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Tetrazolium violet – a new spectrophotometric reagent for molybdenum determination

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The interaction of Mo(VI) and tetrazolium violet (TV) has been examined. The ion-associate formed is extracted into 1,2-dichloroethane. The tetrazolium violet : molybdenum(VI) : 3,4,5-trihydroxybenzoic acid ratio (R) is 2 : 1 : 2. The optimum conditions have been established. Beer's law is obeyed in the range of 0.2–5 μg ml^{-1} molybdenum(VI). The molar absorptivity of the complex is $(1.30 \pm 0.08) \times 10^5$ L mol^{-1} cm^{-1}. The sensitivity of the method is 7.4×10^{-4} μg cm^{-2}. It is possible to extract and determine molybdenum in the presence of a large number of cations and anions. The characteristic values for the extraction and the aqueous phase equilibria were determined. A sensitive and selective method for determination of molybdenum(VI) microquantities in soils has been developed.

Key words: molybdenum determination, extraction-spectrophotometry, tetrazolium violet, soils

INTRODUCTION

The concentration of certain trace elements in the soil is an important factor for their entry into plants. Low levels cause some functional diseases in plants. It has been established that molybdenum takes part in the fixing and assimilation of atmospheric nitrogen. Complete information about the molybdenum content in soils and plants is needed to study its importance and to establish the need for molybdenum in fertilizers.

Spectral and chemical methods are being constantly used for molybdenum determination. Therefore, new organic reagents for a selective and sensitive photometric determination of molybdenum are of particular interest.

Numerous reagents have been suggested for the determination of this element: triphenyltetrazolium chloride [1, 2], sodium pentamethylene dithiocarbamate [3], 2'-hydroxyacetophenone benzoylhydrazone [4], dithiopyrilmethane [5], neotetrazolium chloride [6], N-phenylbenzohydroxamic acid [7], triocthylammonium chloride [8], 4-/2-pyridylazo/-resorcin [9], tribenzylamin [10], bythyltriphenyl phosphonic bromide [11], N-n-tolyl-N'-n-chlorphenyl-o-chlorbenzamidine hydrochloride [12], pyrogallol [13], N-hydroxy-N-n-chlorphenyl-N'-n-chlorphenyl-o-chlorbenzamidine chlorhydrate [14], lignocain [15], mercapto-propionic acid [17], 2-hydroxy-4-n-propoxyachetophenone oxime [18], 8-mercaptoquinoline [19], isonicotinoylhydrazone salicylic aldehyde [20], malachite green [21], 1,5-diphenylcarbazone [22], pyrocatechol [23], 2,4-diaminophenol [24], 2,4-dihydroxyacetophenone [25], 8-hydroxyquinoline [26], capheic acid [27], N-benzoylphenylhydroxylamine [28], pyrocatechine violet [29], 9-(2,4-dihydroxyphenyl)-2,3,7-trihydroxyl-6-fluorone [30], 3,5-dibromo-4-hydroxyphenylfluorone [31], 2,3,7- trihydroxyphenylfluorone [32]. Of these methods, 4, 16, 19, 24–27 are of low selectivity; methods 3–8, 15, 17, 18, 23, 25 are characterized by a low sensitivity ; methods 1, 20–22, 27 are of high sensitivity; methods 1, 2, 9, 11, 13, 15, 28 are time-consuming; and the rest 12, 14, 18 show a low stability of the ion-association complex.

Tetrazolium salts have been used to provide cations for the formation of ion-associates of various anions in the determination of a number of metals. Tetrazolium violet (TV) as a cation forms ion-association complexes of Mn(VII), Zn(II), Co(II) and Cr(VI) [33–36]. No data on determination of molybdenum with TV can be found in the literature. The tetrazolium salt TV we suggest is a new reagent for determination of microquantities of molybdenum(VI). The present work for the first time examines TV employment for the determination of molybdenum microquantities.

EXPERIMENTAL

Apparatus

All measurements were carried out in 1-cm quartz cells with measurements at 230 nm on a VSU spectrophotometer (Germany).

Reagents

All the chemicals used were of analytical grade.
All solutions were prepared with distilled demineralized water.

A 1×10^{-3} M stock solution of TV 2,5-diphenyl-3-2-naphthyl-tetrazoliumchloride (Fluka) was prepared by dissolving 0.0192 g in distilled water and diluting to 50 ml. Other TV concentrations were prepared by appropriate dilution. The solutions were stable for several months.

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Stock molybdenum(VI) solution. A 1 × 10^{-3} M solution of molybdenum(VI) was prepared by dissolving 0.0242 g of $Na_2MoO_4 2H_2O$ (Fluka) in 100 ml of distilled water. Solutions of lower concentrations were prepared from the stock solution by dilution. Further dilutions were performed in order to fit a calibration curve.

3,4,5-trihydroxybenzoic acid (R), p. a. A 1 × 10^{-2} M aqueous solution was prepared by dissolving 0.17 g of acid in 100 ml of distilled water.

Phosphoric, hydrochloric, sulfuric and nitric acids were used at concentrations of 0.2, 0.12 , 0.2 and 0.15 M, respectively.

1,2-dichloroethane, p. a. (laboratory safety rules should be obeyed).

Solutions of ions for interference studies were prepared by dissolving the amount of each compound needed to give 10 mg ml^{-1} of the ion concerned.

Recommended procedure

Transfer a known volume of Mo(VI) solution containing 2–50 μg of molybdenum to a 100 ml separating funnel, and add 1.2 ml of 1 × 10^{-4} M TV, 0.2 ml of 1 × 10^{-2} M 3,4,5-trihydroxybenzoic acid (R). Dilute to a volume of 10 ml with distilled water and extract with 3 ml of 1,2-dichloroethane for 30 s. The organic layer is then transferred through filter paper into a 1-cm cell and measured at 230 nm. A blank solution containing all reagents except molybdenum was prepared and treated in the same way.

RESULTS AND DISCUSSION

Absorption spectra and solvent selection

The absorption spectra of the complexes formed by molybdenum(VI) – 3,4,5-trihydroxybenzoic acid (R) – tetrazolium violet (TV) (Series 1) and TV – R (Series 2) were compared under identical conditions and are shown in Fig. 1. The absorption spectra of TV solutions and the ion-association complex of molybdenum(VI) show that the absorption maximum is around 230 nm. The absobance additivity of both components, without a significant shift of the absorption maximum, indicates formation of an ion-association complex.

The extraction of the Mo(VI)–R–TV ion-association complex was tested in several organic solvents: hydrocarbons (benzene and toluene), ethers, ketones, alcohols, dichloroethane, chloroform and tetrachloroethane. The extraction was complete and quantitative with dichloroethane, and a clear separation was obtained.

Optical properties

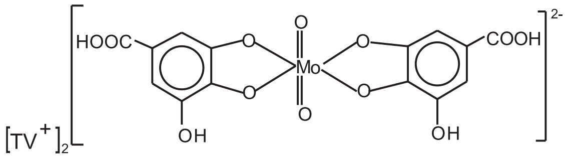
The molar absorptivity of the associate, calculated by the method of Komar-Tolmatchov [37], was (1.30 ± 0.08) × 10^5 L mol^{-1} cm^{-1}. This indicates a high sensitivity of the given reaction. Under optimum reaction conditions, Beer's law was obeyed at a concentration of 0.2–5 μg ml^{-1} Mo(VI). The sensetivity of the method according to Sandell is 7.4 × 10^{-4} μg cm^{-2} [38]. The relative standard deviation (6 determinations with 5 μg of Mo(VI) , 95% confidence level) is ± 1.4%.

Stoichiometry and stability of the extract

The binary complex formed was retained on an anion-exchanger, indicating that it is negatively charged. Infrared measurements on the solid complex confirmed the presence of both Mo=O and Mo–O bonds [16]. On the basis of these data and earlier reports [39, 40], the stoichiometry of the complex MoR_2 is Mo : R = 1 : 2.

The isomolar series method [37] showed that the extracted species was a 2 : 1 ion-associate of the TV cation and the molybdenum anion.

The ion-associate formed from the cation of the (TV^+) and Mo(VI) with 3,4,5-trihydroxybenzoic acid may be described by the formula:



It was established that the ion-pair had a high stability. The absorption did not change for 45 days.

The rate of formation of the complex was very fast, and the minimum shaking time for complete extraction of the ion-association complex into 1,2-dichloroethane was found to be 10 s at room temperature.

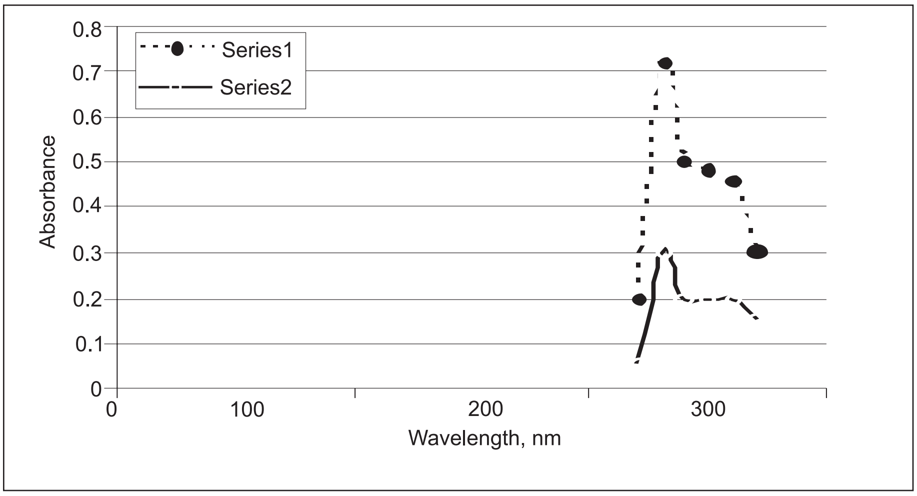


Fig. 1. Absorption spectra in dichloroethane of the ion-association complex of Mo-R-TV (Series 1) and TV-R (Series 2) : $C_{Mo(VI)} = 1 \times 10^{-5}$ M, $C_R = 1 \times 10^{-3}$ M, $C_{TV} = 0.8 \times 10^{-5}$ M

Effect of reagents and conditions on absorbance measurements

The effect of 3,4,5-trihydroxybenzoic acid (R) on the formation and extraction of the ion-association complex was investigated with a sample containing 3×10^{-6} M Mo(VI), keeping the other conditions constant. It was found that a constant and maximum absorbance was obtained when the concentration of $R \geq 2 \times 10^{-4}$ M and a considerable excess of it did not interfere the determination (Fig. 2).

The effect of the type of mineral acid and its concentration upon the interaction of molybdenum(VI) and TV and the effect of various acids (phosphoric, hydrochloric, sulfuric and nitric) on the extraction equilibrium were studied (Fig. 3). The light absorption of the extracts showed constant and maximum values without acids or when $0.1–4 \times 10^{-4}$ M H_3PO_4 and $0.1–3.6 \times 10^{-4}$ M HCl acids were used. Figure 3 indicates that not only the concentration but also the nature of the acid has an effect on the extraction equilibrium.

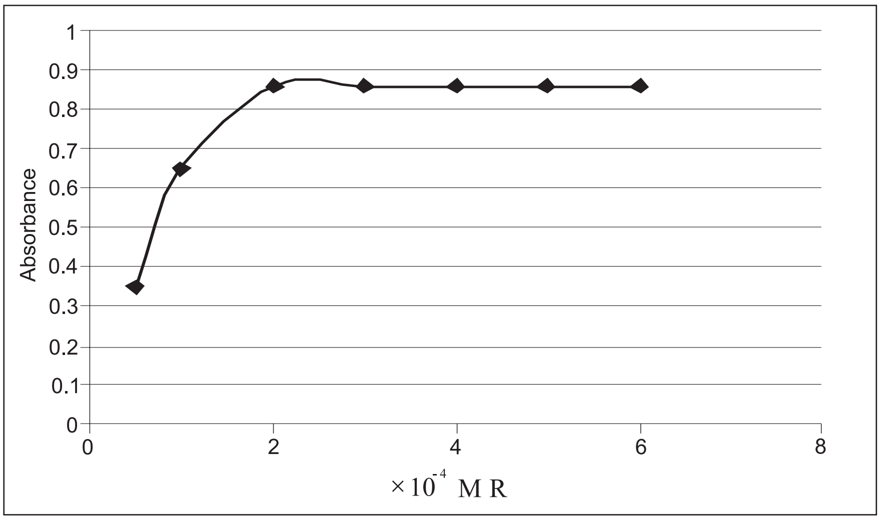


Fig. 2. Dependence of the light absorption change in dichloroethane on the concentration of 3,4,5-trihydroxybenzoic acid (R) : $C_{Mo(VI)} = 3 \times 10^{-6}$ M, $C_{TV} = 1 \times 10^{-5}$ M

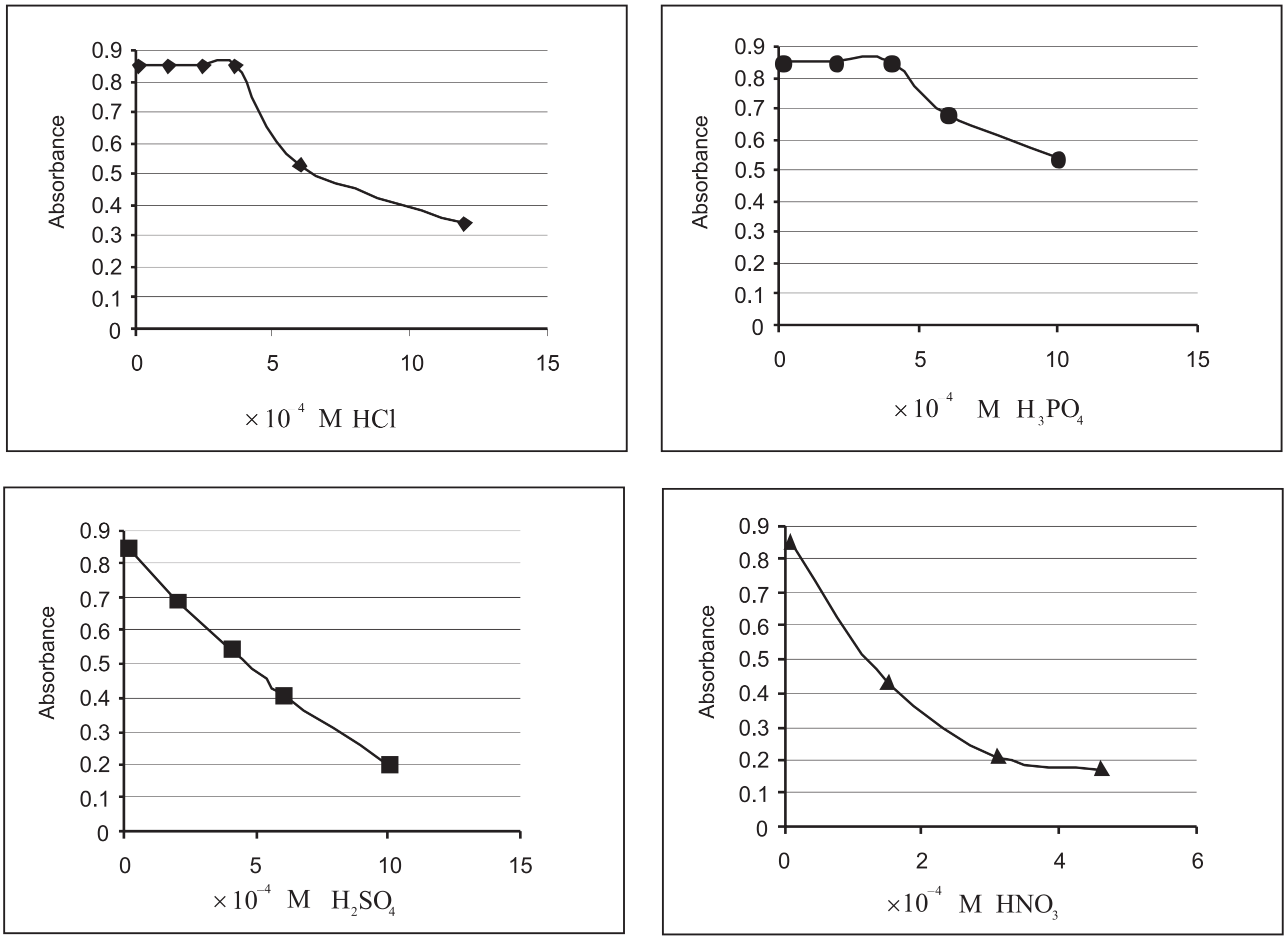


Fig. 3. Investigation of the influence of acidity on the extraction of Mo(VI) with TV in dichloroethane: $C_{Mo(VI)} = 3 \times 10^{-6}$ M, $C_R = 2 \times 10^{-4}$ M, $C_{TV} = 1 \times 10^{-5}$ M

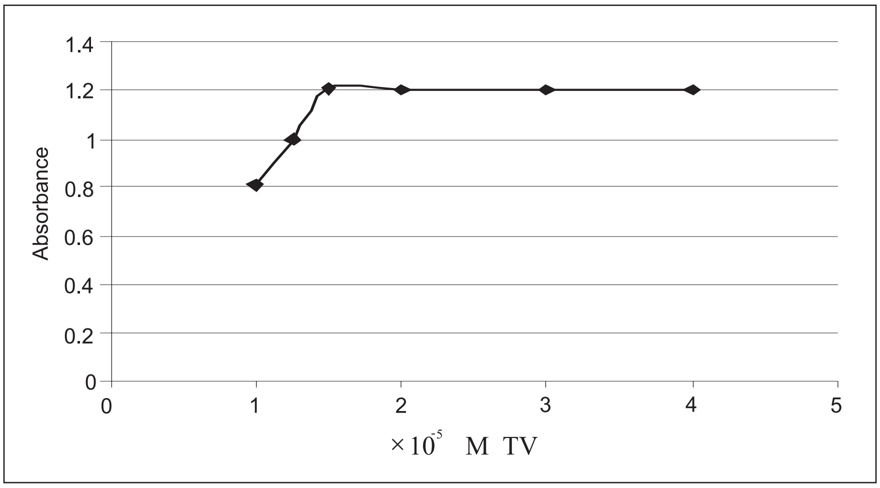


Fig. 4. Dependence of the light absorption change of ion-associate Mo-R-TV on the concentration of TV : $C_{Mo(VI)} = 3 \times 10^{-6}$ M, $C_R = 2 \times 10^{-4}$ M

It was found that 1.5×10^{-5} M solution of TV was adequate for a quantitative extraction of 3×10^{-6} M Mo(VI). The absorbance of the complex was not affected by using an excess of the reagent (Fig. 4).

Effect of diverse components

In Table 1, the effects of various potentially interfering species commonly found in soils on the determination of Mo(VI) are listed. These effects were investigated by adding a known amount of the test ion to the molybdenum solution. The tolerance level is defined as a foreign ion concentration that produces no more than a ± 3% spectrophotometric error in the recovery of Mo(VI). The results show the possibility of extractive-spectrophotometric determination of molybdenum(VI) in the presence of numerous ions without separation beyond the extraction procedure described above. Hg(II) and I^- showed an interfering action. Ammonium fluoride was used to eliminate the interference of Mg(II) and Al(III).

Table 1. **Effect of foreign ions on the determination of 2 µg ml^{-1} of molybdenum**

Foreign ion or species	Limiting conc., µg
K(I), Na(I)	100000
PO_4^{3-}	80000
F^-, citrate	45000
Sr(II), Ni(II)	20000
Mn(II) , Cl^-	10000
Fe(III)*	1500
Cr(VI), SO_4^{2-}, NO_3^-	1000
W(VI)	500
Pb(II)	400
BrO_3^-	200
Cu(II), Zn(II), Al(III)*, Br^-	100
Cd(II), Mg(II)*, NO_2^-	50
Co(II)	30
Ca(II), V(V), CO_3^{2-} , $C_2O_4^{2-}$	20
Hg(II), I^-	Interfere

* In the presence of masking reagents (fluoride and citrate).

EXTRACTION EQUILIBRIA

The following equilibria should be taken into account for the system of molybdenum, 3,4,5-trihydroxybenzoic acid, tetrazolium violet, water and dichloroethane.

1. Formation of the ion-association complex in the aqueous phase, and the corresponding equilibrium constant:

$$\beta = \frac{TV_2[MoR_2]}{[TV^+]^2[MoR_2^{2-}}.$$

2. Distribution of the complex between the aqueous and the organic phases and the corresponding distribution constant:

$$K_D = \frac{\{TV_2[MoR_2]\}_o}{\{TV_2[MoR_2]\}}.$$

3. The entire extraction process and the corresponding extraction constant:

$$K_{ex} = \frac{\{TV_2[MoR_2]\}_o}{[TV^+]^2[MoR_2^{2-}]} = K_D \cdot \beta.$$

The quantitative investigation of the interaction of the aqueous phase and extraction of the ion-association complex with dichloroethane was be performed using a chemical model [41] and the method of Likussar–Boltz [42].

The distribution constant of the ion-associate of $[MoR_2]^{2-}$ and monotetrazolium salt TV^+ were determined using the chemical model [41]. For this purpose, we determined the distribution coefficient of TV as a function of Mo(VI) concentration.

The following solutions were introduced into 100 ml separating funnels: 2 ml of 1×10^{-4} M TV, 2 ml of 1×10^{-3} M 3,4,5-trihydroxybenzoic acid (R), and the corresponding amounts of molybdenum(VI). The volume of the aqueous phase is brought to 10 ml with distilled water, then it is extracted with 3 ml of dichloroethane for 10 s. After the separation of the two phases, the organic phase is transferred through filter paper into a 25 ml flask and is diluted to the mark with dichloroethane. The light absorption of the organic phase is measured at 230 nm with a 1-cm light path cuvette.

To determine the distribution coefficient of TV, the absorptions obtained are compared to the one obtained after three extractions of the aqueous layer:

$D_{TV} = A / A_{max} - A$,
where A – light absorption for a single extraction,
A_{max} – light absorption for three extractions.

Using the logarithmic values of D_{TV} and the concentration of molybdenum, the plot log D_{TV} vs log $C_{Mo(VI)}$ allows determination of the extraction values. The distribution constant is determined graphically by extrapolation of the straight line parallel to the X-axis up to the point of intersection with the Y-axis.

We used the Likussar–Boltz [42] method for determination of the extraction constant K_{ex}.

The association constant of the study compounds is calculated on the basis of the dependence $K_{ex} = K_D \cdot \beta$ [41].

The values of the equilibrium constants characterizing the extraction equilibrium and the stability of the ion-association complex of Mo(VI) with TV were as follows:

$K_D = 17.47 \pm 0.1$,
$K_{ex} = (1.54 \pm 0.13) \times 10^{11}$,
$\beta = (8.81 \pm 0.08) \times 10^{9}$.

They were statistically worked up at a confidence level of 95%. The results show that the monotetrazolium salt TV is superior in terms of extraction completeness.

APPLICATION OF THE METHOD

Determination of molybdenum in soils

An air-dried soil sample (4 g) is weighed and introduced into a platinum crucible and heated for 3 h in a furnace at 500–550 °C to digest the organic matter. After cooling, the sample is transferred to a platinum disk, and 5 ml of concentrated H_2SO_4, 5 ml of concentrated HNO_3 and 2 ml of H_2O_2 are added. The sample is heated until white fumes of SO_3 appear. Next, 1 ml of H_2SO_4 and 3 ml of HNO_3 are added and evaporated to dryness. 5 ml of concentrated HCl and 2 ml of conc. $HClO_4$ are then added to the dry residue and evaporated to dryness again to remove chromium as chromyl chloride and to destroy any organic compounds [43], because they interfere. If any reduction of the molybdenum occurs, it should be oxidized back to Mo(VI) with conc. HNO_3 [44]. The dry residue is then dissolved in double-distilled water and acidified with hydrochloric acid. The solution obtained is then neutralized with conc. ammonia solution to precipitate the iron(III), magnesium(II) and aluminium(III). The precipitate is filtered through filter paper, and the filtrate is evaporated until the volume is about 30 ml. Then the solution is transferred into a 50 ml volumetric flask hydrochloric acid to pH ~4 is added and diluted to the mark with distilled water. To mask the interfering ions, 1.2 ml of 1×10^{-4} M TV, 0.2 ml of 1×10^{-2} M 3,4,5-trihydroxybenzoic acid, 1 ml of fluoride and 1 ml of citrate solutions, both 45 mg ml^{-1} (to mask the interfering ions) were added to 1 ml of soil solution. The solution was diluted to 10 ml with distilled water, then shaken for 30 s with 3 ml 1,2-dichloroethane. The organic phase was filtered through a dry paper into a 1-cm cuvette, and the absorbance was measured at 230 nm. A blank was run in parallel in the absence of the soil sample.

Table 2. **Determination of molybdenum in soils**

Soils	Mo , x 10^{-4}%		
	TV method	RSD* % (n = 6)	Atomic absorption method
Brown	5.15	1.9	5.05
Black	4.00	1.6	4.10
Pseudopodzolic	7.10	1.2	7.18
Leached cinnamonic	4.12	1.4	4.04
Podzolic cinnamonic	5.80	1.8	5.70
Podzolized black	7.55	1.4	7.65
Alluvial	4.50	1.5	4.41

* Relative Standard Deviation (bazed on 6 determination).

On the basis of the data obtained we have developed a method for determination of trace amounts of molybdenum in soils. A few types of soils were studied: black, brown, alluvial, podzolized black, lessive cinnamonic, pseudopodzolic, leached cinnamonic. The samples were collected from a depth of 0–20 cm.

The results of the soil analyses are given in Table 2, and the accuracy of the method was cheked by atomic-absorption spectrometry. The method we developed has a high selectivity and sensitivity. Preisolation of molybdenum or of most other ions is not necessary. The good agreement of the results obtained by this method with AAS method shows that tetrazolium violet can be used as a reagent for determining the microquantities of molybdenum in soils.

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TETRAZOLIO VIOLETINIS – NAUJAS SPEKTROFOTOMETRINIS REAGENTAS MOLIBDENUI NUSTATYTI

Santrauka

Buvo tirta Mo(VI) ir Tetrazolio Violetinio (TV) sąveika. Susidaręs joninis asociatas buvo ekstrahuojamas 1,2-dichloretanu. Naudotas TV : Mo(VI) : 3,4,5-trihidroksibenzoinės rūgšties santykis 2 : 1 : 2. Nustatytos optimalios sąlygos. Bero dėsnis galioja esant Mo(VI) koncentracijai 0,2–5 μg ml^{-1}. Komplekso molinis ekstinkcijos koeficientas sudaro (1,30 ± 0,08) × 10^{5} l mol^{-1} cm^{-1}. Siūlomo metodo jautrumas siekia 7,4 × 10^{-4} μg cm^{-2}. Yra įmanoma molibdeną ekstrahuoti ir nustatyti esant dideliam katijonų ir anijonų kiekiui. Pasiūlytas jautrus ir atrankus metodas tinka Mo(VI) mikrokiekiams dirvoje nustatyti.