

## High performance Liquid Chromatographic separations of Metal-Pyrrolidine Dithiocarbamate Complexes

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**Abstract.** Separations of Pb(II), Ba(II), Mn(II), Cr(III), Ni(II), Cu(II), Hg(II), Zn(II), Fe(III) and Co(II) metal-pyrrolidinedithiocarbamate (PDC) complexes have been carried out using High Performance Liquid Chromatography (HPLC). Prior to the separations, chloroform and acetonitrile have been used to extract the metal complexes by solid phase extraction, using C<sub>18</sub> bonded silica as sorbent. Phenyl bonded silica HPLC column was found suitable to separate metal-PDC complexes. The eluent was acetonitrile : water (66 : 34 ratio by volume) containing 1.5 x 10<sup>-5</sup>M ammonium pyrrolidinedithiocarbamate (APDC) at pH 5.6. The mixtures of metal-PDC complexes that successfully separated are Pb(II), Cr(III), Ni(II), Hg(II) or Cu(II), Zn(II), Fe(III) and Co(II). The separated components were detected by UV detector at 254 nm wavelength. The quantitative determination of NIST 1633a Coal Fly Ash Standard Reference Material (US Department of Commerce 1990) was carried out to validate the method developed.

**Abstrak.** Pemisahan bagi Pb(II), Ba(II), Mn(II), Cr(III), Ni(II), Cu(II), Hg(II), Zn(II), Fe(III) dan Co(II) dalam bentuk kompleks logam-pirolidinditiokarbamat (PDC) telah dilakukan dengan kaedah Kromatografi Cecair Berprestasi Tinggi (KCBT). Sebelum pemisahan kromatografi, kloroform and asetonitril telah digunakan untuk mengekstraks logam-logam kompleks tersebut secara ekstraksi fasa pepejal, yang mana penggerapnya adalah silika terikat C<sub>18</sub>. Turus KCBT silika terikat fenil telah didapati sesuai untuk memisahkan kompleks logam-PDC. Pelarut elusi adalah asetonitril : air (nisbah 66:34 mengikut isipadu) mengandungi amonium pirolidinditiokarbamat (APDC) 1.5 x 10<sup>-5</sup>M pada pH 5.6. Campuran kompleks logam-PDC yang berjaya dipisahkan ialah Pb(II), Cr(III), Ni(II), Hg(II) atau Cu(II), Zn(II), Fe(III) dan Co(II). Komponen-komponen yang terpisah dikesan menggunakan pengesan ultralembayung pada jarak gelombang 254 nm. Penentuan kuantitatif bagi NIST 1633a Coal Fly Ash Standard Reference Material (US Department of Commerce 1990) telah dijalankan untuk mengesahkan (validate) kaedah yang dikembangkan.

Key words : metal-dithiocarbamate complexes, solid phase extraction, HPLC.

### Introduction

In recent years, high-performance liquid chromatography (HPLC) has been employed for the separation and determination of trace metals in form of metal chelates. The most commonly used chelating agents are dithiocarbamates (DTC), since they react with many heavy metals and the metal chelates have large molar absorptivity in the ultraviolet (UV) region. Diethyldithiocarbamate (DDTC) is one of the derivatives commonly used in analysis. The problem with using DDTC is that it is unstable at low pH and consequently may result in incomplete formation and extraction of the metal-DDTC complexes. Based on this reason, ammonium pyrrolidinedithiocarbamate (APDC) has been used.

The determination of metals chelates requires inevitably a preconcentration step prior to HPLC analysis. Conventional solvent extraction is mostly used for preconcentration, but it has many practical and technical limitations. Solid phase extraction (SPE) has gained increasing attention as an alternative to solvent extraction. The principles and methods of

SPE have previously been published [1,2,3] and this technique is widely used [4,5]. The complexation of the metal ions with APDC followed by adsorption on C<sub>18</sub> chemically bonded silica gel for the AAS determinations has been reported [6,7]. However, the use of APDC for metals determination by HPLC after preconcentration on C<sub>18</sub> SPE cartridges has not been reported elsewhere.

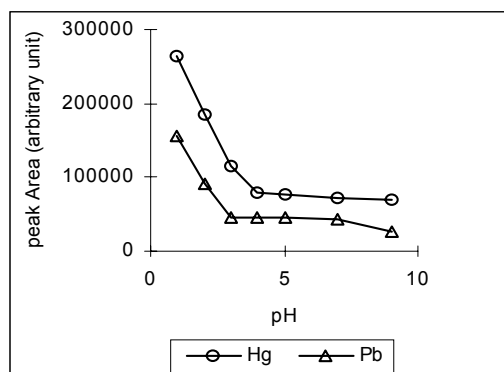
The aim of this work was to separate a number of metal-PDC complexes by HPLC. Prior to that, solid phase extraction method was used for preconcentration of some metal-PDC complexes. The effect of pH on the formation and extraction of the complexes was also studied.

### Experimental

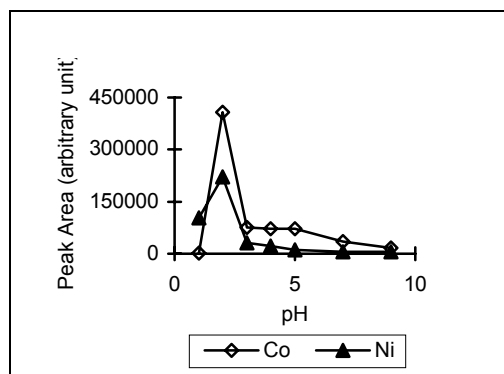
#### Reagents, Chemicals and Apparatus

All chemicals, reagents and solvents were of analytical-reagent grade. Most of the stock solutions were prepared from nitrate, sulfate or chloride salts of

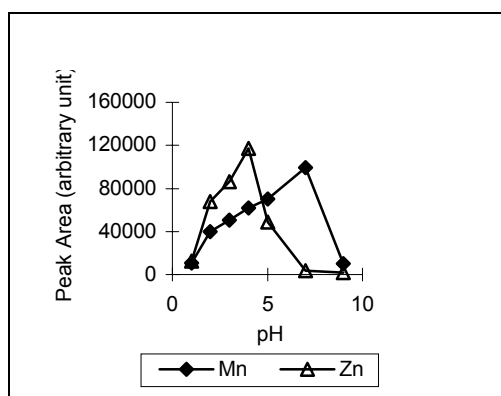
the metals. The salts are mercury (II) chloride,



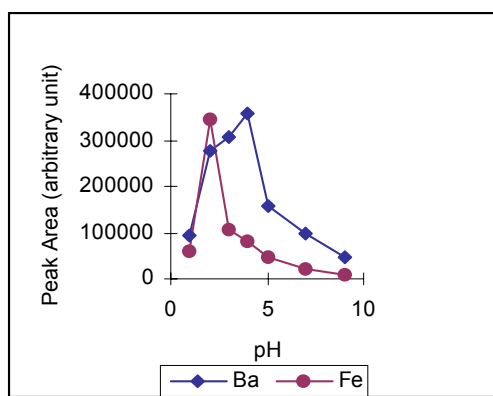
(a)



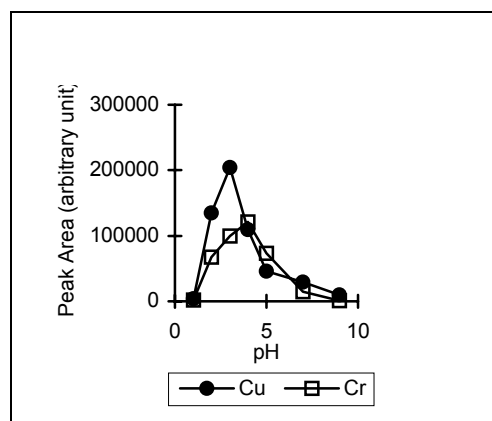
(b)



(c)



(d)



(e)

Figure 1 : pH Dependence on the Complex Formation and Solid Phase Extraction of (a)  $\text{Hg}(\text{PDC})_2$  and  $\text{Pb}(\text{PDC})_2$ , (b)  $\text{Co}(\text{PDC})_2$  and  $\text{Ni}(\text{PDC})_2$ , (c)  $\text{Zn}(\text{PDC})_2$  and  $\text{Mn}(\text{PDC})_2$ , (d)  $\text{Ba}(\text{PDC})_2$  and  $\text{Fe}(\text{PDC})_3$ , (e)  $\text{Cu}(\text{PDC})_2$  and  $\text{Cr}(\text{PDC})_3$

copper(II) nitrate, nickel(II) nitrate, barium(II) chloride (Merck), lead(II) nitrate, manganese(II) nitrate, cobalt(II) nitrate (Riedel-de Haën AG), chromium(III) sulfate, iron(III) sulfate (BDH Chemicals) and zinc(II) chloride (Fluka). The ligand, ammonium pyrrolidinedithiocarbamate was supplied by Sigma Chemical Company. Chloroform and acetonitrile solvents were obtained from Mallinckroft. Ammonium acetate buffer, acetonitrile and methanol for HPLC were supplied by Merck. Distilled deionized water was prepared from Barnstead, E-pure purification system.

HPLC instrument consisting of L-5200 Hitachi pump, L-4000 Hitachi UV spectrophotometric detector, model U6K Water Associates injector, ERC-3522 Erma degasser and D-2500 Hitachi integrator were used. Technopak phenyl RP (300 x 3.9mm I.D) and Spherisorb S5 ODS2 (150 x 4mm I.D) analytical columns, and silica S5 ODS2 guard column were used for the separations. C<sub>18</sub> Sep-Pak cartridges which were obtained from Water Associates was used for solid phase extraction. Shimadzu UV-VIS spectrophotometer model UV-160A was used for determination of UV-spectra of metal-PDC complexes. The pH meter used was a digital meter Delta model 320 fitted with Ingold InLab model TM 405 pH electrode.

#### *Preparation of Metal-PDC Complex*

The PDC complexes of Co(II), Cu(II), Ba(II), Ni(II), Fe(III), Mn(II), Hg(II), Pb(II), Cr(III) and Zn(II) were prepared generally by transferring 1 ml of the standard solution of metal ion (10 ppm) to a clean beaker. The pH level of the solution was adjusted to a suitable value with buffer solution. After that, 0.2 ml of 1% (w/v) APDC aqueous solution was added, and the pH was rechecked. The metal-PDC complexes was immediately formed.

#### *Solid Phase Extraction (SPE)*

The C<sub>18</sub> SPE cartridge was conditioned with methanol to activate the packing before the sample is extracted. Each metal-complex solution was passed through each separate SPE cartridge by using a 10 ml syringe. The C<sub>18</sub> stationary phase was capable of retaining the species of interest, and contaminants passed through the packing "unretained". Impurities and excess APDC were removed by rinsing with distilled-deionized water.

The cartridge was then dried by blowing with compressed air. Finally, the analytes of interest were eluted with 3ml chloroform or acetonitrile. The cleaned and concentrated complexes were then analysed by HPLC and the retention parameter for

each metal complex was recorded and compared with standard metal complexes. To avoid metal contaminants, each Sep-Pak cartridge was used only for one metal complex species.

## **Results and Discussion**

### *Effect of pH :*

During sample preparation of metal-DTC complexes, pH adjustment was one of the most important variable, which means that complete formation and extraction of metal-DTC complexes depend strongly on pH. The effect of pH on the ability of the DTC ligands to form complexes with metal ions and extraction ability by SPE from aqueous solutions varies from metal to metal for the same ligand, and also varies from ligand to ligand for the same metal [7]. So the effect of pH on the formation and extraction of metal-PDC complexes was investigated at pH range of 1 to 9. After solid phase extraction, HPLC analysis was carried out and the relationship between pH and peak area for each metal complex was deduced from the plots in Figures 1(a), (b), (c), (d) and (e).

Figure 1(a) indicates that, the highest peak area for Hg(PDC)<sub>2</sub> and Pb(PDC)<sub>2</sub> is at around pH 1, Figure 1(b) shows the effect of pH on the formation and extraction of Co(PDC)<sub>2</sub> and Ni(PDC)<sub>2</sub> and the greatest peak area for Co(PDC)<sub>2</sub> and Ni(PDC)<sub>2</sub> complexes are achieved at around pH 2, Figure 1(c) shows the optimal pH for Zn(PDC)<sub>2</sub> and Mn(PDC)<sub>2</sub>, are at around pH 4 and 7 respectively. Figure 1(d) indicates the highest peak area for Ba(PDC)<sub>2</sub> and Fe(PDC)<sub>2</sub> are at around pH 4 and 2 respectively and Figure 1(e) for Cu(PDC)<sub>2</sub> and Cr(PDC)<sub>3</sub> which are at around pH 3 and 4 respectively. The ability of APDC to form complexes with Co(II), Fe(III), Ni(II), Hg(II) and Pb(II) followed by solid phase extraction generally tends to decrease at pH greater than 2. For Ba(II), Cu(II), Zn(II) and Cr(III), the ability of formation and extraction of metal-PDC complexes generally decrease at pH greater than 4. Whereas for Mn(II) complex, the optimum pH is at pH 7. This is probably due to formation of hydroxo complexes of certain metals [8].

### *HPLC Conditions for Separation of Metal-PDC Complexes*

#### *Selection of Analytical Column:*

The separation of metal-PDC complexes on the C<sub>18</sub> column were examined with a variety of mobile phases. It was found that, the use of C<sub>18</sub> column resulted in poor separation efficiency which gave rise to broader chromatographic peaks of the complexes.

Improved separation of metal-PDC complexes was achieved when a Technopak phenyl (300 mm x 3.9 mm I.D) column was used. The advantages of using this column instead of C<sub>18</sub> column to separate the metal-PDC complexes are that, phenyl column is slightly less retentive reverse-phase packing than C<sub>18</sub>, more suitable for moderate to highly polar compounds, and more selective for aromatics samples.

*Selection of UV Detector Wavelength:*

The high molar absorptivity of the metal-PDC complexes lends itself to spectrophotometric detection. The UV-VIS spectra of metal-PDC complexes were determined. The wavelength and molar absorptivity are summarized in Table 1. The maximum UV absorption spectra differ from metal to metal and within the range of 203 nm to 284 nm. In the absence of common wavelength of maximum absorbance for all the metal-PDC under study, it was decided to use 254 nm as the detection wavelength, to make the method easily applicable to chromatographic system equipped with fixed UV wavelength detector.

Table 1 : UV Spectroscopic Data of Metal-PDC Complexes

Metal-PDC	Wavelength (nm)	Molar Absorptivity (l mol <sup>-1</sup> cm <sup>-1</sup> )
Pb <sup>2+</sup>	236.0	178.25
	284.0	428.25
Ba <sup>2+</sup>	203.2	612.00
	219.8	614.00
Mn <sup>2+</sup>	222.4	615.25
	228.4	615.25
Cr <sup>3+</sup>	203.8	224.50
	256.6	312.00
Ni <sup>2+</sup>	203.2	613.50
	219.0	615.75
Cu <sup>2+</sup>	211.0	613.50
	203.2	612.00
Hg <sup>2+</sup>	203.2	613.50
	216.8	615.50
Zn <sup>2+</sup>	236.2	157.50
	281.0	428.50
Fe <sup>3+</sup>	213.6	264.50
	258.2	434.75
Co <sup>2+</sup>	203.2	616.00
	216.6	613.50

*Selection of Mobile Phase:*

The separation of metal-PDC complexes was assessed in a variety of mobile phases to determine the best chromatographic selectivities according to Snyder and Kirkland [9]. A single solvent system consisting of methanol, acetonitrile, chloroform; and binary solvent

mixtures (methanol-water, acetonitrile-water) were investigated. Preliminary experiments indicated that a mixture of acetonitrile-water (66:34) containing 1.5x10<sup>-5</sup> M APDC with 2x10<sup>-2</sup> M ammonium acetate buffer, at pH 5.6 gave the best chromatographic selectivity and good peak shape. The advantage of using binary mixtures of solvents is that, increased eluent strength can be obtained without increasing the viscosity. The high viscosity mobile phase would necessitate the use of excessively high pressures.

The effect of APDC ligand in the mobile phase was also investigated. The chromatograms with and without APDC ligand in the mobile phase under identical conditions were compared. The addition of APDC in mobile phase in concentration of 1.5 x 10<sup>-5</sup> M enhanced the peak area reproducibility and the peak shapes of metal complexes which were relatively poor initially. It is concluded that the addition of APDC to the mobile phase prevents on-column decomposition of the complexes. However, a high concentration of ligand added to the mobile phase can cause difficulty in sample components detection at UV detector wavelength of 254 nm, due to high baseline absorption.

*Opimisation of Flow Rate:*

The effect of eluent flow rate on the retention time of metal-PDC complexes were carried out as shown in Figure 2. The plot indicates that the most suitable eluent flow rate for metal-PDC separation was 0.7 ml/min, where seven metal-PDC complexes was successfully separated.

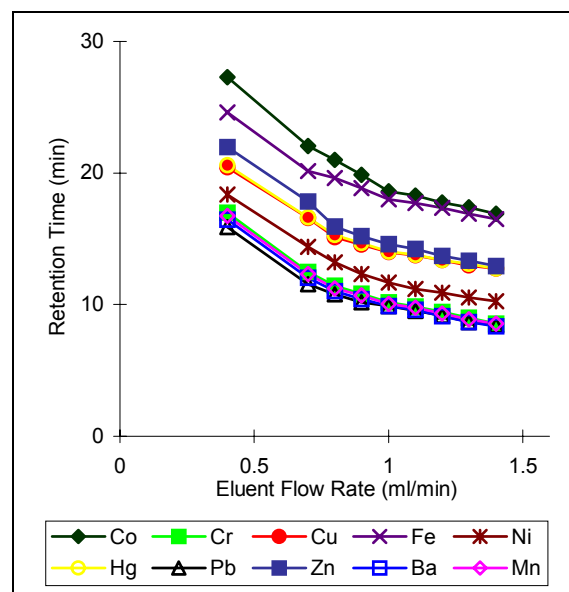


Figure 2 : Effect of Eluent Flow Rate on the Retention Time of Metal-PDC

Table 2 : High Performance Liquid Chromatographic Parameter of Metal-PDC Complexes

Metal-PDC	$t_R$ (min)	$k'$	$N_R$	H (mm)	Elution*
Pb <sup>2+</sup>	11.56	1.26	2138	0.14	S
Ba <sup>2+</sup>	12.04	1.35	6443	0.05	S
Mn <sup>2+</sup>	12.31	1.41	3788	0.08	B
Cr <sup>3+</sup>	12.48	1.44	5086	0.06	B
Ni <sup>2+</sup>	14.30	1.79	6677	0.04	S
Cu <sup>2+</sup>	16.60	2.24	4409	0.07	S
Hg <sup>2+</sup>	16.64	2.25	3661	0.08	S
Zn <sup>2+</sup>	17.83	2.48	3532	0.08	B
Fe <sup>3+</sup>	20.16	2.94	1626	0.18	B
Co <sup>2+</sup>	22.07	3.31	6441	0.05	S

\* S= sharp peak, B= broad peak

Table 3 : Resolution Data for Binary Mixtures of Metal-PDC Complexes

Metal Chelate	Resolution	Metal Chelate	Resolution
Co/MnPDC	9.76	Zn/Mn-PDC	5.52
Co/PbPDC	9.14	Zn/Pb-PDC	5.70
Co/NiPDC	7.77	Zn/Ni-PDC	3.72
Co/HgPDC	4.53	Zn/Hg-PDC	1.03
Co/FePDC	1.12	Zn/Fe-PDC	1.46
		Zn/Ba-PDC	6.09
Fe/MnPDC	5.23	Zn/Co-PDC	3.69
Fe/PbPDC	5.21	Zn/Cr-PDC	5.63
Fe/NiPDC	3.91		
Fe/HgPDC	2.10	Ba/Pb-PDC	0.56
		Ba/Ni-PDC	3.23
Hg/MnPDC	4.33	Ba/Fe-PDC	6.01
Hg/PbPDC	4.42	Ba/Co-PDC	11.14
Hg/NiPDC	2.34	Ba/Cr-PDC	0.63
Hg/Ba-PDC	5.11	Ba/Cu-PDC	5.36
Mn/PbPDC	0.79	Cu/Pb-PDC	5.04
Mn/NiPDC	2.49	Cu/Ni-PDC	2.71
Mn/Ba-PDC	0.36	Cu/Hg-PDC	0.04
Mn/Cu-PDC	4.77	Cu/Fe-PDC	2.37
		Cu/Co-PDC	5.21
Cr/Mn-PDC	0.23	Cu/Zn-PDC	1.12
Cr/Pb-PDC	1.10	Cu/Cr-PDC	4.85
Cr/Ni-PDC	2.60		
Cr/Hg-PDC	4.62	Ni/PbPDC	2.88
Cr/Fe-PDC	5.69		
Cr/Co-PDC	10.66		

*Chromatographic Parameter*

The retention time ( $t_R$ ), number of theoretical plate ( $N_R$ ), the height equivalent to a theoretical plate (H) and capacity factor, ( $k'$ ), for metal-PDC complexes are summarized in Table 2.

*(i) Number of Theoretical Plate, ( $N_R$ ) :*

From the chromatographic data for metal-PDC complexes as listed in Table 2, it is noted that Ni(PDC)<sub>2</sub> gives the highest value of the number of the

theoretical plate ( $N_R=6677$ ) and lowest H value (H=0.04mm). Consequently, it is a highly efficient separation. The less efficient separation was obtained for Fe(PDC)<sub>3</sub> which gives the lowest number of the theoretical plate ( $N_R=1626$ ) and highest value of H (H=0.18mm).

*(ii) Capacity Factor, ( $k'$ ) :*

From the  $k'$  values of metal-PDC complexes in Table 2, it was noted that:

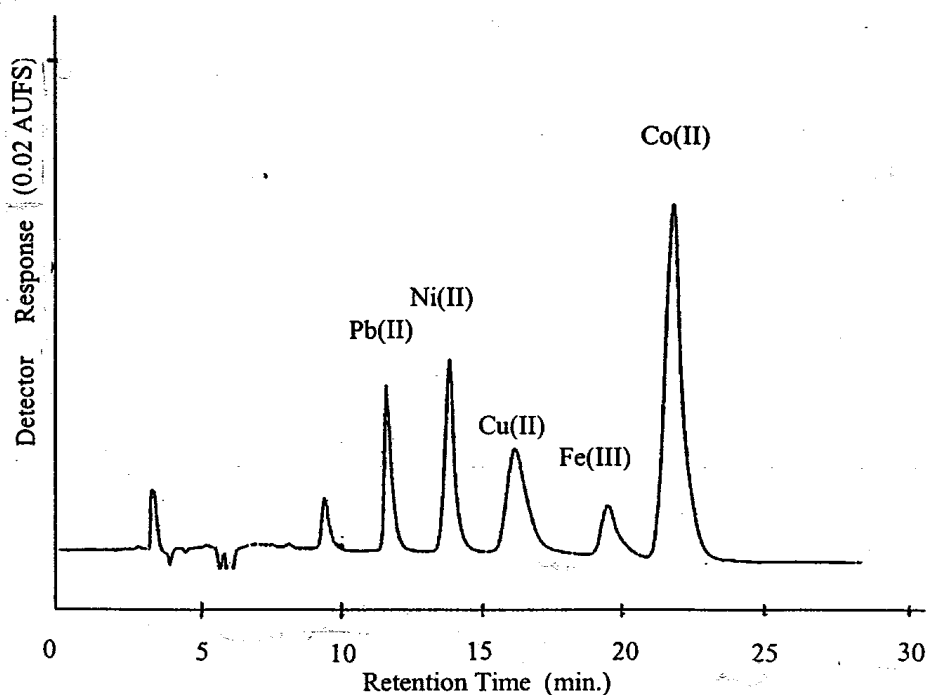


Figure 3 : Chromatogram of Mixture of  $Pb(PDC)_2$ ,  $Ni(PDC)_2$ ,  $Cu(PDC)_2$ ,  $Fe(PDC)_3$  and  $Co(PDC)_2$  (HPLC conditions: Column: Technopak phenyl 300x3.9 mm i.d.; eluent: 66% acetonitrile + 34% water containing  $1.5 \times 10^{-5}$  M APDC,  $2 \times 10^{-2}$  M ammonium acetate buffer (pH 5.6); detector: UV at  $\lambda = 254$  nm; flow rate: 0.7 ml/min.)

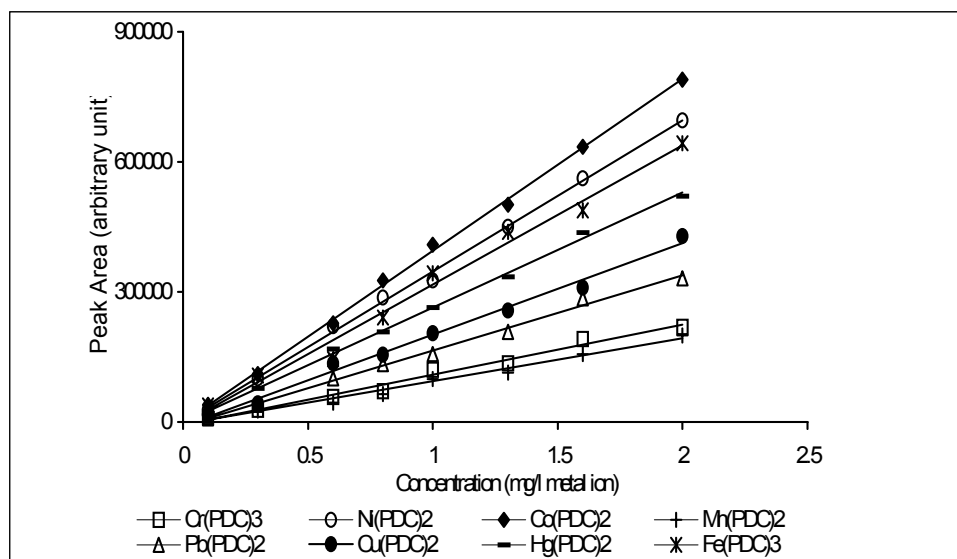


Figure 4 : Calibration Curves of Metal-PDC Complexes

- 1) All  $k'$  values for metal-PDC complexes are greater than 1 and less than 10 which means that these complexes show good retention in the column, between 16 to 20 minutes.
- 2) Complexes of  $Pb(II)$ ,  $Ba(II)$ ,  $Mn(II)$ ,  $Cr(III)$  and  $Ni(II)$  have the  $k'$  values greater than 1 but smaller than 2, which means that these complexes can be separated in shorter time of between 10 to 14 minutes.

- 3) Complexes of Cu(II), Hg(II), Zn(II), Fe(III) and Co(II) have the  $k'$  values higher than 2.2, hence will be retained longer in the column between 16 to 20 minutes.

(iii) Resolution, ( $R_s$ )

The resolution of mixture of each two metal-PDC complexes was calculated and summarized in Table 3.

It was noted from Table 3 that:

- 1) Good resolution with complete separation are obtained for mixture of Co/Fe-PDC, Zn/Fe-PDC, Cr/Pb-PDC, Cu/Zn-PDC and Zn/Hg-PDC.
- 2) Each mixture of Mn/Pb-PDC, Cr/Mn-PDC, Ba/Cr-PDC, Mn/Ba-PDC, Cu/Hg-PDC and Ba/Pb-PDC give incomplete separation (with some overlapping of peaks  $\gg 2\%$ ).
- 3) Complete separation with moderate to long analysis time are obtained for each mixture of Fe/Hg-PDC, Hg/Ni-PDC, Mn/Ni-PDC, Ni/Pb-PDC, Hg/Mn-PDC, Co/Hg-PDC, Cu/Ni-PDC, Mn/Cu-PDC, Ba/Ni-PDC, Zn/Co-PDC, Fe/Cu-PDC, Hg/Pb-PDC, Zn/Ni-PDC, Cu/Cr-PDC, Cr/Ni-PDC, Cr/Hg-PDC and Fe/Ni-PDC.
- 4) The mixtures which give complete separation but long analysis time are: Co/Mn-PDC, Co/Pb-PDC, Fe/Mn-PDC, Fe/Pb-PDC, Co/Ni-PDC, Zn/Cr-PDC, Pb/Zn-PDC, Cr/Fe-PDC, Zn/Mn-PDC, Ba/Fe-PDC, Cr/Co-PDC, Ba/Co-PDC, Cu/Co-PDC, Hg/Ba-PDC, Cu/Pb-PDC, Zn/Ba-PDC and Ba/Cu-PDC.

*Separation of Mixtures of Metal-PDC Complexes*

*Qualitative Separation*

A chromatogram of a mixture of five metal-PDC complexes which show baseline separation is depicted in Figure 3. Pb(PDC)<sub>2</sub> was first eluted, followed by Ni(PDC)<sub>2</sub>, Cu(PDC)<sub>2</sub>, Fe(PDC)<sub>3</sub> and Co(PDC)<sub>2</sub>. The mixture was separated in less than 25 minutes.

*Quantitative Separation*

(i) Calibration Curves :

Calibration curves of metal-PDC complexes were prepared from the chromatographic data obtained after separation of known amounts of each metal ion versus the corresponding peak area as shown in Figure 4.

Quantitative studies were carried out for each of the metal ions to determine the linearity of the calibration curves and range of the response. The

working range in this study was 0.1 to 2 mg/l. Table 4 gives the results for the regression analysis for metal-PDC complexes. The correlation coefficients,  $R^2$ , fell in the range of 0.999 to 0.979 for metal-PDC complexes.

Table 4: Results of Regression Analysis for Metal-PDC

Metal Ion	Regression Equation	$R^2$
Co(II)	$Y = 396690X - 2351.5$	0.9986
Ni(II)	$Y = 349483X - 2621.9$	0.9978
Hg(II)	$Y = 264836X - 1328.7$	0.9977
Pb(II)	$Y = 173468X - 9171.7$	0.9941
Fe(III)	$Y = 320855X - 3576.5$	0.9868
Mn(II)	$Y = 98519X - 4600.6$	0.9794
Cr(III)	$Y = 114776X - 6174.1$	0.9826
Cu(II)	$Y = 211193X - 9846.7$	0.9915

The calibration curves of Co(PDC)<sub>2</sub>, Ni(PDC)<sub>2</sub>, Fe(PDC)<sub>3</sub>, Pb(PDC)<sub>2</sub>, Hg(PDC)<sub>2</sub>, Cu(PDC)<sub>2</sub>, Cr(PDC)<sub>3</sub> and Mn(PDC)<sub>2</sub>, in Figure 4 show excellent linearity, except for Mn(PDC)<sub>2</sub>, Cr(PDC)<sub>3</sub> and Fe(PDC)<sub>3</sub>. The good linearity of Co(PDC)<sub>2</sub>, Ni(PDC)<sub>2</sub>, Pb(PDC)<sub>2</sub>, Hg(PDC)<sub>2</sub> and Cu(PDC)<sub>2</sub> indicate that, these metals reacted with APDC completely and eluted without decomposition. Calibration curves for Fe(PDC)<sub>3</sub> and Cr(PDC)<sub>3</sub> show poorer sensitivity. The low sensitivity for Fe(PDC)<sub>3</sub> may be due to interaction of the complex with silanol groups on the column and/or some decomposition of the complex during the analysis. For Cr(PDC)<sub>3</sub>, the poor sensitivity may due to decomposition of the complex in the HPLC system. For Mn(PDC)<sub>2</sub>, the low gradient might be due to the use of higher wavelength (254 nm) of detection, where Mn(PDC)<sub>2</sub> complex have low absorbance. Therefore, in order to achieve higher absorbance for Mn(PDC)<sub>2</sub>, wavelength of 220 nm must be used instead. Statistical analysis indicates that most of the calibration curves intercept very near to the origin. This is in accordance with the results of Moriyasu and Hashimoto [10] who found that at low concentration ( $< 1 \times 10^{-5}$  M), most metal-DTC complexes dissociate, and therefore their calibration graphs intercept are non-zero and the graphs are not linear at low concentrations.

(ii) Detection Limits :

The detection limit for each metal complex was determined separately at 254 nm using the component HPLC system. Peak areas were measured rather than peak heights because they are more reproducible. Table 5 shows the detection limit in mg/l for each metal-PDC complexes. The higher detection limit for

Mn(PDC)<sub>2</sub> may be due to the use of 254 nm wavelength at which the complex gives low absorbance. The higher detection limits for Fe(PDC)<sub>3</sub> and Cr(PDC)<sub>3</sub> were probably caused by interaction of the complexes with silanol groups in the column and/or some decomposition of the complexes in the HPLC system.

Table 5 : Detection Limits of Metal-PDC Complexes

Metal Ion	Detection Limits (mg/l)
Pb <sup>2+</sup>	0.03
Mn <sup>2+</sup>	0.08
Cr <sup>3+</sup>	0.05
Ni <sup>2+</sup>	0.01
Cu <sup>2+</sup>	0.03
Hg <sup>2+</sup>	0.02
Fe <sup>3+</sup>	0.04
Co <sup>2+</sup>	0.01

(iii) Analysis of Standard Reference Material:

In order to test the accuracy of the HPLC method developed for metal ions determination, it was validated by analysing the NIST 1633a Coal Fly Ash Standard Reference Material. The results of the analyses are listed in Table 6 which shows that the recovery range of 63 to 100 % for the metal-PDC complexes.

Since Fe<sup>2+</sup> is less stable and easily oxidised to Fe<sup>3+</sup>, the Fe<sup>2+</sup> in the reference standard was oxidised to Fe<sup>3+</sup> and determined as total iron(III). The lower recovery of some of the metal-PDC complexes might be contributed by stability of the complexes and efficiency during HPLC separations.

Table 6 : HPLC Analysis of PDC Complexes of Metals in NIST 1633a Coal Fly Ash Standard Reference Material

Element	Certified Value	Determined Value	% Recovery
Cobalt	46 ppm	45.4 ppm	98.7
Chromium	196 ppm	125 ppm	63.1
Copper	118 ppm	98 ppm	83.1
Iron	9.4 %	6.8 %	72.3
Lead	72.4 ppm	68.7 ppm	95.0
Nickel	127 ppm	133 ppm	104.7

**Conclusion**

The effect of pH on formation and extraction of metal-PDC complexes were carried out in range of pH 1 to 5, 7 and 9. The results indicate that APDC is a good chelating agent at low pH value. An efficient sample preparation method using solid phase extraction was also developed for enhancing the concentration of some heavy metals for HPLC analysis. A mixture of five important metal ions, i.e. Pb(II), Ni(II), Hg(II), Fe(II) and Co(II) can be separated as their PDC complexes under isocratic elution condition within 22 minute on phenyl column. Validation of the method developed was carried out by analysing NIST 1633a Coal Ash Standard Reference Materials (US Department of Commerce 1990).

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