Talanta 96 (2012) 185–189

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

# A non-extractive sequential injection method for determination of molybdenum

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ARTICLE INFO

*Article history:*
Received 28 October 2011
Received in revised form 12 January 2012
Accepted 18 January 2012
Available online 30 January 2012

*Keywords:*
Molybdenum
Polymethine dye
Sequential injection analysis
Spectrophotometric detection

ABSTRACT

A non-extractive sequential injection spectrophotometric method for the determination of molybdenum has been developed. The method is based on the reaction between the thiocyanate complex of molybdenum and the polymethine dye (2-[2-(4-dipropylamino-phenyl)-vinyl]-1,3,3-trimethyl-3*H*-indolium chloride) in a hydrochloric acid medium. The calibration plot in the SIA system was linear from 0.080 mg $L^{-1}$ to 1.92 mg $L^{-1}$ of Mo; LOD was 0.021 mg $L^{-1}$. The method was applied for the determination of molybdenum in spiked drinking water and mineral water.

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## 1. Introduction

Molybdenum is a biologically and physiologically significant element. It is present in living organisms in trace amounts and is vital for plants, animals and humans. It plays a part in the regulation of body processes, assists in the elimination of harmful substances from the human body, helps reduce the content of toxic agents in blood, regulates digestion, suppresses cancer-causing agents and is a component of the enzyme systems responsible for the degradation of amino acids, drugs and toxic substances. Mo and its compounds are commonly used in the metallurgical, chemical and electrotechnical industries as well as in space technology, jewellery making and other industries. Mo is preferentially used for the production of special alloys and steels, so-called "Mo steels", which are excellent materials for the manufacture of weapons and high-pressure boilers and pipes. Mo compounds are also used as catalysts in the processing of oil [1–3].

Due to its unique properties and its importance in nature, Mo has been the subject of a great deal of attention in terms of developing methods for its determination. Methods currently known include various optical methods [4–7], chromatography [8], electroanalytical techniques [9,10] and a combination of different methods [11–13]. The methods for determination of molybdenum in environmental samples are discussed [14]. Determination of Mo by flow injection analysis followed by various detection techniques such as ETAAS [15], ICP-AES [16], chemiluminescence [17] and spectrophotometry [18–23] has also been developed. However, we found only one article in the literature devoted to SIA determination of molybdenum [24]. In it, Mo with thiocyanate is first extracted into a toluene film containing tetraheptylammonium bromide as an ion-pairing reagent. Then, the thiocyanate ligand is displaced by 1,5-diphenylcarbazone (in a back-extraction solvent, methanol) to form a colored complex. The main difficulty of this approach is the thickness and stability of the wetting film, as they are important for the reproducibility, sensitivity and extraction capacity [25]. The aim of the present work was to develop a non-extractive SIA method for determination of Mo using the polymethine dye 2-[2-(4-dipropylamino-phenyl)-vinyl]-1,3,3-trimethyl-3*H*-indolium chloride (DETI) (Fig. 1) [26] with spectrophotometric detection. The method is based on the reaction between the thiocyanate complex of Mo and DETI in a hydrochloric acid medium. The developed method was applied for the determination of Mo in spiked water samples.

## 2. Experimental

### 2.1. Apparatus

Absorbance measurements were carried out using an Agilent 8453 UV–vis spectrophotometer with 1 cm quartz cell. The SIA manifold (Fig. 2) was constructed from a FIA-lab® 3500 system (FIAlab Instruments, USA) with an 8-port selection valve, a 5 mL syringe pump, a holding coil and a reaction coil. The detection part of the system consisted of a fiber-optic spectrophotometer with a charge-coupled USB 2000 detector equipped with a

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doi:10.1016/j.talanta.2012.01.040

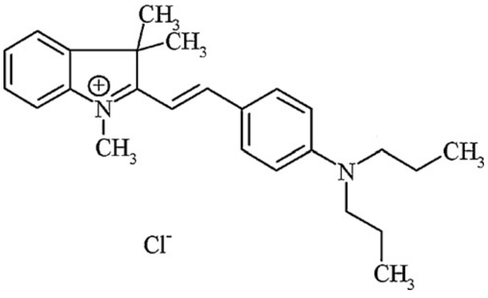

**Fig. 1.** The structure of DETI.

flow cell of 1 cm optical path length and an LS-1 VIS tungsten lamp (Ocean Optics, Inc., USA). The operational software FIAlab for Windows 5.9 was used for system control, data acquisition and storage.

### *2.2. Reagents and solutions*

All solutions were prepared from analytical grade reagents. Ultra pure water (Millipore, USA) was used throughout. A stock Mo solution (0.1 mol L$^{-1}$) was prepared by dissolving $Na_2MoO_4{\cdot}2H_2O$ in water. Working solutions were prepared by appropriate dilution of the stock solution with water. The solution of DETI reagent was prepared by dissolving the compound in a small amount of methanol (0.3 mL) and diluting with water. A 1% aq. solution of the polyethylene glycol ether of alkyl alcohol (OC-20), $CH_3(CH_2)_4(OCH_2CH_2)_{10}OH$, was applied as a non-ionic surfactant in batch measurements.

### *2.3. Procedures*

Preliminary studies were carried out in batch mode by manual mixing of the solutions. The following solutions were added into the tubes: 1 mL of sample, 0.4 mL of 1.44 mol L$^{-1}$ KSCN, 0.4 mL of $8 \times 10^{-4}$ mol L$^{-1}$ DETI, 2 mL of 0.5 mol L$^{-1}$ HCl, 1 mL of 1% OC-20 and enough water to dilute the solution up to 5 mL. The mixture was shaken and then transferred into a 1 cm quartz cell for the absorbance measurement.

The operating program of the SIA system for the analysis of Mo is presented in Table 1. The analytical cycle started with the aspiration of carrier into the syringe pump reservoir. Next, the DETI solution, the sample solution and the solutions of KSCN, HCl and water were aspirated into the holding coil. The flow was reversed to mix all solutions (3 flow reversals), and the mixture was propelled through the detector flow cell. Each point of the analytical plot represents the average peak height for four successive injections.

## 3. Results and discussion

### *3.1. Absorption spectra*

Determination of Mo is based on the formation of a suspension of the ion associate, which cannot be detected by spectrophotometry. Therefore, in order to stabilize the suspension, it is necessary to add a non-ionic surfactant. The extraction of ion associate is another way of overcoming problems associated with the formation of a suspension; however, this requires the use of an organic solvent. In contrast to batch mode, the ion associate is stable in a dynamic system as in the SIA technique, and can be detected without adding a non-ionic surfactant or performing an extraction.

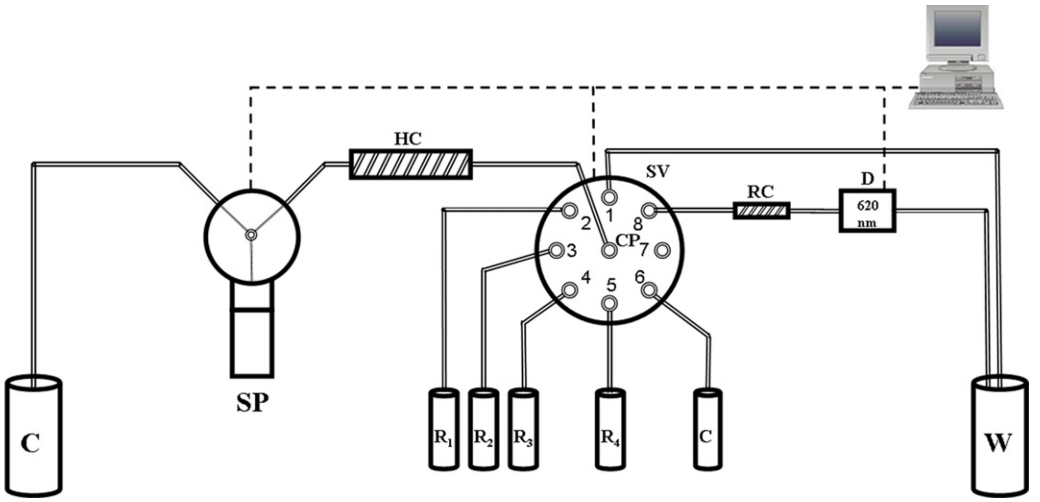

**Fig. 2.** A schematic view of the sequential injection manifold. SP – syringe pump, HC – holding coil, SV – selection valve, $R_1$ – $8 \times 10^{-4}$ mol L$^{-1}$ DETI, $R_2$ – sample, $R_3$ – 1.44 mol L$^{-1}$ KSCN, $R_4$ – 0.5 mol L$^{-1}$ HCl, C – water as carrier, RC – reaction coil, D – detector, W – waste.

**Table 1**
Sequence of key steps for determination of Mo.

| Step | Port no. | Flow rate, μL s$^{-1}$ | Operation | Description |
|---|---|---|---|---|
| 1a | – | 100 | Aspirate, 3000 μL | Aspiration of water as carrier (syringe pump in the Valve In position) |
| 1b | | | | Returning the syringe pump to the Valve Out position |
| 2 | 2 | 30 | Aspirate, 68 μL | DETI ($8 \times 10^{-4}$ mol L$^{-1}$) |
| 3 | 3 | 30 | Aspirate, 50 μL | Sample |
| 4 | 4 | 30 | Aspirate, 26 μL | KSCN (1.44 mol L$^{-1}$) |
| 5 | 5 | 30 | Aspirate, 36 μL | HCl (0.5 mol L$^{-1}$) |
| 6 | 6 | 30 | Aspirate, 300 μL | Water |
| 7 | 8 | 100 | Dispense-aspirate, 100 μL | Mixing procedure |
| 8 | 8 | 70 | Empty | Empty the whole volume through the flow cell into the detector |

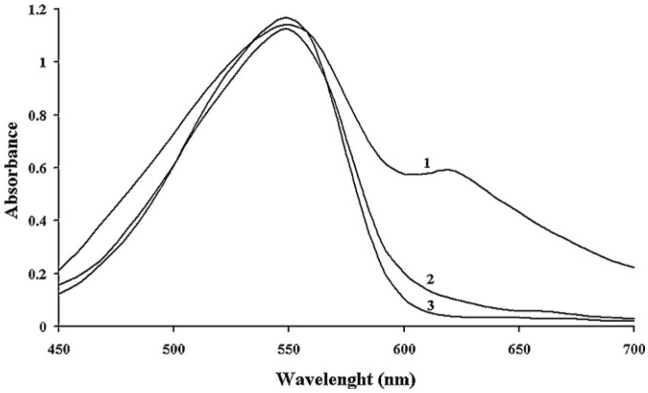

**Fig. 3.** Absorption spectra of DETI (3), binary mixture of the DETI with thiocyanate (2), and ternary mixture of the DETI, thiocyanate ion and Mo in an acidic medium (1). $6.4 \times 10^{-5}$ mol L$^{-1}$ DETI (1, 2, 3); 0.12 mol L$^{-1}$ SCN$^{-}$ (1, 2); $4 \times 10^{-5}$ mol L$^{-1}$ Mo (1).

The absorption spectra of the DETI (line 3), the binary mixture of DETI with thiocyanate (line 2), and the ternary mixture of DETI, thiocyanate and Mo (line 1) in an acidic medium are shown in Fig. 3. The maximum absorption peak of DETI and of DETI with thiocyanate appears at 550 nm, whereas the Mo-SCN-DETI complex also shows a new a peak at 620 nm, the wavelength difference thus being 70 nm.

### 3.2. *Optimization of conditions for complex formation*

#### 3.2.1. *Batch method*

The experimental conditions, such as the reagents sequence, concentrations and volumes, were optimized using univariate optimization method. The optimal order of reagents was found to be: sample, KSCN, HCl, DETI, OC-20 and water; and the selected values were found to be: $0.6 \times 10^{-4}$ mol L$^{-1}$ DETI, 0.12 mol L$^{-1}$ KSCN, 0.2 mol L$^{-1}$ HCl and 0.2% OC-20 (Fig. 4).

#### 3.2.2. *SIA method*

The optimal sequence of zones aspiration to the SIA system was: DETI, sample, KSCN, HCl, water. The effect of the concentration of reagents was investigated in the ranges: 0–$2 \times 10^{-3}$ mol L$^{-1}$ DETI, 0–3.5 mol L$^{-1}$ KSCN and 0–3 mol L$^{-1}$ HCl, and based on the results obtained (Fig. 5) the following conditions were selected: $8 \times 10^{-4}$ mol L$^{-1}$ DETI, 1.44 mol L$^{-1}$ KSCN, 0.5 mol L$^{-1}$ HCl.

For the optimization of reagents volumes and the flow rate to the detector a modified Simplex method was used, varying the four parameters–DETI volume, KSCN volume, HCl volume and flow rate to the detector–until the optimal value was found. The following optimal data were obtained after 20 optimization steps: 68 μL DETI, 26 μL KSCN, 36 μL HCl and a flow rate through the optical cell of 70 μL s$^{-1}$.

A comparison of the optimal reaction conditions for the batch and SIA methods is shown in Table 2. In the batch method it was necessary to stabilize the complex formed by the addition of a non-ionic surfactant. In contrast, the addition of surfactant was not necessary for the SIA method. The SIA method requires ten-times less reagent volumes than the batch method. Automation and a decrease in the volume of reagents required are the main advantages of the SIA method.

### 3.3. *Figures of merit*

The calibration range, limits of detection, accuracy, precision and selectivity for the developed method were all evaluated (Table 3). The LOD value in the SIA system is lower than that of the batch system, which can be explained by the fact that the reaction

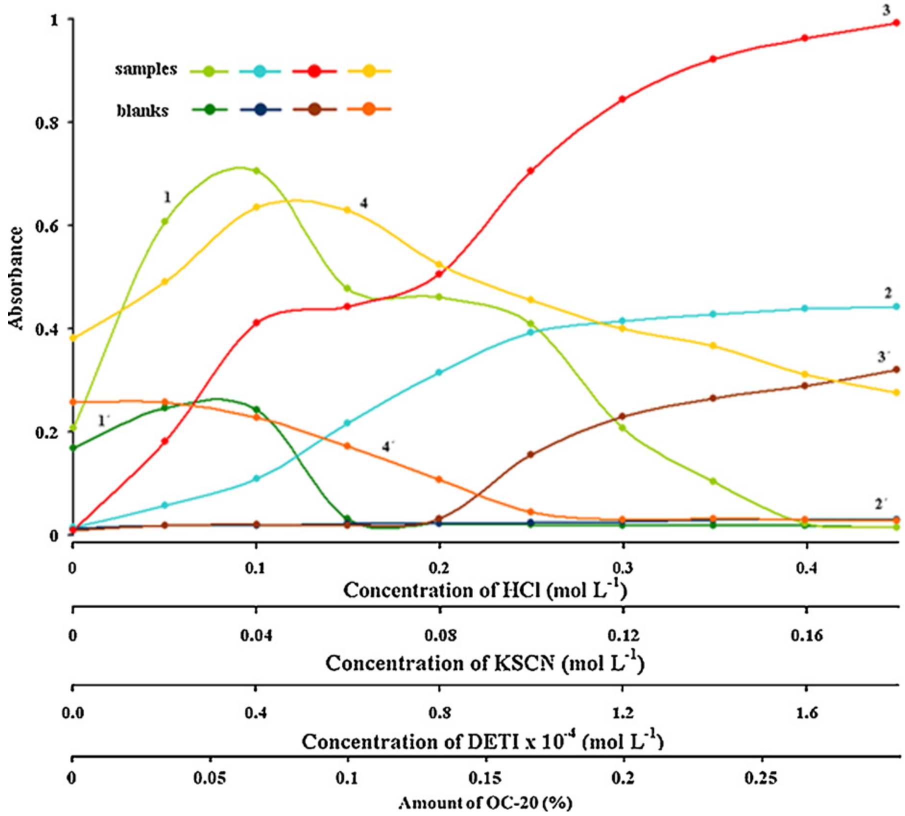

**Fig. 4.** Effect of reagents concentration with the batch method: HCl (1); KSCN (2); DETI (3); OC-20 (4); (1′–4′) are blank tests.

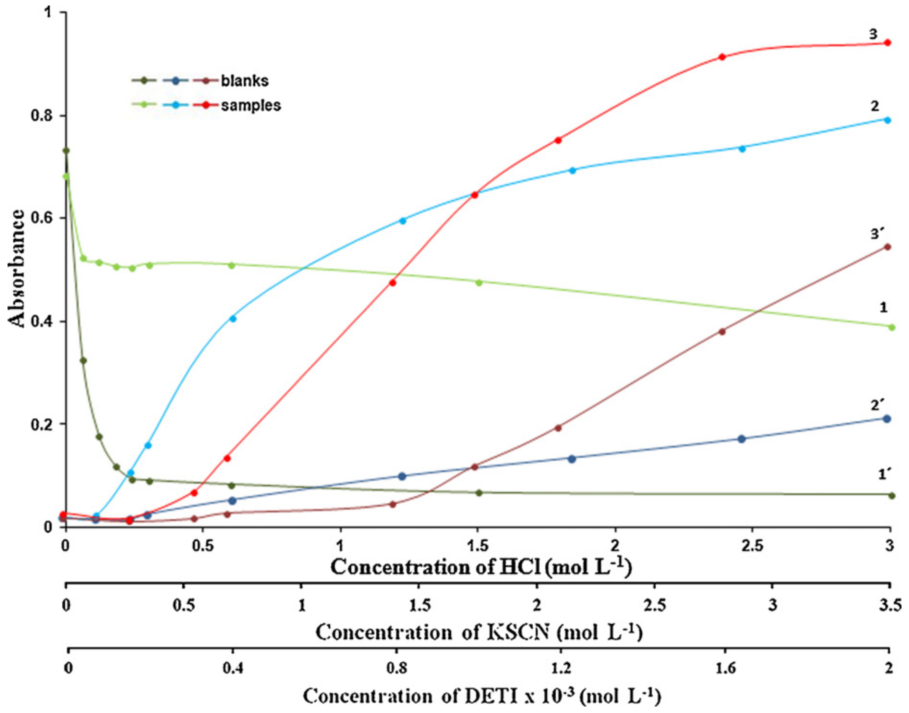

**Fig. 5.** Effect of reagents concentration with the SIA method: HCl (1); KSCN (2); DETI (3); (1′–3′) are blank tests.

**Table 2**
Comparison of the batch and SIA methods.

| Parameter | Batch method | SIA method |
|---|---|---|
| Reagent order | Sample; KSCN; HCl; DETI; OC-20; water | DETI; sample; KSCN; HCl; water |
| Sample volume | 1000 | 50 |
| KSCN concentration, mol $L^{-1}$ | 1.44 | 1.44 |
| KSCN volume, μL | 400 | 26 |
| DETI concentration mol $L^{-1}$ | $8 \times 10^{-4}$ | $8 \times 10^{-4}$ |
| DETI volume, μL | 400 | 68 |
| HCl concentration mol $L^{-1}$ | 0.5 | 0.5 |
| HCl volume, μL | 2000 | 36 |
| OC-20 concentration, % | 1 | 0 |
| OC-20 volume, μL | 1000 | Not necessary |

**Table 3**
Comparison of the batch and SIA methods.

| Parameter | Batch method | SIA method |
|---|---|---|
| Regression equation, mg $L^{-1}$ | $A = b + aC$ | |
| *a* (slope) | 0.1587 | 0.1796 |
| *b* (intercept) | −0.0450 | 0.0012 |
| Correlation coefficient, $r^2$ | 0.997 | 0.999 |
| Linear range, mg $L^{-1}$ | 0.440–1.92 | 0.080–1.92 |
| LOD, mg $L^{-1}$ | 0.331 | 0.021 |
| ε, $cm^{-1}$ $mol^{-1}$ $dm^3$ | $1.53 \times 10^4$ | – |

*A* means the absorbance and *C* the concentration of Mo in mg $L^{-1}$.

in a flow system is easier and faster in comparison with batch mode. The stability of the ion associate in the batch system is achieved by adding OC-20. On the other hand, in the SIA system the ion associate forms quickly, and its stability is achieved by the dynamic system of SIA. The repeatability of the signal at 1.92 mg $L^{-1}$ of Mo level was 2.12% ($n = 10$).

### 3.4. *Interference study*

The effect of potential interfering ions was studied by the analysis of the samples containing $1 \times 10^{-5}$ mol $L^{-1}$ of Mo in the presence of varying amounts of diverse ions. The limiting value of the concentration of foreign ions was indicated as an amount that causes an error of 5%. A selectivity coefficient higher than $10^3$ was obtained for $NH_4^+$, $Na^+$, $K^+$, $Mg^{2+}$, $Ca^{2+}$, $F^-$, $Cl^-$, $Br^-$, $I^-$, $SO_4^{2-}$, $NO_3^-$, $PO_4^{3-}$, $ClO_4^-$ and thiourea; and higher than $10^2$ for $Fe^{2+}$, $Ni^{2+}$, $Mn^{2+}$, $Cd^{2+}$, $Al^{3+}$, $Cr^{3+}$, $Cr^{6+}$ citrate and tartrate; and higher than 10 for oxalate, $Cu^{2+}$, $Co^{2+}$ $V^{5+}$ and $Zn^{2+}$. The interfering effect of $W^{6+}$, $Zn^{2+}$ and $Fe^{3+}$ could be eliminated by the addition of sodium-potassium tartrate or ascorbic acid.

### 3.5. *Analytical application*

The precision and accuracy of the developed method were tested by analysis of model samples over three consecutive days (Table 4). The developed method was applied for the analysis of spiked water

**Table 4**
Inter-day precision and accuracy study ($n = 5$, $P = 0.95$).

| | Mo(VI) added mg $L^{-1}$ | SIA determination | | | Batch determination | | |
|---|---|---|---|---|---|---|---|
| | | $C \pm ts/\sqrt{n}$, mg $L^{-1}$ | RSD, % | R, % | $C \pm ts/\sqrt{n}$, mg $L^{-1}$ | RSD, % | R, % |
| 1st day | 0.38 | 0.37 ± 0.01 | 2.17 | 97.4 | 0.36 ± 0.02 | 4.50 | 94.7 |
| | 0.76 | 0.77 ± 0.02 | 2.09 | 101.3 | 0.78 ± 0.03 | 3.09 | 102.6 |
| 2nd day | 0.38 | 0.39 ± 0.01 | 2.06 | 102.6 | 0.40 ± 0.02 | 4.03 | 105.3 |
| | 0.76 | 0.75 ± 0.01 | 1.07 | 98.7 | 0.75 ± 0.03 | 3.23 | 98.7 |
| 3rd day | 0.38 | 0.38 ± 0.02 | 4.24 | 100.0 | 0.36 ± 0.03 | 6.72 | 94.7 |
| | 0.76 | 0.77 ± 0.02 | 2.09 | 101.3 | 0.73 ± 0.03 | 3.20 | 96.1 |

**Table 5**
Comparison of the determination of Mo in spiked water samples by SIA, batch and ICP-AES methods.

| Sample | Mo(VI) added, mg L$^{-1}$ | SIA determination, mg L$^{-1}$ | Batch determination, mg L$^{-1}$ | ICP-AES determination, mg L$^{-1}$ |
|---|---|---|---|---|
| Drinking water | 0 | n.d. | n.d. | n.d. |
| | 0.095 | 0.092 ± 0.006 | n.d. | 0.105 |
| | 0.19 | 0.18 ± 0.01 | 0.17 ± 0.02 | 0.179 |
| | 0.38 | 0.38 ± 0.02 | 0.39 ± 0.02 | 0.372 |
| Mineral water | 0 | n.d. | n.d. | – |
| | 0.095 | 0.098 ± 0.008 | n.d. | – |
| | 0.19 | 0.18 ± 0.02 | 0.21 ± 0.02 | – |
| | 0.38 | 0.38 ± 0.02 | 0.36 ± 0.02 | – |

n.d. – Not determined.

samples, and the results of the determination in the batch and SIA methods were compared (Table 5). According to the *t*-test, no significant difference between the results was found. However, the repeatability of the SIA determination was better (1.07–4.24%) than the batch method (3.09–6.72%). The rate of analysis in the SIA method was 3 min for one analytical cycle, i.e. 20 injections/h. The ICP-AES was used as reference method for determination of Mo.

## 4. Conclusions

Spectrophotometric methods for the determination of heavy metals commonly require a separation or pre-concentration step, and liquid–liquid extraction (LLE) is frequently used for this purpose. Despite the advantages of LLE, it also has drawbacks, the primary ones being the necessity of using high volumes of environmentally unfriendly organic solvents and the need sometimes to perform the technique manually. Therefore, at present so-called miniaturized LLE-based methods with low-solvent consumption are being intensively developed [27–34]. Also, non-extractive methods are obviously much more suitable to the requirements of green chemistry. In the present work, a non-extractive SIA method for the determination of Mo using the polymethine dye 2-[2-(4-dipropylamino-phenyl)-vinyl]-1,3,3-trimethyl-3*H*-indolium chloride (DETI) has been developed. The performance of the batch method and the SIA method are compared. The SIA method is fully automated and has a higher frequency and accuracy of determination, and the consumption of reagent and sample is lower when compared with the batch method. The developed method did not achieve a detection limit as low as that of more sophisticated techniques such as AAS and AES; however, the SIA system employed low-cost instrumentation and could be applied for on-site measurements. Moreover, it can offer a green alternative to a previously published automated extraction technique [24] that applied toluene and methanol for wetting-film extraction.

## Acknowledgments

This work has been supported by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic, the Slovak Academy of Sciences VEGA (Grant No. 1/1096/12) and the Czech Ministry of Education, MSM 0021620822. The authors would like to thank the analytical laboratory of State Geological Institute of Dionyz Stur in Spišská Nová Ves for providing the results of ICP-AES.

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