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Some Observations on Thin Layer Chromatography Technique

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Abstract: ***The present study is to report the various problems faced during TLC methodology. Although used regularly some technical aspects must be kept in mind to get better and uniform results. During our experiments with TLC methods we came across some problems and here these aspects of TLC methodology are being highlighted. It is suggested that the solvent use as mobile phase should also be used for extraction of any particular phytochemical. TLC plates should be 3 to 4 mm thick, to be dried for at least 72 hrs. It is also suggested that potassium permanganate solution gives better clarity in visualizing the spots.***

Key words: TLC, Extraction. Methodology, Phytochemical, Potassium Permanganate

I. INTRODUCTION

Chromatography is a perfect analytical technique for the identification, isolation and separation of compounds based on differences in affinity for stationary phase and mobile phase. In Chromatography the stationary phase maybe solid, liquid, gel or a solid liquid mixture and the mobile phase may be liquid or gas TLC is a chromatography technique which is used for basic separation. This technique is carried out by making thin layer of adsorbent materials, generally Silica Gel, Aluminium oxide are commonly used as a adsorbent materials over the glass plate, plastic etc. Within a short period of time large number of sample can be analyzed simultaneously with the help of thin layer chromatography technique, material which are non-volatile or having low volatility and which are not responding by liquid chromatography and gas chromatography then for those kind of material TLC is useful. Separation of substance is based on the solute and the mobile phase on the stationary phase. If the substance is more polar, Rf value getting more than that of less polar material.

Principle of chromatography: The principle of chromatography is adsorption and partition, while mixture of substance is allowed to pass through stationary and mobile phase, a compound become separated according to their Rf.

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Rf = distance travelled by the sample salute /distance travelled by solvent front
Rf value is depend on some factors such as type of adsorbent, quantitative mixture of solvent,
Volatility, temperature. If temperature is more than quick evaporation will take place so better to keep running chamber away from the heat, sunlight etc, thickness of the material, moisture content, running tank situation, quantity of samples.

Application of thin layer chromatography: Thin layer chromatography of amino acids pharmaceuticals and drugs- identification, purity test and evaluating the concentration of active ingredients, preservative in drug, preparation process control in multi-potent Pharmaceutical formulation, qualitative analysis of alkaloid, in cosmetology, food analyses, petroleum product, aromatic compound isolation, molecular distillation, characterization of vitamin antibiotics and definitions of drugs and inorganic ions.
The present study deal with modification of thin layer chromatography by different alterations to the regular methods to get better results. (1- 4)

II. METERIALS AND METHODS

Sample Preparation:
Leaves of plants were taken and soacked in various organic solvents separately to obtain the phytochemicals. The extracts were dried and the powders were used for identification of phytochemicals by TLC using different solvent mixtures. The various spots were visualized by putting the slides in Crystal Iodine chamber. The respective standards were also used to compare the spot movement and rf values for each phytochemical, such as steroids, alkaloids, flavonoids, terpenoids etc. we have used Potassium Permanganate solution also for visualizing the spots and the present study indicates some advantages of using this methods as compared to using Crystal Iodine chamber.

III. RESULTS AND DISCUSSION

The observations regarding techniques and modifications are mentioned hereunder.
1. During TLC plate preparation by the silica gel, the thickness of the TLC plate should be maintained between 3 to 4mm.
a. If the layer is thick it gets cracks during heating.
b. If the layer is very thin, drawing the line for charging the sample becomes difficult and there is a chance that the portion of the gel which is in touch with the solvent can dissolve.

c. If the plates are not dried properly the gel slips into the solvent causing difficulty in charging and separation.
d. It was observed that the plates should be dried in closed rooms or chambers and should be used after 72 hrs for best results. If they are used immediately after drying or at intervals of 12, 24 and 48 hrs. there is a chance that that the gels may slip or lead to other problems. (Figure 1 and Figure 2)

IV. SAMPLE SPOT: FOR ANALYSIS OF BIOLOGICAL SAMPLE

a. It was observed that the samples should be dissolved in the respective solvents for any phytochemical, allowed to settle for 10 to 15 hrs and should be centrifuged for 8m to 10 min at 6000 to 7000 rpm to get good and clear spots. (Figure 3)

TIME CONSUMPTION

It was found that if the mobile phase solvent is very old by 5, 24, 36 and 48 hrs than running of TLC plates takes too much time than freshly prepared solvent for the running of chromatography plates. So it is better to use freshly prepared solvent or mixture of solvents.

V. VISUALIZATION OF SPOTS ON TLC PLATES

Generally visualization of TLC plates is carried out by UV, iodine chamber, sulphuric acid and potassium permanganate etc. for developing the spots. In the present experiment we used both Iodine chamber and Potassium permanganate solution for visualizing the spots. It was observed that Potassium permanganate indicated clearer spots when compared to Iodine Chamber treatment. The plates were kept in chamber containing Potassium permanganate solution and it was observed that in few min. the whole plate was stained with Potassium permanganate. The plates were removed, dried for a day and then kept in chamber dipped in distilled water. The excess Potassium permanganate was removed by the distilled water and the spots were visualized very clearly as shown in Figures 3-9.

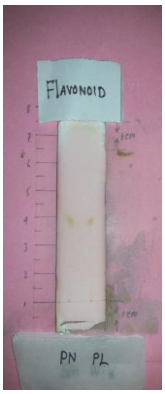

Figure 1

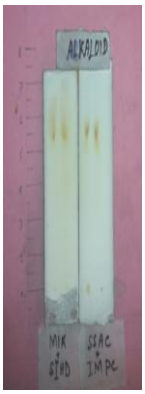

Figure 2.

Figure 3

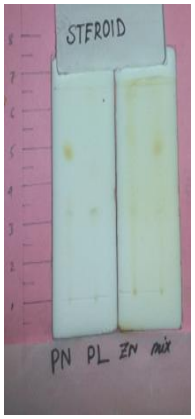

Figure 4

Figure 5

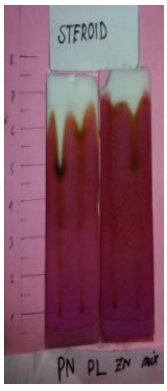

Figure 6

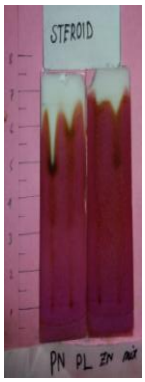

Figure 7

Figure 8

Figure 9

Figure legends:
Figure 1: Broken TLC layer due to its use just after drying.
Figure 2. Broken TLC layer due to its use just after drying.
Figure 3. Sample after centrifugation shown clear spot (left) as compared to the one without cent ifugation.
Figure 4 to 9.Indicate the process of using Potassium permangatnate solution for staining the spots as compared to Crystal Iodine Chamber exposure (Figure 4)

REFERENCES

1. Mudiganti Ram Krishna Rao, Sampad Shil. Thin Layer Chromatography - as a tool for standardization of Ayurvedic medicine, Triphala churna. Indo American J of Pharmaceutical Sciences, Vol. 5(05), pp. 3804-3817, 2018.
2. Sampad Shil, Mudiganti Ram Krishna Rao, Prabhu K, Amuthvalli K Thin Layer Chromatography - as a tool for standardization of Ayurvedic medicine, Trikatu churna. Indo American J of Pharmaceutical Sciences, Vol. 5(6), pp. 5039-5046, 2018.
3. Mudiganti Ram Krishna Rao, Balasubramanuam M. TLC, GC MS and antibacterial study of methanol extracts of *Tribulus terrestris* thorns and *Morinaga oleifera* flowers. Indo American J of Pharmaceutical Sciences, Vol. 5(5), pp. 3300-3308, 2018.
4. N. Vijayalakshmi, Mudiganti Ram Krishna Rao. The antioxidant studies of two medicinal plants, *Sphaeranthus indicus and Psophocarpus tetragonolobus*. Asian J of pharmaceutical and Clinical Res, Vol. 12(1), pp. 321-327.2019.