. The Rf value of the unknown sample was found to be 0.31 for spot 3 which corresponds to Alogliptin. In conclusion, this has laid the foundation for robust laboratory practices, ensuring precision, accuracy, and reliability in pharmaceutical analysis and quality control processes. The established SOPs, calibrated instruments, and analytical techniques contribute to the advancement of research and development in the pharmaceutical industry, ultimately enhancing the safety and efficacy of medical treatments for consumers.

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V. REFERENCES

1. Torres y Torres, J. L.; Hiley, S. L.; Lorimor, S. P.; Rhoad, J. S.; Caldwell, B. D.; Zweerink, G. L.; Ducey, M. “Separation of Caffeine from Beverages and Analysis Using Thin Layer Chromatography and Gas Chromatography-Mass Spectrometry”. J. Chem. Educ. 2015, 92 (5), 900– 902.
2. Sjursnes, B. J.; Kvittingen, L. K.; Sch"mid, R.” Normal and Reversed-Phase Thin Layer Chromatography of Green Leaf Extracts”. J. Chem. Educ. 2015, 92 (1), 193– 196,
3. A . Archana, Bele and Anubha Khale, “An overview on thin layer chromatography”, IJPSR, 2(2), 2011, 256-267.

4. 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.
5. A.V. Kasture, K.R. Mahadik, S.G.Wadodkar, H.N. More, "A textbook of pharmaceutical analysis, instrumental methods" , Nirali Prakashan, 9th edition, 2, 2005, 18-30.
6. A.H. Beckett, J.B.Stenlake, "Practical pharmaceutical chemistry, thin layer chromatography" , CBS publishers, 4th edition, 2005, 115-128.
7. B. Fried, J. Sharma, "Thin-layer chromatography, fourth edition, revised and expanded." Marcel Dekker inc., New York - Basel, 1999, 499.
8. B.K.Sharma, Instrumental methods of chemical analysis, Goel publishing house, Meerut, 5th edition, 2007, 241-264.
9. D.A.Skoog, F.J.Holler and T.A. Nieman, "Principles of instrumental analysis, Saunders college publishing, 5th edition, 2006: 761-766.
10. E. Dreassi, G. Ceramelli, P. Corti, "Thin-layer chromatography in pharmaceutical analysis. In practical thin-layer chromatography—a multidisciplinary approach". Fried b., Sharma J. (eds.) Crc press, Boca Raton, fl, 1996, p.
11. Sharma G, Fried B (eds.) Marcel Dekker, Steroids. "In handbook of thin layer chromatography, Sharma G, Fried B (eds.) Marcel Dekker, New York, 1991, 907.
12. Sharma G, Fried B (eds.) Marcel Dekker, Steroids. In handbook of thin layer chromatography". New York, 1996, 971.
13. Bele AA, Khale A. An overview on thin layer chromatography. International Journal of Pharmaceutical Sciences and Research. 2011 Feb 1;2(2):256.
14. Tavallali H, Zareiyan J SF, Naghian M. "An efficient and simultaneous analysis of caffeine and paracetamol in pharmaceutical formulations using TLC with a fluorescence plate reader". Journal of AOAC International. 2011 Jul 1;94(4):1094-9.