Plain and ion pair thin layer chromatography of some herbicides and related compounds on admixture of silica gel G and barium sulphate in a single solvent system

Aditya K Misra*, Sonu Pachauree & Upasana Gupta
Department of Chemistry, NMSN Dass (PG) College, Badaun 243 601, India
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Some carboxylic acid herbicides and related compounds such as - p-chlorophenoxy acetic acid, benzoic acid, cinnamic acid, β-naphthoxy acetic acid, α-naphthoxy acetic acid, β-naphthalene acetic acid, indo 3-acetic acid, indole propionic acid and gallic acid have been separated on silica gel G-barium sulphate coatings in different ratio using acetone as mobile phase. The ion pair thin layer chromatography (IPTLC) has shown the better possibilities of separating ternary mixtures of a herbicides and related compounds in comparison to the normal phase TLC.

Herbicides are used either singly or in admixture for weed control in numerous crops such as sugar beet, fodder beet, winter oil seed rape and carrot. They are used largely in commonly grown crops such as wheat, barley, oats, corn, paddy and sugarcane and are very effective. The toxic effect of some of these herbicides has been studied in rats which indicates that the mammalian toxicity of herbicides under study is not of primary importance. However, as they tend to accumulate, their concentration in soil, plants, water and marine animals increases day by day. Thus, there is a growing interest in new methods of herbicide analysis.

A recent survey of literature reveals that herbicides have been chromatographed on silufol, admixture of silicagel and kieselguhr, impregnated silica gel, calcium sulphate, admixture of calcium sulphate and barium sulphate and on coconut oil impregnated calcium sulphate layers. However, the separation potential of silica gel G-barium sulphate coatings with ion pair reagent has not been investigated so far. Although ion pair reverse phase (IPRP) layers of calcium sulphate have been used for the separation of herbicides by Rathore et al.

Herbicides either do not move or give bad tailing on silica gel G and hence separation is not feasible but when mixed with barium sulphate gives better results. Admixture of silica gel G and barium sulphate gives uniform, smooth and stable layers and have good separation potential for herbicides. Barium sulphate is more insoluble than calcium sulphate, it is non-toxic, cheap and chemically inert material that can also be used as an adsorbent. Barium sulphate has been used in TLC for the separation of sulpha drugs and pharmaceuticals. Calcium sulphate thin layers have been successfully utilised for the separation of phenols and admixture of silica gel G and barium sulphate has been used in normal phase and ion pair thin layer has been used chromatography for the separation of synthetic dyes.

The use of ion pair (IP) reagents is a useful method for obtaining acceptable chromatography of polar, ionisable compounds. The use of such reagents is possible with a wide range of stationary phase. By altering the type and concentration of the ion-pair reagents and composition of the eluent, it is possible to vary the retention of the sample substances and the selectivity of the chromatographic system.

This paper reports the separation of some herbicides and related compounds on coatings of silica gel G alone and with barium sulphate with and without using cetrimide as ion pair reagent. Better results are obtained by using ion pair reagent which gave decreased Rf values, compact and symmetrical spots in comparison to the plain silica gel G layers.

Experimental Procedure

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 (15x3.0 cm), glass jars (20x5 cm), graduated
micropipette with vaccupet control and temperature controlled electric oven (Jindal Ind., India) were used.

**Chemicals and reagents**

Silica gel G powder, barium sulphate, ethanol, acetone and pyridine were obtained from E. Merck India. Bromophenol blue and herbicides were obtained from Sigma, USA. Ion pairing reagent cetrimide (Cetrilak) from Shalaks Pharmaceuticals Pvt. Ltd., India. All other reagents used were of analytical grade.

**Preparation of plates**

Plates of 0.25 mm thickness have been prepared by a slurry of pure silica gel G and mixing silica gel G and barium sulphate powder in different ratio with and without 1% ethanolic solution of ion pairing reagent and distilled water (DW). The plates were first allowed to dry at room temperature and then at 80°C for one hour in an hot air oven.

Ten different coatings made to prepare thin layer chromatographic plates were as follows:

- **Coating A<sub>1</sub>:** Plain silica gel G
- **Coating A<sub>2</sub>:** Plain silica gel G + 1% ethanolic solution of ion pairing reagent + DW
- **Coating B<sub>1</sub>:** Silica gel G + Barium sulphate (80:20) + DW
- **Coating B<sub>2</sub>:** Silica gel G + Barium sulphate (80:20) + 1% ethanolic solution of ion pairing reagent + DW
- **Coating C<sub>1</sub>:** Silica gel G + Barium sulphate (60:40) + DW
- **Coating C<sub>2</sub>:** Silica gel G + Barium sulphate (60:40) + 1% ethanolic solution of ion pairing reagent + DW
- **Coating D<sub>1</sub>:** Silica gel G + Barium sulphate (40:60) + DW
- **Coating D<sub>2</sub>:** Silica gel G + Barium sulphate (40:60) + 1% ethanolic solution of ion pairing reagent + DW
- **Coating E<sub>1</sub>:** Silica gel G + Barium sulphate (20:80) + DW
- **Coating E<sub>2</sub>:** Silica gel G + Barium sulphate (20:80) + 1% ethanolic solution of ion pairing reagent + DW

The plates of pure barium sulphate cannot be made due to cracking on drying.

**Preparation of test solutions**

The solutions of herbicides were prepared in 1% ethanol.

**Preparation of detection reagent**

The reagent is prepared by mixing 40 mL of distilled water and 0.5 mL of 15% NaOH in 100 mL of pyridine.

**Method**

The test solutions (1% ethanolic) were spotted onto the plates, ca 1.5 cm above in lower edge by means of a micropipette. The spots were dried with the help of hot air and plates were developed with acetone mobile phase. The solvent ascent was fixed to 10 cm above the point of application. After development the plates were removed from the jars, dried at room temperature.

**Detection method**

The herbicides on plates were visualized by spraying ethanolic alkaline solution of bromophenol blue (0.1%). The rear limit (R<sub>r</sub>) and the front limit (R<sub>f</sub>) values were measured in usual way<sup>16</sup> and R<sub>f</sub> values were calculated as follows:

\[
R_f = \frac{R_r + R_t}{2}
\]

while \( hR_f = R_t \times 100 \)

**Results and Discussion**

The results obtained have been presented in Tables 1 and 2. The separations achieved on plain and ion pair impregnated silica gel G-barium sulphate coatings using single solvent system are recorded in Table 2. The \( hR_f \) values of some herbicides and related compounds with and without ion pair reagent have been plotted against different coatings in Fig.1.

A close examination of the Table 1 shows that gallic acid can be separated from other herbicides and related compounds on coatings of silica gel G and barium sulphate (80:20). The plant growth regulators indole-3 acetic acid and indole propionic acid have high \( hR_f \) values on most of the coatings while gallic acid shows a large variation in \( hR_f \) values on various coatings and enables the possibility of its separation from other. However, long tailing has been seen for gallic acid in fifty per cent cases. The \( \alpha \)- and \( \beta \)-isomers of naphthoxy acetic acid shows almost same \( hR_f \) values on different coatings except on admixture of silica gel G and barium sulphate (60:40) i.e. C<sub>1</sub> layers, it may be due to the hydrogen bonding on the mixed adsorbent of definite ratio. It is also evident from the table that coating B<sub>2</sub>, C<sub>1</sub> and C<sub>2</sub> shows high variation in \( hR_f \) values of different compounds of agricultural importance. The \( \alpha \)- and \( \beta \)-isomers of naphthoxy
Table 1 — $hR_t$ values of herbicides and related compounds on different coatings in single solvent system (acetone)

<table>
<thead>
<tr>
<th>Herbicides and related compounds</th>
<th>Code used</th>
<th>Coatings*</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-chlorophenoxy acetic acid</td>
<td>PCA</td>
<td>A1, A2, B1, B2, C1, C2, D1, D2, E1, E2</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>BA</td>
<td>91, 96, 95, 84, 51, 52, 81, 90, 89, 93</td>
</tr>
<tr>
<td>Cinamic acid</td>
<td>CA</td>
<td>89, 89, 96, 65, 78, 55, 85, 85, 79, 93</td>
</tr>
<tr>
<td>$\beta$-naphthoxy acetic acid</td>
<td>$\beta$-NTA</td>
<td>95, 96, 97, 95, 58, 97, 97, 95, 96, 95</td>
</tr>
<tr>
<td>$\alpha$-naphthoxy acetic acid</td>
<td>$\alpha$-NTA</td>
<td>91, 95, 98, 95, 72, 61, 85, 76, 94, 75</td>
</tr>
<tr>
<td>$\beta$-napthalene acetic acid</td>
<td>$\beta$-NAA</td>
<td>90, 95, 98, 73, 70, 61, 83, 95, 87, 73</td>
</tr>
<tr>
<td>Indole 3-acetic acid</td>
<td>IAA</td>
<td>79, 93, 95, 85, 87, 50, 95, 95, 85, 85</td>
</tr>
<tr>
<td>Indole propionic acid</td>
<td>IPA</td>
<td>93, 95, 97, 85, 87, 50, 95, 95, 85, 85</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>GA</td>
<td>79, 91, 63, 46, 40, 32, 73, 40, 96, 97</td>
</tr>
</tbody>
</table>

*As per experimental.

a: Tailing; b: Long tailing; -: Not detected.

Table 2 — Separations of herbicides and related compounds on different coatings in a single solvent system (acetone)

<table>
<thead>
<tr>
<th>Coatings*</th>
<th>Separations achieved</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>GA(63) – PCA(95)/BA(95)/CA(96)/$\beta$-NTA(97)/$\alpha$-NTA(98)/$\beta$-NAA (98)/IAA(95)/IPA(97)</td>
</tr>
<tr>
<td>B2</td>
<td>PCA(13) – BA(84)/CA(65)/$\beta$-NTA(95)/$\alpha$-NTA(95)/IAA(84)/IPA(85)</td>
</tr>
<tr>
<td>C1</td>
<td>PC(42) – $\beta$-NTA(58)/$\alpha$-NTA(72)/$\beta$-NAA(70)/IAA(88)/IPA(87)</td>
</tr>
<tr>
<td>C2</td>
<td>PCA(32) – IAA(69)/$\beta$-NTA(97)</td>
</tr>
<tr>
<td>D1</td>
<td>PCA(64) – CA(85)/$\beta$-NTA(97)/$\alpha$-NTA(85)/IAA(85)/IPA(95)</td>
</tr>
<tr>
<td>D2</td>
<td>$\alpha$-NTA(76) – $\beta$-NTA(95)</td>
</tr>
<tr>
<td>E2</td>
<td>$\alpha$-NTA(75)/$\beta$-NAA(73) – $\beta$-NTA(95)/IAA(97)/GA(97)</td>
</tr>
</tbody>
</table>

*As per experimental.

Acetic acid indicates the large difference in $hR_t$ values on coating C4 and shows the possibility of their mutual separation. Table 2 presents some analytically important and interesting separations actually achieved on different coating i.e. gallic acid from all other herbicides and plant growth regulators on coatings B1; Separation of cinamic acid from benzoic acid on coating B2 and separation of $\beta$-naphthoxy acetic acid from $\alpha$-naphthoxy acetic acid on coating C1, D2 and E2.

Results show that thin layers of silica gel G alone is not as good as that of silica gel G and barium sulphate admixture. Admixture of silica gel G and barium sulphate have good separation potential for herbicides and related compounds.

The time of development of TLC plates increases with the increasing percentage of barium sulphate in the admixtures. The admixture containing silica gel G-barium sulphate (60:40) is most suitable for thin layer chromatographic studies for separating herbicides and related compounds in single solvent systems. The separation potential is limited due to tailing nature of the herbicides in some of the stationary phases i.e. silica gel G-barium sulphate in 20:80 ratio and in the silica gel G alone (i.e. 100%) with and without ion pair reagent. However,
compact spots were obtained in silica gel G-barium sulphate (80:20) layers with IP reagents and silica gel G-barium sulphate 60:40 ratio without IP reagents.

Fig. 1 depicts that most of the compounds have lower $hR_f$ values on pure silica gel G than on ion pair impregnated silica gel G layers which shows that these compounds form ion pairs with ion pairing reagent. As the concentration of barium sulphate increases, the $hR_f$ values of the compounds show increase on plain layers (i.e. 80:20 ratio) and decrease on impregnated layers until the ratio becomes 60:40 and then further sharp increase in $hR_f$ values for all the compounds on both type of layers have been seen.

Cetrimide is unsymmetrical and has its quaternary nitrogen placed at one end of a long, lyophillic, alkyl chain. So its water solubility is lower than other quaternary ammonium salts. Therefore, cetrimide gives quite different layers when coated onto TLC plates. The silica gel G has silanol groups which are available to interact ion pairing reagents, so a lyophillic layer is formed on the surface of the silica gel G in which the tails of further cetrimide molecules might sit with their hydrophilic and ion pairing ends protruding into the aqueous mobile phase. This enables the cetrimide to act with an ion-exchange (or ion interaction) type of mechanism for the carboxylic acid herbicides and related compounds chromatographed on such plates. This is due to the fact that $hR_f$ values increase when silica gel G is impregnated with cetrimide in comparison to the pure silica gel G layer while in case of admixtures of silica gel G - barium sulphate in ratio 40:60, the silanol groups are not much available for
the interaction of cetrimide molecules and \( hR_f \) values increases further in comparison to the silica gel G impregnated layers. It may also be possible that cetrimide is soluble in water (0.1 M) and sufficiently polar to migrate with the solvent front and is able to form ion pairs with the herbicides and related compounds which are not affected by the admixture in ratio 40:60 and 20:80 when the proportion of barium sulphate increases by more than fifty per cent. This is further evident by the fact that the admixture ratio 60:40 have lowest \( hR_f \) value for all the compounds except chlorophenoxy acetic acid and \( \beta \)-naphthoxy-acetic acid which shows slightly increase in \( hR_f \) values indicating the decrease in ion-exchange behaviour of the silica gel G and increase in adsorption properties of the layers.

Some ternary separations were achieved on silica gel G-barium sulphate (80:20) layers impregnated with ion pairing reagent and on plain silica gel G and barium sulphate (60:40) layers. Table 2 reveals silica gel G-barium sulphate can be used for several binary and ternary separations of herbicides and related compounds using single solvent system i.e. acetone.

**Conclusion**

Admixture of silica gel G-barium sulphate (80:20) with IP reagent and silica gel G-barium sulphate (60:40) with and without IP coatings have good separation potential in thin layer chromatographic studies of carboxylic acid herbicides. It can be used for some binary and ternary separations of some herbicides and related compounds in a single solvent system acetone which is easily available and inexpensive. The method is very simple, non-technical and inexpensive and can be arranged easily.

Thus, it seems that ion pair thin layer chromatography (IPTLC) on admixture of silica gel G-barium sulphate provides a more convenient and simpler method to separate and detect these compounds in multicomponent system in comparison to the normal phase thin layer chromatography. The method can be applied for the separation of these herbicides from fruits, leaves and other parts of the plants and from crops because slight excess of 2,4-D and 2,4,5-T compounds separated here has severe toxic effect on human health. The work on quantitative separation from a large number of samples like fruits, leaves and plants extracts is under investigation.

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**References**