Monatsh Chem (2011) 142:131–136
DOI 10.1007/s00706-010-0442-x

ORIGINAL PAPER

Determination of molybdenum in biological samples by flame atomic spectrometry after preconcentration on activated carbon

Ergul Yigmatepe · Mehmet Yaman

Received: 27 February 2010 / Accepted: 12 December 2010 / Published online: 14 January 2011

Abstract Molybdenum is well known for its toxic effects, although it is also essential for N_2-fixing cyanobacteria and several enzymes. This study proposes a simple and accurate procedure for separation, preconcentration, and determination of trace amounts of molybdenum in biological samples by flame atomic absorption spectrometry. It is based on complexation of Mo by cupferron and sorption onto activated carbon. Effects of parameters such as pH, stirring time, and amounts of activated carbon and cupferron on recovery were examined. The results demonstrated that Mo at pH range of 2.5–3.5 was quantitatively sorbed onto activated carbon as its cupferron complex. The optimum stirring time was found to be 30 min. The relative standard deviation was found to be 12% for 200 cm^3 50 ng/cm^3 Mo using 10 replicate preconcentration procedures. The limits of detection and quantification were found to be 1.0 and 3 ng/cm^3, respectively, by preconcentration of 200 cm^3 initial sample to 2 cm^3 final volume. As a result, an enrichment factor of 100-fold was achieved. The proposed preconcentration procedure was applied to determine Mo in biological samples such as vegetables, milk, and animal liver. The molybdenum concentrations were found (as μg/dm^3 or μg/kg) in the range of 70–5,500 for plants, 3–124 for milk and milk powder, and 960 for liver samples.

M. Yaman (✉)
Department of Chemistry, Science and Art Faculty,
Firat University, Elazig, Turkey
e-mail: myaman@firat.edu.tr

E. Yigmatepe
Cement Factory, Tatvan, Bitlis, Turkey

Keywords Spectroscopy · Metals · Ligands · Solid-phase extraction · Metal complexes

Introduction

Molybdenum is an essential element at trace level for plants, animals, and humans. It is a cofactor for several enzymes such as xanthine oxidase, sulfite oxidase, and aldehyde oxidase, and its role is strongly associated with the levels of Cu, Fe, and Zn. Furthermore, it is responsible for the initial stages of nitrogen, carbon, and sulfur metabolism of plants, animals, and human. Mo requirement for humans is approximately 25 μg or possibly less for each day, while intake of 150 μg/kg body weight may be toxic [1–5]. Molybdenum is added in trace amounts to fertilizers to stimulate plant growth. Molybdenum is also used as a component in glass, fertilizer, catalyst, and lubricant, and as an alloying element in steel. As a result, industrial effluents contain excess molybdenum that causes detrimental effects in animals and humans. Molybdenum poisoning causes severe gastrointestinal irritation with diarrhea, coma in ruminants, and death from cardiac failure. Excessive amounts of Mo may cause bone deformation, vulnerability of teeth, and disturbances in the metabolism of fats and proteins [6, 7]. Thus, monitoring of molybdenum in environmental and biological samples is necessary in order to know the exposure level of this element. Molybdenum as a microelement is of great importance because high concentrations of Mo in soils, sediments, and sludges, either from natural sources or through pollution, can enter the food chain through plant uptake, direct ingestion of soil by animals, or through water supplies [6]. Therefore, accurate determination of Mo at trace levels in environmental and biological samples is necessary.

There are many analytical techniques available for determination of Mo such as neutron activation analysis (NAA), inductively coupled plasma-mass spectrometry (ICP-MS), inductively coupled plasma-atomic emission spectrometry (ICP-AES), flame and flameless atomic absorption spectrometry (FAAS and ETAAS), voltammetry, spectrofluorimetry, and spectrophotometry [7–17]. Some of these techniques, including NAA and ICP-MS, are costly, and use of them is rather sophisticated. In comparison with FAAS, flameless AAS has some disadvantages such as its high cost, slowness, and greater proneness to interferences despite its advantage of high sensitivity. Therefore, it is recognized that FAAS should be preferred to flameless AAS if both of these methods can be applied for any sample [18, 19].

In environmental, geological, and biological samples the low concentration of molybdenum together with the high concentration of interfering matrix components often requires a preconcentration step combined with a matrix separation. Solid-phase extraction methods have been mainly developed and applied for molybdenum preconcentration and separation prior to its detection. The selection of a suitable preconcentration procedure depends considerably on the analyte concentration and the measurement method for its final determination. Thus, many separation and preconcentration procedures have been proposed, mainly based on sorption processes with activated carbon and chelating reagents. Other sorbents used for preconcentration procedures include amberlite XAD, muromac A-1, silica gel, polyurethane foam, and chelex 100 [7–10].

Activated carbon has been preferred for preconcentration of trace metals due to its advantages such as high sorptive properties, extensive porosity, and large size of available surface area. Adsorption of ionic species from solution is mainly controlled by electrostatic forces, which is related to the various surface functional groups. Selectivity and capacity of metal retention can be improved by using complexing agents in the sorption processes [18]. Reagents commonly used as complexing agent for this purpose include xylenol orange, thiocyanate, chlorosulfophenol S, toluene-3,4-dithol, 2-amino-4-chlorobenzenethiol, phenylfluorone, catechol violet, morin, 8-hydroxyquinoline, thiazolylazo compounds, 7,8-dihydroxy-4-methylcoumarin, bromopyrogallol red, and cupferron.

The present study aims at the determination of Mo in biological samples by FAAS using separation and preconcentration procedures. This method is based on sorption processes with activated carbon and cupferron. The parameters affecting recovery were optimized. In digestion of samples, aqua regia, HCl, H_2O_2, HNO_3, and $HClO_4$ and their mixtures were examined to extract Mo from studied matrices and to elute adsorbed Mo complexes on activated carbon. The optimum conditions were applied to determination of Mo in various biological samples including vegetables, milk, and liver.

Results and discussion

In developing the analytical scheme, the parameters including pH of solution, amounts of complexing agent and activated carbon, and stirring time were investigated for 100 cm^3 100 ng/cm^3 Mo solution containing matrix components.

Effects of parameters on recovery

The influence of solution pH on recovery was investigated using a model solution of 100 cm^3 100 ng/cm^3 Mo. For this purpose, the solutions were preconcentrated using pH values in the range of 2.0–7.0. It can be seen from Fig. 1 that the highest recovery of 85% was obtained by using 40 mg cupferron (20 cm^3 of 0.2%) within the pH range from 2.5 to 3.5. Taking into consideration these results, pH 3.0 ± 0.2 was chosen. The obtained recoveries less than 100% may be explained by dissociation of the Mo–cupferron complex, and/or insufficient adsorption of this complex onto activated carbon. However, the relatively low recoveries are not a defect, because of the good precision of recoveries of 85%. On the other hand, insufficient recoveries were found (Fig. 1) when using 20 cm^3 of the other studied complexing reagents: 4-(2-pyridylazo)resorcinol (PAR) 60% at pH 2.5, 1-(2-pyridylazo)-2-naphthol (PAN) 50% at pH 3.5, and oxine (8-hydroxyquinoline) 30% at pH 3.0. Accordingly, cupferron was chosen in all subsequent studies. To determine the optimum amount of cupferron, it was found that 30 mg cupferron was sufficient for maximum recovery (Fig. 2). Thus, 40 mg cupferron

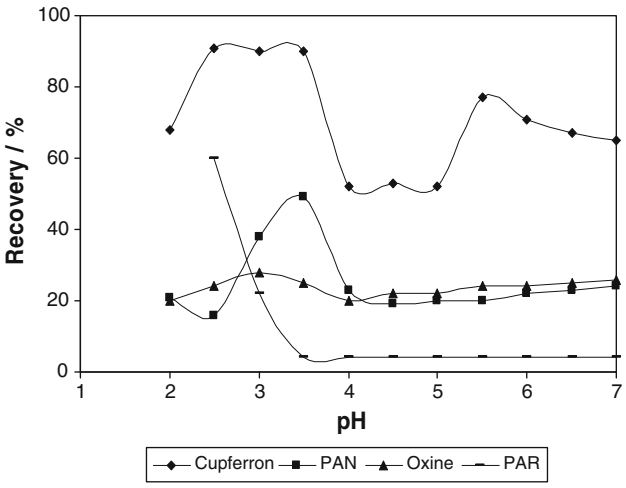

Fig. 1 Effect of pH and complexing agent on recovery

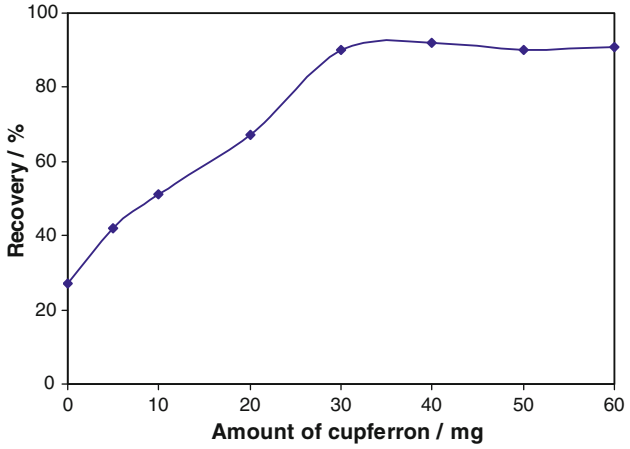

Fig. 2 Effect of cupferron amount on recovery

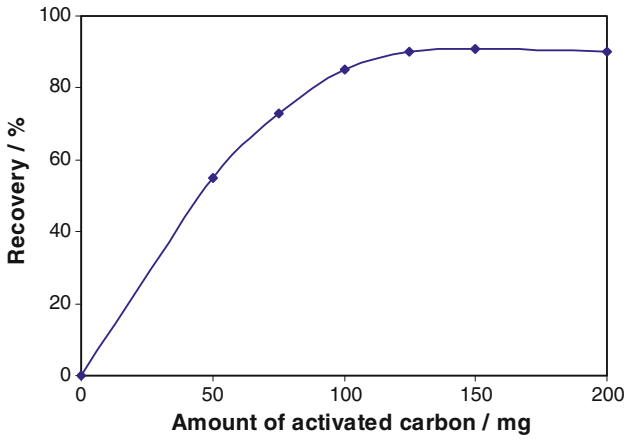

Fig. 3 Effect of activated carbon amount on recovery

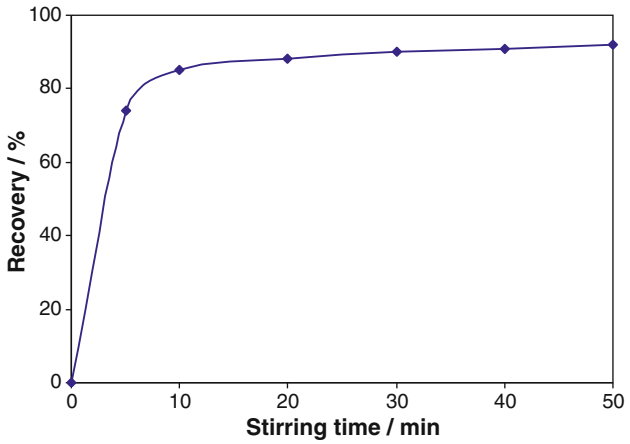

Fig. 4 Effect of stirring time on recovery

was used in subsequent studies. From Fig. 3, it was observed that 125 mg activated carbon was sufficient for maximum recovery. The stirring time required to reach equilibrium and sorption of the complex was found to be 30 min (Fig. 4). As a result, stirring time of 30 min was applied in subsequent studies. The influence of buffer amount was tested by keeping all other experimental variables constant. The results showed that, by adding 10.0–50.0 cm^3 buffer solution, no obvious variation in recovery occurred. Accordingly, for all subsequent experiments, 20 cm^3 citrate buffer solution was used.

To determine which digestion reagent is better in the elution step, different volumes between 2.0 and 8.0 cm^3 of aqua regia, concentrated HNO_3, HCl, H_2O_2, and $HClO_4$ and their mixtures were tested for back-extraction from activated carbon. The results demonstrated that 4 cm^3 aqua regia can be used for desorption of adsorbed molybdenum before the evaporation step in the preconcentration procedure.

Analytical performance

The relative standard deviation (RSD) was found to be 12% for 200 cm^3 of 50 ng/cm^3 using 10 replicate preconcentration procedures. The limits of detection and quantification were 1 and 3.0 ng/cm^3, respectively, for initial volume of 200 cm^3. The achieved preconcentration factor was found to be 100, which can be further improved by using more samples. The possibility of sample contamination was examined by subtracting the values obtained for blanks. Adsorption losses can be excluded, as the procedure was followed in exactly the same way, using the same glassware and the same reagents throughout the study. The results showed that there was no contamination or adsorption loss under the conditions studied. It is known that the reliability of a method can be checked by using standard reference materials and/or independent analytical methods and/or recovery tests. In this study, the accuracy of the method was checked by examining a standard reference material (bush branches and leaves-NCS DC73348). The results are given in Table 1. It can be seen that the recovery values were found to be at least 92%. Because the standard reference leaves have included elements such as Ca, Na, K, Si, Mg, Al, and Fe at major and minor level, it can be said that there is no interference from these metals at those levels. Furthermore, the accuracy of the optimized method was studied by carrying out recovery tests from plant samples fortified with Mo. The following amounts of molybdenum were added: 0.5 mg/kg for white cabbage, 0.2 mg/kg for red cabbage, 0.9 mg/kg for canned peas, and 1.7 mg/kg Mo for soybean. After application of the optimized method as described above, recoveries of 94–100% were found (Table 1).

Applications

The optimized preconcentration and separation method was applied to the determination of Mo in biological samples including cabbages, canned peas, soybean, garlic,

Table 1 Obtained Mo concentrations in biological samples by the optimized preconcentration method ($n = 3$)

Sample	Dry matter (%)	Ashing time at 550 °C (h)	Added Mo (μg/kg)	Found Mo ± SD (μg/kg)	Recovery (%)
White cabbage	8.8	5	0	900 ± 55	96.7
			500	1,350 ± 120	
Red cabbage	9.0	5	0	290 ± 30	94.3
			200	460 ± 20	
Canned peas	25	7	0	1,400 ± 86	100.5
			900	2,310 ± 210	
Soybean (dry)	95	5	0	5,500 ± 280	98.8
			1,700	7,110 ± 570	
Garlic-1	30	5		90 ± 10	
Leaf of garlic-1	15	2		70 ± 10	
Garlic-2	50	5		140 ± 14	
Strawberry	9	5		250 ± 22	
Liver				960 ± 110	
Cow's milk				3.0 ± 0.2 μg/dm^3	
Pasteurized milk				7.0 ± 0.5 μg/dm^3	
Milk powder				124 ± 7	
SRM (bush branches and leaves)	Certified: 0.26 μg/g			0.24 μg/g	92.3

SD standard deviation

strawberry, milk, and liver. The obtained results are given in Table 1. These results are mean values of three different portions of the same sample. The Mo concentrations were found to be in the range of 70–5,500 μg/kg for plant samples, the lowest of which was recorded for the leaf of garlic while the highest for soybean. Mo levels in soybean samples were determined as 1,080, 1,250, and 2,950 μg/kg by Comitre and Reis [20], Zaijun et al. [21], and Lavado et al. [22], respectively. Koplik et al. [23] determined Mo levels in pea samples in the range of 1,230–3,010 μg/kg. Lopez-Garcia et al. [8] determined Mo levels in ranges of 0.7–4.1 μg/dm^3 for breast milk, 14.7–17.3 μg/dm^3 for commercial cow's milk, and 140–260 μg/kg for milk powder, by using ion-exchange chromatography and ETAAS. Wappelhorst [24] determined Mo levels in human milk between 0.27 and 1.62 μg/dm^3 with a mean value of 0.72 μg/dm^3. Zou et al. [25] determined Mo levels in a liver sample as 7,560 μg/dm^3, and Jiang et al. [12] obtained Mo contents in pig liver as 4,000 μg/kg. Investigating analytical parameters such as sensitivity in the preconcentration procedure, Agrawal and Sharma achieved 30-fold increase in sensitivity by using liquid–liquid extraction and ICP-AES [9]. Similarly, Madrakian and Ghazizadeh and Lopez-Garcia et al. [7, 8] obtained 20- and 30-fold increases in sensitivity using cloud-point extraction and ion-exchange chromatography methods for preconcentration, respectively. A 100-fold increase in sensitivity was achieved by dos Santos et al. [10] by using calmagite and activated-carbon ICP-AES. In this study, 100-fold increase in sensitivity was achieved by using 200 cm^3 initial volume and 2.0 cm^3 final elution volume. Our Mo levels in soybean were found to be higher than the levels in the literature [20–22]. Mo concentration in canned peas was found to be 1,400 μg/kg. This value matches well with the values reported by Koplik and co-workers [29]. Mo contents were found to be 3.0 μg/dm^3 for cow's milk, 7.0 μg/dm^3 for pasteurized milk, and 124 μg/kg for milk powder. These results are similar to the results reported by Lopez-Garcia [8] and Wappelhorst [24].

In conclusion, a simple and reliable preconcentration method was modified using adsorption on activated carbon for determination of Mo in biological samples. The sensitivity of FAAS was increased up to 100 times by using the optimized method, which can be increased further if necessary. The proposed preconcentration and separation procedure can also be applied to determination of trace molybdenum in various biological samples. Selectivity of the method was increased by a preconcentration procedure in the low pH range of 2.5–3.5. The validity of the procedure was confirmed by recoveries of Mo spiked to the samples.

Experimental

Apparatus

An ATI UNICAM model 929 FAAS, equipped with ATI UNICAM hollow cathode lamp and deuterium background

Table 2 Operating parameters for FAAS

Parameters	Mo
Wavelength (nm)	313.3
Lamp current (mA)	10
Type of flame	C_2H_2–N_2O
Background correction	DL
Slit width (nm)	0.5
C_2H_2 flow rate (dm^3/min)	4.2
N_2O flow rate (dm^3/min)	4.7

DL deuterium lamp

correction, was used for molybdenum determinations. The optimum conditions for FAAS are given in Table 2. The other apparatus used were magnetic stirrer (Velp Scientifica), pH meter (SCHOTT LabStar), and centrifuge (Heittich EBA III).

Reagents

Unless otherwise stated, all chemicals (Merck) used were of analytical-reagent grade. Throughout all analytical work, double-distilled water was used. All Pyrex glassware was kept permanently filled with 1 M nitric acid when not in use. In digestion work, aqua regia (1 volume HNO_3 plus 3 volumes HCl) was used. Stock standard Mo solution (1,000 mg/dm^3) was prepared by dissolving $(NH_4)Mo_7O_{24}{\cdot}4H_2O$ in diluted (1/20) aqua regia.

Buffer solutions in the pH range of 2.0–7.0 ± 0.2 were prepared by using 0.1 M citric acid plus 0.1 M HCl/0.1 M NaOH; 0.1 M potassium phthalate ($C_8H_5KO_4$) plus 0.1 M HCl; and 0.1 M KH_2PO_4 plus 0.1 M NaOH solutions.

Solution of 0.2% cupferron (*N*-nitrosophenylhydroxylamine) was prepared by dissolving 0.20 g of the substance in 100 cm^3 ethyl alcohol. Oxine (8-hydroxyquinoline) solution of 0.2% was prepared by dissolving 0.20 g oxine in 100 cm^3 distilled water. Solution of 0.05% 1-(2-pyridylazo)-2-naphthol (PAN) was prepared by dissolving 0.05 g in 100 cm^3 ethyl alcohol. Solution of 0.05% 4-(2-pyridylazo)resorcinol (PAR) was prepared by dissolving 0.05 g PAR in 100 cm^3 distilled water.

Activated carbon (Merck, analytical grade) passing through a 325 mesh sieve (Taylor) was purified by means of treatment with concentrated HCl for 3 h, washing with distilled water, drying at 110 °C, and treating with aqua regia for 24 h. The mixture was filtered through filter paper (Advantec Toyo 5B, white ribbon), washed with distilled water, and dried at 110 °C. A suspension of 25 mg/cm^3 in distilled water was prepared from the dried activated carbon as described elsewhere [26].

Sample preparation

The studied samples include cabbage, canned peas, soybean, garlic, strawberry, milk, and liver. Plant samples were taken from major agricultural areas of Elazig, Turkey, known to be uncontaminated with Mo. The plant samples were collected in plastic bags. They were washed separately and thoroughly with running tap water and further rinsed twice with distilled water, then left on a filter paper for draining. The samples were cut into small pieces, and dried at 85 °C for 24 h.

Dry ashing of plants

After grinding and homogenizing, approximately 2.0–10.0 g of the dried plant samples depending on their Mo concentration was placed into evaporating dishes. Then they were ashed at 550 °C in an ashing furnace for 2–7 h (Table 1) using different ashing time depending on sample species. A 4.0 g portion of the standard reference material (bush branches and leaves-NCS DC73348) was also digested by using dry ashing as described above. The residue was digested in aqua regia and diluted to 100 cm^3 with distilled water. Then, the optimized preconcentration procedure was applied to the sample.

Wet ashing of milk samples

The cow's milk samples were collected in precleaned polyethylene bottles. After transferring 200 cm^3 milk sample into a Gorsuch flask [27], 40 cm^3 aqua regia was added. The mixture was heated and evaporated by stirring at 100 °C until obtaining a clear digest as described elsewhere [27]. The clear digest was diluted to 200 cm^3 with distilled water. Then, the optimized preconcentration procedure was applied to the samples by using 2 cm^3 final volume. For the milk powder, a 5.00 g sample was digested in 10 cm^3 concentrated aqua regia in the same way. The liver samples were digested similarly to the milk powder. The clear digest was diluted to 100 cm^3 with distilled water. A blank digest was carried out in the same way.

Preconcentration procedure

The batch method was applied usefully for preconcentration and determination of trace elements by FAAS [28–32]. For this purpose, 100 cm^3 100 ng/cm^3 Mo was used as model solution. The pH of the solution was adjusted to the desired value by adding solution of 0.1 M HCl and/or NaOH. After adding the necessary buffer solution (20 cm^3), 20 cm^3 0.2% cupferron was added. Then, 5 cm^3 activated carbon suspension was added, and the pH of the mixture was adjusted to the desired value again, if necessary. The mixture was stirred mechanically for 30 min and filtered through a filter paper (Advantec Toyo 5B, white

ribbon). The residue was dried at 105 °C. After transferring the residue to a glass beaker, 4 cm^3 concentrated aqua regia was added and evaporated to near dryness. Finally, 2.0 cm^3 diluted (1/20) aqua regia was added and centrifuged two times. The clear solution was measured by FAAS. The blank was treated in the same way.

Optimization of parameters

Parameters thought to affect the preconcentration and measurement steps in the analytical procedure were examined, including acidity of solution, amounts of ligand and adsorbent, and stirring time. In developing the analytical scheme, each parameter was investigated for 100 cm^3 100 ng/cm^3 Mo solution containing matrix components described below. The effect of each parameter listed was tested three times.

Calibration

It is important to study the effect of matrix components because some of these components form chelates with complexing agents, and can interfere in the adsorption steps. In the preconcentration procedure, matrix components were added to all Mo solutions at the following concentrations (μg/cm^3): Ca^{2+} 200, Mg^{2+} 100, Zn^{2+} 50, Fe^{3+} 4, Cu^{2+} 4, Mn^{2+} 4 to represent the plant samples. These solutions were referred to as model solutions. After applying the optimized preconcentration procedure, calibration curves were examined at two different concentration ranges depending on initial sample volume. Firstly, a calibration graph was obtained for Mo solutions from 3.0 to 50 ng/cm^3 using initial sample volume of 200 cm^3. Secondly, a calibration graph was obtained for Mo solutions from 6.0 to 100 ng/cm^3 using initial sample volume of 100 cm^3. The obtained graphs were rectilinear in the concentration range described above. The equations of the curves for solutions prepared in both diluted (1/20) aqua regia and 1 M nitric acid, separately, were as follows (Y absorbance and X concentration):

$$Y = 27.94X - 3.8 \quad R^2 = 0.999; \quad \text{in diluted } (1/20) \text{ aqua regia} \tag{1}$$

$$Y = 4.497X - 1.5 \quad R^2 = 0.996; \quad \text{in 1 M of nitric acid} \tag{2}$$

Using initial volume of 200 cm^3, the difference in the slopes of equations may be attributed to the formation of thermally stable oxide compounds such as MoO_2, MoO_3, and Mo_4O_{11} in spite of using a N_2O/C_2H_2 flame. The formation of these compounds can be hindered in aqua regia medium. Consequently, calibration solutions prepared in diluted aqua regia medium were used in subsequent studies due to the high Mo sensitivity in this medium.

Using initial volume of 100 cm^3, the equation of the curve for solutions prepared in diluted (1/20) aqua regia was as follows:

$$Y = 13.73X + 0.62 \quad R^2 = 0.996 \tag{3}$$

The calibration graph obtained by using initial volume of 200 cm^3 was used for determination of Mo in cow's and pasteurized milk samples. The calibration graph obtained by using initial volume of 100 cm^3 was used for determination of Mo in other samples.

Acknowledgments This study was supported by the Scientific Investigate Projects of Firat University (FUBAP-758).

References

1. Vyskocil A, Viau C (1999) J Appl Toxicol 19:185
2. Seifert M, Dorn W, Muller R, Holzinger S, Anke M (2009) Acta Aliment Hung 38:471
3. Pyrzynska K (2007) Anal Chim Acta 590:40
4. Patriarca M, Menditto A, Di Felice G, Petrucci F, Caroli S, Merli M, Valente C (1998) Microchem J 59:194
5. Holzinger S, Anke M, Rohrig B, Gonzalez D (1998) Analyst 123:447
6. Das AK, Chakraborty R, Cervera ML, de la Guardia M (2007) Talanta 71:987
7. Madrakian T, Ghazizadeh F (2008) J Hazard Mater 153:695
8. Lopez-Garcia I, Vinas P, Romero-Romero R, Hernandez-Cordoba M (2007) Anal Chim Acta 597:187
9. Agrawal YK, Sharma KR (2005) Talanta 67:112
10. dos Santos HC, Korn MGA, Ferreira SLC (2001) Anal Chim Acta 42:79
11. Burguera JL, Burguera M, Rondon C (2002) Talanta 58:1167
12. Jiang C, Wang J, He F (2001) Anal Chim Acta 439:307
13. Yigmatepe E, Avci H, Yaman M (2010) Asian J Chem 22:1829
14. Felipe-Sotelo M, Carlosena A, Fernandez-Fernandez E, Muniategui S, Lopez-Mahıa P, Prada D (2004) Anal Chim Acta 524:329
15. Gil RA, Pasini-Cabell S, Takara A, Smichowski P, Olsina RA, Martinez LD (2007) Microchem J 86:156
16. Ensafi AA, Khayamian T, Atabati M (2002) Talanta 57:785
17. Jiao K, Jin W, Metzner H (2001) Anal Chim Acta 260:35
18. Senkal BF, Ince M, Yavuz E, Yaman M (2007) Talanta 72:962
19. Kaya G, Yaman M (2008) Talanta 75:1127
20. Comitre ALD, Reis BF (2003) Anal Chim Acta 479:185
21. Zaijun L, Yuling Y, Jian T, Jiaomai P (2005) J Food Compos Anal 18:561
22. Lavado RS, Porcelli CA, Alvarez R (2001) Soil Till Res 62:55
23. Koplik R, Borkova M, Mestek O, Kominkova J, Suchanek M (2002) J Chromatogr B 775:179
24. Wappelhorst O (2002) Nutrition 18:317
25. Zou X, Li Y, Li M, Zheng B, Yang J (2004) Talanta 62:719
26. Avci H, Yaman M (2006) At Spectrosc 27:117
27. Yaman M (1998) Microchim Acta 129:115
28. Kaya G, Akdeniz I, Yaman M (2008) At Spectrosc 29:150
29. Yaman M, Ince M (2008) At Spectrosc 27:186
30. Yaman M, Gucer S (1995) Analusis 23:168
31. Gucer S, Yaman M (1992) J Anal At Spectrom 7:179
32. Yaman M (2001) Spectrosc Lett 34:763