

EXPERIMENT NO.13

THIN-LAYER CHROMATOGRAPHY OF THE GIVEN HERBAL EXTRACT.

AIM OF THE EXPERIMENT: To perform Thin-layer chromatography of the given herbal extract.

REFERENCES:-----

REQUIREMENTS (APPARATUS):

a) Flat glass plates of appropriate dimensions which allow the application at specified points of the necessary quantities of the solution being examined and appropriate reference solutions

b) An aligning tray or a flat surface on which the plates can be aligned and rested when the coating substance is applied.

The adsorbent or coating substance consisting of finely divided adsorbent materials, normally 5µm to 40µm in diameter is suitable for chromatography.

d) A spreader which, when moved over the glass plate, will apply a uniform layer of adsorbent of desired thickness over the entire surface of the plate.

e) A developing chamber that can accommodate one or more plates and can be properly closed and sealed. Graduated micro-pipettes capable of delivering microlitre quantities say 10 µl and less. A reagent sprayer that will emit a fine spray and will not itself be attacked by the reagent.

h) A ultra-violet light, suitable for observation at short (254 nm) and long (365 nm) ultra-violet wavelengths.

Theory:

Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, stationary phase acting through adsorption and a mobile phase in the form of liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet or plate. Pre-coated plates are most commonly used. Separation may also be achieved on the basis of partition or a combination of partition and adsorption, depending on the particular type of support,

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its preparation and its use with different solvent. Identification can be effected by observation of spots of identical Rf value and about equal magnitude obtained, respectively, with an unknown and a reference sample chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation.

Preparation of plates:

Unless otherwise specified in the monograph, the plates are prepared in the following manner. Prepare a suspension of the coating substance in accordance with the instructions of the supplier and, using the spreading device designed for the purpose, spread a uniform layer of the suspension, 0.20 to 0.30 mm thick, on a flat glass plate 20 cm coated plates to dry in air, heat at 1000 to 1050 for at least 1 hour (except in the case of plates prepared with cellulose when heating for 10 minutes is normally sufficient) and allow to cool, protected from moisture. Store the plates protected from moisture and use within 3 days of preparation. At the time of use, dry the plates again, if necessary, as prescribed in the monographs. Now a day, pre coated plates of silica gel on glass/aluminium/ plastic sheets long. Allow the are also available.

Method:

Unless unsaturated conditions are prescribed, prepare the tank by lining the walls with sheets of filter paper pour into the tank, saturating the filter paper in the process, sufficient of the mobile phase to form a layer of solvent 5 to 10 mm deep, close the tank and allow to stand for 1 hour at room temperature. Remove a narrow strip of the coating substance, about 5 mm wide, from the vertical sides of the plate. Apply the solutions being examined in the form of circular spots about 2 to 6 mm in diameter. Allow the solvent to evaporate and place the plate in the tank, ensuring that it is as nearly vertical as possible and that the spots or bands are above the level of the mobile phase. Close the tank and allow to stand at room temperature, until the mobile phase has ascended to the marked line. Remove the plate and dry and visualize, where a spraying technique is prescribed it is essential that the reagent be evenly applied as a fine spray.

Visualisation:

The phrases ultra-violet light (254 nm) and ultra-violet light (365 nm) indicate that the plate should be examined under an ultra-violet light having a maximum output at about 254 or at about 365 nm, as the case may be. Measure and record the distance of each

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Calculation

$$R_f \text{ value} = \frac{4.8}{5.8} \\ = 0.82$$

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spot from the point of its application and calculate the Rf value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

Solution: prepare 1% solution of the extract to be identified in methanol, shake well and filter.

RESULT-

The Rf value of the given natural product was found to be
0.62



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