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Determination of Cobalt in Some Plants by Reversed-Phase High Performance Liquid Chromatography

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The separation and determination of cobalt chelate with 4-(2-pyridylazo)resorcinol (PAR) by reversed-phase HPLC was investigated. The Co(III)-PAR chelate was separated on a C_{18} siloxane bonded phase and eluted within 5.4 min with methanol-water-acetonitrile (1×10^{-2} mol L^{-1}) and sodium acetate (55:40:2.5:2.5 v/v) containing 5×10^{-3} mol L^{-1} ethylenedi-aminetatraacetic acid (EDTA) (pH 7.5). The detection limits of Co(III) at 520 nm are 1.2 μg L^{-1}. The method was applied to the determination of cobalt in some samples of plants. The contents of cobalt in the plants samples were found in the ranges, 0.941-0.141 mg/kg (dry matter). Rosa canina is rather rich in cobalt and is recommended as a source.

Key Words: HPLC, 4-(2-Pyridylazo)resorcinol, UV-Visible, Cobalt, PAR, Resorcinol, Rosa canina.

INTRODUCTION

Cobalt in the form of vitamin B12 (hydroxo-cyanocobalamin) is essential for humans. Vitamin B12 supports important synthetic reactions in metabolic processes and is essential for the production of red blood cells and several enzymes[1].

Total cobalt is present in most soils in the range of 0.1-50 μg/g and available cobalt (*i.e.*, the proportion of cobalt which is taken up by vegetation) between[2] 0.1-2 μg g^{-1}. The average intake of cobalt in foods by adults in the United States has been estimated at 300 μg/d. Daily intake from water is estimated at 6 μg and intake from air is estimated at less than 0.1 μg. The major source of cobalt is food, in the form of green leafy vegetables, which may contain as much as 0.5 mg/kg dry weight[3].

The determination of trace element in environmentally has been carried out by such instrumental techniques as neutron activation analysis (NAA), inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS), X-ray fluorescence spectroscopy[4] and atomic absorption spectrometry (AAS)[5]. 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. On the other hand, highly sensitive and stable UV-Vis detectors, which give the low noise level ± 2 + 10^{-5} absorbance have been developed. The spectrophotometric methods are combining reversed-phase high performance liquid chromatography (RP-HPLC) are used in the determination of various trace metal ions[6]. One of them, the precolumn derivatization RP-HPLC method, is based on the chelation of metal ions with a ligand and the subsequent RP-HPLC separation and spectrophotometric detection of the metal chelates. It has been used as a powerful technique for the simultaneous determination of metal ions[7]. This method shows the advantages of low cost, excellent selectivity and sensitivity. Several groups of ligands such as azo compounds[8], oxine and its derivatives[9] and dithiocarbamic acid[10] were used as derivatizing agents for the RP-HPLC determination of various metal ions.

In this work a high performance liquid chromatography (HPLC) method for determination a distribution of cobalt in vegetables cultivated in Elazig, Turkey without used preconcentration steps is described.

EXPERIMENTAL

Atomic absorption standard solutions of Fe(III), Co(II), Co(III), Mg(II), Ca(II), Cu(II) and Zn(II) (Merck, Germany, 1000 ± 2 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, USA) Milli-Q water purification system to yield a final conductance of 18 MΩ. HPLC grade methanol and acetonitrile were obtained from Merck (Darmstadt, Germany). All other reagents used, acetic acid (BDH, Poole, UK), hydrochloric acid (BDH), concentrated nitric acid, perchloric acid (Merck, Germany), sodium hydroxide (Merck, Germany) and EDTA (Fluka, USA) were of analytical grade.

Mobile phases were prepared by dissolving 1×10^{-3} mol L^{-1} EDTA in water, adding required amount of methanol and acetonitrile, adjusting the pH (7.5) with acetic acid and sodium hydroxide.

The chromatographic system was equipped with a Shimadzu LC-9A pump, SPD-10AV UV-Visible spectrophotometric detector. Shimadzu SPD-M10AVP photodiode array detector. Luna reversed-phase column (4.6 mm × 200 mm 5 μm, phenomex, USA). Spectral measurements were used

Scomam 750 UV spectrophotometer. HI 8418 Hanna pH meter was used for pH measurements. Weighing of plants samples was performed with JL 180 Chyo electronic balance (readability: 0.1 mg). The flow-rate of the mobile phase was maintained at 0.6 mL min^{-1}, while the column temperature was kept at 30 °C. Microwave-assisted acid digestions have been made using a Premier microwave system. Teflon bumb and various glassware were used throughout the experimental work.

Plants samples to be grown in Turkish were digested microwave system. 2.00 g portion of each sample dried at 80 °C was accurately and 0.50 g directly weighed into teflon bumb. For the samples decomposition concentrated 4 mL HNO_3 and 1 mL $HClO_4$ acid were added. In a tightly closed system, the following six-step microwave digestion program was applied according to the manufacturer's operating guideline: 1 st step, 1 min 150 W; 2 st step, 2 min 0 W; 3 st step, 4 min 150 W; 4 st step, 3 min 300 W; 5 st step, 3 min 480 W; 6 st step, 2 min 650 W. Teflon bumb was halted 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 10 mL with water.

The standard solutions were prepared as follows: 2 mL of 1×10^{-3} mol L^{-1} acetate solution, 2 mL of PAR 1×10^{-3} mol L^{-1} solution, 2 mL of 1×10^{-3} mol L^{-1} EDTA solution was added to a 20 mL volumetric flask and then a standard solution of cobalt(III) was added, the pH was adjusted to 7.5 with dilute acetic acid. EDTA was found to be a useful ligand to mask metal ion contaminants from the chromatographic system and for buffer solution. Afterwards, final volume was diluted 20 mL with water. Then inject an aliquot of the solution into the chromatograph with a 50 µL loop injector. The concentration of the metal ions was determined by measuring the peak area.

The plants samples were treated as follows: 2 mL of 1×10^{-3} mol L^{-1} acetate solution, 2 mL of 1×10^{-3} mol L^{-1} PAR solution, 2 mL of 1×10^{-3} mol L^{-1} EDTA solution 20 mL volumetric flask and then 10 mL the digested samples solution was added. The pH was adjusted to 7.5 with dilute acetic acid solution and 2 mol L^{-1} sodium hydroxide. Afterwards, final volume was diluted 20 mL with water. The sample solution was filtered through a 0.45 µm filter before a 50 µL 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) can produce colour reaction with PAR in pH 6.0-7.5 sodium acetate-EDTA buffer solution. The absorption spectra of PAR and Co(III)-PAR chelate are shown in Figs. 1 and 2, respectively.

The maximum absorption wavelengths of PAR and Co(III)-PAR chelate are 414 and 520 nm. Co(III)-PAR can be separated by RP-HPLC with spectrophotometric detection at 520 nm.

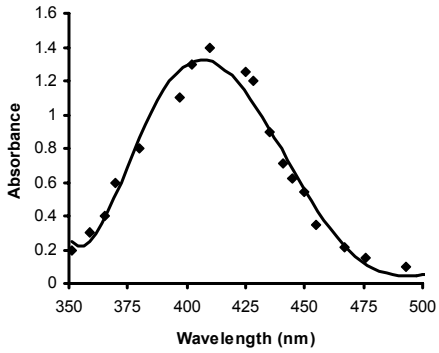

Fig. 1. Absorption spectra of PAR

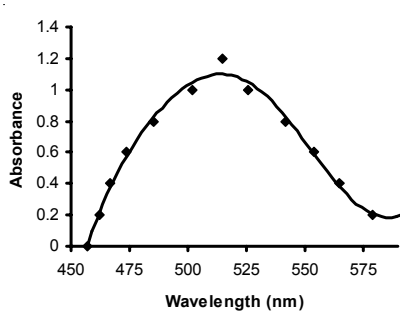

Fig. 2. Absorption spectra of Co(III)-PAR chelate

Effect of variation in pH on absorption spectra of the PAR and Co(III)-PAR: The PAR reacts with Co(III) ion in aqueous methanolic solution to develop colour immediately in pH range 5-9 (Fig. 3).

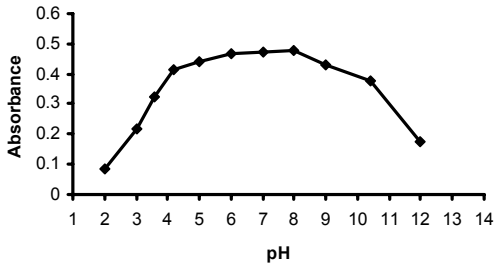

Fig. 3. Effect of variation in pH on the formation of Co(III)-PAR (Final concentration of Co(III)-PAR 2×10^{-5} mol L^{-1})

Effect of variation in molar ratio of Co(III) and PAR for the formation of maximum Co(III)-PAR complex: The composition of Co(III)-PAR chelate was investigated by changing the metal: PAR mole ratio spectrophotometrically. The absorbance of Co(III)-PAR was 520 nm in aqueous methanolic solution. The result, show in Fig. 4, suggest that Co(III):PAR form a 1:2 complex.

Effect of variation in times for the formation of maximum Co(III)-PAR complex: Complex formation period was found 10 s for Co(III)-PAR in ambience methanol-water (Fig. 5).

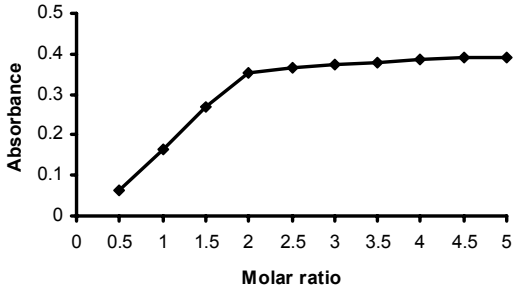

Fig. 4. Effect of variation in molar ratio of Co(III) (2×10^{-5} mol L^{-1}) and PAR (1×10^{-5} mol L^{-1}-10×10^{-5} mol L^{-1})

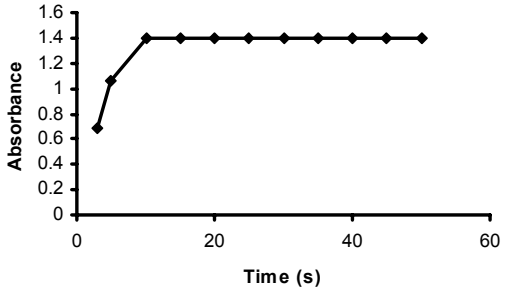

Fig. 5. Effect of variation in times for the formation of Co(III)-PAR (5×10^{-5} mol L^{-1})

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 Co(III)-PAR chalete. Fig. 6 shows the effect of the methanol concentration on the retention time of the chelate. If methanol concentration > % 65 (v/v), Co-PAR and PAR peaks are overlapped.

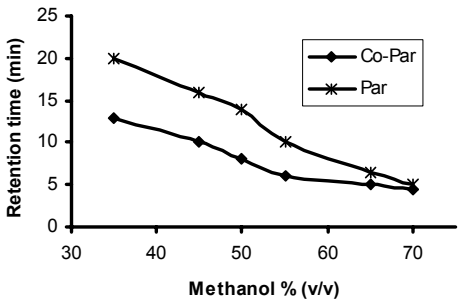

Fig. 6. Effect of the concentration of methanol on retention time of Co(III)-PAR

The effect of the mobile phase pH on the retention time of the Co-PAR chelate was investigated in methanol-water-acetonitrile-acetate solution (55:40:2.5:2.5 v/v) (Fig. 7).

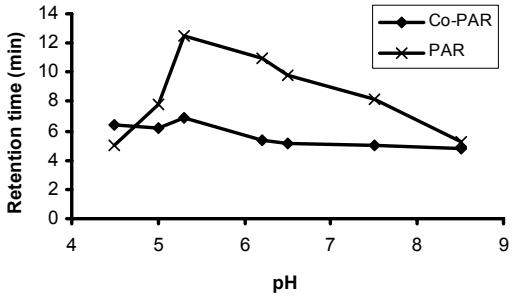

Fig. 7. Effect of pH on retention time

If pH < 5, the peak area of Co(III)-PAR chelate will decrease. If pH > 8, Co-PAR and PAR peaks are overlapped. Therefore, the most suitable pH values for complex separation were found between pH 5.5-7.5.

Effect of foreign ions: Under optimum condition, the effects of foreign ions showed that more than 1000 mg L^{-1} excess of Ca^{2+}, K^{+}, Na^{+}, Mg^{2+}, Cr^{3+}, Mn^{2+}, Al^{3+}, Ba^{2+}, Fe^{3+}, Ni^{2+}, Zn^{2+} and Cu^{2+} did not interfere. However 200 mg L^{-1} of Pb^{2+} and Cd^{2+} ions are interfering. Therefore, the method has high selectivity.

Calibration graph and detection limits: The use different concentrations of Co(III) in accordance with the general procedure; construct calibration graphs with the peak areas against the concentrations of Co(III) ions. The linear range is 1.5-1800 μg L^{-1}. The detection limits were calculated at a signal level of Three times the background noise (signal:noise ratio = 3), the result being 1.2 μg L^{-1} for Co(III)-PAR.

Application for some plants analysis: Five kinds of plants samples were analyzed according to the general procedure. Fig. 8 shows the chromatography of Rosa Cannine sample. The analytical results for cobalt in five kinds plants are summarized in Table-1.

TABLE-1
DETERMINATION OF COBALT IN SOME PLANTS

Plant	Level of cobalt (mg kg^{-1} dry matter)
Rosa canina	0.941 ± 0.106
Sweet basil	0.302 ± 0.038
Daisy	0.242 ± 0.018
Cummin	0.203 ± 0.013
Salvia	0.141 ± 0.021

Results are mean ± SD of three replicate analyses.

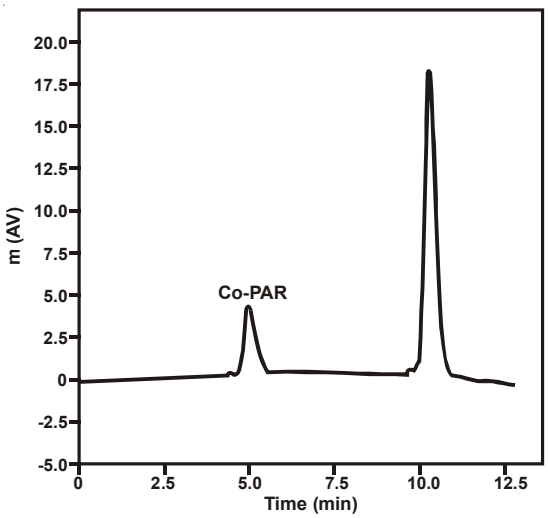

Fig. 8. Chromatography of rosa canina sample

As it is seen in Table-1, the most cobalt amounts were determined in Rosa canina. The amount of cobalt in five kind of plants have the order Rosa canina > Sweet basil > Daisy > Cummin > Salvia.

Cobalt ion may mimic or replace other essential divalent metal ions (*e.g.*, magnesium, calcium, iron, copper or zinc), thus altering many important cellular reactions and functions. There is good evidence that cobalt ions can inhibit DNA repair processes or interact with hydrogen peroxide to form reactive oxygen species that can damage DNA. These mechanisms may contribute to the genotoxic and carcinogenic effects reported for cobalt sulfate and other cobalt compounds[11].

The uptake of cobalt by plants is species-dependent *e.g.*, cobalt is hardly detectable in green beans and the level is exceedingly low in radishes[12]. Leafy plants, such as lettuce, cabbage and spinach, have a relatively high cobalt content, whereas the content is low in grasses and cereals[13]. It is yet unknown whether cobalt is essential for plants. In some cases, small amounts of cobalt produce positive growth effects, but these are dose-dependent and may be indirect[12]. It has been suggested that the element is necessary for the fixation of nitrogen in vegetables that are relatively rich in cobalt. Cobalt concentrations in pastures vary according to season and the use of fertilizers[13].

Plant foods such as cabbage, onions, spinach, tomato and pears contain about 0.2 μg g^{-1} Co while foods containing low amounts of cobalt (0.05 μg g^{-1}) are apples, apricots, carrots, potatoes, oats, wheat and rice. Green leafy vegetables and fresh cereals contain the highest concentration of cobalt (0.2-0.6 μg g^{-1} d.w.)[14].

Rosa canina is rather rich in cobalt and is recommended as a source. Various plants tea is recommended for treatment of some diseases in literature[15]. The present resource show that to consume Rosa canina tea may be help to avoid cobalt deficiency as well as consuming various plants with diet.

HPLC system, for lots of metal chelates which are obtained different ligands at the same time, photodiode series and with flouresance detections and regardless off interference eliminate and preconcentration step, at the some time, very low sensitive and analysis at high selection can be used.

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