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DETERMINATION OF TRACE AMOUNTS OF VANADIUM IN PLANT MATERIALS BY REVERSED-PHASE LIQUID CHROMATOGRAPHY

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SUMMARY

The determination of trace amounts of vanadium by high-performance liquid chromatography is described. The vanadium-PAR [4-(2-pyridylazo)resorcinol] chelate, which was formed at pH 5.5, was separated on a C_{18} column by methanol–water (60:40, v/v) containing phosphate buffer (pH 5.5), 1,2-cyclohexanediamine-N,N,N′,N′-tetraacetic acid and tetrabutylammonium bromide as mobile phase and was spectrophotometrically determined at 555 nm. This technique was applied to the determination of vanadium in plant materials after wet ashing of the sample and extraction of the vanadium as the N-benzoyl-N-phenylhydroxylamine complex. The results obtained for the determination of vanadium in two reference standard samples NIES (National Institute for Environmental Studies, Japan)-chlorella and NIES-pepperbush showed a good agreement with the reported values for the samples.

INTRODUCTION

Although many methods have been used for the determination of vanadium, most of them necessitate more than a few micrograms of vanadium per one analysis. Consequently, 5–20 g of sample must be used for the determination of vanadium in plant materials, because plants usually contain vanadium at the 10^{-2} ppm level.

Recently, several reports on the determination of trace amounts of heavy metals by high-performance liquid chromatography (HPLC) have appeared[1–4]. For example, Hoshino *et al.*[1] reported that the quantitative separation of cobalt(III)-, iron(III)-, and nickel(II)-PAR [4-(2-pyridylazo)resorcinol] chelates could be accomplished on a C_{18} column with tetrabutylammonium bromide (TBA-Br) as an ion-pair reagent by reversed-phase ion-pair partition liquid chromatography. The PAR also reacts with vanadium to form a red chelate even in the presence of 1,2-cyclohexanediamine-N,N,N′,N′-tetraacetic acid (CyDTA) and is used for the spectrophotometric determination of vanadium. The method using both PAR and CyDTA is known as one of the most selective and sensitive method for vanadium. If the vanadium-PAR chelate can be separated from the other chelates or reagents, a highly selective and sensitive analytical method for vanadium will become available. Therefore, we have investigated the determination by HPLC of vanadium in the form of PAR-chelate.

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In this paper, the determination of vanadium by HPLC and its application to the determination of vanadium in plant materials are described.

EXPERIMENTAL

Apparatus and chemicals

The HPLC system consisted of a Twincle pump unit, a Uvidec-100III spectromonitor from Japan Spectroscopic, a Rheodyne Model 7125 injection valve (100-μl loop) and a Shimadzu C-R1A recorder. A 5-μm particle size Cosmosil C_{18} chemically bonded silica gel column (150 × 4.6 mm I.D.) supplied from Nakarai (Kyoto, Japan) was employed. Standard vanadium solutions were prepared by dissolving ammonium vanadate(V), NH_4VO_3, in water. The chelate reagents, PAR, CyDTA and N-benzoyl-N-phenylhydroxylamine (N-BPHA), were obtained from Dojindo (Kumamoto, Japan). The PAR solution (10^{-3} *M*) and the CyDTA solution were prepared by dissolving each compound in small amount of sodium hydroxide solution and diluting the solutions to the desired volume with water. The N-BPHA solution (0.1%) was prepared by dissolving the reagent in chloroform. The ion-pair reagent, TBA-Br of HPLC grade, was supplied by Nakarai. The TBA-Br solution was prepared immediately before use. All the other chemicals used were of reagent grade.

Mobile phase

The mobile phase consisted of 10^{-2} *M* phosphate buffer (pH 5.5, NaH_2PO_4–$NaHPO_4$), 10^{-3} *M* CyDTA and 0.25% TBA-Br in a mixture of 60% (v/v) methanol and 40% (v/v) deionized water. The solution was filtered through a 0.45-μm membrane filter and degassed before use.

Procedure for HPLC of vanadium

To a neutral sample solution containing less than 0.4 μg of vanadium(V), 1 ml of 0.1 *M* phosphate buffer (pH 5.5) and 1 ml of 10^{-3} *M* PAR solution were added, and the solution was diluted to 10 ml with deionized water. The solution was prepared immediately before analysis, and 100 μl were injected on to the column through a 0.45-μm membrane filter. The mobile phase flow-rate was 0.7 ml/min and the eluted vanadium-PAR chelate was detected at 555 nm, the sensitivity being set at 0.02 a.u.f.s. The amount of vanadium was determined by measuring the peak height or the peak area.

Procedure for plant analysis

Weigh 0.5–1 g of fine-powdered plant sample into a conical beaker and digest with a mixture of nitric, perchloric and sulphuric acids. After digestion, dilute the digest to 25 ml with water. Transfer a 20-ml aliquot of the digestate solution to a 50-ml separating funnel, add 0.5 ml of 0.1% cerium(IV) sulphate, $Ce(SO_4)_2$, solution as an oxidizing agent and mix thoroughly. Stand the solution for 15 min, and then add 10 ml of concentrated hydrochloric acid. Immediately add 10 ml of 0.1% N-BPHA chloroform solution and vigorously shake the mixture for 1 min. Allow the two phases to separate, and withdrawn the organic phase. Shake the aqueous phase again with a further 8 ml of the N-BPHA chloroform solution. Combine the two extracts and take 15 ml of the combined extract into a beaker. Evaporate the extract

on a hot plate to dryness and then ash the residue for 20 min in an electric furnace at 550°C. After cooling, dissolve the residue with 1 ml of 0.1 *N* sodium hydroxide solution and a small amount of water by heating. Neutralize the solution with 1 ml of 0.1 *N* sulphuric acid and determine the vanadium in the solution according to the procedure for HPLC of vanadium described above.

RESULTS AND DISCUSSION

HPLC determination of vanadium

When methanol–water (60:40) was used as the mobile phase, the vanadium-PAR chelate rapidly eluted without retention on the C_{18} column, because the chelate probably exists in the form of negatively charged $[VO_2(PAR)]^{-1}$. Thus, reversed-phase liquid chromatography was applied, with TBA-Br as an ion-pair reagent. As expected, the concentrations of methanol and TBA-Br in the mobile phase affected the elution rate of vanadium-PAR chelate: the retention of the chelate increased with decreasing concentration of methanol and/or with increasing amounts of TBA-Br in the mobile phase. Thus, in this work, 0.25% TBA-Br and 60% (v/v) methanol in the mobile phase were chosen. Under these conditions, the vanadium-PAR chelate eluted in a position at *ca.* 1.4 of the capacity factor. To avoid contamination with metal ions, CyDTA was also added to the mobile phase to give a concentration of 10^{-3} *M*.

To choose the wavelength for the detection of eluted vanadium-PAR chelate, the absorption maximum of the chelate was at 555 nm, whereas that measured in the solution without addition of methanol was at 545 nm. Thus, the detection wavelength was set at 555 nm.

The effect on the chelate peak height of varying the concentration of PAR from $2 \cdot 10^{-6}$ to $3 \cdot 10^{-4}$ *M* in sample solutions, containing 0.04 ppm of vanadium, was examined. A constant peak height was obtained for concentrations greater than $5 \cdot 10^{-5}$ *M*. The effect of the pH of the sample solution was also examined. As seen in

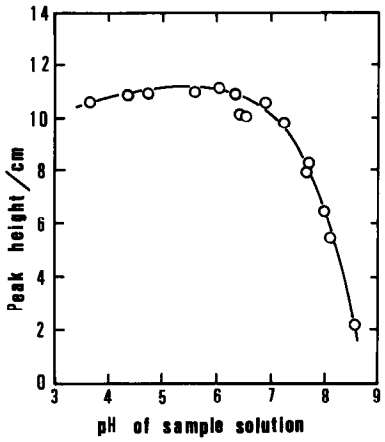

Fig. 1. Effect of pH of sample solution on peak height. HPLC conditions: column, Cosmosil C_{18} (150 mm × 4.6 mm I.D.); flow-rate, 0.7 ml/min; detection wavelength, 555 nm; sensitivity, 0.04 a.u.f.s. Mobile phase, methanol–water (60:40, v/v) containing 10^{-2} *M* phosphate buffer (pH 5.5), 10^{-3} *M* CyDTA and 0.25% TBA-Br. Amount of vanadium injected, 0.002 μg.

Fig. 1, a constant peak height was obtained in the pH range 4.5–6.5, but above pH 6.5 the peak height was reduced with increasing pH. In view of these results, PAR was added to the sample solution to give a concentration of 10^{-4} *M* and the solution was buffered at pH 5.5 with phosphate buffer.

The vanadium-PAR chelate was stable for at least 30 min, during which there was no significant change on the chromatogram. However, if the solution containing the chelate was allowed to stand for longer, the peak height of the chelate gradually became lower. The effect was also produced by the addition of hydroxylamine, a reducing reagent, to the solution. It may be that the peak height is lowered because of the reduction of vanadium in the chelate. Accordingly, the sample solution should be analysed immediately after preparation.

A typical chromatogram is shown in Fig. 2. The elution peaks whose retention times were 5.3 and 10 min at a flow-rate of 0.7 ml/min were vanadium-PAR chelate and free PAR, respectively. The calibration curve was linear over the range 0–0.004 μg at 0.02 a.u.f.s., when either peak height or peak area was measured. The detection limit (at a signal-to-noise ratio of 2:1) was 17 pg for vanadium at 0.005 a.u.f.s. The relative standard deviation, calculated from six replicate analyses of a sample containing 0.02 ppm of vanadium, was 1.75%.

The interference by foreign ions on the determination of vanadium was examined. Thus, 0.04 ppm of vanadium could be determined within 5% of relative error in the presence of 10 ppm each of Al^{3+}, Ca^{2+}, Cd^{2+}, Cu^{2+}, Ga^{3+}, Mg^{2+}, Mn^{2+}, Mo^{6+}, Ni^{2+}, Pb^{2+} or Zn^{2+} and 5 ppm each of Co^{2+}, Cr^{6+}, Sn^{2+}, Ti^{4+} or W^{6+}. However, among the metals tested, Fe^{3+} caused serious interference in the determination of vanadium.

Application to the determination of vanadium in plant materials

The proposed method was applied to the determination of vanadium in plant materials. Plants usually contain *ca.* 100 ppm of iron which interferes with the de-

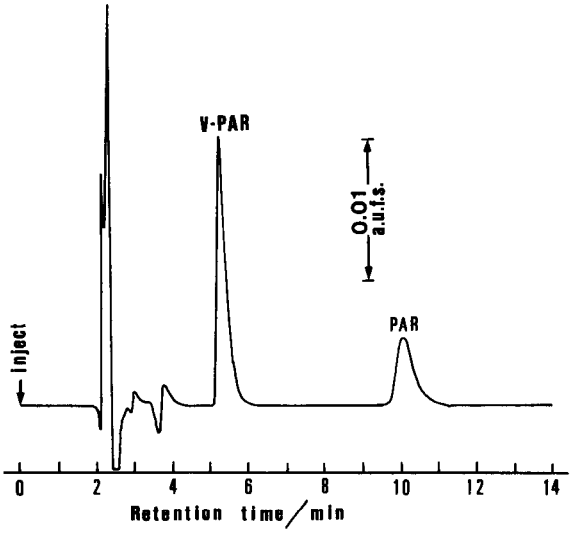

Fig. 2. Typical chromatogram. See Fig. 1 for conditions.

termination of vanadium by the proposed HPLC method. Therefore, after sample digestion, it is necessary to separate vanadium from other elements present in the digestate solution by extraction with N-BHPA. N-BPHA reacts with vanadium(V) in strongly acidic solution to form a violet complex, which can be quantitatively extracted into chloroform. N-BPHA can also extract molybdenum, titanium, tungsten and zirconium. However, these elements, which may be present in plant materials

TABLE I

REPRODUCIBILITY AND RECOVERY OF THE ANALYSES

Sample	*Sample taken (g)*	*Vanadium added (μg)*	*Vanadium found (μg)**		*Vanadium content in original sample (μg/g)*	*Reported[5,6] value (μg/g)*
Spinach	0.484	0	0.099	$X = 0.0964$ S = 0.0035 C.V. = 3.6%	0.20	–
	0.484	0	0.096			
	0.484	0	0.100			
	0.484	0	0.096			
	0.484	0	0.091			
	0.484	0.10	0.19	$X = 0.193$ Recovery = 96.6%		
	0.484	0.10	0.20			
	0.484	0.10	0.19			
Tangle	0.486	0	0.44	$X = 0.428$ S = 0.0126 C.V. = 2.9%	0.88	–
	0.486	0	0.41			
	0.486	0	0.43			
	0.486	0	0.43			
	0.486	0.50	0.90	$X = 0.923$ Recovery = 98.9%		
	0.486	0.50	0.92			
	0.486	0.50	0.94			
	0.486	0.50	0.93			
NIES-chlorella	0.480	0	0.20	$X = 0.19$ S = 0.0082 C.V. = 4.3%	0.40	0.51
	0.480	0	0.18			
	0.480	0	0.19			
	0.480	0	0.19			
	0.480	0.20	0.39	$X = 0.398$ Recovery = 104%		
	0.480	0.20	0.39			
	0.480	0.20	0.41			
	0.480	0.20	0.40			
NIES-pepperbush	0.458	0	0.26	$X = 0.237$ S = 0.021 C.V. = 8.8%	0.52	0.23–0.58 0.61
	0.458	0	0.23			
	0.458	0	0.22			
	0.458	0.20	0.43	$X = 0.443$ Recovery = 103%		
	0.458	0.20	0.44			
	0.458	0.20	0.46			
	0.458	0.20	0.44			

* X = mean; S = standard deviation; C.V. = coefficient of variation.

in concentrations comparable with that of vanadium, did not interfere with the HPLC determination of vanadium. To return the vanadium extracted in chloroform into aqueous solution, a dry ashing technique was applied after evaporation of the solvent. The vanadium oxide formed on dry ashing could be easily dissolved in a small amount of sodium hydroxide solution. On the basis of these results, we have established the procedure for plant analysis described in the Experimental section.

The reproducibility and recovery tests for the proposed method were studied using four kinds of plant sample: two commercial foods (spinach and tangle) and two reference standard samples issued from the National Institute for Environmental Studies, Japan, NIES-chlorella and NIES-pepperbush. The spinach and tangle were analysed after washing, drying and pulverizing. The vanadium recovery tests were carried out by adding a known amount of vanadium as ammonium vanadate. The results are summarized in Table I. The determined values were reproducible with relative standard deviations of 2.9–8.8%, and satisfactory recoveries (96.6–104%) of vanadium were obtained. In addition, the vanadium contents obtained for the reference standard samples showed good agreement with the reported values for the samples[5,6].

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