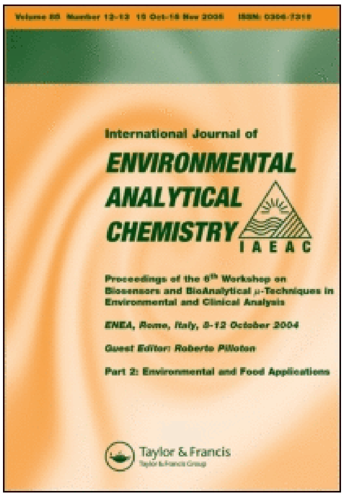

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Separation and Detection of Some Phenoxyacid Herbicides and Plant Growth Regulators by Ion-Pair Reversed-Phase Thin-Layer Chromatography

H. S. Rathore[a]; I. Ali[a]; S. R. Sharma[a]; S. K. Saxena[a]
[a] Chemistry Section, Z. H. College of Engineering and Technology, Aligarh Muslim University, Aligarh, India

To cite this Article Rathore, H. S. , Ali, I. , Sharma, S. R. and Saxena, S. K.(1988) 'Separation and Detection of Some Phenoxyacid Herbicides and Plant Growth Regulators by Ion-Pair Reversed-Phase Thin-Layer Chromatography', International Journal of Environmental Analytical Chemistry, 33: 3, 209 — 217

To link to this Article: DOI: 10.1080/03067318808081672

URL: http://dx.doi.org/10.1080/03067318808081672

Intern. J. Environ. Anal. Chem., Vol. 33, pp. 209–217
Reprints available directly from the publisher
Photocopying permitted by license only

Printed in Great Britain

Separation and Detection of Some Phenoxyacid Herbicides and Plant Growth Regulators by Ion-Pair Reversed-Phase Thin-Layer Chromatography

H. S. RATHORE, I. ALI, S. R. SHARMA and S. K. SAXENA
Chemistry Section, Z.H. College of Engineering and Technology, Aligarh Muslim University, Aligarh-202001, India.

(*Received 2 September 1987; in final form 12 December 1987*)

Separation and detection of phenoxyacid herbicides such as 4-chlorophenoxyacetic, 2,4-dichlorophenoxyacetic, phenoxyacetic, 2,4,5-trichlorophenoxyacetic, trichloroacetic acids and plant growth regulators such as benzoic, cinnamic, gallic, indole-3-acetic, indole-3-propionic, α-naphthaleneacetic, β-naphthalenaecetic, β-naphthoxyacetic acids have been made by ion-pair reversed-phase thin-layer chromatography on calcium sulphate coating impregnated with an ion-pair reagent cetrimide and different oils such as coconut oil, olive oil, paraffin oil and silicon oil using distilled water as a solvent and bromophenol blue as a detector.

KEY WORDS: Phenoxy acids, plant growth regulators, phenoxyacid herbicides, reversed phase TLC, TLC.

INTRODUCTION

Phenoxyacid herbicides, such as 2,4-D and 2,4,5-T and trichloroacetic acid (TCA) are widely used to control dicot weeds in the crops and forests whereas plant growth regulators like benzoic, cinnamic, indole-3-acetic, gallic and α-naphthaleneacetic acids are used to control the plant growth in crops. These herbicides and plant growth regulators mix up in natural water either by accidental fallout of sprays from agricultural treatment or by surface run-off from agricultural land. Work has been done to study the environmentally deleterious effects of the herbicides on the aquatic organisms,[1] their phototoxicity to plants,[2] to fish,[3] pollen grains[4] and to rats.[5]

The mentioned compounds are difficult to analyse by the frequently used and highly sensitive technique of gas-chromatography due to their non-volatile nature. High performance liquid chromatography (HPLC) has proved to be a good alternative to clean-up and determine acid herbicides from different samples with great accuracy[6–9] but is a sophisticated and expensive technique which requires technically skilled operators. These compounds have been separated by thin-layer chromatography (TLC) on calcium sulphate coating in our laboratory by previous workers.[10] Reversed-phase thin-layer chromatography (RP-TLC) is a useful alternative to normal-phase thin-layer chromatography (NP-TLC) on silica gel for the separation of polar compounds.

Lewis and Wilson[11] studied the organic acids by ion-pair reversed-phase thin-layer chromatography (IP-RP-TLC) on both paraffin-coated and C_{18} bonded silica gel plates using six ion-pair reagents. Hui and Taylor[12] studied reversed-phase ion-pair high-performance liquid-chromatography (RP-IP-HPLC) procedure for the determination of histamine and its metabolites in rat urine using 1-pentane sulphonic acid as an ion-pair reagent. Ruane *et al.*[13] have also studied normal-phase ion-pair thin-layer chromatography (NP-IP-TLC) and RP-IP-TLC of hydroxy benzoic acids, genestic acid and salicylic acid with bifunctional (bolafarm) bis trimethylammonium ion-pair reagent.

Literature survey shows that attempts have been made to use IP-RP-TLC in organic acids,[14] peptides,[15] amino acids[16] and nitrogen bases.[17] This paper describes the results on calcium sulphate coating using an ion-pair reagent cetrimide (tetradecyltrimethylammonium-bromide) impregnating with oils such as coconut, paraffin, olive and silicon oils. Ternary and quaternary separations of phenoxyacid herbicides and plant growth regulators have been achieved using distilled water as a solvent. Efforts have been made to detect phenoxyacid herbicides from water samples after extraction with ethers.

EXPERIMENTAL

Apparatus

A Stahl apparatus with a universal applicator (adjustable thickness of the applied layer from 0.25–2.00 mm), hot air electric drier, glass plates (20 × 4 cm), glass jars (25 × 5 cm), separating funnel, graduated micro pipette with vaccupet control and temperature controlled electric oven were used.

Chemicals

Calcium sulphate precipitated powder (E. Merck, India), cetrimide, olive oil (Shalaks Chemicals, India), silicon oil (Central Drug House, India), coconut oil (Tata product, India), benzoic acid, 4-chlorophenoxyacetic acid, cinnamic acid, 2,4-dichlorophenoxyacetic acid, gallic acid, indole-3-acetic acid, indole-3-propionic acid, α-naphthaleneacetic acid, β-naphthaleneacetic acid, β-naphthoxyacetic acid, phenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid (Sigma, USA), trichloroacetic acid (CDH, India) and all other reagents of analytical grade were used.

Preparation of solutions

Solutions (1 %) of benzoic, 4-chlorophenoxyacetic, cinnamic, 2,4-dichlorophenoxyacetic, gallic, indole-3-acetic, indole-3-propionic, α-naphthaleneacetic, β-naphthaleneacetic, β-naphthoxyacetic, phenoxyacetic and 2,4,5-trichlorophenoxyacetic acids were prepared in ethanol. Solution (1 %) of trichloroacetic acid was prepared in distilled water.

Preparation of plates

Plates of 0.5 mm thickness have been prepared by a slurry obtained by mixing calcium sulphate (30 g), 1 % ethanolic solution of cetrimide (5 ml), oil (2 ml) and distilled water (DW) (70 ml). The plates were first allowed to dry at room temperature and then at 80 °C for half an hour in an oven.

Four different coatings made to prepare thin-layer chromatographic plates are as follows:

Coating A: Calcium sulphate (30 g) + 1% ethanolic solution of cetrimide (5 ml) + coconut oil (2 ml) + DW (70 ml).
Coating B: Calcium sulphate (30 g) + 1% ethanolic solution of cetrimide (5 ml) + olive oil (2 ml) + DW (70 ml).
Coating C: Calcium sulphate (30 g) + 1% ethanolic solution of cetrimide (5 ml) + paraffin oil (2 ml) + DW (70 ml).
Coating D: Calcium sulphate (30 g) + 1% ethanolic solution of cetrimide (5 ml) + silicon oil (2 ml) + DW (70 ml).

Procedure

Test solutions were spotted on the plates with the help of fine capillary. The solvent was removed by hot air drying, the plates were developed up to 10 cm in the solvent and then dried and acids were detected with 0.1% ethanolic alkaline bromophenol blue. For tailing, the front limit (RI) and the real limit (RT) were measured, while for compact spot R_f values were taken as usual,

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

Ternary and quaternary separations were achieved by spotting mixtures of three or four acids in water and detected by the same procedure. Phenoxy acid herbicides were extracted from water samples with diethyl ether, isopropyl ether and petroleum ether and quaternary separations were achieved successfully on coating B.

RESULTS

Tables 1 and 2 show that many ternary and quaternary separations have been achieved on different coatings. Some of the important separations are shown in photo print Nos. 1 and 2. Diethyl ether was the best extractant in comparison to petroleum ether and isopropyl ether due to herbicide's higher solubilities in the former. Recovery figures are given in Table 3.

Table 1 Ternary separations

Acid	*Separated from*	*Coating*
CPX (0.0)	2,4-D (3-6) and PHX (9-10)	D
CIN (0.0)	2,4-D (5-7) and GA (1.0)/TCA (1.0)	C
CIN (0-2)	CPX (5-7) and TCA (1.0)	B
CIN (0.0)	2,4,5-T (3-5) and GA (9-10)/ β-NPX (1.0)/TCA (1.0)	C
CIN (0.0)	BN (4-6) and β-NPX (1.0)/PHX (1.0)/ TCA (1.0)/GA (1.0)	C and B
β-NPX (0-1)	CPX (4-5) and GA (1.0)/PHX (1.0)/ TCA (1.0)	B
β-NPX (0.0)	2,4-D (0.5) and BN (1.0)/CIN (1.0)/ TCA (1.0)	D
β-NPX (0-2)	BN (3-5) and GA (1.0)/PHX (9-10)	B
β-NPX (0.0)	IAA (3-5) and GA (1.0)/PHX (9-10)/ TCA (1.0)/BN (1.0)	B and D
α-NPA (0.0)	CPX (5-6) and GA (1.0)/PHX (9-10)	B
α-NPA (0.0)	2,4-D (5-7) and β-NPX (1.0)/TCA (1.0)	C
α-NPA (0.3)	BN (5-6) and GA (1.0)/β-NPX (0.9)/ PHX (1.0)	A, C and D
β-NPA (0.0)	CPX (3-5) and TCA (1.0)	B
β-NPA (0-1)	BN (0.3) and PHX (9-10)	A, B and D
β-NPA (0.0)	2,4-D (0.2) and PHX (9-10)	A, B and D
β-NPA (0.0)	IAA (6-7) and GA (1.0)/PHX (1.0)/ TCA (1.0)	B
2,4,5-T (0-1)	CPX (3-5) and GA (1.0)/PHX (1.0)/ TCA (1.0)	B
2,4,5-T (0-1)	2,4-D (2-3) and GA (1.0)/PHX (1.0)/ TCA (1.0)	B and D
2,4,5-T (0-2)	BN (5-6) and GA (1.0)/PHX (1.0)/ TCA (1.0)	A and B
2,4,5-T (1-2)	IAA (0.7) and GA (1.0)/PHX (9-10)/ TCA (1.0)	B and D
2,4,5-T (0-1)	IPA (5-6) and GA (1.0)/PHX (9-10)/ TCA (1.0)	A

Abbreviations used: Benzoic acid—BN; 4-chlorophenoxyacetic acid—CPX; cinnamic acid—CIN; 2,4-dichlorophenoxyacetic acid—2,4-D; gallic acid—GA; indole-3-acetic acid—IAA; indole-3-propionic acid—IPA; α-naphthaleneacetic acid-α—NPA; β-naphthaleneacetic acid-β—NPA; β-naphthoxyacetic acid—β-NPX; phenoxyacetic acid—PHX; trichloroacetic acid—TCA; 2,4,5-trichlorophenoxyacetic acid—2,4,5-T.

Table 2 Quaternary separations

Acid	*Separated from*	*Coating*
CIN (0.0)	BN (2-3)—IAA (0.8) and β-NPX (1.0)	C
α-NPA (0.0)	2,4-D (0.3)—BN (0.6) and GA (1.0)/ PHX (1.0)	B
α-NPA (0.0)	2,4-D (3.5)—PHX (7-8) and β-NPX (1.0)/ TCA (1.0)	C
α-NPA (0.0)	BN (0.2)—IPA (0.6) and PHX (1.0)	A
β-NPA (0.0)	2,4-D (0.2)—CPX (5-6) and GA (1.0)/PHX (9-10)	B
β-NPA (0.0)	2,4-D (2-3)—IAA (5-7) and GA (1.0)/PHX (1.0)/TCA (1.0)	B
β-NPX (0.0)	2,4-D (0.2)—CPX (4-5) and PHX (9-10)	B
β-NPX (0.0)	2,4-D (2-3)—IAA (5-6) and GA (1.0)/ PHX (9-10)	B
2,4,5-T (0.0)	BN (2-3)—IPA (5-6) and GA (1.0)/ PHX (1.0)/TCA (1.0)	A
2,4,5-T (0.0)	2,4-D (2-3)—BN (0.6) and TCA (1.0)	B
2,4,5-T (0.0)	2,4-D (2-3)—CPX (7-8) and GA (1.0)/ PHX (1.0)/TCA (1.0)	B
2,4,5-T (0-1)	2, 4-D (0.3)—IAA (0.7) and GA (1.0)/ PHX (1.0)/TCA (1.0)	B

Abbreviations are defined in Table 1.

Table 3 Recovery figures

Sl. No.	*Extractant*	*Herbicides present in water sample*	*Lower limit of detection (μg)*
1.	Di-ethyl ether	PHX, CPX, 2,4-D, 2,4,5-T	20
2.	Iso-propyl ether	PHX, CPX, 2,4-D, 2,4,5-T	40
3.	Petroleum ether	PHX, CPX, 2,4-D, 2,4,5-T	60

Abbreviations are defined in Table 1.

DISCUSSION

Ion-suppression reversed-phase high-performance liquid-chromatography (IS-RP-HPLC) analysis of gibberillins and its conjugates on glycosides and glucosyl esters using as a C_{18} support was done by Jensen *et al.*[18] Reversed-phase ion-pair high-performance

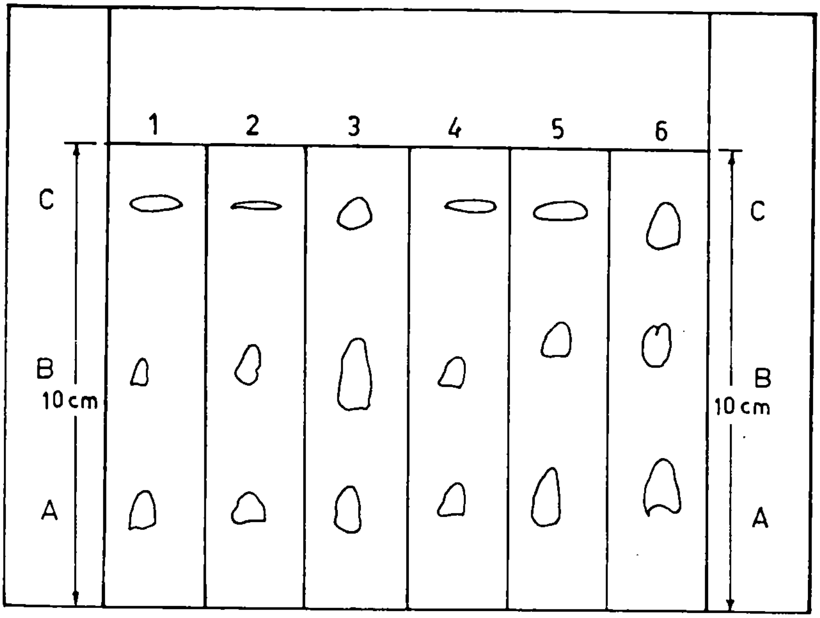

Figure 1 Ternary separation achieved on coating B. Spot A—2,4,5-Trichlorophenoxyacetic acid in 1 and 2; α-Naphthaleneacetic acid in 3 and 4; β-Naphthaleneacetic acid in 5 and 6. Spot B—4-Chlorophenoxyacetic acid in 1 and 3; 2,4-Dichlorophenoxyacetic acid in 2 and 4; Indole-3-acetic acid in 5; Indole-3-propionic acid in 6. Spot C—Phenoxyacetic acid in 1 and 2; Trichloroacetic acid in 3 and 4; Gallic acid in 5 and 6.

liquid-chromatography (RP-IP-HPLC)[19] has been found very useful in quantitative analysis of choline in plant extracts and choline esters in pharmaceutical preparations. The chromatographic properties of 132 compounds[20] were studied in two ion-pair reagent systems on reversed-phase thin-layer plates. The retention power of polar ionic compounds is achieved using ion-pair reagent and some applications of the use of such ion-pair reagent compounds in RP-TLC have been described by Bieganouskal *et al.*[21] and Grossini-Starazza *et al.*[22]

In our laboratory calcium sulphate has been developed as an excellent TLC material, acids used to separate plant growth regulators, phenoxy acids and carboxylic acids.[10] Therefore an attempt is made

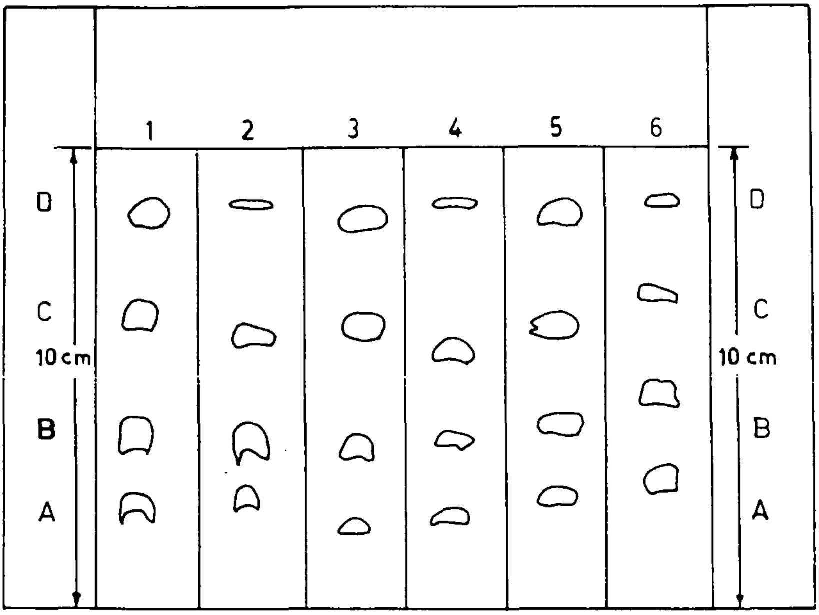

Figure 2 Quaternary separation achieved on coating B. Spot A—2,4,5-Trichlorophenoxyacetic acid in 1, 2 and 6; β-Naphthoxyacetic acid in 3; β-Naphthaleneacetic acid in 4 and 5. Spot B—2,4-Dichlorophenoxyacetic acid in 1, 2, 3, 4, 5 and 6. Spot C—4-Chlorophenoxyacetic acid in 1, 2, 3, 4, 5 and 6. Spot D—Phenoxyacetic acid in 1 and 5; Trichloroacetic acid in 2 and 4; Gallic acid in 3 and 6.

to expose the utility of IP-RP-TLC in separating and detecting these compounds from water samples. The coatings of calcium sulphate impregnated with cetrimide cracked on drying. Addition of 2 ml of coconut/olive/paraffin/silicon oil prevented the cracking of the coatings. However, the R_f values of 4-chlorophenoxyacetic, 2,4-dichlorophenoxyacetic and 2,4,5-trichlorophenoxyacetic acids significantly decreased in the presence of the oils, indicating that ion-pair formation had occurred, e.g. R_f values of 4-chlorophenoxyacetic, 2,4-dichlorophenoxyacetic and 2,4,5-trichlorophenoxyacetic acid decreased from 1.0 to 5-6, 1.0 to 3-4 and 1.0 to 0.0 respectively.

The coatings A, B, C and D impregnated with coconut, olive, paraffin and silicon oil respectively were found to be very useful for ternary and quaternary separations. Results indicate the novelty of the method in ternary and quaternary separations of the same group of compounds like 2,4-D, 2,4,5-T, CPX and PHX from water samples. Separation of plant growth regulators is also possible. The best results were obtained on coating B.

Thus it seems that IP-RP-TLC on calcium sulphate provides a more convenient and simpler method to separate and detect these compounds in multi-component systems in comparison to NP-TLC.

Acknowledgement

We are grateful to the Council of Scientific and Industrial Research, New Delhi, for the award of financial assistance to one of the authors, S. K. Saxena.

References

1. M. Tscheu-Schlueter and W. D. Skibba, *Acta Hydrochim. Hydrobiol.* **14**(6), 627 (1986).
2. A. Man and E. S. Lim, *Pertanika* **9**(1), 23 (1986).
3. I. Y. Mustafa, Alba-Ezz, El-Arab and S. M. A. D. Zayed, *J. Environ. Sci. Health, Part B*, **B22**(2), 235 (1987).
4. S. A. Salgare, *Curr. Pollut. Res. India*, 317 (1985).
5. F. K. Mohammad, St. Omer and E. V. Vincent, *Neurobehav. Toxicol. Teratol.* **8**(5), 551 (1986).
6. K. Ramsteiner, *Int. J. Environ. Anal. Chem.* **25**(1–3), 49 (1986).
7. J. E. Woodraw, M. S. Mujwski and J. N. Seiber, *J. Environ. Sci. Health, Part B*, **B21**(2), 143 (1986).
8. C. A. Chang, Q. Wu and L. Tan, *J. Chromatogr.* **361**, 199 (1986).
9. M. D. Osselton and R. D. Snelling, *J. Chromatogr.* **368**, 265 (1986).
10. S. R. Ahmad, I. Ali, H. S. Rathore and S. Gupta, *J. Liq. Chromatogr.* **7**, 1321 (1984).
11. S. Lewis and I. D. Wilson, *J. Chromatogr.* **312**, 133 (1984).
12. J. Y. Hui and S. L. Taylor, *J. Chromatogr.* **312**, 443 (1984).
13. R. J. Ruane, I. D. Wilson and J. A. Torke, *J. Chromatogr.* **368**(1), 163 (1986).
14. I. D. Wilson, *J. Chromatogr.* **354**, 99 (1986).
15. D. J. Poll, D. R. Knighton, D. R. K. Harding and W. S. Hancock. *J. Chromatogr.* **236**, 244 (1982).
16. L. Lepri, P. G. Desideri and D. Heimler, *J. Chromatogr.* **195**, 65 (1980).
17. W. Jost and H. E. Hauck, *J. Chromatogr.* **264**, 91 (1983).
18. E. Jensen, A. Corizer and A. M. Monterio, *J. Chromatogr.* **367**, 377 (1986).
19. J. Garharn, *J. Chromatogr.* **362**, 243 (1986).
20. H. M. Ruigten, R. H. Van Amsterdam and H. deBree, *J. Chromatogr.* **252**, 193 (1982).
21. M. Bieganouskal, E. Soczewinski and M. Janowska, *Chromatographia* **18**(2), 99 (1984).
22. G. Grossini-Starazza, I. Nicoletti and C. M. Polcaro, *J. Chromatogr.* **367**, 323 (1986).