

## SEPARATION AND IDENTIFICATION OF TOXIC HEAVY METAL IONS BY THIN LAYER CHROMATOGRAPHY

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**Abstract**-Thin layer chromatographic method has been developed for the separation of metal ions such as Cr (VI), Cr (III), Ni (II), Co (II), Cu (II), Fe(III), Zn (II) and Mo (VI) from their two, three and four component mixtures. Silicagel- G is acting as a good adsorbent so various separations were performed on thin layer of silica gel G using aqueous humic acid and DMF i.e. - dimethylformamide as a mobile phase. The effect of concentration and pH of mobile phase on the R<sub>f</sub> values of individual metal ions were studied and the optimum conditions for separation of metal ions from their mixture were determined. By using surfactant and with various additives mixed in mobile phase, metal ions such as Cu<sup>2+</sup>, Cr<sup>+6</sup> and Co<sup>2+</sup>, Ni<sup>2+</sup> were separated from their binary mixture.

### INTRODUCTION

Thin layer chromatography (TLC) is a very convenient and effective technique for the separation and identification of inorganic ions. It permits selective separations, simple detection and easy manipulation of the mobile phase. As a result, numerous sorbents and even greater number of mobile phases have been developed for. From literature [1,2] Heavy metals have received considerable attention from analysts, because of their physical and environmental importance [3, 4]. Metals such as As, Cr, Hg, Tl, Cd, Ni, Tl, Cu, Fe, Zn, Pb, Co, Mo is toxic and harmful to human health. These metal ions can form complexes with bio-ligands containing Oxygen, Nitrogen and Sulphur atoms, which causes many problems by their redox processes in living organisms [5]. The use of aqueous solution as a mobile phase in TLC was pioneered by Armstrong and Terrill [6]. Using a surfactant as the mobile phase gained popularity and became more widely applied due to its operational simplicity, cost effectiveness, relative non-toxicity and enhanced separation efficiency [7-10]. The use of silica gel and an alumina layer with surfactant-mediated mobile phase systems [11-16] has been used to separate various inorganic species. Organic matter soil amendments have been known by farmers to be beneficial to plant growth for longer than recorded history [17]. However, the chemistry and function of the organic matter have been a subject of controversy since humans began their postulating about it in the 18th century. Until the time of Liebig, it was supposed that humus was used directly by plants, but, after Liebig had shown that plant growth depends upon inorganic compounds, many soil scientists held

the view that organic matter was useful for fertility only as it was broken down with the release of its constituent nutrient elements into inorganic forms. At the present time, soil scientists hold a more holistic view and at least recognize that humus influences soil fertility through its effect on the water-holding capacity of the soil. Also, since plants have been shown to absorb and translocate the complex organic molecules of systemic insecticides, they can no longer discredit the idea that plants may be able to absorb the soluble forms of humus; [18] this may in fact be an essential process for the uptake of otherwise insoluble iron oxides. A study on the effects of Humic acid on plant growth was conducted at Ohio State University which said in part "humic acids increased plant growth" and that here were "relatively large responses at low application rates" [19]. In Ancient Egypt, according to archeology, straw was mixed with mud in order to produce building bricks. Straw produces stronger bricks that are less likely to break or lose their shape. Modern investigations have found that humic acid is released from straw when mixed with mud, basically a mixture of sand and clay. Humic acid increases clay's plasticity. **Humic acid** is a principal component of humic substances, which is produced by biodegradation of dead organic matter. By definition, humic acids are brownish-black, alkali-soluble solids which can either be recovered from naturally weathered coals or alternatively, be prepared by controlled oxidation. This paper deals with the rapid separation of heavy metal ions present in three, as well as four component mixtures on non-impregnated silica gel 'G' coated plates, using aqueous solution of Humic acid and DMF (dimethylformamide) as a mobile phase.

### EXPERIMENTAL SECTION:-

#### Apparatus

Glass plates of 4 x 20 cm size (coated with silica gel 'G'), 20 x 25 cm glass jars for the development of glass plates, glass sprayer for spraying reagents & EI pH meter.

#### Chemicals and Reagents

Humic acid (E. Merck; India), DMSO (dimethylsuccinamide), Silica gel- G (E. Merck; India); Hydrochloric acid and Sodium hydroxide.

**Metal ion studied :** Cr (VI), Cr (III), Ni (II), Co (II), Cu (II), Fe(III), Zn (II) and Mo (VI)

#### Stock Solutions

Stock solutions of 1% of following salts were prepared in the 0.1 M Hydrochloric acid.

1. Potassium salt of Cr(VI),
2. Chloride of Cr(III), Ni(II), Zn(II) and Fe(III),
3. Sulphate of Cu(II).
4. Trioxide of Mo(VI).
5. Nitrate of Co(II).

The mobile phase was prepared in double distilled water.

#### Detection Reagents:

For the detection of various cations, the following reagents were used

- 1) 0.05 % Dithiozone in Carbon tetra chloride.
- 2) Saturated Alcoholic AgNO<sub>3</sub>.
- 3) Saturated Alcoholic Alizarin red.
- 4) 1% Alcoholic solution of DMG i.e. Dimethylglyoxime.
- 5) 1% Aqueous potassium ferrocyanide

**Stationary Phase:** Silica gel –G.

**Mobile Phase:** The aqueous solution of Humic acid and DMSO (dimethylsuccinamide) as the mobile phases.

#### Thin - layer chromatography

##### (1) Preparation of plates:-

Slurry was prepared by mixing silica gel 'G' in double distilled water in the ratio of 1:2 with constant steering for about 10 minutes. It was then immediately applied to the glass plate by the dipping method and dried over night at room temperature.

##### (2) Running of TLC plates:-

The test solutions were spotted on the silica gel-G plates using fine glass capillaries and they were blow-dried with hot air. The Humic acid & DMSO of varying concentration was adjusted to the desired pH using sodium hydroxide and hydrochloric acid solution. The plates were developed for about 15 min in the glass jar containing 15 ml Humic acid and DMSO solution. Approximately 2 -3 ml of solvent was required to run the sample per plate.

##### (3) Development of TLC plates:-

Plates were dried and different cations were detected by spraying various spot test reagent, which are saturated alcoholic silver nitrate, saturated alcoholic alizarin red, dithiozone in carbon tetra chloride, dimethylglyoxime & potassium ferrocyanide for Cr(VI), Cr(III), and other metal ions i.e. Mo(VI), Zn(II), Ni(II), Co(II), Cu(II), & Fe(III) respectively. All experiments were carried out at room temperature. The R<sub>f</sub> values were measured in triplicate for each set of determinations. Various experiments were carried out to study the mobile phase (0.005M - 0.1 M); pH (1.0 -7.0) and time (5 - 20 min) for the R<sub>f</sub> values of the individual cations.

#### RESULTS AND DISCUSSION:

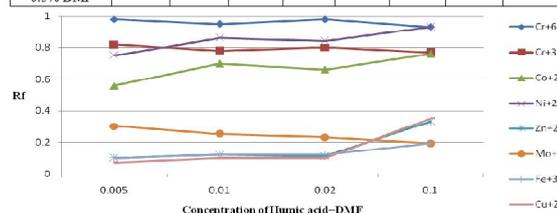
This section deals with the separation of Cr(VI), Cr(III), Mo(VI), Co(II), Zn(II), Fe(III), Cu(II) and Ni(II). Various experiments were carried out at different run time, different pH and at different concentration of Humic acid in DMF

(dimethylformamide) for determining optimum conditions for separation of the metal ions. The variation in the R<sub>f</sub> values of metal ions with run time are shown in table 1 and graphically represented in Fig. 1. The R<sub>f</sub> values have been measured at pH 4.0 and 0.1 M concentration of Humic acid. From the data obtained it revealed that, when the migration time was kept at 10 minutes, the metal ion shows remarkable difference in their R<sub>f</sub> values which was found out to be good for the binary separation, but for the ternary and quaternary mixtures of metal ions it was not effective. When the development time was increased to 15 minutes, separation of metals has been found out to be good. Further increase in the runtime did not affect the separation, and hence, the run time of 15 minutes was considered for further R<sub>f</sub> measurements.

#### Effect of Concentration :-

This section deals with the separation of Cr (vi); Cr (iii); Ni (ii); Co (ii); Fe (iii); Cu(ii); Zn(ii); and Mo(vi). Various experiments were carried out at different pH and at different concentration of Humic acid and DMF (dimethylformamide) for determining optimum separation concentration for the metal ions. The results dealing with the effect of concentration of mobile phase, i.e. Humic acid and DMF on the R<sub>f</sub> values of different metal ions such as Cr (vi); Cr (iii); Ni (ii); Co (ii); Fe (iii); Cu(ii); Zn(ii); and Mo(vi) are tabulated in table 1. The variations in the R<sub>f</sub> values with concentration in the range of 0.005 to 0.1 M were studied in pH range of 1 to 7.

Concentration	Metal ion							
	Cr(VI)	Cr(III)	Co(II)	Ni(II)	Zn(II)	Cu(II)	Fe(III)	Mo(VI)
0.1% Humic acid + 5% DMF	0.93	0.77	0.76	0.93	0.33	0.35	0.19	0.32
0.01% Humic acid + 1% DMF	0.95	0.78	0.70 (sp)	0.86 (sp)	0.12	0.10	0.12	0.28
0.02% Humic acid + 1% DMF	0.98	0.80	0.66	0.84	0.11	0.10	0.12	0.26
0.005% Humic acid + 0.5% DMF	0.98	0.82	0.56 (T)	0.75 (T)	0.10 (T)	0.07	0.10	0.26



**Fig.[1] Effect of Humic acid on the R<sub>f</sub> value of metal ion**

It was observed that, at low concentration of 0.005% HA+ 0.5%DMF Cr (vi); Zn (ii); and Ni(ii) shows tailing, at 0.01 M concentration all metal ions shows little tailing. As the concentration of humic acid and dmf was increased to 0.05 M, clear and distinct spots were seen. It was also observed that, there is an increase in the R<sub>f</sub> values with increase in the concentration, but at 0.1% humic acid +1% dmf spots are not compact and shows little spreading, specially Cr (vi); Mo(VI). (ii). However 0.01 M concentration was selected as the optimum concentration for further studies.

## Effect of pH: -

Table 2:- Effect of pH on the Rf values of metal ions

Metal Ion	pH of Humic acid+ DMF									
	2	2.5	3	3.5	4	4.5	5	5.5	6	7
Cr(VI)	0.94	0.94	0.96	0.98spr	0.99 T	0.95 T	0.95 T	0.96 T	0.98 T	0.98 T
Cr(III)	0.77	0.66	0.58	0.57	0.57	0.56	0.57	0.55	0.34	0.34
Co(II)	0.83	0.84	0.85	0.57	0.58	0.58	0.57spr	0.57pr	0.55	0.56
Ni(II)	0.98	0.96	0.95	0.96spr	0.98spr	0.98spr	0.95spr	0.98spr	0.98	0.98
Zn(II)	0.63	0.62	0.42	0.19T	0.18 T	0.22 T	0.22 T	0.20 T	0.17 T	0.16 T
Cu(II)	0.74spr	0.73spr	0.14	0.22	0.24	0.23	0.24	0.15	0.1	0.1
Fe(III)	0.76spr	0.75spr	0.47	0.2	0.18	0.19	0.18	0.17	0.16	0.16
Mo(VI)	0.63 N.D	0.44 N.D	0.54	0.43T	0.42 T	0.35 T	0.35T	0.34T	0.53	0.53

Notation: - T- Tailing; Spr- Spreading; N.D. - Not Detected

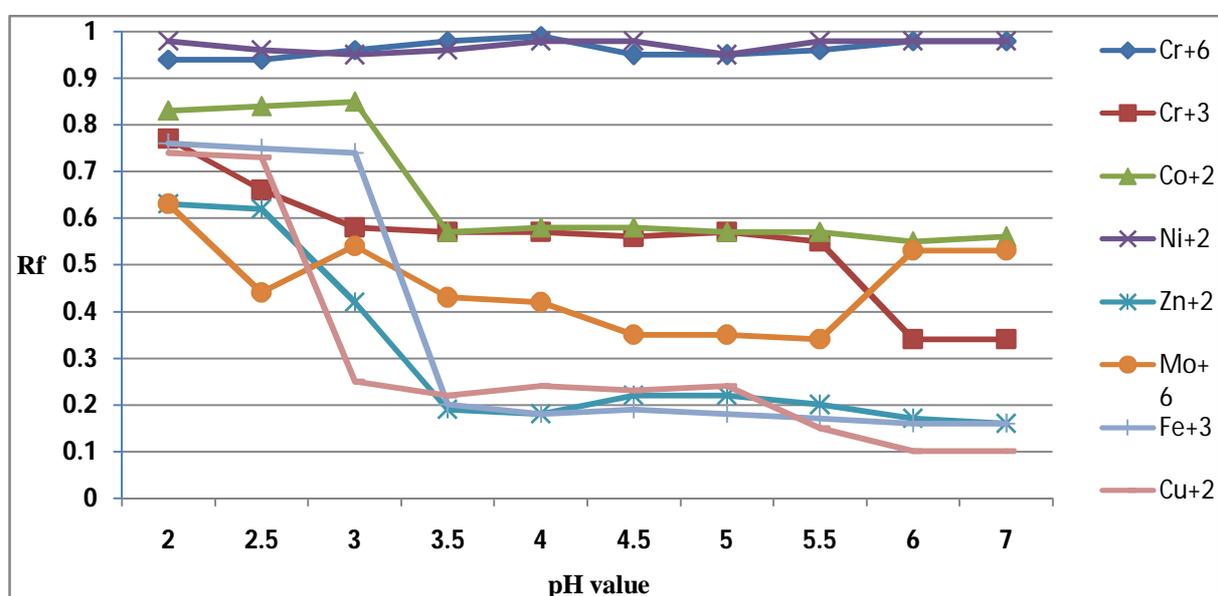


Fig. [2] Effect of pH on the Rf value of metal ion

The effect of pH on the Rf values of different metal ions was carefully studied by carrying repeated experiments. The results are graphically represented in Fig. 2, and tabulated in table 2 which reveal variations in the Rf values with pH of Humic acid and DMF. The Rf value measurements were done in the pH range of 2.0 to 7.0 at 0.01% HA and 1% DMF concentration and 15 minutes of run time.

It can be observed from fig. [1] and [2] that all metal ions showed very little difference in the Rf values at pH 3, but as we increase the pH to 3.5, maximum difference in the Rf values of different metal ions could be achieved, which was required for better separation. However, the behavior of cations changed after the increase above pH 4.0 and especially at pH 5.0 and above and the metal ions Cr(VI); Cr(III); Zn(II); Mo(VI); & Cu(II) show tailing whereas Ni(II) shows spreading, But Co (ii) and Cu(ii) shows compact spot at all pH except pH 1 &

pH 2 and sometimes Mo(VI) could not be detected. From the observed values, pH 4.0 has been found out to be ideal for bringing about maximum separation. Hence, separation measurements have not been carried beyond pH 7.0. Therefore, pH 4.0 has been fixed for further Rf measurements in 0.01% HA+ 1 %DMF as a media.

Table No 3:- Binary separations

Sr. No	Components of Binary mixture	Metal ions with their Rf Values
1	Ni(II); Co(II);	Ni(II) - 0.95;; Co(II)- 0.44
2	Ni(II); Zn(II)	Ni(II) - 0.76; Zn(II) - 0.22
3	Cr(VI); Ni(II);	Cr(VI) - 0.95; Ni(II) - 0.74
4	Co(II); Cr(VI);	Co(II) - 0.67; Cr(VI) - 0.95

5	Cr(VI); Zn(II)	Cr(VI) - 0.95; Zn(II) - 0.20
6	Cr(VI);Cr(III)	Cr(VI) - 0.95;Cr(III) - 0.23
7	Ni(II); Cu(II);	Ni(II) - 0.73; Cu(II) - 0.11

**Table No 4:- Ternary separations**

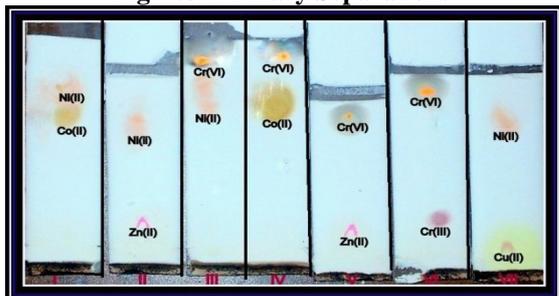
Sr. No.	Components of Ternary mixture	Metal ions with their Rf Values
1	Ni(II); Cr(VI); Zn(II)	Ni(II) - 0.76; Cr(VI) - 0.90; Zn(II) - 0.18
2	Ni(II); Co(II); Cr(III)	Ni(II) - 0.80; Co(II) - 0.69; Cr(III) - 0.23
3	Ni(II); Cr(VI); Mo(VI)	Ni(II) - 0.79; Cr(VI) - 0.92; Mo(VI) - 0.14
4	Ni(II); Cr(VI); Cu(II)	Ni(II) - 0.80; Cr(VI) - 0.90; Cu(II) - 0.16
5	Ni(II); Co(II); Zn(II)	Ni(II) - 0.85; Co(II) - 0.73; Zn(II) - 0.15

**Table No 5:- Quaternary separations**

Sr. No	Components of Quaternary mixture	Metal ions with their Rf Values
1	Cr(VI); Ni(II); Co(II) Cr(III)	Cr(VI) - 0.91;
		Ni(II) - 0.75;
		Co(II) - 0.66;
2	Cr(VI); Ni(II); Co(II) Cu(II)	Cr(III) - 0.22
		Cr(VI) - 0.89;
		Ni(II) - 0.56;
3	Cr(VI); Ni(II); Co(II) Cr(III)	Co(II) - 0.40;
		Cu(II) - 0.10
		Cr(VI) - 0.95;
4	Cr(VI); Ni(II); Cu(II) Zn(II)	Ni(II) - 0.82;
		Co(II) - 0.72;
		Cr(III) - 0.32
5	Cr(VI); Ni(II); Co(II) Mo(VI)	Cr(VI) - 0.89;
		Ni(II) - 0.55;
		Cu(II) - 0.10;
		Zn(II) - 0.20
		Cr(VI) - 0.91;
		Ni(II) - 0.74;
		Co(II) - 0.60;
		Mo(VI) - 0.11

Pictures for the achieved separations using above mention optimum separating conditions.

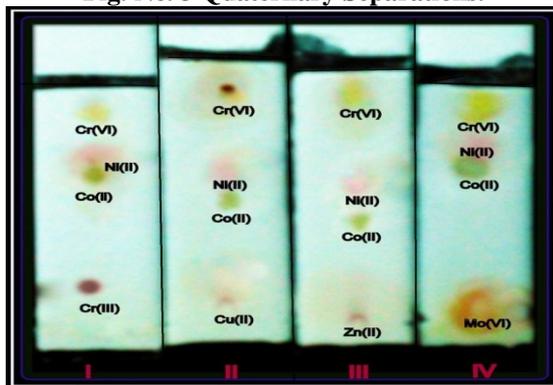
**Fig. No. 1 Binary Separations**



**Fig. No. 2 Ternary Separations.**



**Fig. No. 3 Quaternary Separations.**



**CONCLUSION:-**

Using the above mentioned optimum separating conditions, that is 0.01% Humic acid+1%DMF at pH 4, qualitative separation of Seven binary mixtures; Four ternary mixtures; and Four quaternary mixtures of metal ions have been carried out. The Rf values of various binary, ternary and quaternary separations have been listed in Table no 3, 4, and 5 respectively. The Rf values of metal cations are given in top to bottom format, as they appear on the chromatographic plate. Photograph of achieved binary separations were given in figure number 1, for ternary separations in figure number 2 and quaternary separations in figure number 3. Rf values of experimentally achieved separations on silica gel 'G' layers developed with aqueous Humic acid in DMF as a mobile phase.

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