

## Optimization of Analytical Parameters for Determination of Iron, Nickel and Cobalt in Plants with RP-HPLC

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This study optimized the analytical parameters such as combination of mobile phase, pH of mobile phase, attempt effect of strange ions and current rate for simultaneous determination of iron, nickel and cobalt. 4-(2-Pyridylazo)resorcinol (PAR) ligand is used for metal chelates. The metal-PAR chelates were separated on a Luna C<sub>18</sub> siloxane bonded phase and eluted within 7.4 min of Co-PAR, 9.6 min of Fe-PAR and 12.4 min of Ni-PAR with methanol-water-acetonitrile-(1 × 10<sup>-2</sup> mol L<sup>-1</sup>) sodium acetate (55:40:2.5:2.5 v/v) (pH 5.3). Detection limits of process for Fe-PAR, Ni-PAR and Co-PAR under optimized conditions (500 nm) were found as 4.45, 3.26 and 1.86 µg L<sup>-1</sup>, respectively. The analysis of Fe, Ni and Co ions in plant samples under optimized conditions were carried out by HPLC.

**Key Words:** Trace element, 4-(2-Pyridylazo)resorcinol, UV-Visible, Cobalt, Iron, Nickel, HPLC.

### INTRODUCTION

Precolumn derivation HPLC is not only a sensitive but also a selective and rapid method for the separation and determination of metal ions<sup>1</sup>. Various azo derivatives are interesting complexing reagents, because they form highly stable and intensely coloured complexes immediately (1-40 s), by bonding through the hydroxyl and nitrogen atoms, which are ideally suited for spectrophotometric detection<sup>2</sup>.

Iron is found component of peroxides, catalyzes, myoglobin and hemoglobin. In its shortage it causes not only anemia but also behaviour and teaching problems at children, animals in catecholamine metabolism. It is necessary for adults to receive between 1-1.4 mg in daily diet<sup>3</sup>. Nickel is a toxic trace element<sup>4</sup> of widespread distribution in the environment. It is emitted to the atmosphere from volcanoes and windblown dusts and there are numerous man-made sources. The nickel content of soil may range widely (2 to 50 mg/kg or more) depending on mineral composition<sup>3</sup>. This metal is readily absorbed by plant roots and is highly mobile in plants.

Cobalt in the form of vitamin B<sub>12</sub> (hydroxo-cyanocobalamin) is essential for humans. Vitamin B<sub>12</sub> supports important synthetic reactions in metabolic processes and is essential for the production of red blood cells and several enzymes<sup>5</sup>.

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Because of quick development at HPLC, besides for various analysis of trace elements, various vitamins<sup>6</sup> and oil acid, HPLC have been begun frequently.

The determination of trace element in environmentally has been carried out by such instrumental techniques as neutron activation analysis (NAA)<sup>7</sup>, inductively coupled plasma atomic emission spectrometry (ICP-AES)<sup>8</sup>, inductively coupled plasma mass spectrometry (ICP-MS)<sup>9</sup>, atomic absorption spectrometry (AAS)<sup>10</sup> and X-ray fluorescence spectroscopy<sup>11</sup>. The methods ICP-AES, ICP-MS and especially NAA, show good sensitivity, but are limited because of expensive instrumentation and high cost for routine analysis. In ICP-MS, the direct measurement of metal ions in the solutions obtained from the acid digestion, which often contain large amount of salts, is difficult. The introduction of saline solutions to the plasma frequently causes blockage of the nebulizer and chemical interferences, with resulting decreases in sensitivity and precision. AAS and X-ray fluorescence spectroscopy often suffer the problem of low sensitivity<sup>12</sup>. On the other hand, high performance liquid chromatography (HPLC) has been used frequently determination of many ions which are level of trace<sup>13</sup>. The possibility of determination of many metal ions in the same time, being removed attempts with optimization different conditions, being low of limit of determination, being low cost of maintenance and study cause HPLC more advantages than other methods.

In this study, it is optimized analytical parameters for determination of iron, nickel, cobalt by RP-HPLC.

## EXPERIMENTAL

Atomic absorption standard solutions of Ni(II), Fe(III), Co(II), Mg(II), Ca(II), Cu(II) and Zn(II) (Merck,  $1000 \pm 2 \text{ mg L}^{-1}$ ). The PAR monosodium salt hydrate was obtained from Aldrich and solutions of the dye were freshly prepared in water-methanol before use. All water used was distilled and then deionized using a Millipore (Bedford, MA) Milli-Q water purification system to yield a final conductance of  $18 \text{ M}\Omega$ . Other chemical reagents used in the analysis were analytical grade and obtained from Merck (Darmstadt, Germany). HPLC was performed with the Shimadzu LC-9A and Shimadzu SPD-M10AVP photodiode array detector, Luna reversed-phase column ( $4.6 \text{ mm} \times 250 \text{ mm } 5\mu\text{m}$ , phenomex, USA). Spectral measurements were used Scomam 750 UV spectrophotometer. HI 8418 Hanna pH meter was used for pH measurements. Microwave-assisted acid digestions have been made using a Premier microwave system. Teflon bomb and various glassware.

Plants samples were digested microwave system. 2.00 g portion of each sample dried at  $80^\circ\text{C}$  was accurately and 0.50 g directly weighted into teflon bomb. For the samples decomposition concentrated 4 mL  $\text{HNO}_3$  and 1 mL  $\text{HClO}_4$  acid were added. In a tightly closed system, the following 6-step microwave digestion program was applied<sup>14</sup>. Teflon bomb was waited for 1 h to cool and was carefully opened. Colourless solution was transferred into beaker and evaporating to dryness with hot plate. Afterwards final volume was diluted 20 mL with water.

The standard solutions were prepared as follows: 2 mL of  $1 \times 10^{-3}$  mol L<sup>-1</sup> acetate solution, 2 mL of PAR  $5 \times 10^{-3}$  mol L<sup>-1</sup> solution were added to a 20 mL volumetric flask and then a standard solution of Fe(III), Co(III) and Ni(II) was added, the pH was adjusted to 5.3 with dilute acetic acid solution. Afterwards, final volume was diluted 20 mL with water. Then inject an aliquot of the solution into the chromatograph with a 50  $\mu$ L loop injector. The concentrations of the metal ions were determined by measuring the peak area.

The plants samples were treated as follows: 2 mL of  $1 \times 10^{-3}$  mol L<sup>-1</sup> acetate solution, 2 mL of  $5 \times 10^{-3}$  mol L<sup>-1</sup> PAR solution were added 20 mL volumetric flask and then 10 mL the digested samples was added. The pH was adjusted to 5.3 with dilute acetic acid solution and 1 mol L<sup>-1</sup> sodium hydroxyl. Afterwards, final volume was diluted 20 mL with water. Then sample solution was filtered through a 0.45  $\mu$ m filter before a 50  $\mu$ L aliquot was injected into the HPLC. For quantitative analysis, the analyte concentrations were determined by comparison peak area of standard solution.

## RESULTS AND DISCUSSION

At room temperature Co(III), Fe(II) and Ni (II) can produce colour reaction with PAR in pH 5-6 sodium acetate-acetic acid buffer solution. The absorption spectra of PAR, Co(III)-PAR, Fe(III)-PAR and Ni (II)-PAR chelates are shown in Fig. 1. The maximum absorption wavelengths are 414, 520, 490 and 510 nm, respectively. Metal-PAR chelates can be separated by RP-HPLC with spectrophotometric detection at 500 nm.

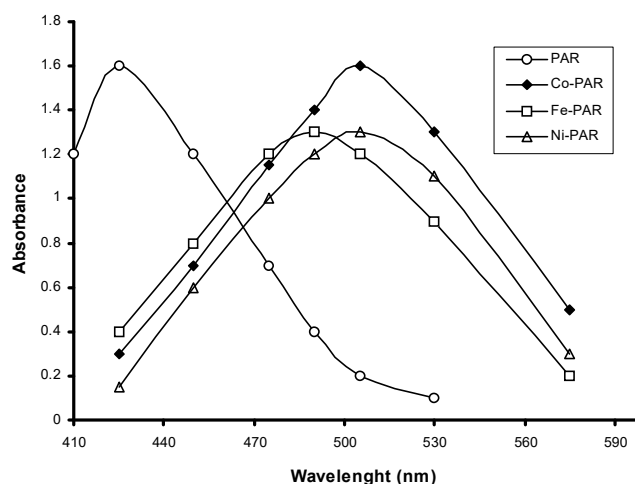


Fig. 1. Absorption spectra of PAR and Metal-PAR chelates

The PAR reacts with metal ions in aqueous methanolic solution to develop colour immediately in pH range 5-9 (Fig. 2).

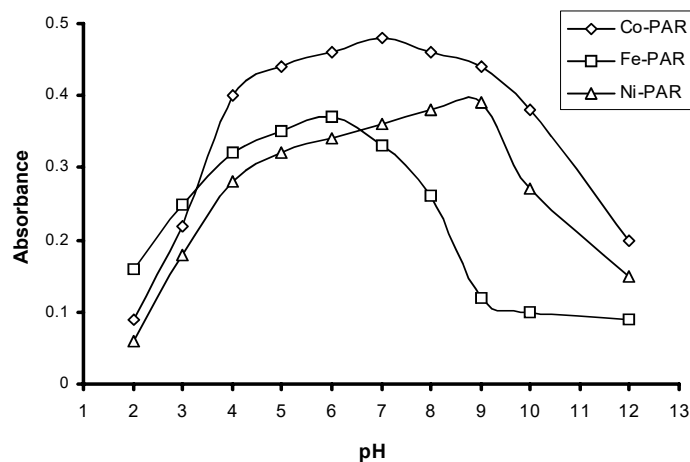


Fig. 2. Effect of variation in pH on the formation of metal-PAR (final concentration  $2 \times 10^{-5}$  mol L<sup>-1</sup>)

The composition of Co(III)-PAR, Fe(III)-PAR and Ni(II)-PAR chelate were investigated by changing the metal: PAR mole ratio spectrophotometrically. The absorbance of Co(III)-PAR, Fe(III)-PAR and Ni(II)-PAR were 500 nm in aqueous methanolic solution. The results shown in Fig. 3, suggest that metal:PAR form a 1:2 complexes.

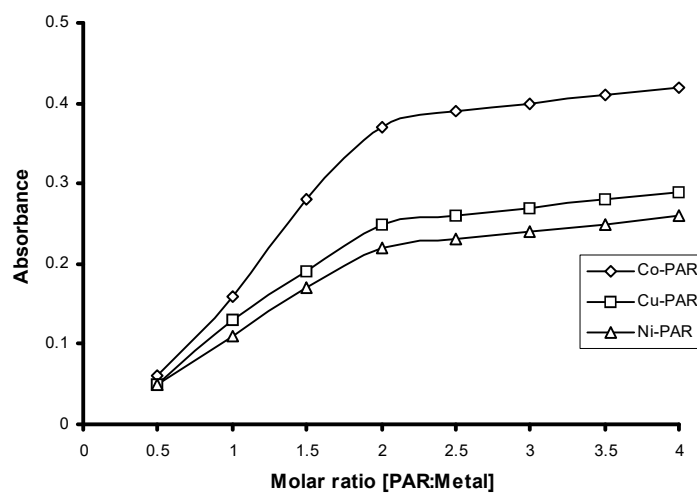


Fig. 3. Effect of variation in molar ratio of Ni(II), Co(III), Fe(III) ( $2 \times 10^{-5}$  mol L<sup>-1</sup>) and PAR ( $2 \times 10^{-5}$ – $10 \times 10^{-5}$  mol L<sup>-1</sup>)

Complex formation period was found 8, 10 and 30 seconds for Fe-PAR, Co-PAR and Ni(II)-PAR in ambience methanol-water (Fig. 4).

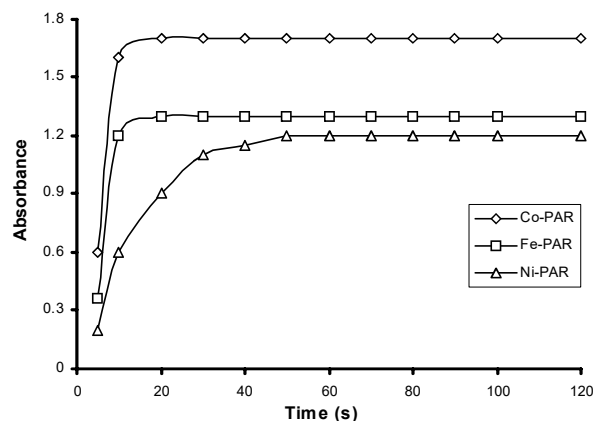


Fig. 4. Effect of variation in times for the formation of metal-PAR ( $8 \times 10^{-5} \text{ mol L}^{-1}$ )

**Effect of the composition and pH of the mobile phase:** The mobile phase was selected from among some organic solvent-water mixtures, such as acetonitrile-water, methanol-water and methanol-acetonitrile-water among which methanol-water-acetonitrile-acetate solution (55:40:2.5:2.5 v/v) is the most satisfactory for separation of the metal-PAR chalet. Fig. 5 shows the effect of the methanol concentration on the retention time of the chelate. If methanol concentration  $> 60 \%$  (v/v), Ni(II)-PAR, Fe(III)-PAR, Co(III)-PAR and PAR peaks are overlapped. If methanol concentration  $< 45 \%$  (v/v), the peak area of metal-PAR chaletes are decrease and retention times of metal-PAR peak are increase.

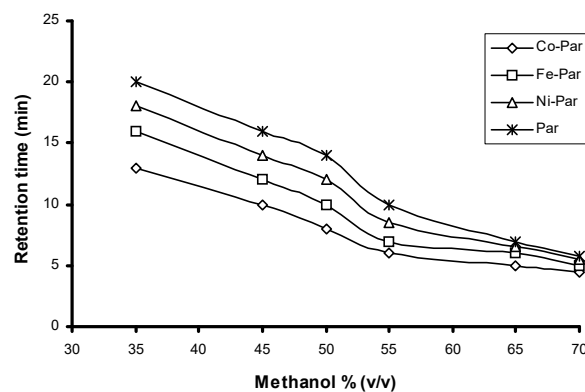


Fig. 5. Effect of the concentration of methanol on retention time

Using the methanol-water-acetonitrile system but in absence of any acetate solution as mobil phase, the peaks for metal-PAR and PAR are overlapped.

The effect of the mobile phase pH on the retention time of the metal-PAR chelates was investigated in methanol-water-acetonitrile-acetate solution (55:40:2.5:2.5 v/v) (Fig. 6).

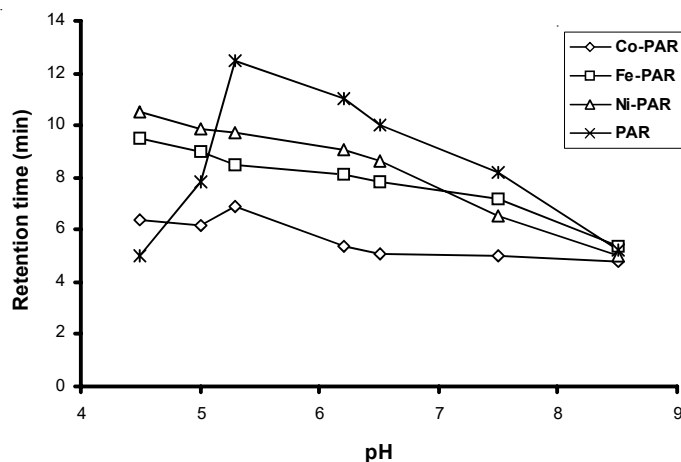


Fig. 6. Effect of pH on retention time

If  $\text{pH} < 5.3$ , the peak area of Co(III)-PAR, Fe(III)-PAR and Ni(II)-PAR chelates were decrease, in addition Ni(II)-PAR, Fe(III)-PAR and PAR peaks are overlapped. If  $\text{pH} > 6.8$ , Ni(II)-PAR and Fe(III) PAR peaks are overlapped. If  $\text{pH} > 8$ , all peaks are overlapped. Therefore, the most suitable pH values for complex separation were obtained between pH 5.3–6.3.

**Effect of foreign ions:** Under optimum condition, the effects of foreign ions showed that more than  $1000 \text{ mg L}^{-1}$  excess of  $\text{Ca}^{2+}$ ,  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{Ba}^{2+}$  and  $\text{Cu}^{2+}$  did not interfere. However  $200 \text{ mg L}^{-1}$  of  $\text{Fe}^{3+}$  was interfered for  $1 \text{ mg L}^{-1}$   $\text{Co}^{3+}$  and  $\text{Ni}^{2+}$  ions. If sodium citrate added into digest samples,  $1000 \text{ mg L}^{-1}$  excess of  $\text{Fe}^{3+}$  not interfere for  $\text{Co}^{3+}$  and  $\text{Ni}^{2+}$ . Therefore, the method has high selectivity. Co(II)-PAR complex was gradually oxidized by oxygen in air to form the stable Co(III)-PAR complex and strong oxidants and higher temperature could accelerate the oxidation.

**Calibration graph and detection limits:** Use of different concentrations of Ni(II), Fe(III) and Co(III) in accordance with the general procedure; construct calibration graphs with the peak areas against the concentrations of metal ions. The calibration curves of metals are given Figs. 7-9.

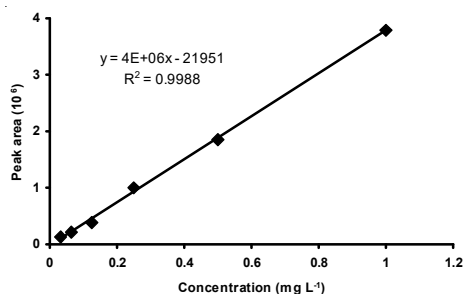


Fig. 7. Calibration curve of cobalt

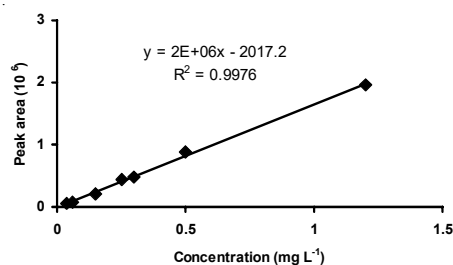


Fig. 8. Calibration curve of nickel

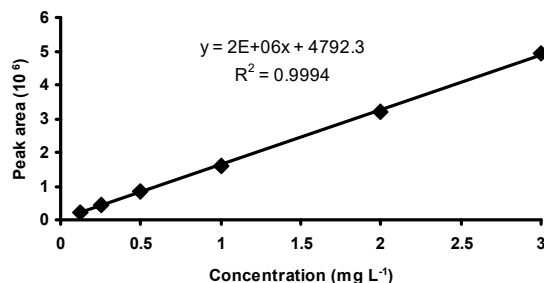


Fig. 9. Calibration curve of iron

TABLE-1  
OPERATING PARAMETERS FOR HPLC WHICH ARE USED IN  
ANALYSIS OF TRACE ELEMENTS

|                                   |                                                                                                             |
|-----------------------------------|-------------------------------------------------------------------------------------------------------------|
| Mobile phase (v/v)                | Methanol:water:acetonitrile / ( $1 \times 10^{-2}$ mol L <sup>-1</sup> )<br>acetate solution: 55:40:2.5:2.5 |
| Column                            | LUNA C <sub>18</sub> (250 mm × 4.6 mm 5 μ) ODS                                                              |
| Column temperature (°C)           | 30                                                                                                          |
| Detector                          | Photo diode array (PDA)                                                                                     |
| pH of mobile phase                | 5.3                                                                                                         |
| Wavelength (nm)                   | 500                                                                                                         |
| Flow rate (mL min <sup>-1</sup> ) | 0.6                                                                                                         |
| Injection volume (μL)             | 20-50                                                                                                       |

**Application for plants analysis:** Eight kinds of plants samples were analyzed according to the general procedure. Fig. 10 shows the blank signal. Fig. 11 show the chromatograms of standard test solutions 0.25 mg L<sup>-1</sup> Co(III), Fe(III) and Ni(II).

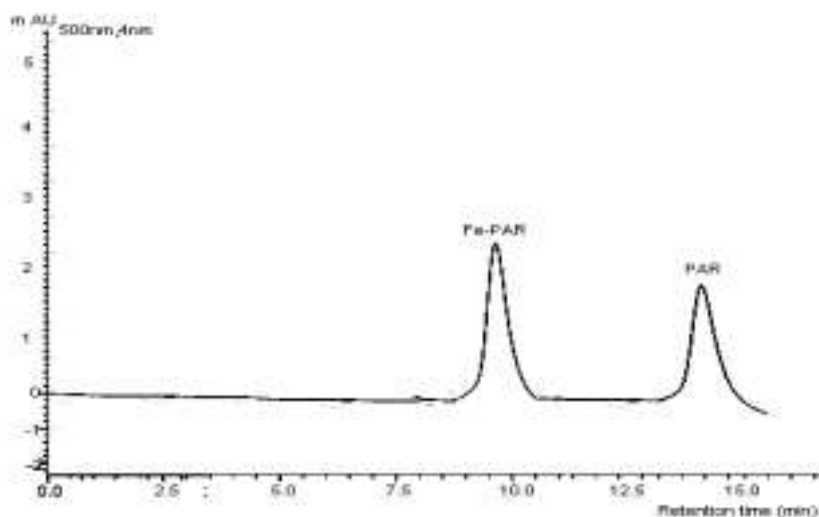


Fig. 10. Chromatography of blank

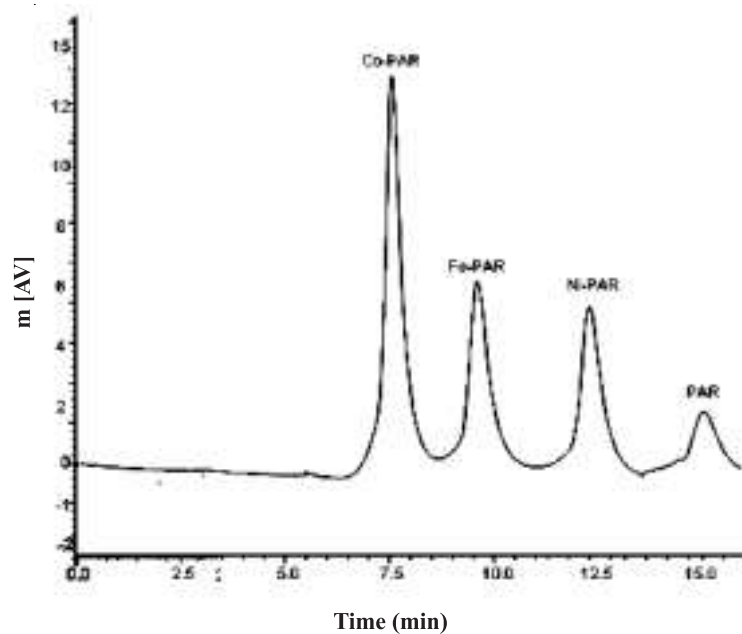


Fig. 11. Chromatography of standard chelates PAR of Co(III), Fe(III) and Ni(II) ions (50  $\mu$ L injection, 0.25 mg L<sup>-1</sup>)

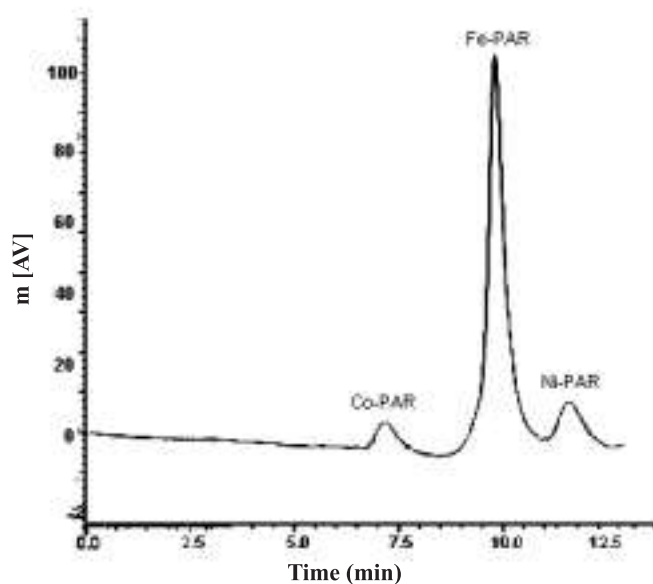


Fig. 12. Chromatography of wormwood sample

The analytical results for cobalt, nickel and iron in 8 kinds plants are summarized in Table-2.

TABLE-2  
TRACE ELEMENT LEVELS OF VARIOUS PLANTS (mg kg<sup>-1</sup> DRY MATTER)

| Plant                                         | Cobalt      | Nickel      | Iron         |
|-----------------------------------------------|-------------|-------------|--------------|
| Coffee                                        | 0.072±0.006 | 1.476±0.156 | 51.63±4.25   |
| Ginger ( <i>Rhizome zingiberis</i> )          | ND          | 0.356±0.024 | 136.97±12.77 |
| Common Mallow ( <i>Malva rotundifolia</i> L.) | 0.087±0.006 | 0.782±0.055 | 273.03±35.62 |
| Rice                                          | ND          | 0.576±0.048 | 41.58±3.45   |
| Wormwood ( <i>Artemisia absinthium</i> L.)    | 0.168±0.015 | 3.728±0.026 | 264.53±21.98 |
| Tobacco of cigarette                          | 0.542±0.065 | 1.511±0.012 | 497.12±55.61 |
| Sumac ( <i>Cotinus coggygria</i> )            | ND          | 0.918±0.058 | 158.63±8.56  |
| Mint ( <i>Mentha piperita</i> )               | ND          | 2.111±0.189 | 257.13±36.47 |

ND = Not detective, Results are mean ± SD of three replicate analyses.

The contents of iron, nickel and cobalt in samples of plant and food were found to be 41.58- 497.12, 0.356- 3.728 and 0.072- 0.542 mg kg<sup>-1</sup>, respectively.

The highest and lowest levels of cobalt were found in tobacco and coffee. The cobalt levels can not determined in mint, sumac, rice and ginger.

Minimum nickel concentration was 0.356 mg kg<sup>-1</sup> in ginger. Maximum nickel concentration was 3.728 mg kg<sup>-1</sup> in wormwood.

The highest iron content was 497.12 mg kg<sup>-1</sup> in tobacco; the lowest was 41.58 mg kg<sup>-1</sup> in rice.

Zhank *et al.*<sup>12</sup> have found between 0.107-0.139 and 0.50-4.27 mg kg<sup>-1</sup> for levels of cobalt and nickel in different marks of tobacco samples by HPLC. In present paper, levels of cobalt and nickel in tobacco were obtained as 0.542 and 1.511 mg kg<sup>-1</sup>.

In a study, levels of nickel and iron were determined as 0.9 and 51.0 mg kg<sup>-1</sup> in coffee<sup>15</sup> by flame atomic absorption spectrometry. In this study, levels nickel and iron were found as 1.476 and 51.63 mg kg<sup>-1</sup> in coffee.

In study of Ekholm *et al.*<sup>16</sup>, the levels of cobalt and nickel were determined as less than 0.01 and 0.21 mg kg<sup>-1</sup> in rice samples by ICP-MS. Although, we have found as 0.576 mg kg<sup>-1</sup> cobalt level in rice, nickel can not detective.

The level of iron was determined as 264 mg kg<sup>-1</sup> in wormwood that is used as medical plant, Razic *et al.*<sup>17</sup> have founded as 182 mg kg<sup>-1</sup> level of iron in this plant.

## Conclusion

The developed method proved to be an alternative procedure for measuring Co, Ni and Fe metals in plants samples. Its main advantage lays on the simultaneous determination of three elements of great importance in plants analysis being carried out with the widely available and inexpensive HPLC instruments.

## ACKNOWLEDGEMENT

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