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### Sequential Thin-Layer Chromatography of 2,4-D and Related Compounds

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## SEQUENTIAL THIN-LAYER CHROMATOGRAPHY OF 2,4-D AND RELATED COMPOUNDS

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### SUMMARY

Separations and identification of carboxylic herbicidal substances such as 2,4-dichlorophenoxyacetic acid, trichloroacetic acid, 2,4,5-trichlorophenoxyacetic acid and plant growth regulators such as benzoic acid, cinnamic acid, indole-3-acetic acid,  $\beta$ -naphthaleneacetic acid,  $\beta$ -naphthoxyacetic acid, phenoxyacetic acid have been made by sequential thin-layer chromatography on calcium sulphate layer with acetone, benzene, carbon tetrachloride, chloroform, ethyl acetate, dioxan, propanol as solvents and bromophenol blue as detector.

### INTRODUCTION

Plant growth regulators<sup>1</sup> such as benzoic acid, cinnamic acid, indole-3-acetic acid and its derivatives, gallic acid, TCA and 2,4,5-T are difficult to analyse by largely used and highly sensitive technique, "Gas chromatography" due to their non-volatile nature. Therefore simple and commonly used methods<sup>2</sup> such as spectrophotometry

and thin-layer chromatography have been proved to be the readily available tool for their analysis. Amongst the above listed compounds 2,4-D, TCA and 2,4,5-T are toxic, their  $LD_{50}$  values<sup>3</sup> are 370 mg, 5000 mg and 500 mg/kg respectively and they are being used as herbicides for destroying dicots.

Our previous work<sup>4</sup> shows that these compounds can be separated by plain thin-layer chromatography (PLC) on calcium sulphate coating in common solvents, acetone, benzene, carbon tetrachloride, chloroform, dioxan, ethyl acetate, propanol etc. Abou-Donia and Komeil<sup>5</sup> have claimed that the newly developed "sequential thin-layer chromatography" (STLC) is a rapid and fast method of analysis of complex mixtures. Therefore an attempt has been made to explore the utility of calcium sulphate for the separation, detection and determination of herbicides by this procedure.

As a result many new ternary separations which were not possible by plain thin-layer chromatography on calcium sulphate, have now been achieved. The details of the procedure and the results obtained are reported in this paper.

## EXPERIMENTAL

### Apparatus

A sathal apparatus with a universal applicator (adjustable thickness of the applied layer from 0.0-2.00 mm), hot air electric drier, glass plates (20 x 4 cm), glass jars (25 x 5 cm), temperature controlled electric oven, centrifugation machine and spectrophotometer were used.

### Chemicals

Acetone (BDH, India), benzene (S.M. Chemicals, India), calcium sulphate precipitated powder, carbon tetrachloride, chloroform,

dioxan, ethyl acetate, propanol (E.Merck,India), benzoic acid, p-chlorophenoxyacetic acid, cinnamic acid, citric acid, 2,4-dichlorophenoxyacetic acid, gallic acid, indole-3-acetic acid, maleic acid, malic acid, malonic acid,  $\beta$ -naphthaleneacetic acid,  $\beta$ -naphthoxyacetic acid, oxalic acid, phenoxyacetic acid, salicylic acid, tartaric acid, trichloroacetic acid, 2,4,5-trichlorophenoxyacetic acid (Sigma,USA) were used.

#### Preparation of solutions

Solutions (2%) of benzoic, p-chlorophenoxyacetic, cinnamic, 2,4-dichlorophenoxyacetic, gallic, indole-3-acetic,  $\beta$ -naphthaleneacetic,  $\beta$ -naphthoxyacetic, phenoxyacetic, salicylic and 2,4,5-trichlorophenoxyacetic acids were prepared in ethanol. Solutions (2%) of citric, maleic, malic, malonic, oxalic, tartaric and trichloroacetic acids were prepared in distilled water.

Reagent for the spectrophotometric determination of indole-3-acetic acid was prepared by mixing 1 ml of 0.5M ferric chloride solution in 35% (v/v) perchloric acid. Prepared fresh reagent before use.

#### Preparation of plates

A slurry of calcium sulphate obtained by mixing calcium sulphate (30 g) with distilled water (70 ml) was applied on the glass plates with the help of applicator so that the thickness of calcium sulphate slurry would be 0.75 mm. The plates were first allowed to dry at room temperature and then in a temperature controlled electric oven at 110°C for one hour.

#### Detection of acids

The acids under study were located on the plates by 1% ethanolic, alkaline bromophenole blue solution.

### Spotting of test solutions

Test solutions were spotted on the plates with the help of a fine capillary. The solvent was removed by hot air drying, the plate was developed up to the length of 5 cm in solvent A, the plate was taken out from the jar and A was removed as above, the plate was again developed upto the length of 10 cm in solvent B, B was also removed and then the spot was located with bromophenole blue by spray method. For tailing, the front limit (RF) and the real limit (RR) were measured while for compact spot  $R_f$  values were taken as usual.

$$R_f = \frac{\text{Distance travelled by substance (cm)}}{\text{Distance travelled by solvent front (cm)}}$$

### Qualitative separations

To achieve the qualitative separation of acids, one of the acids was spotted firstly and the solvent was removed, second acid solution was spotted and the solvent was removed and so on so far and then the plates was developed, dried and acids were located as above.

### Quantitative separations

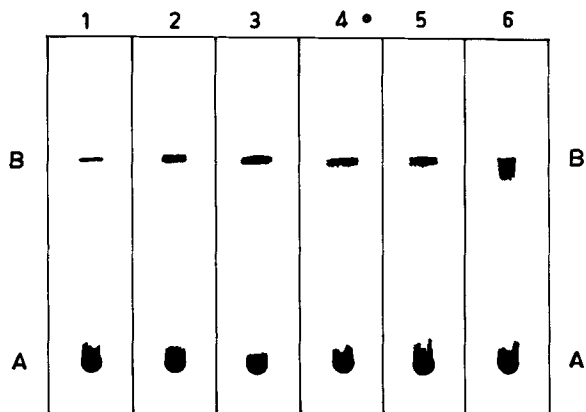
A known volume of standard acid solution was spotted on the plate with the help of a graduated micro pipette with vaccupet control, the solvent was removed, plates were developed and spots were located as above. The demarcated area of the plate was scratched out, the acid was extracted with methanol (3 ml) and the solid was removed by centrifugation method. The acid in the centrifugate was determined spectrophotometrically by the following procedure.

In the extract (3 ml) containing indole-3-acetic acid, 2 ml of freshly prepared reagent were added dropwise but rapidly with continuous agitation, it was placed in dark for one hour for colour development. Finally the absorbance was measured at 510 nm against a blank containing methanol (3 ml) and reagent (2 ml).

RESULTS

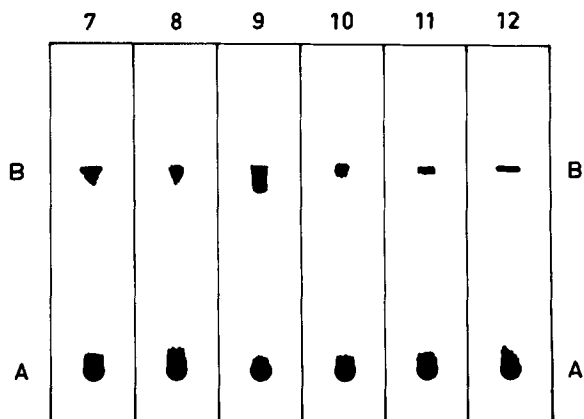
Many separations have been achieved by using different sets of solvents. Some of the important separations are shown in photoplates 1 to 8 and other separations are given below. The first solvent that was allowed to ascend for 0-5 cm is listed first and the second solvent that was allowed to ascend for 0-10 cm listed at the second place.

Binary separations by FTLC: Benzoic acid (1.0) from citric (0.0), oxalic (0.0) and tartaric acid (0-2) in acetone, ethylacetate; p-chlorophenoxyacetic acid (1.0) from citric (0.0), oxalic (0.0) and tartaric acid (0-2) in acetone, ethyl acetate; cinnamic acid (1.0) from citric (0.0), maleic (0-3), malic (0-7), malonic (0.0), oxalic (0.0), and tartaric (0-2) in acetone, benzene, ethyl acetate; 2,4-dichlorophenoxyacetic acid (1.0) from citric (0.0), oxalic (0.0) and tartaric acid (0-2) in acetone, ethylacetate; gallic acid (1.0) from citric (0.0), oxalic (0.0) and tartaric (0-2) in acetone, ethylacetate; indole-3-acetic acid (1.0) from citric (0.0), maleic (0-3), malonic (0.0), oxalic (0.0) and tartaric (0-2) in acetone, benzene, ethyl acetate; malonic acid (1.0) from citric (0.0), oxalic (0.0) and tartaric acid (0-2) in ethylacetate;  $\beta$ -naphthalene-acetic acid (1.0) from citric (0.0), maleic (0.3), malonic (0.0), oxalic (0.0) and tartaric acid (0-2) in chloroform, ethyl acetate;  $\beta$ -naphthoxyacetic acid (3-10) from citric (0.0), oxalic (0.0) and tartaric acid (0-2) in acetone, carbon tetrachloride, propanol; phenoxyacetic acid (1.0) from citric (0.0), maleic (0-3), malic (0-7), malonic (8-10), oxalic (0.0) and tartaric acid (0-2) in acetone, benzene, chloroform; salicylic acid (1.0) from citric (0.0), malonic (0.0), oxalic (0.0) and tartaric acid (0-2) in benzene, carbontetrachloride, ethylacetate; trichloroacetic acid (1.0) from citric (0.0), oxalic (0.0) and tartaric acid (0-2) in acetone, ethylacetate; 2,4,5-trichlorophenoxyacetic acid (1.0) from citric (0.0), oxalic (0.0) and tartaric acid (0-2) in acetone, benzene, ethylacetate.



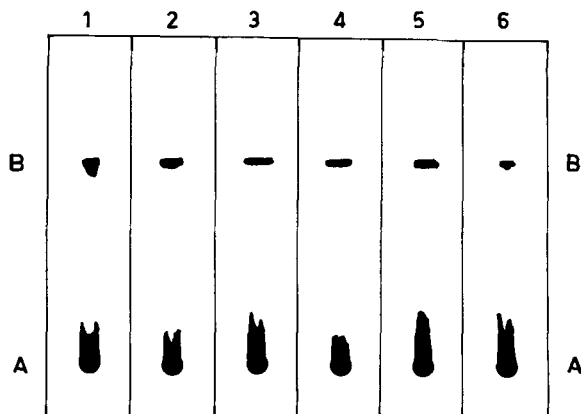
Photoplate No. 1

Separation of tartaric acid (Spot A) from 1) 2,4,5-trichlorophenoxyacetic acid from 2) 2,4-dichlorophenoxyacetic acid from 3) benzoic acid from 4) trichloroacetic acid from 5) p-chlorophenoxyacetic acid and from 6) gallic acid (Spot B) on calcium sulphate coating developed in dioxan (5 cm) followed by ethyl acetate (10 cm).



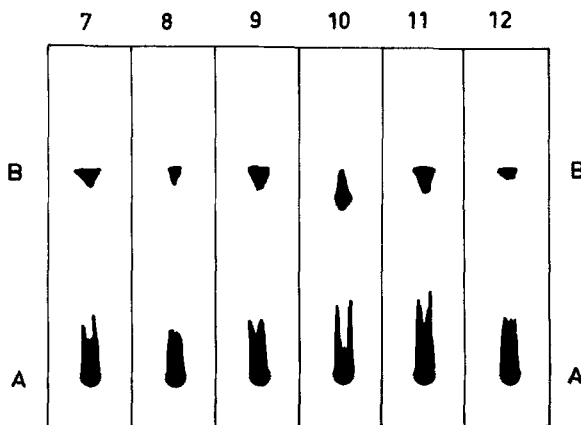
Photoplate No. 2

Separation of tartaric acid (Spot A) from 7) cinnamic acid; from 8) indole-3-acetic acid; from 9) B-naphthaleneacetic acid; from 10) B-naphthoxyacetic acid; from 11) phenoxyacetic acid from 12) salicylic acid (Spot B) on calcium sulphate coating, developed in dioxan (5 cm) followed by ethyl acetate (10 cm).



Photoplate No. 3

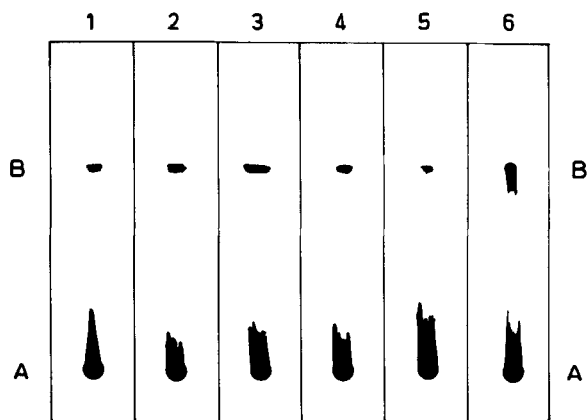
Separation of oxalic acid (Spot A) from 1) 2,4,5-trichloroacetic acid, from 2) 2,4-dichlorophenoxyacetic acid from 3) benzoic acid, from 4) trichloroacetic acid, from 5) p-chlorophenoxyacetic acid and from 6) gallic acid (Spot B) on calcium sulphate coating, developed in dioxan (5 cm) followed by ethyl acetate (10 cm).



Photoplate No. 4

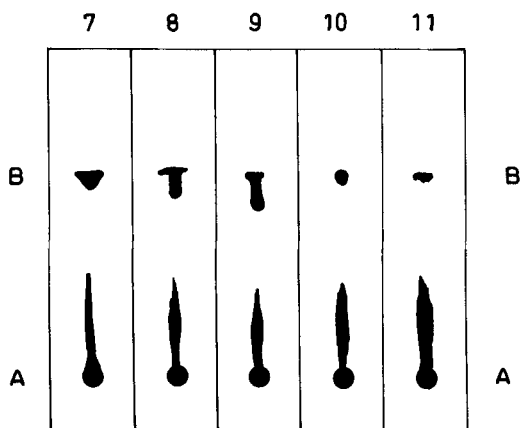
Separation of oxalic acid (Spot A) from 7) cinnamic acid, from 8) indole-3-acetic acid, from 9) B-naphthaleneacetic acid, from 10) B-naphthoxyacetic acid, from 11) phenoxyacetic acid, and from 12) salicylic acid, (Spot B) on calcium sulphate coating, developed in dioxan (5 cm) followed by ethyl acetate (10 cm).





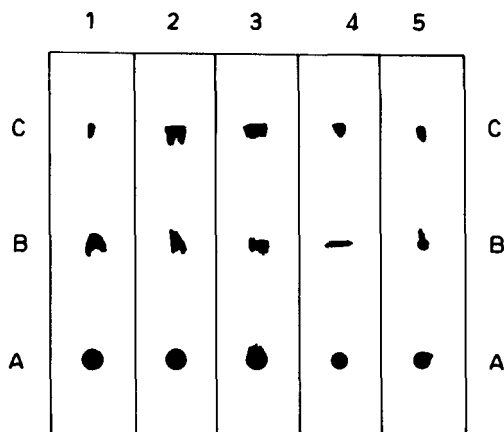
Photoplate No. 5

Separation of citric acid (Spot A) from 1) 2,4,5-trichlorophenoxyacetic acid, from 2) 2,4-dichlorophenoxyacetic acid, from 3) benzoic acid, from 4) trichloroacetic acid from 5) p-chlorophenoxyacetic acid and from 6) gallic acid (Spot B) on calcium sulphate coating, developed in dioxan (5 cm) followed by ethyl acetate (10 cm).



Photoplate No. 6

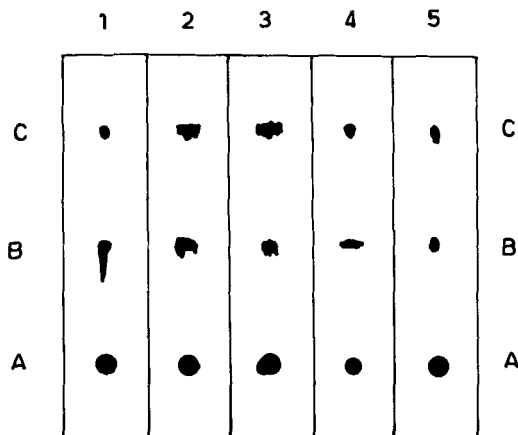
Separation of citric acid (Spot A) from 7) cinnamic acid, from 8) B-naphthaleneacetic acid, from 9) B-naphthoxyacetic acid, from 10) phenoxyacetic acid and from 11) Balicyclic acid (Spot B) on calciumsulphate coating, developed in chloroform (5 cm) followed by ethyl acetate (10 cm).



Photoplate No. 7

Ternary Separations: Oxalic acid (Spot A) from 1) malic acid (Spot B) and cinnamic acid (Spot C) in chloroform (5 cm) and ethylacetate (10 cm); from 2) indole-3 acetic acid (Spot B) and cinnamic acid (Spot C) in ethyl acetate (5 cm) and carbon tetrachloride; from 3) indole-3-acetic acid (Spot B) and phenoxyacetic acid (Spot C) in ethyl acetate (5 cm) and carbon tetrachloride (10 cm); maleic acid (Spot B) and cinnamic acid (Spot C) in acetone (5 cm) and benzene (10 cm); from maleic acid (Spot B) and phenoxyacetic acid (Spot C) in acetone (5 cm) and benzene (10 cm) on calcium sulphate coated plates.

Binary separations by STLC: Cinnamic acid (1.0) from indole-3-acetic (0-5), maleic (0-3), malonic acid (0.0) in carbon tetrachloride-chloroform, ethylacetate-chloroform, chloroform-benzene; indole-3-acetic acid (6-10) from citric (0-2), maleic (0-3) and malonic acid (0.0) in chloroform-benzene, propanol-benzene; maleic acid (8-10) from citric (0-2), oxalic (0.0) and tartaric acid (0.0) in chloroform-ethyl acetate, dioxan-ethyl acetate; maleic acid (7-10) from citric (0-2), oxalic (0.0) and tartaric acid (0.0) in chloroform-ethyl acetate, dioxan-ethyl acetate; malonic acid (7-10) from citric (0-2), oxalic (0.0) and tartaric acid (0-2) in chloroform-ethyl acetate, dioxan-ethyl acetate;  $\beta$ -naphthaleneacetic acid (6-10) from maleic (0-3), malonic acid (0.0) in chloroform-benzene;  $\beta$ -naphthoxy-acetic acid (5-8) from malic (0-3) and malonic acid (0-0) in chloroform-benzene; phenoxyacetic acid (8-10) from indole-3-acetic (0-5), maleic (0-3),



Photoplate No. 8

Ternary Separations: Tartaric acid (Spot A) from 1) maleic acid (Spot B) and cinnamic acid (Spot C) in chloroform (5 cm) and ethyl acetate (10 cm); from 2) indole-3-acetic acid (Spot B) and cinnamic acid (Spot C) in ethylacetate (5 cm) and carbon tetrachloride (10 cm); from 3) indole-3-acetic acid (Spot B) and phenoxy acetic acid (Spot C) in ethyl acetate (5 cm) and carbon tetrachloride (10 cm); from 4) maleic acid (Spot B) and cinnamic acid (Spot C) in acetone (5 cm) and benzene (10 cm); from 5) maleic acid (Spot B) and phenoxyacetic acid (Spot C) in acetone (5 cm) and benzene (10 cm) on calcium sulphate coated plates.

malic (0-5) and malonic acid (0.0) in chloroform-benzene, propanol-benzene, propanol-carbon tetrachloride; salicylic acid (1.0) from maleic (0-3) and malonic acid (8-10) in chloroform-benzene, propanol-benzene; 2,4,5-trichloro phenoxyacetic acid (8-10) from maleic acid (0-3) in propanol-benzene.

Ternary separations: Oxalic acid (0.0) from maleic acid (0.5) and  $\beta$ -naphthaleneacetic acid (1.0) in ethylacetate-chloroform; oxalic acid (0.0) from maleic acid (4-5) and  $\beta$ -naphthoxyacetic acid (9-10) in propanol-chloroform; tartaric acid (0.0) from  $\beta$ -naphthoxyacetic acid (0.5) and cinnamic acid (8-10) in ethyl acetate-benzene; tartaric acid (0.0) from indole-3-acetic acid (3-5) and salicylic acid (9-10) in ethyl acetate-benzene; tartaric acid (0.0) from malic acid (0.5) and salicylic acid (9-10) in ethyl acetate-carbon tetra chloride.

The results of quantitative separations are given in table I, II and III. The analytical parameters are calculated by the following expression

$$\sigma = \sqrt{\frac{(x_1 - \mu)^2 + (x_2 - \mu)^2 + \dots}{N - 1}} \quad \dots \quad (1)$$

$$\text{C.V.} = \frac{\sigma \times 100}{\mu} \quad \dots \quad (2)$$

where  $x_1, x_2, \dots$  = measured values

$\mu$  = average value and

$N$  = number of sets.

#### DISCUSSION

Thin-layer chromatography<sup>6</sup> is particularly useful to herbicides containing carboxylic group because of their polar nature and insusceptibility to gas chromatography. Therefore it has been used<sup>7,8</sup>

Table I. Binary quantitative separations of indole-3-acetic acid (IAA) from oxalic acid (OA) and from tartaric acid (TA).

Acid	N	Separated from	Absorbance at 510 nm $\mu \pm \sigma$	C.V.
50 ug of IAA	6	200 ug of TA	0.215 $\pm$ 0.005	2.32
50 ug of IAA	6	400 ug of OA	0.212 $\pm$ 0.0098	4.64

Thickness of calcium sulphate layer = 0.75 mm, ethyl acetate 0-5 cm followed by carbon tetrachloride 0-10 cm.

Table II. Ternary quantitative separations of indole-3-acetic acid (IAA) from oxalic acid (OA) and cinnamic acid (CIA).

Acid	N	Separated from	Absorbance	C.V.
			at 510 nm $\mu \pm \sigma$	
50 ug of IAA	6	400 ug of OA and 400 ug of CIA	0.205 $\pm$ 0.0081	3.99
50 ug of IAA	6	800 " 800 "	0.205 $\pm$ 0.0137	6.68
50 ug of IAA	6	1200 " 1200 "	0.191 $\pm$ 0.0075	3.95
50 ug of IAA	6	1600 " 1600 "	0.193 $\pm$ 0.0126	6.50

Thickness of calcium sulphate = 0.75 mm, ethyl acetate 0-5 cm followed by carbon tetrachloride 0-10 cm.

Table III. Ternary quantitative separations of indole-3-acetic acid (IAA) from tartaric acid (TA) and cinnamic acid (CIA).

Acid	N	Separated from	Absorbance	C.V.
			at 510 nm $\mu \pm \sigma$	
50 ug of IAA	6	200 ug of TA and 400 ug of CIA	0.216 $\pm$ 0.0081	3.75
50 ug of IAA	6	400 " 800 "	0.207 $\pm$ 0.0081	3.91
50 ug of IAA	6	600 " 1200 "	0.205 $\pm$ 0.0055	2.68
50 ug of IAA	6	800 " 1600 "	0.215 $\pm$ 0.0104	4.83

Thickness of calcium sulphate = 0.75 mm, ethylacetate 0-5 cm followed by carbon tetrachloride 0-10 cm.

for the detection, separation and determination of carboxylic herbicides. Zawadzka et al. have determined 2,4-D, dalapon, MCFE, dichlorprop, mecoprop, DNOC, dinoseb and FCA herbicides in water and sewage by TLC on silicagel G-Kieselguhr G (2:3) or silicagel G-H<sub>3</sub>PO<sub>4</sub> (5g of silicagel G in 17 ml of 0.3% H<sub>3</sub>PO<sub>4</sub> solution) layers with light petroleum-isopropyl ether (1:2) as solvent. Ethanolic diphenylamine have been used as a location reagent. Thielmann<sup>10</sup> has separated and identified carboxylic herbicidal substances on Uv 254 sheets with benzene-acetone (2:3) or (4:2) as the solvent and 0.02% ethanolic Rhodamine B as the spray reagent. Bogacka and Taylor<sup>11</sup> have determined these pesticides in water by TLC on silicagel G-Kieselguhr G (2:3) with paraffin as solvent and 0.5% AgNO<sub>3</sub> solution as spray reagent. Ahmed<sup>4</sup> et al. have claimed that calcium sulphate is an excellent TLC material. They have separated plant growth regulators such as benzoic, cinnamic, indole-3-acetic, β-naphthaleneacetic, β-naphthoxyacetic and phenoxyacetic acids from many carboxylic acids present in plants fruits and soils. The results obtained from the present study show that the separations of 2,4-D, FCA and 2,4,5-T shown in photoplates 1, 5 and 5 which were not studied by Ahmed et al. can also be achieved on calcium sulphate by the sequential development of chromatograms with common organic solvents. It is also clear that separations of plant growth regulators such as cinnamic, benzoic, indole-3-acetic, gallic, β-naphthaleneacetic, β-naphthoxyacetic, and phenoxy acetic acids shown in photoplates 2, 4 and 6 and mentioned in the result section which are not possible by FTLC can be achieved by STLC. Ternary separations of indole-3-acetic, maleic, malic, oxalic, phenoxyacetic, salicylic and tartaric acids shown on photoplates 7 and 8 and mentioned in result section can be achieved successfully by STLC while they are not possible by FTLC. Hence it is obvious STLC on calcium sulphate gives better results than FTLC for separating complex and multicomponents system.

**ACKNOWLEDGEMENTS**

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