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Note

Determination of molybdenum and tungsten with tiron by reversed-phase liquid chromatography

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Molybdenum and tungsten are one of the critical pairs of elements that have similar chemical properties. Although many methods have been proposed for the determination of molybdenum or tungsten, mutual interference between molybdenum and tungsten is often experienced. Only a few methods for determining molybdenum and tungsten simultaneously are known; for example, with toluene-3,4-dithiol as colorimetric reagent, simultaneous determination of molybdenum and tungsten is made possible by measurements at two wavelengths[1].

Recently, several reports on the application of high-performance liquid chromatography (HPLC) to separate and determine various metal chelates have been published[2–8]. HPLC seems to be a convenient method for the separation and simultaneous determination of elements that have similar chemical properties, such as molybdenum and tungsten. Therefore, we have investigated the possibilities of separation and determination of molybdenum and tungsten chelates by HPLC. As a result, when tiron as a chelating reagent and tetrabutylammonium bromide (TBA-Br) as an ion-pair reagent were used, the separation of molybdenum and tungsten chelates could be achieved on a column packed with bonded-phase supports by reversed-phase partition liquid chromatography.

In this paper, the separation and simultaneous determination of molybdenum and tungsten in the form of tiron chelates by HPLC are described.

EXPERIMENTAL

Apparatus and chemicals

The HPLC system consisted of a TWINCLE pump unit, a UVIDEC-100III spectromonitor from Japan Spectroscopic and a Rheodyne Model 7125 injection valve (100-μl loop). A 5-μm particle size Cosmosil C_{18} column (150 mm × 4.6 mm I.D.) as a main column and a 10-μm particle size Cosmosil C_{18} column (50 mm × 4.6 mm I.D.) as a precolumn were employed. The short precolumn was placed immediately before the main column. These columns were supplied by Nakarai-Chemical Co. A Hitachi 100-60 type spectrophotometer and a Hitachi-Horiba F-7 type pH meter were used. Standard molybdenum(VI) and tungsten(VI) solutions were prepared by dissolving ammonium molybdate, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, and sodium

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tungstate, $Na_2WO_4 \cdot 2H_2O$, respectively, in water. The chelating reagent, tiron (1,2-dehydroxybenzene-3,5-disulphonic acid, disodium salt), was obtained from Dojindo Labs. (Japan). The tiron solution was prepared by dissolving the compound in water. The ion-pair reagent, TBA-Br of HPLC grade, was supplied from Nakarai-Chemical Co. 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 $1.5 \cdot 10^{-3}$ *M* tiron, $3 \cdot 10^{-2}$ *M* TBA-Br and $1.5 \cdot 10^{-3}$ *M* acetic acid–sodium acetate buffer (pH 3.8) in a mixture of 57% (v/v) methanol and 43% (v/v) deionized water. The solution was filtered through a 0.45-μm membrane filter and degassed before use.

Procedure

To a neutral sample solution containing less than 10 μg each of molybdenum and tungsten, 1 ml of $1.5 \cdot 10^{-2}$ *M* tiron solution and 1 ml of 0.2 *M* acetate buffer solution (pH 3.8) were added, and the solution was diluted to 10 ml with deionized water. An aliquot of the solution was injected on to the column through a 0.45-μm membrane filter. The mobile phase was pumped at a rate of 0.7 ml/min. Detection was effected with the UV detector operated at 315 nm, the sensitivity being set at 0.02 or 0.01 absorbance units at full scale (a.u.f.s.). The amount of each metal was determined by measuring the peak heights.

RESULTS AND DISCUSSION

HPLC separation of molybdenum- and tungsten–tiron chelates

Using a methanol–water mixture as mobile phase, the elution peaks of molybdenum- and tungsten–tiron chelates could not be observed. Because of the negative charges of their chelates, probably their chelates rapidly eluted together with the other components, such as tiron, without retention on the hydrophobic support in the column. Therefore, to apply reversed-phase partition liquid chromatography, TBA-Br as an ion-pair reagent was added to the mobile phase. However, even if TBA-Br was added, only the peak of tungsten–tiron chelates appeared on the chromatogram; but, by adding tiron to the mobile phase, well-resolved peaks of molybdenum- and tungsten–tiron chelates could be obtained. On the basis of these elementary compositions of mobile phase, optimum conditions for analysis were studied in detail.

Effect of pH of buffer added to mobile phase

Preliminary experiments revealed that the pH of the acetate buffer solution to be added to the mobile phase had effects on the elution patterns of tiron chelates. The effect of buffer pH was examined in the pH range 3–6 by using acetic acid–sodium acetate buffer. Constant peak height of each chelate and good resolution (R_s) were obtained in the pH range 3–4 (Table I), but above pH 4, the resolution and the peak heights became worse with increasing pH. The formation of higher chelates, which had tiron:metal ratios of 2:1 or 3:1, probably occurred above pH 4, while at pH 3–4 the ratio of tiron to molybdenum or tungsten was estimated to be 1:1 as

TABLE I

EFFECT OF pH ON SEPARATION OF MOLYBDENUM–TIRON AND TUNGSTEN–TIRON CHELATES

HPLC conditions: column, Cosmosil C_{18} (main column, 150 mm × 4.6 mm I.D.; precolumn, 50 mm × 4.6 mm I.D.; flow-rate, 0.7 ml/min; detection wavelength, 315 nm; sensitivity, 0.04 a.u.f.s.; mobile phase: methanol–water (57:43, v/v) containing $1.5 \cdot 10^{-3}$ *M* tiron, $3 \cdot 10^{-2}$ *M* TBA-Br and $1.5 \cdot 10^{-3}$ *M* acetate buffer. Sample: 100 µl, containing 1 ppm each of molybdenum and tungsten.

pH		*Tungsten–tiron chelate*		*Molybdenum–tiron chelate*		*Separation factor* (α)	*Resolution* (R_s)
Buffer	*Mobile phase*	*Retention time (min)*	*Peak height* (cm)*	*Retention time (min)*	*Peak height* (cm)*		
3.0	4.6	12.3	11.1	14.0	11.1	1.20	1.87
3.5	4.7	12.3	11.3	14.1	11.9	1.21	1.87
4.0	5.0	12.5	10.8	14.3	12.3	1.21	1.90
4.5	5.5	12.1	8.0	13.8	11.9	1.21	1.53
5.0	6.0	11.3	6.7	13.0	11.4	1.22	1.53
5.5	6.5	11.9	4.1	14.7	6.2	1.34	1.46
6.0	6.6	11.7	4.8	14.7	4.1	1.26	1.20

* Peak height 12.5 cm is equivalent to 0.02 absorbance unit.

described later in this paper. Many chelates having different ratios of tiron to molybdenum or tungsten were formed in the mobile phase; overlapping of elution peaks of these many chelates might affect the elution pattern. Therefore, acetate buffer solution of pH 3.8 was added to the mobile phase to give a concentration of $3 \cdot 10^{-3}$ *M*.

Effect of tiron concentration in mobile phase

If there was no tiron in the mobile phase, the peak for the molybdenum–tiron chelate disappeared. As the stability of the molybdenum–tiron chelate is probably lower than that of the tungsten–tiron chelate, so the molybdenum–tiron chelate injected may be immediately dissociated into tiron and molybdate ions owing to dilution by a large volume of mobile phase, without addition of tiron. Therefore, to suppress the dissociation of the molybdenum–tiron chelate and to establish an optimum concentration of tiron, the latter compound was added to the mobile phase in varying concentrations from $3.75 \cdot 10^{-4}$ to $3 \cdot 10^{-3}$ *M*. The almost constant peak heights of molybdenum– and tungsten–tiron chelates and good resolution of the two peaks were obtained at a tiron concentration greater than $1 \cdot 10^{-3}$ *M*. According to this result, we chose, as optimum tiron concentration in the mobile phase, $1.5 \cdot 10^{-3}$ *M*.

Effect of concentration of TBA-Br and methanol in mobile phase

The effects of concentration of TBA-Br and methanol were examined. As expected, the greater the concentration of TBA-Br, the longer the retention time of each chelate and the better the resolution of peaks for molybdenum– and tungsten–tiron chelates became. On the other hand, the greater the concentration of methanol, the shorter the retention time and the worse the resolution of the two peaks became. Therefore, $3 \cdot 10^{-2}$ *M* TBA-Br and 57% (v/v) methanol in the mobile phase were chosen.

Detection wavelength and composition of chelates

To choose the wavelength for the detection of eluted molybdenum– and tungsten–tiron chelates, the absorption spectrum of each chelate was measured in the same solution as the mobile phase, but without the addition of tiron. Both chelates exhibited maximum absorption at 315 nm, while the reagent blank showed negligible absorption at this wavelength. Thus, the detection wavelength was set at 315 nm.

The composition of molybdenum– and tungsten–tiron chelates, which were formed at pH 3.8, was studied by Job's method of continuous variations. As a result, the ratio of tiron to molybdenum or tungsten was to be 1:1.

Preparation of sample solution

The effect of varying the concentration of tiron from 0 to $3 \cdot 10^{-3}$ *M* in sample solutions, containing 20 μg each of molybdenum and tungsten, on the peak heights of their tiron chelates was examined. Constant peak heights for molybdenum– and tungsten–tiron chelates were obtained for concentration $> 6 \cdot 10^{-5}$ *M*. The effect of pH of the sample solution was also examined by using acetate buffer. Both peak heights of the chelates were almost constant in the pH range 3–5, and buffer concentrations $< 8 \cdot 10^{-2}$ *M* did not affect to the peak heights. Considering these results, $1.5 \cdot 10^{-3}$ *M* for tiron and $2 \cdot 10^{-2}$ *M* for acetate buffer (pH 3.8) were chosen as the concentration in sample solution.

At room temperature, both molybdenum and tungsten reacted readily with tiron forming chelates, which were stable for at least one month and showed no significant change on the chromatogram.

Chromatogram and calibration curve

A typical chromatogram is shown in Fig. 1. The tiron chelates were eluted in the order of tungsten, then molybdenum, and the retention times for each chelate were 12.1 and 14.1 min, respectively, at a flow rate of 0.7 ml/min. The calibration curves for the determination of molybdenum and tungsten were linear over the range $2 \cdot 10^{-7}$ to $1 \cdot 10^{-5}$ *M* for molybdenum and $1 \cdot 10^{-7}$ to $5 \cdot 10^{-6}$ *M* for tungsten at 0.01 a.u.f.s., when peak heights were measured. The detection limits (at a signal-to-noise ratio of 2:1) for the metals in tiron chelates were 1.6 ng for molybdenum and 2.2 ng for tungsten at 0.01 a.u.f.s. Relative standard deviations calculated from ten replicate analyses of a sample containing 1 μg/ml each of molybdenum and tungsten were 2.4 and 1.7%, respectively.

Effect of foreign ions

The effect of the presence of other ions on the determination of molybdenum and tungsten was examined. The following ions affected the peak heights of molybdenum and tungsten chelates, with an error of less than ±5%, in determining 10 μg each of molybdenum and tungsten: K^+, Cu^{2+}, Mn^{2+}, Zn^{2+}, Mg^{2+}, BO_3^{3-}, $H_2PO_4^-$, F^- and SiO_3^{2-} (1000 μg); and Al^{3+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+} and AsO_3^- (100 μg). The presence of Fe^{3+}, V^{5+}, Cr^{6+}, Ti^{4+} and Sn^{2+} caused serious interference in the determination of molybdenum and tungsten. Therefore, before applying this HPLC method to actual samples, these interfering ions must be removed by using a suitable masking or extracting agent. Fortunately, both molybdenum and tungsten can be selectively extracted, with use of a suitable reagent for masking of

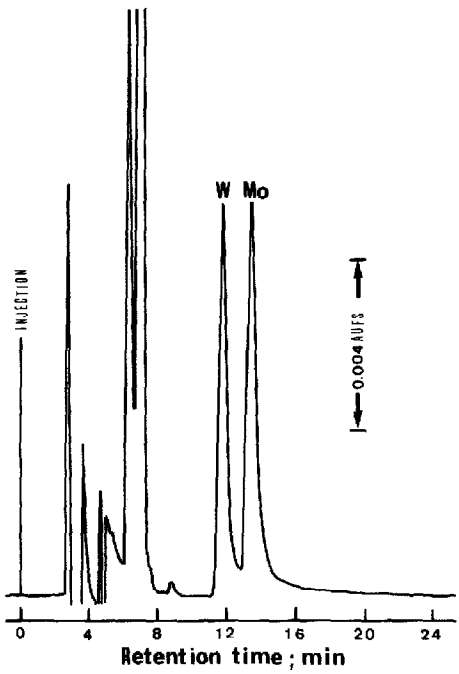

Fig. 1. Typical chromatogram. Mobile phase: methanol–water (57:43, v/v) containing $1.5 \cdot 10^{-3}$ *M* tiron, $3 \cdot 10^{-2}$ *M* TBA-Br and $1.5 \cdot 10^{-3}$ *M* acetate buffer (pH 3.8); chart speed, 5 mm/min. Other conditions are the same as described in Table I.

V^{5+} and Cr^{6+}, from acidic aqueous solution into chloroform or ethyl acetate by α-benzoin oxime (cupron) as chelating reagent.

To determine molybdenum and tungsten in an environmental sample, such as soils or plants, by using the HPLC method described in this paper, the separation of molybdenum and tungsten from the sample by extraction with α-benzoin oxime is now under investigation.

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