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## **Comparative study of different mobile phase for separation of some toxic heavy metal ions by thin layer Chromatography**

**SM Wanjari, LJ Paliwal and Lata Deshmukh**

### **Abstract**

Thin layer chromatographic method has been developed for the separation of metal ions such as  $\text{Cr}^{6+}$ ,  $\text{Cr}^{3+}$ ,  $\text{As}^{3+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Tl}^{3+}$  and  $\text{Hg}^{2+}$  from their two, three and four component mixtures and there comparative study in different mobile phases. As well as their separation ability was studied comparatively with respect to each other. The separations were performed on thin layer of silica gel ‘G’ using aqueous Oxalic acid and L-valine as mobile phase. The effect of concentration and pH of mobile phase on the  $R_f$  values of individual metal ions were studied and the optimum conditions for separation of metal ions from their mixture were determined separately for each mobile phase, then data of both mobile phase has been compared.

**Keywords:** Thin layer chromatography, separation, silica gel-G, oxalic acid, L-valine, toxic heavy metal ions

### **Introduction**

Heavy metals have received considerable attention from analysts, because of their physical and environmental importance [1-2]. Metal such as As, Cr, Hg, Tl, Cd, Ni, Tl, Cu, Fe, Zn, Pb are toxic and harmful to human health. These metal ions can form complexes with bio-ligands containing Oxygen, Nitrogen and Sulphur atoms [3], which causes many problems by their redox processes in living organisms. In the past few decades, there is substantial increase in the use of heavy metals, due to the industrialization; this resulted in an increased flux of metallic substances. Industrial waste is the major source of different kinds of metal pollution in aquatic systems. The major sources of pollution of Chromium in the aquatic environment are electroplating stainless steel industries, metal finishing industrial effluents, sewage and waste-water treatment plants discharge and chromates from cooling water. Chromium occurs in several oxidation states such as di, tri, penta and hexavalent, but only  $\text{Cr}^{3+}$  and  $\text{Cr}^{6+}$  are biologically important. Chromium in the aquatic tends to speciate into  $\text{Cr}^{3+}$  and  $\text{Cr}^{6+}$ , with the trivalent ion being oxidized to the hexavalent form or precipitating from solution.

There are different analytical techniques of separation and detection of chromium including graphite furnace atomic-absorption spectroscopy [4-5], neutron activation analysis [6-7], atomic emission spectroscopy [8], normal phase and reverse phase thin layer chromatography [9-11], ion chromatography [12-13], precipitation floatation [14], titrimetry [15, 16], and hyphenated techniques such as ion-exchange chromatography - flame atomic absorption spectroscopy [17], solid - phase extraction - flame atomic emission spectroscopy [18]. Out of these different separation procedures, thin layer chromatography is probably the most versatile, because it can be used for the selective separation of metal cations on the micro and as well as macro scales. Exhaustive survey off the literature published in the last thirty years [19] shows that

much progress has been made in developing rapid and selective thin layer chromatographic method for separation of toxic heavy metals from interfering elements, by use of a variety of acidic developer containing mineral or carboxylic acid as one of the component.

All studies with oxalic acid and L-valine separately as mobile phase has been performed using conventional, laboratory made TLC plates coated with silica gel-G as stationary phase for analysis of heavy metal ions. Several important separations of heavy metals were conducted from different combinations of metal ions solutions.

Separation of the chromium is industrially important, because  $\text{Cr}^{+3}$  are converted to  $\text{Cr}^{+6}$  in alkaline peroxide medium.

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 oxalic acid and L-valine as a mobile phase.

### Experimental procedure

#### 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 digital pH meter.

#### Chemicals and reagents

Oxalic acid (E. Merck; India), L-valine (E. Merck; India), Silica gel-G (E. Merck; India); Hydrochloric acid and Sodium hydroxide to maintain the pH.

#### Stock solutions and metal ions studied

Stock solutions of 0.05 M of following salts were prepared in the 0.1 M Hydrochloric acid.

1. Potassium salt of  $\text{Cr}^{6+}$
2. Chloride of  $\text{Cd}^{2+}$  and  $\text{Hg}^{2+}$
3. Sulphate of  $\text{Cr}^{3+}$
4. Trioxide of  $\text{As}^{3+}$  and  $\text{Ti}^{3+}$

The mobile phase was prepared in double distilled water.

#### Detection reagents

For the detection of various cations, the following reagents [20] were used

1. 0.05 % Dithiozone in Carbon tetra chloride.
2. Saturated Alcoholic  $\text{AgNO}_3$ .
3. Saturated Alcoholic Alizarin red.

#### 1. Preparation of TLC plates

Slurry was prepared by mixing silica gel 'G' in double distilled water in the ratio of 1:2 with constant steering for about 20 minutes. It was then immediately applied to the glass plate by the dipping method [21] 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 oxalic acid of varying concentration 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 oxalic acid 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 and dithiozone in carbon tetra-chloride, for  $\text{Cr}^{6+}$ ,  $\text{Cr}^{3+}$  and other metal ions i.e.  $\text{As}^{3+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Ti}^{3+}$  &  $\text{Hg}^{2+}$  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

This section deals with the separation of  $\text{Cr}^{6+}$ ,  $\text{Cr}^{3+}$ ,  $\text{As}^{3+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Ti}^{3+}$  and  $\text{Hg}^{2+}$ . Various experiments were carried out at different concentration, pH, and run time of oxalic acid and L-valine for determining optimum conditions for separation of the metal ions [22-23].

#### Effect of concentration

The results dealing with the effect of concentration of mobile phase on the  $R_f$  values of different metal ions such as  $\text{Cr}^{6+}$ ,  $\text{Cr}^{3+}$ ,  $\text{As}^{3+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Ti}^{3+}$  and  $\text{Hg}^{2+}$  are tabulated in table 1. The variations in the  $R_f$  values with concentration in the range of 0.005 to 0.1 M of both mobile phase were studied in pH range of 1 to 7.

In case of L-valine, It was observed that, at low concentration of 0.005 M,  $\text{Cr}^{6+}$ ;  $\text{Cd}^{2+}$ ; and  $\text{Ti}^{3+}$  show long tailing, at 0.01 M concentration, all metal ions shows short tailing. At 0.05 M concentration of L-valine, clear and distinct spots were seen. But at 0.1 M. L-valine spots were not compact and shows little spreading, especially  $\text{Cr}^{6+}$ ;  $\text{Cd}^{2+}$ ;  $\text{Hg}^{2+}$ .

In case of oxalic acid at 0.005 and 0.01 M. concentration, most of the metal ions show tailing or spreading of spots. At concentration of 0.05 and 0.1M spots are compact but at 0.05 M. concentration difference in  $R_f$  values is remarkable which is required for separation of metal ions.

However 0.05 M concentration was selected as the optimum concentration for both mobile phase for further chromatographic studies.

**Table 1:** Effect of concentration of mobile phase on the  $R_f$  values of metal ions

Sr. No.	Metal ions combinations along with their $R_f$ values	
	L-valine	Oxalic acid
1	$\text{Cr}^{6+}$ - 0.97; $\text{Cr}^{3+}$ - 0.38.	$\text{Cr}^{6+}$ - 0.97; $\text{Cr}^{3+}$ - 0.21.
2	$\text{Cr}^{6+}$ - 0.97; $\text{As}^{3+}$ - 0.80.	$\text{Cr}^{6+}$ - 0.97; $\text{As}^{3+}$ - 0.64.
3	$\text{Cr}^{6+}$ - 0.97; $\text{Cd}^{2+}$ - 0.44.	$\text{Cr}^{6+}$ - 0.98; $\text{Cd}^{2+}$ - 0.25.
4	$\text{Cr}^{6+}$ - 0.98; $\text{Ti}^{3+}$ - 0.18.	$\text{Cr}^{6+}$ - 0.97; $\text{Ti}^{3+}$ - 0.23.
5	$\text{Hg}^{2+}$ - 0.71; $\text{Cr}^{3+}$ - 0.35	$\text{Hg}^{2+}$ - 0.98; $\text{Cr}^{3+}$ - 0.18.
6	$\text{As}^{3+}$ - 0.85; $\text{Cd}^{2+}$ - 0.44.	$\text{As}^{3+}$ - 0.62; $\text{Cd}^{2+}$ - 0.32.

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

#### Effect of pH

This section deals with effect of pH on  $R_f$  values of metal ions. The effect of pH on the  $R_f$  values of different metal ions was carefully studied by conducting several sets experiments. The plates were run near about to 10 cm above from the base line. The results are tabulated in table 2 which reveal variations in the  $R_f$  values with pH of L-valine and Oxalic acid. The  $R_f$  value measurements were done in the pH range of 1.0 to 7.0 at 0.05 M concentration.

**Table 2:** Effect of pH on the R<sub>f</sub> values of metal ions at conc. 0.05 m L-valine and oxalic acid

Mobile phase	Metal ions	pH of mobile phase									
		1	2	2.5	3	3.5	4	4.5	5	6	7
L-valine	Cr <sup>6+</sup>	0.95	0.95	0.95	0.95	0.96	0.93	0.93	0.92D.S	0.94D.S	0.94D.S
	Cr <sup>3+</sup>	0.7	0.76	0.54	0.27	0.2	0.15	0.16	0.06T	0.07T	0.08T
	As <sup>3+</sup>	0.83	0.85	0.84	0.86	0.89	0.88	0.87	0.84	0.87	0.79
	Cd <sup>2+</sup>	0.9	0.83	0.69	0.57	0.46	0.37T	0.38T	0.35T	0.33T	0.37T
	Tl <sup>3+</sup>	0.8	0.85	0.61	0.43	0.32	0.22T	0.24T	0.18T	0.22T	0.16T
	Hg <sup>2+</sup>	0.95	0.93	0.91	0.79	0.71	0.72	0.73	0.67spr	0.69spr	0.66spr
Oxalic acid	Cr <sup>6+</sup>	0.95	0.95	0.93	0.96	0.95	0.9	0.93	0.93T	0.94T	0.94T
	Cr <sup>3+</sup>	0.66	0.65	0.52	0.49	0.48	0.43	0.3	0.15	0.12T	0.18T
	As <sup>3+</sup>	0.8	0.8	0.8	0.78	0.78	0.78	0.73	0.84	0.84	0.81
	Cd <sup>2+</sup>	0.64	0.63	0.69	0.7	0.68	0.63	0.61	0.66T	0.67T	0.70T
	Tl <sup>3+</sup>	0.85	0.85	0.7	0.56	0.54	0.52	0.35T	0.24T	0.18T	N.D
	Hg <sup>2+</sup>	0.93	0.93	0.96	0.98	0.96	0.93	0.95	0.96	0.96spr	0.91spr

Notation: T - Tailing; Spr - Spreading; D.S - Double Spot; N.D. - Not detected.

It can be observed from table 2 that, at low pH, all the six metal ions move with the solvent front. It is noted that all metal ions showed very small difference in the R<sub>f</sub> values at pH 1.0 and 2.0, but as pH increases from 2.5 to pH 3, maximum difference in the R<sub>f</sub> values of different metal ions were seen, which was required for better separation. From the observed values, pH 3 was ideal pH to carry out maximum separation pH 3 fixed for further R<sub>f</sub> measurement in aqueous media of L-valine.

In case of oxalic acid, at pH to 4.0, maximum difference in the R<sub>f</sub> values of different metal ions was observed. From the observed values, pH 4.0 was ideal pH for bringing about maximum separation. Separation measurements have not been carried beyond pH 7.0. Therefore, pH 4.0 has been fixed for further R<sub>f</sub> measurements in oxalic acid media.

However, in case of both mobile phases, the behavior of cations changed after optimum pH shown by individual mobile phase. Increase pH above 4.0 and especially at pH 5.0 the metal ions Cr<sup>6+</sup>, Cr<sup>3+</sup>, As<sup>3+</sup>, Cd<sup>2+</sup>, Tl<sup>3+</sup> show tailing, whereas Hg<sup>2+</sup> shows spreading and As<sup>3+</sup> could not be detected.

**Effect of runtime**

Using optimum condition of 0.05M concentration of both mobile phase and pH 3 for L-valine and pH 4 for Oxalic acid, effect of run time was determine for both mobile phase separately at 5, 10, 15 and 20 minutes..

From obtained data it revealed that, In case of L-valine, at 5 minute, metal ion is very close to each other. When the migration time was kept at 10 minutes, the metal ion shows remarkable difference in there 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 run time did not remarkable difference in metal ions spots, hence does not affect the separation, hence, the run time of 15 minutes was considered for further R<sub>f</sub> measurements.

Same observation was seen in case of oxalic acid.

**Conclusion**

Using above optimum condition of metal ions on chromatogram different combinations of binary, ternary and quaternary mixtures were separated successfully. There were eight binary mixtures, seven ternary mixtures and five quaternary mixtures were separated in both the mobile phases. But most of successful speciation separations of Cr<sup>3+</sup> and Cr<sup>6+</sup> are possible in L-valine as compared to oxalic acid. In L-valine, one binary, two ternary and two quaternary combination of speciation separations of Cr<sup>3+</sup> and Cr<sup>6+</sup> are possible along with different important separation. Along with that six binary, five ternary and four quaternary separations along with chromium ions was separated.

In oxalic acid, one binary combination, two ternary and only one quaternary combination of speciation separations were achieved. As well as five binary, five ternary and four quaternary separations along with chromium ions was separated.

Hence by observing number and type of metal ion combinations, L-valine is better mobile phase as compared to oxalic acid for the separations of metal ion by thin layer chromatography.

Results are tabulated in table no. 3 for binary; table no. 4 for ternary and table no. 5 for quaternary separations are given.

**Table 3:** Binary separations

Sr. No.	Metal ions combinations along with their R <sub>f</sub> values	
	L-valine	Oxalic acid
1	Cr (VI) - 0.97; Cr (III) - 0.38.	Cr (VI) - 0.97; Cr (III) - 0.21.
2	Cr (VI) - 0.97; As (III) - 0.80.	Cr (VI) - 0.97; As (III) - 0.64.
3	Cr (VI) - 0.97; Cd (II) - 0.44.	Cr (VI) - 0.98; Cd (II) - 0.25.
4	Cr (VI) - 0.98; Tl (III) - 0.18.	Cr (VI) - 0.97; Tl (III) - 0.23.
5	Hg (II) - 0.71; Cr (III) - 0.35	Hg (II) - 0.98; Cr (III) - 0.18.
6	As (III) - 0.85; Cd (II) - 0.44.	As (III) - 0.62; Cd (II) - 0.32.
7	Hg (II) - 0.74; Cd (II) - 0.53.	Hg (II) - 0.97; As (III) - 0.66.
8	Cr (VI) - 0.97; Hg (II) - 0.76	Hg (II) - 0.98; Cd (II) - 0.36

**Table 4:** Ternary separations

Sr. No.	Metal ions combinations along with their R <sub>f</sub> values	
	L-valine	Oxalic acid
1	Cr <sup>6+</sup> - 0.96; As <sup>3+</sup> - 0.29; Cr <sup>3+</sup> - 0.37.	Cr <sup>6+</sup> - 0.99; As <sup>3+</sup> - 0.77; Cr <sup>3+</sup> - 0.27.
2	Cr <sup>6+</sup> - 0.98; Cd <sup>2+</sup> - 0.59; Cr <sup>3+</sup> - 0.64.	Cr <sup>6+</sup> - 0.97; Cd <sup>2+</sup> - 0.48; Cr <sup>3+</sup> - 0.20.
3	Hg <sup>2+</sup> - 0.85; Cd <sup>2+</sup> - 0.34; Tl <sup>3+</sup> - 0.23.	Hg <sup>2+</sup> - 0.98; Cd <sup>2+</sup> - 0.32; Tl <sup>3+</sup> - 0.14.
4	Cr <sup>6+</sup> - 0.97; Cd <sup>2+</sup> - 0.39; Tl <sup>3+</sup> - 0.25.	Cr <sup>6+</sup> - 0.97; Cd <sup>2+</sup> - 0.51; Tl <sup>3+</sup> - 0.22.
5	As <sup>3+</sup> - 0.83; Hg <sup>2+</sup> - 0.62; Cd <sup>2+</sup> - 0.36	Hg <sup>2+</sup> - 0.98; As <sup>3+</sup> - 0.48; Tl <sup>3+</sup> - 0.18.
6	Cr <sup>6+</sup> - 0.98; As <sup>3+</sup> - 0.80; Cd <sup>2+</sup> - 0.50.	Cr <sup>6+</sup> - 0.98; As <sup>3+</sup> - 0.48; Tl <sup>3+</sup> - 0.18.
7	As <sup>3+</sup> - 0.81; Cd <sup>2+</sup> - 0.50; Cr <sup>3+</sup> - 0.31.	Hg <sup>2+</sup> - 0.97; As <sup>3+</sup> - 0.55; Cr <sup>3+</sup> - 0.19.

**Table 5:** Quaternary separations

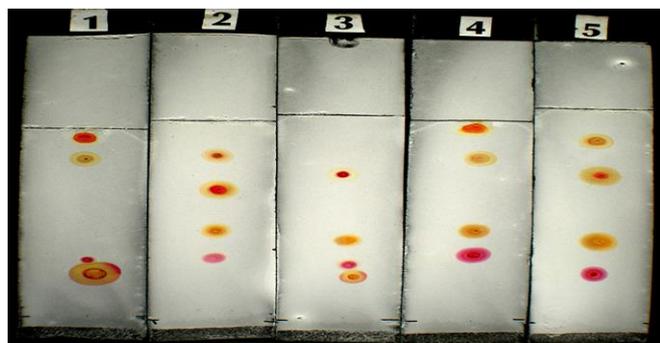
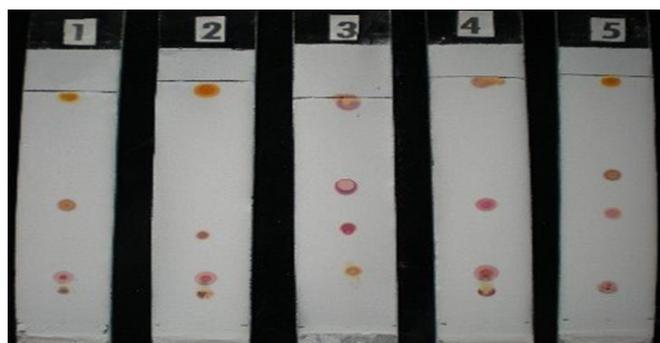
Sr. No.	Metal ions combinations along with their R <sub>f</sub> Values	
	L-valine	Oxalic acid
1	Cr <sup>6+</sup> - 0.97; As <sup>3+</sup> - 0.23; Cr <sup>3+</sup> - 0.22; Tl <sup>3+</sup> - 0.30.	Cr <sup>6+</sup> - 0.98; As <sup>3+</sup> - 0.51; Cr <sup>3+</sup> - 0.20; Tl <sup>3+</sup> - 0.13.
2	Hg <sup>2+</sup> - 0.67; As <sup>3+</sup> - 0.83; Cd <sup>2+</sup> - 0.46; Tl <sup>3+</sup> - 0.32.	Cr <sup>6+</sup> - 0.98; Cd <sup>2+</sup> - 0.37; Cr <sup>3+</sup> - 0.20; Tl <sup>3+</sup> - 0.13;
3	Hg <sup>2+</sup> - 0.73; Cd <sup>2+</sup> - 0.40; Cr <sup>3+</sup> - 0.22; Tl <sup>3+</sup> - 0.26.	Hg <sup>2+</sup> - 0.97; As <sup>3+</sup> - 0.62; Cd <sup>2+</sup> - 0.41; Tl <sup>3+</sup> - 0.23.
4	Cr <sup>6+</sup> - 0.96; As <sup>3+</sup> - 0.81; Cd <sup>2+</sup> - 0.45; Cr <sup>3+</sup> - 0.33.	Hg <sup>2+</sup> - 0.98; Cd <sup>2+</sup> - 0.49; Cr <sup>3+</sup> - 0.21; Tl <sup>3+</sup> - 0.13;
5	Hg <sup>2+</sup> - 0.70 As <sup>3+</sup> - 0.86; Cd <sup>2+</sup> - 0.49; Cr <sup>3+</sup> - 0.23;	Cr <sup>6+</sup> - 0.98; As <sup>3+</sup> - 0.60; Cd <sup>2+</sup> - 0.44; Cr <sup>3+</sup> - 0.14;

The R<sub>f</sub> values of metal cations are given in top to bottom format, as they appear on the chromatographic plate.

There is slight difference in the R<sub>f</sub> values of metal ions during the separations from their mixtures, as compared to individual R<sub>f</sub> values, due to the mutual interaction of metal ions on chromatogram.

Photograph of achieved quaternary separations were given in picture no 1, using L-valine as mobile phase in picture no. 2, using oxalic acid.

**Figure for the achieved separations using above mention optimum separating conditions**

**Fig 1:** For quaternary separations using L-valine as mobile phase**Fig 2:** For quaternary separations using oxalic acid as mobile phase

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