



# CHARACTERISTIC STUDY OF HEAVY METAL ION BY USING THIN LAYER CHROMATOGRAPHY WITH HUMIC ACID AND L-METHIONINE AS A MOBILE PHASE

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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. The separations were performed on thin layer of silica gel 'G' using aqueous Humic acid with L-methionine as mobile phase. Thin layer of Silica Gel-G was used to study the chromatographic behavior of metal ions in surfactant mixed solvents. Effect of presence of humic acid at various concentrations, presence of strong and weak electrolytes, and effect of concentration of surfactant, effect of acidity and basicity of aqueous surfactant on mobility of metal cations were also studied. By using surfactant and with various additives mixed in mobile phase, metal ions such as Cu (II), Mo(VI), Zn(II) and Ni(II) were separated from their binary mixture.**

## Introduction

Thin layer chromatography ie. (TLC) is superior and versatile method for the separation and identification of inorganic metal ions compared to other methods available for separation. Number of methods these days is available to recuperate thin layer chromatography such as use of support having characteristics different than those normally used in TLC. It has a number of basic advantages over other chromatographic techniques. While a methodology is being developed for a specific separation; it uses less solvent and polarity of the solvent or the type of a solvent mixture can be changed in a matter of minutes. Thus, because of short development time and easy change of mobile phase it is the

easiest method for separation and identification of metal ions. It has been successfully utilized in the analysis of waste water for heavy metal contents [Volynets. *et al.*, 1986.], characterization of hazardous waste[Stephans. *et al.*, 1980], estimation of toxic metal ions in industrial sewage[Thielemann. *et al.*, 1977], separation of heavy metal cations[Mohammad. *et al.*, 2004], separation of metal ion in tube well water sample[Deshmukh. *et al.*, 1989].

In recent years, there has been an increasing ecological and global public health concern associated with environmental contamination by heavy metals ions. Normal phenomena such as weathering and volcanic eruptions have also been reported to significantly contribute to heavy metal pollution [ Bradl 2002 & HeZL. *et al.*, 2005 ) Industrial sources include metal processing in refineries, coal burning in power plants, petroleum combustion, nuclear power stations and high tension lines, plastics, textiles, microelectronics, wood preservation and paper processing plants [Sträter. *et al.*, 2010]. From the pesticides contain different concentration of heavy metals ions which is available in considerable amount in the soil once they are being used.

Using of amino acid as mobile phase is not common for chromatographic separations of heavy metal ion using conventional laboratory made TLC plates. Therefore it was favoured to use the analytical potential of amino acid that is aqueous solution of L - Methionine as mobile phase and silica gel-G as stationary phase for analysis of heavy metal ions. As a result distinct analytically important separations of heavy metals were performed. The importance of methionine in animal feed and food was discovered in the 1950s; and the first production

plant was fabricated by the Deutsche Gold - und Silber - Scheideanstalt (Degussa AG, since 1980 part of Evonik). After all that time, the various publications increased continuously on methionine. The history of industrial amino acid production since the early 1970s was recently reviewed by Udaka. Methionine was produced by fermentation were reviewed by Roy et al., Mondal et al., Gomes and Kumar., Kumar and Gomes. (Bisaria . 2003., Kumar .2005., Udaka.2008 )

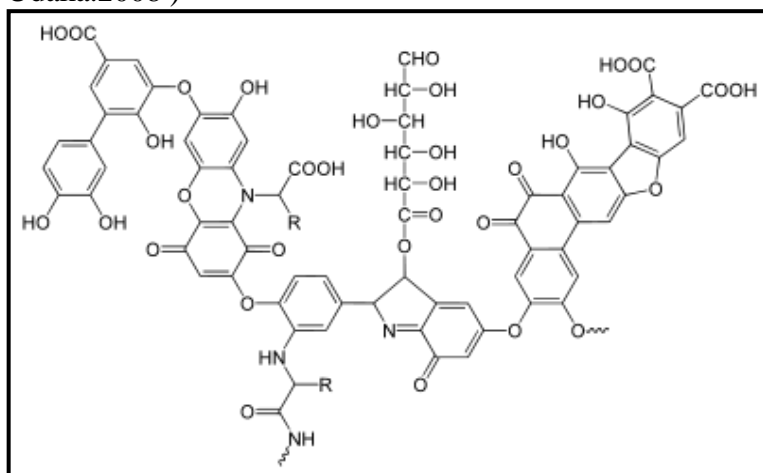


Fig.1 – Structure of Humic acid

This paper deals with the effect of pH, effect of concentration on the Rf value obtained for various metal ions also it deals with the rapid separation of heavy metal ions present in binary, ternary and quaternary form of mixtures on non - impregnated silica gel 'G' coated plates, using aqueous solution of L - Methionine as a mobile phase. The structure of humic acid, and L-methionine is given below.

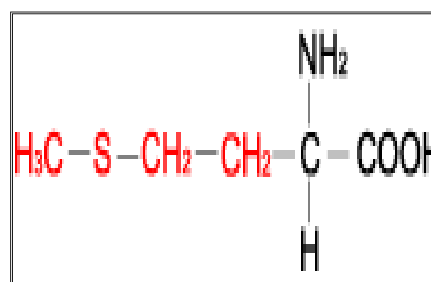


Fig. 2 – Structure of L-methionine

## Experimental

### 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 and EI pH meter.

### Chemicals and Reagents

Humic acid ( Merck, India), (Merk India), silica gel- G (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 tetrachloride.
- 2) Saturated alcoholic AgNO<sub>3</sub>.
- 3) Saturated alcoholic alizarin red.
- 4) 1% Alcoholic solution of DMG ie. Dimethylglyoxime.
- 5) 1% Aqueous potassium ferrocyanide

### Stationary phase

Silica gel -G.

### Mobile phase

The aqueous solution of humic acid with L-methionine as the mobile phases.

### Thin - layer chromatography (TLC)

#### 1. Preparation of plates

Slurry was prepared by mixing silica gel 'G' in double distilled water in the ratio of 1:2 with constant stirring for about 10 minutes. It was then immediately applied to the glass plate by the dipping method [21] and dried overnight 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 varying concentration of mobile phase was adjusted to the desired pH using sodium hydroxide and hydrochloride acid solution. The plates were developed for about 15 min in the glass jar containing 15 ml aqueous humic acid with L-methionine 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 and potassium ferrocyanide for Cr(IV), Cr(III), and other metal ions i.e. Mo(VI), Zn(II), Ni(II), Co(II), Cu(II) and Fe(III) respectively. All experiments were carried out at room temperature. The  $R_f$  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  $R_f$  values of the individual cations.

## Results and Discussion

### Effect of pH

The effect of pH on the  $R_f$  values of different metal ions was carefully studied by conducting several sets of experiments. The results are graphically summarized in Fig. 3 and tabulated in Table 1.1 which revealed the variation in the  $R_f$  value with pH by using aqueous humic acid with L- methionine as a mobile phases. The measurement of  $R_f$  value were done in the range of 1.0 to 7.0.

It was revealed that from Fig.1.1 at low pH all the eight metal ions move along with solvent front. It was noted that metal ions shows a little difference in the  $R_f$  value of pH 3.0 but as the pH was increased to 4.0 there was maximum difference in the  $R_f$  value of different metal ions. This was required for better separation. However the behavior of metal ions changes after pH 5.0 Cr(III), Mo (VI), Zn(II), Fe(III), Cu(II) shows tailing Ni(II) and Co(II) shows spraying and Cr(VI) shows double spots. The clear separation was observed only at pH 4.0. Thus separation measurement has been carried out at pH 4.0, which has been considered as optimum separating pH in aqueous humic acid with L- methionine as a media

**Table 1.1: Effect of pH on the  $R_f$  Value of Metal Ions**

Metal Ion	pH of Humic acid + L-methionine										
	1	2	2.5	3	3.5	4	4.5	5	5.5	6	7
Cr(VI)	0.98 Spr	0.98 Spr	0.98	0.96	0.92	0.92	0.93	0.93 DS	0.93 DS	0.94 DS	0.94 DS
Cr(III)	0.74	0.71	0.69	0.69	0.43	0.13	0.10	0.12	0.11	0.11	0.12
Ni(II)	0.94 Spr	0.92	0.90	0.90	0.65	0.64 spr	0.60 spr	0.59 spr	0.58 spr	0.58 spr	0.57 spr
Co(II)	0.93 Spr	0.91	0.89	0.88	0.54	0.54 spr	0.50 spr	0.51 spr	0.52 spr	0.52	0.53
Zn(II)	0.92 T	0.90	0.87	0.88	0.52T	0.19 T	0.18 T	0.19 T	0.17 T	0.16 T	0.16 T
Cu(II)	0.83	0.80 spr	0.77 spr	0.78	0.42	0.39	0.40	0.38 T	0.38 T	0.37 T	0.37 T
Fe(III)	0.82 Spr	0.79 Spr	0.76 Spr	0.76	0.21	0.20	0.15	0.12	0.11	0.11 T	0.10 T
Mo(VI)	0.81	0.79	0.77	0.77	0.50 T	0.24	0.20 T	0.19 T	0.19 T	0.18	0.17

Notation: T – Tailing, Spr – Spreading, DS – Double spot

Table 1.2: Effect of Concentration on the  $R_f$  Value of Metal Ions

Concentration M	Metal ion							
	Cr(VI)	Cr(III)	Co(II)	Ni(II)	Zn(II)	Cu(II)	Mo(VI)	Fe(III)
0.1% Humic acid + 0.1N L- methionine	0.87 DS	0.30	0.52 DS	0.85 DS	0.55	0.81	0.55 T	0.37 T
0.02% Humic acid + 0.02N L- methionine	0 .9 0	0.10 T	0.55	0.78	0.29 T	0.56	0.24 T	0.17 T
0.01% Humic acid + 0.01N L- methionine	0.90	0.10 T	0.58 T	0.78 Spr	0.28	0.55 T	0.22 T	0.16 T
0.005% Humic acid + 0.005N L- methionine	0.94	0.13	0.66	0.73 T	0.26	0.58	0.23	0.14 T

### Effect of Concentration

The effect of concentration of mobile phases i.e. aqueous humic acid with L- methionine on the  $R_f$  value of different metal ions such as of Cr(VI), Cr(III), Ni(II), Cu(II), Mo(VI), Zn(II), Co(II) and Fe(III) are presented graphically in Fig.4 and tabulated in Table 1.2.

It is seen from the Fig. 1.2 at low concentration in the range of 0.01% concentration of humic acid with 0.01M methionine, Cr (III), Fe (III), Mo (VI), Zn (II), Co (II) and Ni (II) shows tailing. But as concentration of aqueous humic acid with L- methionine was increased to 0.01 to 0.02% all the metal ions have same  $R_f$  value but better separation was achieved with clear and distinct spots at 0.005% humic acid with 0.005M L- methionine. At 0.1 % concentration of aqueous humic acid with 0.1M L- methionine, metal ions does not show clear and distinct spots especially Cr(VI) and Ni(II) shows double spots and spreading. It was also observed that there is an increase in  $R_f$  values with increase in concentration of mobile phase. Hence 0.005% concentration of aqueous humic acid with 0.005M concentration of L- methionine was selected as optimum concentration for further studies at pH 4.0.

### Separation Studies in Synthetic Sample

The present work of separation in aqueous humic acid with L- methionine media was applied to the quantitative separation and detection of metal ions in the Effluent Treated Sludge sample obtained from Indian Bureau of Mines and Fack Agrochem PVT (I), Hingna Industrial area, Nagpur. The result is summarized in Table 1.6 and 1.7 respectively. The metals ions present in the sample solution were finally eluted and determined quantitatively by AAS method. The AAS studies of synthetic sample of metal ions were done before and after running chromatogram for the comparative study which is listed in Table 1.3 and 1.4 respectively.

### Separation Studies in Mixture

After establishing optimum conditions, quantitative separation of twelve binary mixtures, nine ternary and six quaternary mixtures of metal ions has been carried out in synthetic samples. The pictures of binary, ternary and quaternary separations using above optimum separating conditions have been depicted in Plate 1.1, 1.2 and 1.3 and tabulated in Table 1.3, 1.4 and 1.5 respectively.

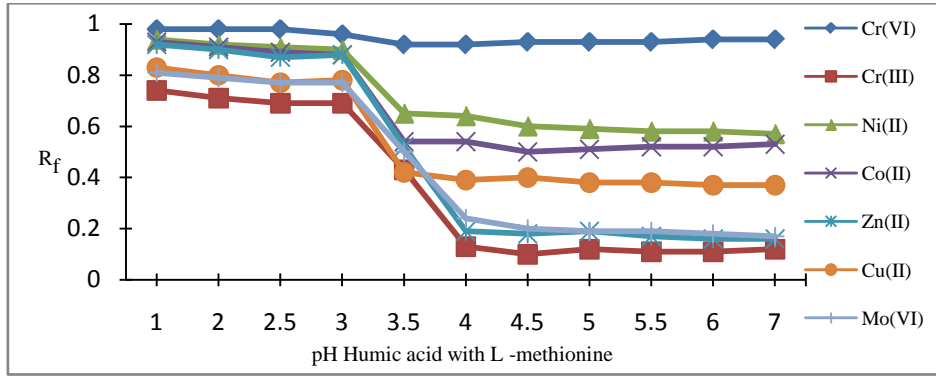


Fig. 3 – Effect of pH on the R<sub>f</sub> value of metal ions

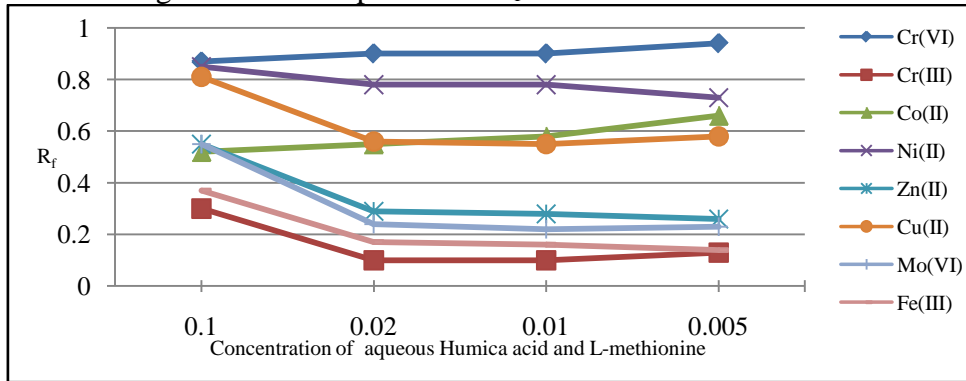


Fig. 4 – Effect of Concentration on the R<sub>f</sub> value of metal ions

**R<sub>f</sub> Values of Experimentally Achieved Separations of Various Mixture using Optimum Separating Conditions**

Mobile Phase: 0.005% aqueous humic acid + 0.005M L-methionine, Adsorbent: Silica gel –G, Developing time: 20 min., pH = 4.0

**Table 1.3 Binary Separations**

Sr. No	Components of Binary mixture	Metal ions with their R <sub>f</sub> Values
1	Cr(VI); Co(II)	Cr(VI) - 0.96; Co(II) - 0.71
2	Ni(II); Zn(II)	Ni(II) – 0.83; Zn(II) – 0.14
3	Ni(II); Mo(VI)	Ni(II) – 0.81; Mo(VI) – 0.10
4	Cr(VI); Cr(III)	Cr(VI) – 0.92; Cr(III) – 0.10
5	Co(II); Zn(II)	Co(II) - 0.61; Zn(II) – 0.11
6	Cr(VI); Zn(II)	Cr(VI) – 0.93; Zn(II) – 0.15
7	Ni(II); Co(II)	Ni(II) – 0.78; Co(II) – 0.58
8	Ni(II); Cu(II)	Ni(II) – 0.76; Cu(II) – 0.40
9	Ni(II); Cr(III)	Ni(II) - 0.81; Cr(III)– 0.10
10	Cr(VI); Ni(II)	Cr(VI) - 0.93; Ni(II) - 0.76
11	Co(II); Fe(III)	Co(II) - 0.60; Fe(III) – 0.09
12	Cu(II); Zn(II)	Cu(II) – 0.40.; Zn(II)– 0.13

**Table 1.4:- Ternary Separations**

Sr.No.	Components of Ternary mixture	Metal ions with their $R_f$ Values
1	Ni(II); Co(II); Cr(III)	Ni(II) – 0.82; Co(II) – 0.71; Cr(III) – 0.10
2	Ni(II); Co(II); Zn(II)	Ni(II) – 0.83; Co(II) - 0.69; Zn(II) – 0.11
3	Ni(II); Cr(VI); Cu(II)	Ni(II) - 0.82; Cr(VI) – 0.95; Cu(II) – 0.41
4	Ni(II); Cr(VI); Mo(VI)	Ni(II) – 0.83; Cr(VI) – 0.95; Mo(VI) – 0.11
5	Ni(II); Cr(VI); Co(II)	Ni(II) – 0.79; Cr(VI) – 0.93; Co(II) – 0.64
6	Ni(II); Cr(VI); Zn(II)	Ni(II) – 0.78; Cr(VI) – 0.92; Zn(II) – 0.13
7	Co(II); Cu(II); Fe(III)	Co(II) – 0.73; Cu(II) -- 0.43; Fe(III) – 0.12
8	Cr(VI); Ni(II); Fe(III)	Cr(VI)-- 0.93; Ni(II) --0.80; Fe(III)-- 0.11
9	Co(II); Cu(II); Zn(II)	Co(II) – 0.71; Cu(II) -- 0.42; Zn(II)– 0.14

**Table No 1.5:- Quaternary Separations**

Sr. No	Components of Quaternary mixture	Metal ions with their $R_f$ Values
1	Cr(VI); Ni(II); Co(II); Zn(II)	Cr(VI) – 0.94; Ni(II) – 0.76; Co(II) – 0.61; Zn(II) – 0.10
2	Cr(VI); Ni(II); Co(II); Cr(III)	Cr(VI) – 0.93; Ni(II) – 0.77; Co(II) – 0.61; Cr(III) – 0.10
3	Cr(VI); Ni(II); Mo(VI); Zn(II)	Cr(VI) – 0.93; Ni(II) – 0.77; Mo(IV) – 0.09; Zn(II) – 0.14
4	Cr(VI); Ni(II); Zn(II); Cr(III)	Cr(VI) -0.93; Ni(II) – 0.77; Zn(II) – 0.15; Cr(III) -0.10
5	Cr(VI); Co(II); Zn(II); Fe(III)	Cr(VI) – 0.94; Co(II) – 0.64; Zn(II) – 0.15; Fe(III) -0.09
6	Cr(VI); Ni(II); Cu(II); Zn(II)	Cr(VI) – 0.90; Ni(II) – 0.72; Cu(II) – 0.34; Zn(II) – 0.15

**Table 3.62 Quantitative Separations of Metal Ions in Synthetic Samples**

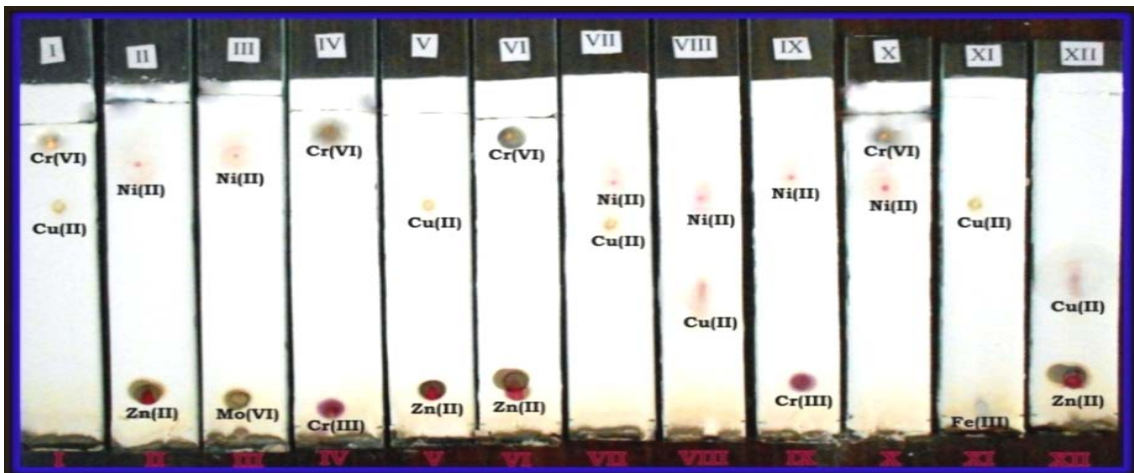
Components	Metal ion analysis	Metal ion present in mixture (ppm) before chromatogram	Metal ion Analysis by AAS (ppm) after chromatogram
Cr(VI), Ni(II) Co(II), Cr (III)	Cr(VI)	Cr(VI) – 11.07	Cr(VI) – 10.95
	Ni(II)	Ni(II) – 8.79	Ni(II) – 8.64
	Co(II)	Co(II) – 7.01	Co(II) – 6.59
	Cr (III)	Cr(III) – 18.83	Cr(III) – 18.79

Zn(II), Mo(VI) Cu(II), Fe(III)	Zn(II) Mo(VI) Cu(II) Fe(III)	Zn(II) – 10.22 Mo(VI) – 5.03 Cu(II) – 10.67 Fe(III) – 25.85	Zn(II) – 9.18 Mo(VI) – 4.95 Cu(II) – 10.62 Fe(III) – 25.56
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**Table 1.7 Quantitative Separation of Metal Ions in Effluent treated sludge sample**

Name of Sample	Metal ion analysis	Metal ion present in mixture (ppm) before chromatogram	Metal ion Analysis by AAS (ppm) after chromatogram
Effluent treated sludge sample	Cr(VI) Cr (III) Ni(II) Co(II) Zn(II) Mo(VI) Fe(III) Cu(II)	Cr(VI) – 0.0258 ppm Cr (III) – 0.0162 ppm Ni(II) – 0.0361 ppm Co(II) – 0.0212 ppm Zn(II) – 0.0131 ppm Mo(VI) – 0.0209 ppm Fe(III) – 0.192 ppm Cu(II) – 0.0453 ppm	Cr(VI) – 0.0243 ppm Cr (III) – 0.0153 ppm Ni(II) – 0.0352 ppm Co(II) – 0.0210 ppm Zn(II) – 0.0111 ppm Mo(VI) – 0.0189 ppm Fe(III) – 0.189 ppm Cu(II) – 0.0449 ppm

**Pictures of Binary, Ternary and Quaternary Separations using Optimum Separating Conditions**



**Plate 1.1: Binary Separation**



**Plate 1.2: Ternary Separation**

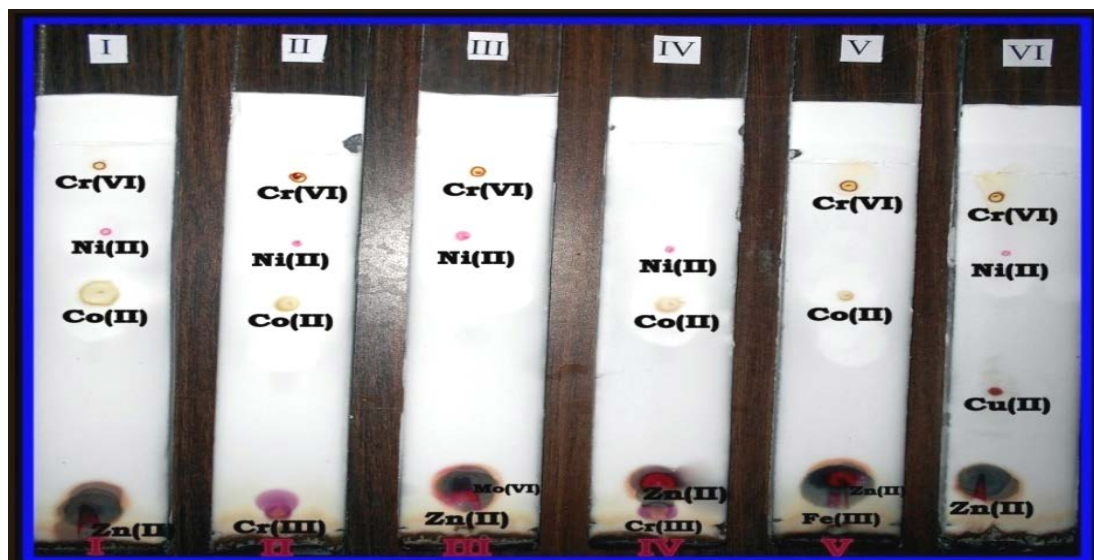


Plate 1.3: Quaternary Separation

### Conclusion

Using the above mentioned conditions qualitative separation of twelve binary mixtures, nine ternary mixtures and six quaternary mixtures of metal ions have been carried out. The  $R_f$  values of metal cations are given in top to bottom format, as they appear on the chromatographic plate. Experimentally achieved separations on silica gel 'G' layers developed Humic acid with L-methionine as mobile phase. The  $R_f$  values of metal ions in their mixture are slightly change from their individual  $R_f$  values because of mutual interactions.

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