An Improved Thiocyanate Method for Determination of Molybdenum using Thioglycollic Acid as Reductant

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Abstract: An improved method for determination of Mo by thiocyanate has been standardized by employing thioglycollic acid, instead of currently employed stannous chloride, as reductant of Mo. The method ensures stability of the colored complex in aqueous acidic medium for about 3.5 hours which is otherwise unachievable and the color unstable while using stannous chloride. The method is linear over 0.5 to 15 ppm Mo in a 2 mL sample with regression estimate in units of absorbance value per µg Mo, b ± s.e., 0.0268 ± 0.0002 and r ± s.e., 0.9998 ± 0.0002 (n = 30). The detection limit can be further reduced to 0.1 ppm by using 8 mL sample and extracting the complex into an organic solvent: 3 mL diethyl ether and 1 mL petroleum ether. The method has been coupled to an extraction protocol employing 0.5M NaOH to extract added Mo from soil, and from water in presence of high metal load. Per cent recovery of added Mo has been 97.3 ± 0.8 (n= 30) with mean per cent accuracy (n=30) and COV (n=4) about 4 each. The method is suitable for monitoring Mo in environmental and other samples including biosolid wastes having toxicological implications.
Keywords: Molybdenum, Thiocayanate, Thioglycollic acid, Technique.

INTRODUCTION

Molybdenum is important from nutritional, industrial, environmental and toxicological considerations. It is present in water, soil, plant materials, mineral ores and environmental bio-solid wastes. Nutritionally, it is required in traces by animals, humans and nitrogen fixing microbs in soil[1-3]. High levels of Mo in soil are toxic to plants, and to animals through plants. High dietary intake causes molybdenosis, teart disease, in domestic animals, especially to bovine species[2-4], and has been linked epidemiologically to human disorders[5]. The determination of Mo is required for evaluation of natural[6-9], industrial[10, 11] and toxicologically important environmental biosolid waste samples[12]. Two colorimetric techniques are commonly recommended for determination of Mo: a) dithiol method[10, 13] and b) thiocyanate method [7, 10, 11]. The thiocyanate method is sensitive and more selective than dithiol method[10, 11]. Consequently, the method is widely used, constitutes an official method[7] and has been recommended for determination of micro quantities of Mo in industrial samples[11]. The major limitation with the technique is very high instability of the color complex in acidic medium necessitating rapid extraction of the complex into organic solvents to prevent fading of color. A need to develop an alternative protocol was mooted. Initially, a number of reducing agents were screened for their potential applications. Thioglycollic acid was chosen for standardization on three considerations. Firstly, it is a

selective and sensitive reagent for Mo, iron, tin and silver[14], and the color complex with iron is measurable only in alkaline medium[1]. Secondly, it has a good reducing property owing to –SH function and the reducing potential is maintained even following its oxidation to final end product oxalic acid. Thirdly, during the course of investigations it was observed to mask the color produced by interaction of thiocyanate and iron (ferrous and ferric) while intensifying the color produced by interaction of Mo and thiocyanate under identical conditions. Consequently, the experiments were designed to optimize the technique for anticipated applications.

MATERIALS AND METHODS

The chemicals used were of analytical-reagent grade. The experiments were carried out at 26 ± 2 ^{0}C (range 14 to 33 ^{0}C). Double-distilled water was used for dilutions, washings and preparation of reagents.

Reagents and materials

Standard molybdenum solution: Stock solution of 1000 ppm Mo in water was prepared by dissolving 185 mg ammonium molybdate in 100.5 ml water. Appropriate working solutions were made by dilution in water.

Thioglycollic acid solution (TGA): Freshly prepared 10% or 20 % (v/v) thioglycollic acid in water.

Potassium thiocyanate solution: 10% (w/v) in water

Sodium hydroxide solution: Approximately 2.5 M NaOH was made by dissolving 10 g sodium hydroxide in water to make 100 mL. Appropriate strengths were made by dilution in water.

Concentrated hydrochloric acid: The strength of the acid was approximately 11.7 M.

Ferrous ammonium sulfate: Freshly prepared at the time of use as 1 % (w/v) in 0.1 % sulfuric acid.

Stannous chloride: Freshly prepared as10 % (w/v) in 10 %(v/v) HCl. The solution was lightly warmed to get a clear solution.

Ferrous sulfate solution: Freshly prepared as 0.2M in water.

Activated charcoal: Acid and alkali washed activated charcoal was thoroughly washed with deionized water and dried to free flowing powder over hot plate.

Metal cocktail solution: Metal cocktail was prepared from zinc sulfate, copper sulfate, ferrous sulfate, ferric chloride, aluminum sulfate, cadmium chloride, mercuric chloride and manganese chloride by dissolving appropriate masses of salts in water to provide approximately 1000 ppm of each of the metals, zinc, iron (ferrous and ferric ratio 1:1), copper, manganese, aluminum, cadmium and mercury.

Soil samples: Random topsoil samples, within 10 cm soil depth, were collected from the Faculty premises, and from arable land of local village, Alesteng. The samples were air-dried, finely pulverized and sieved to remove visible debris.

Optimization studies

The reagents were optimized with respect to requirements of thioglycollic acid and hydrochloric acid, and of alkali hydroxide for facilitating extraction of Mo from *in vitro* simulation experiments. Consequently, TGA and HCl were tested, respectively, over 1 to 50 mg, and 0.8 to 2.0 mL in a 4 mL reaction volume. The effect of varying NaOH from 0.5 to 2 M on absorbance values while using standard mass of 1.5 mL HCl was determined. The necessity of reduction process for development of the color complex was evaluated by monitoring alteration in absorbance values in presence of oxidizing agent, hydrogen peroxide. Organic solvent mixtures were also screened for their efficiency in extracting thiocynanate-Mo color complex for determining low Mo contents. For this study two solvent mixtures were selected and compared, on the basis of trial experiments. Solvent mixture-I included diethyl ether and petroleum ether (3:1). Solvent mixture-II included iso-amyl alcohol, chloroform and diethyl ether 2:2:3. The mixtures were used, respectively, in 4 and 3 mL volumes per extraction.

Comparative stability

The stability of thiocyanate-Mo complex developed in presence of TGA, and stannous chloride was determined by monitoring absorbance values over three and a half hour, and after 48 hours standing at room temperature while using 30 µg Mo. One set was subjected to the standardized protocol (*vide infra*). The second set was assayed by standard stannous chloride method[10] with reagents scaled down to match the standardized method: each 1.5 mL sample was added 0.4 mL HCl, 1 mL ferrous ammonium sulfate solution, 0.6 mL thiocyanate solution and 0.5 mL stannous chloride solution. The absorbance values were monitored at 465 nm ((UV-Visible Spectrophotometer SL-150, Elico (India) Ltd, Hyderabad).

Standard analytical procedure

A two milliliter aqueous sample containing 0, 1, 3, 10, 20 and 30 ppm Mo in 0.5 M NaOH was added 0.1 mL 10% TGA followed by 1.5 mL HCl. The samples were added 0.4 mL potassium thiocyanate, gently mixed and allowed standing at room temperature for about one hour for color development. The color was monitored spectrophotometrically at 465 nm against reagent blank.

Protocol for low Mo concentration

The outlined protocol was adopted to measure Mo in concentrations 0.1 through 1 ppm by taking 8 or 10

mL sample volume. The following reagents were added per mL sample in that order: NaOH 2.5M, 0.25 mL; 20% TGA, 0.05 mL; HCl, 0.75 mL and 10% KSCN, 0.2 mL. The samples were mixed, and allowed standing at room temperature for 30 minutes. The addition of the hydroxide was omitted if the sample *per se* was in 0.5M alkali hydroxide. The colored complex was monitored spectrophotometrically following its extraction into organic solvent mixture-I chosen for application.

Recovery of Mo from water in presence of metallic salts

Recovery studies were conducted in presence of metal load to test efficiency of extraction protocol to extract Mo in presence of high metal load. Each 10 mL water sample, containing Al, Cd, Cu, Mn, Fe, Hg and Zn as 500 ppm with 0, 5, 10 and 50 ppm Mo was processed for extraction of Mo. The samples were added 2.5 M alkali hydroxide @ 0.25 mL per mL solution, allowed to stand 5 minutes following thorough shaking, and centrifuged at 6000 rpm for 5 minutes. Two milliliter aliquots of decanted supernatant were assayed as per outlined protocol using reagent blank and appropriate corresponding standards in 0.5M NaOH.

Recovery of Mo from soil

Each 5 g soil sample was added 0 and 30 µg Mo to provide 0 and 6 ppm Mo. The soil samples were extracted with 15 mL of 0.5 N NaOH for about an hour at room temperature with intermittent shakings. Each sample was added activated charcoal @ 50 mg mL^{-1} solution, thoroughly mixed, and centrifuged (6000 rpm x 5 min). An aliquot of 2 ml was processed for Mo determination as per outlined protocol. Standard samples were prepared in 0.5 N NaOH, and assayed against reagent blank. Mo treated soil samples were read against untreated soil blank, since the soil did not contain detectable Mo.

Mo content in top soil of arable land

The sample sizes varied from 6 to 8 g per extraction. The samples were visibly rich in humus. The alkali hydroxide extract obtained was greenish even upon charcoal treatment. Consequently, the depigmentation was facilitated following verification by trial experimentations by addition of 0.5 mL 0.2M NaOH followed by 0.5 mL 0.2 M ferrous sulfate solution for each 4 mL extract. The ferrous hydroxide matrix provided clear extract with faint palish tinge that disappeared on acidification during the conduct of the assay. To improve detection of low levels of Mo in the local soil, the sample size was increased from 6 to 8 g per extraction ensuring the content of nearly 3 g soil in organic phase. The color complex was extracted at 30th minute with 4 mL of organic solvent, and monitored spectrophotometrically with respect to simultaneously run standards subjected to identical treatments.

Table I. Stability of molybdenum thiocyanate complex in presence of thioglycollic acid and stannous chloride

Time, hr	Absorbance values at 30 µg Mo	
	Thioglycollic acid	**Stannous chloride**
0.25	0.782 ± 0.006^{a}	0.733 ± 0.006^{c}
0.5	0.778 ± 0.006^{a}	0.640 ± 0.005^{c}
1	0.777 ± 0.005^{a}	0.532 ± 0.007^{c}
2	0.758 ± 0.006^{a}	0.415 ± 0.006^{c}
3.5	0.752 ± 0.007^{a}	0.298 ± 0.007^{c}
48	0.665 ± 0.006^{b}	0.075 ± 0.003^{c}

The values are Mean ± S.E. of six observations each; [a] the values are not significantly different (P>0.1); [b] the value is significantly different from preceding values (P<0.01); [c] the values are significantly different (P<0.01).

Table-II. Linear relationship between absorbance values and Mo mass

Molybdenum mass, $µg.ml^{-2}$	Absorbance values[a]
1	0.023 ± 0.002
3	0.081 ± 0.001
10	0.277 ± 0.001
20	0.542 ± 0.004
30	0.803 ± 0.005
Statistical analysis	
r ± S.E.	**b ± S.E.**
0.9998 ± 0.0002	0.0268 ± 0.0002

[a]The absorbance values are Mean + S.E. of six observations

Table-III. Absorbance values at low Mo concentration with organic solvent extraction

Mo ppm	Mo total	Absorbance values	
		Solvent-I	Solvent-II
0.05	0.4	-	0.013 ± 0.003
0.1	0.8	0.033 ± 0.003	0.033 ± 0.003
0.3	2.4	0.090 ± 0.007	0.120 ± 0.006
0.5	4.0	0.146 ± 0.008	0.213 ± 0.012
1.0	8.0	0.302 ± 0.012	0.377 ± 0.012
Statistical features			
r ± s.e.		0.9996 ± 0.0004	0.9968 ± 0.0029
b ± s.e.[a]		0.0374 ± 0.0005	0.0483 ± 0.0017
b ± s.e.[b]		0.1498 ± 0.0021	0.1448 ± 0.0052
r ± s.e[c].		0.9974 ± 0.0017	
b ± s.e[c]		0.1461 ± 0.0035	

Solvent-I: 4 mL (diethyl ether and petroleum ether 3:1), n = 4 each; solvent-II: 3 mL (iso-amyl alcohol, chloroform and diethyl ether 2:2:3), n=3 each; [a]Mo(µg total) vs. mean absorbance values; [b]Mo (µg mL^{-1} solvent) vs. mean absorbance values; [c]over all Mo (µg mL^{-1} solvent) vs. mean absorbance values

Table-IV. Recovery of Mo added to soil and water samples

Sample	Mo added, µg	Treatment	Mo Recovered µg	%	A[b]	COV	n
Water	5	Metal load	4.7 ± 0.05	93.2 ± 1.0	6.8 ± 1.0	2.4	5
	10	Metal load	9.8 ± 0.1	98.3 ± 1.1	2.9 ± 0.6	3.5	12
	50	Metal load	49.2 ±1.0	98.4 ± 2.1	4.0 ± 0.9	4.5	5
Soil	30	Charcoal[a]	29.3± 0.5	97.7 ± 1.7	4.5 ± 1.0	4.8	8

Metal load Al, Cu, Cd, Hg, Mn, Fe (1:1 ferrous and ferric) and Zn 500 ppm each; [a] charcoal added @ 50 mg mL^{-1} extractant 0.5 M NaOH; A[b] is per cent accuracy; COV is coefficient of variation; n , number of observations.

RESULTS AND DISCUSSION

Thiocynate technique for determination of Mo is based on the principle of reduction of Mo in acidic solution from oxidation state (VI) to reduced state (V) using stannous chloride. Reduced Mo complexes with thiocyanate to form dominantly a reddish complex, Mo (SCN) $_5$. The complex is immediately extracted into either iso-amyl alcohol [6-8, 10] or ether[11, 15] to prevent fading of the color due to its instability. Milder reductants like hydrazine sulfate, ascorbic acid, potassium iodide or even photochemical reduction with high acidity have been employed to overcome the difficulties in using stannous chloride which reduces Mo (VI) to a lower grade than Mo (V)[16, 17]. However, the popularity of the stannous chloride continues to remain unsurpassed. The present investigation aimed to identify an alternative reducing agent that would improve the stability of the color complex in acidic medium. A number of reducing agents were screened for the purpose including zinc dust, hydroxylamine hydrochloride, thiosulfate, metol, potassium ferrocyanide, ferrous sulfate and TGA. Out of these, only ferrous sulfate and TGA produced stable color complex over a range of Mo concentrations , 4 to 40µg, with perfect linearity (r=0.999, b= 0.015 and 0.014, respectively). Ferrous sulfate was rejected as it *per se* reacted with thiocyante to form light greenish ferrous thiocyanate complex and, therefore, required addition of thioglycollic acid to mask the background color. Subsequently, thioglycollic acid alone was retained and optimized for use.

Optimization studies

Thioglycollic acid, 10 to 30 mg in 4 mL reaction volume, provided optimal and consistent response. The concentrations beyond the range were either less effective or detrimental to the complex. The mean absorbance value produced with 10 mg TGA, 0.025 ± 0.002, did not differ from the value, 0.022 ± 0.002 (n=4 each), produced with 20 mg TGA.The strength of HCl required for obtaining optimal magnitude of absorbance values (range 0.518 ± 0.003 to 0.563 ± 0.003 at 20 µg Mo) ranged from ca. 2.4 to 4.4 M, provided by 0.8 to 1.5 mL HCl in 4 mL volume. Increase in acid concentration to 5.9 M, 2 mL HCl, caused significant reduction in absorbance value,

0.408±0.003 (P<0.01, n= 4 each). Based on these findings, 1.5 mL HCl and 10 mg thioglycollic acid were chosen for the study. In presence of hydrogen peroxide, ca. 9 mM, the mean absorbance value at 3 μg Mo, 0.048±0.002, was significantly lower than the value, 0.083 ±0.002, observed in absence of the oxidizing agent (P<0.1, n=5 each). The observation implied necessity of maintaining reduced environment, and absence of oxidizing agents from the reaction mixture for conduct of the method.

Stability study
Evaluation of the data (Table 1) reveals significant reduction in absorbance values with each chosen sampling time while employing stannous chloride as reductant (P<0.01) with about 59 per cent reduction over 3.5 hr observation period compared to about 4 per cent alteration observed with TGA with no significant difference over the sampling times (P>0.1). The mean absorbance value with TGA, even following 48 hr standing, showed hardly 15 per cent decline compared to 90 per cent reduction observed with stannous chloride. Perusal of the data reveals 30 to 60 minute time period as most appropriate for monitoring absorbance values while using the proposed method.

Linearity studies with and without organic extraction
The optimized method is linear over 1 to 30 ppm Mo in 2 ml sample aliquots with perfect linearity (r ± s.e., 0.9998 ± 0.0002; b ± s.e., 0.0268 ± 0.0002) (Table 2). This suggests minimum detection limit of 0.5 ppm Mo. With organic extraction and while using increased sample volume, the method is linear over 0.1 to 1 ppm Mo
(r = 0.9999 ±0.0001; b =0.0347 ± 0.0002) (Table 3).
The color complex is quite stable in acidic medium and obviates the use of organic extraction to overcome instability. The extraction of the complex into an organic phase will be required only for determination of low levels of Mo, less than 1 ppm down to 0.05 ppm. This would require an increase in sample volume with a proportional increase in required reagents. An 8 to 10 mL aliquot is quite satisfactory for detection of 0.1 ppm Mo. The standard rates of additions per mL sample solution include: i) NaOH, in case the sample is not in 0.5 M alkali *per se*, 0.25 mL 2.5M NaOH, ii) TGA   0.05 mL 20% solution, iii) concentrated HCl 0.75 mL, and iv) 0.2 mL 10% KSCN solution.

Organic solvent extraction
Based on the report that organic solvents with donor oxygen function are most suited for extraction of reduced thiocyanate Mo color complex[10], diethyl ether was employed to extract the color complex for measuring low Mo concentrations. The solvent posed

problems due to its high volatility. Consequently, it was screened in combination with other organic solvents with the objectives: to allow organic phase to be on top of the aqueous phase for easy aspiration, to provide maximal absorbance with single extraction, and to reduce overall volatility of organic phase. Two formulations performed excellently: diethyl ether and petroleum ether (3:1), and isoamyl alcohol, chloroform and diethyl ether (2:2:3). The experience with isoamyl alcohol alone has not been satisfactory. It exhibited poor extraction ability, and the phase was usually opalescent necessitating centrifugation or filtration to remove the adherent water droplets. Petroleum ether, benzene and chloroform *per se* failed to extract the color complex. Diethyl ether and petroleum ether in 3:1 ratio provided significantly better mean absorbance, 0.409 ± 0.007, compared to the value provided by 2 : 2 ratio, 0.328 ± 0.003 (P<0.01, n= 4 each). The linearity with either of the two solvent combinations has been quite satisfactory (Table 3). Since the two extractants were employed in either 3 or 4 mL volumes per extraction, it was felt worth to find overall linearity by relating Mo concentration (μg mL^{-1} solvent) versus corresponding mean absorbance values obtained with either solvent formulations. The results exhibited perfect linearity (r ± s.e = 0.9974 ± 0.0017) with regression estimate (b ± s.e = 0.1461 ± 0.0035). The correlation coefficient did not differ significantly from corresponding value obtained on similar lines in aqueous medium (Table 2 data; P>0.1) while regression estimate has been about 1.4 times better than the value obtained in aqueous medium, 0.1075 ± 0.0007) (P<0.01).

Extraction and recovery
The samples were routinely prepared in 0.5M NaOH since alkali hydroxide extraction is known to extract maximum of Mo from test samples[9] and serves to precipitate most metals as hydroxides including amongst others Zn, Cd, Hg, Cu, Pb and Fe that are otherwise likely to interact or interfere with functioning of thioglycollic acid and thiocyanate. Optimization studies revealed maximum recovery of Mo in presence of metals with 0.5 M NaOH. The recoveries have been lower at < 0.5 M alkali hydroxide, ca. 73% and ca.84 % at 0.1 M and 0.3 M alkali hydroxide respectively. Alkali hydroxide as high as 2 M in 2mL sample volume is accommodated by the employed HCl concentration. The absorbance values at 3 μg Mo in 2 mL sample in 0.5, 1.0 or 2 M NaOH have been, respectively, 0.086 ± 0.002, 0.084 ±0.002 and 0.086 ± 0.002 (P>0.1, n=5 each) This also suggests that overall acid concentration of about. 3.4 to 4.1 M is optimal for conduct of the method.
The efficiency of the technique has proved quite satisfactory when applied to aqueous samples

containing added Mo in presence of various metallic species or from soil samples by employing alkali hydroxide to provide overall 0.5 M concentration Overall per cent recovery of added Mo has remained 97.3 ± 0.8 (n= 30) with mean per cent accuracy (n=30) and COV (n=4) about 4. Blank soil extracts containing equivalent of about 0.67 g soil from non-arable land or about 2 g from arable land did not contain detectable Mo. This implied the Mo content in the soil was either absent or less than 0.05 ppm. Reagent blanks were clear indicating reagents were free of molybdenum. Activated charcoal ensured colorless soil extract that was otherwise colored in presence of alkali hydroxide. In separate experiments, it was observed that activated charcoal did not adsorb Mo in presence of alkali hydroxide, while acidic pH was found to reduce recovery of Mo in presence of the charcoal (unpublished data). Activated charcoal has been ineffective in depigmenting alkaline soil extracts prepared from soils of arable agricultural area possibly due to reactivity of phenolics in humus with alkali hydroxide. For such soil sample extracts, depigmentation was efficiently facilitated by using ferrous hydroxide matrix instead of activated charcoal. This provided clearer extract. Any faint tinge got eliminated during acidification while using the outlined standard protocol.

Thioglycollic acid has proved an efficient reducing agent to substitute stannous chloride for thiocyanate technique for determination of Mo. Stannous chloride solution in concentrated HCl is a powerful reducing agent, and is capable of reducing Mo to lower reduced states[16, 17]. In contrast, TGA provides controlled reduction. The technique has obvious advantages as the colored complex is stable under test conditions and obviates the routine use of organic solvents for extracting the color complex. The use of organic solvent is reserved for facilitating determination of low concentrations of Mo. The practical applications of the method are obvious. Mo content in forages is estimated as 1 to 2 ppm with maximum content in legumes, estimated as 20 to 40 ppm. Legumes, such as Berseem, in affected regions in Punjab (India) often contain Mo exceeding 10 ppm, and blood of poisoned Buffaloes has contained over a thousand ppm of Mo compared to 0.1 ppm in normal bovine[4]. Maximum permissible dietary Mo load, as ppm, in highly susceptible ruminant livestock is 10, range 2 to 50[18]. An average Mo content in environmental biosolids is estimated to be 9.24 ppm[12, 18]. In view of these observations, the improved technique would be quite useful in monitoring samples for Mo with environmental and toxicological implications.

CONCLUSIONS

Thioglycollic acid has been demonstrated to provide stable thiocyanate Mo color complex compared to that obtained with stannous chloride. Alkali extraction procedure has benefit of eliminating most interfering metals as hydroxides. The method is quite suitable for determining a wide range of Mo concentrations, 0.1 through 30 ppm, in environmental, industrial and biological samples.

REFERENCES

1. Oser BL. Hawk's Physiological Chemistry, Tata McGraw-Hill Publishing Co., New Delhi, 1971, p.565.
2. Phillips RW. Molybdenum. .Jones LM, Booth NH, McDonald LE (eds). In: Veterinary Pharmacology and Toxicology, Oxford & IBH Publishing Company, New Delhi, 1981, pp.806-807.
3. Uppal RP. Toxicity of Metals. Garg SK (eds.). In: Veterinary Toxicology, pp. 37-59, CBS Publishers & Distributors, New Delhi, 2000, pp. 37-59.
4. Takkar PN, Randhawa SS. Specific Geomedical Problems in Asia. Jul Lag (eds.). In: Geomedicine, CRC Press, Boston, 1990, pp.245-255.
5. Anonymous. 2005. Toxicity Summary for Molybdenum, Risk Assessment Information System,http://rais.ornl.gov/tox/profiles/ molybdenum_c_V1.shtm.
6. Tandon HLS. Methods of Analysis of Soils, Plants, Waters and Fertilizers, Fertilizer Development and Consultation Organization, New Delhi, 1999, p. 67.
7. Horwitz W. (ed.). AOAC Official Methods of Analysis, Volume 1, 17th Edition, AOAC International, Maryland, USA, 2000, pp.9-10.
8. Upadhyay RM, Sharma NL. Manual of Soil, Plant, Water and Fertilizer Analysis, Kalyani Publishers, Ludhiana, India, 2002, pp. 109-110.
9. Jaiswal PC. Soil, Plant and Water Analysis, Kalyani Publishers, Ludhiana, 2003, pp. 238, 334.
10. Bassett J, Denney RC, Jeffrey GH, Mendham J. Vogel's Textbook of Quantitative Inorganic Analysis, 4th Edn. ELBS and Longman, London.1978, pp 160, 747.
11. Willard, H.2007. Advanced Quantitative Analysis, Read Books, 2007, pp. 222-228. http://books. google.co.in/books?
12. Anonymous. 2008. Biosolids Management: Biosolids Quality (Nutrients, Metals & Pathogens). http://www.toronto.ca/water/biosolids/quality.htm

13. Williams CH. A Colorimetric Method for the Determination of Molybdenum in Soils. J. Sci. Food Agr 1954; 6(2):104-110.

14. Windholz M. The Merck Index, 10th Edn, Merck & Co., USA, 1983.

15. Purvis ER, Peterson NK. Methods of Soil and Plant Analysis for Molybdenum. Soil Sci. 1956; 81:223-228.

16. Prasad J, Suryanarayana M. Colorimetric Determination of Molybdenum by the Reaction with Thiocyanate. J. Anal. Chem 1966; 219(4):346-349

17. Melwanki MB, Seetharamappa J, Masti SP. SpectrophotometricDetermination of Molybdenum (VI) using Isothipendyl Hydrochloride and Pipazethate Hydrochloride in Alloy Steels and Soil Samples. Anal. Sc. 2001; 17:1121-1123.

18. O'Connor GA., Brobst RB, Chaney RL, Kincaid RL, McDowell LR, Pierzynski GM, Rubin A, Van Riper GG. A Modified Risk Assessment to Establish Molybdenum Standards for Land Application of Biosolids. J. Environ. Qual. 2001; 30:1490-1507.

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