

PULSE STRIPPING VOLTAMMETRIC DETERMINATION OF NICKEL  
AND MOLYBDENUM IN MEDICINAL HERBS SAMPLES

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ZASTOSOWANIE PULSOWEJ WOLTAMPEROMETRII INWERSYJNEJ  
W OZNACZANIU NIKLU I MOLIBDENU W SUROWCACH LECZNICZYCH

W pracy przeprowadzono oznaczanie zawartości niklu i molibdenu w surowcach zielarskich przy użyciu adsorpcyjnej woltamperometrii inwersyjnej z wzbogacaniem anodowym (DPAdSV) po uprzednim przeprowadzeniu reakcji kompleksowania z dimetyloglioksymem i 8-hydroksychinoliną. Wszystkie próbki (oczyszczone, umyte i zmielone) mineralizowano przy użyciu energii promieniowania mikrofalowego w układzie  $\text{HNO}_2 + \text{H}_2\text{O}_2$ . Uzyskano następujące zakresy zawartości odpowiednio dla Ni i Mo: 0,7–5,0; 0,2–3,6  $\text{mg kg}^{-1}$  (s.m.). W badaniach zastosowano certyfikowany materiał odniesienia: tytoń orientalny (CTA-ATL-1).

Summary

The method for determination of nickel and molybdenum in medicinal herbs by differential pulse adsorptive stripping voltammetry (DPAdSV) using dimethylglyoxime and 8-hydroxyquinoline as complexing agents after the microwave-assisted digestion has been presented. Samples (segregated, cleaned and powdered) were digested by wet method in a mixture of  $\text{HNO}_2 + \text{H}_2\text{O}_2$ . Typical Ni and Mo concentrations in the ranges of 0.7–5.0; 0.2–3.6  $\text{mg kg}^{-1}$  of dry matter, respectively, were found from replicate measurements of the samples following dry ashing. The oriental tobacco (CTA-ATL-1) as the certified reference material was applied in those investigations.

INTRODUCTION

Bioindicative methods are commonly used in the evaluation of environmental purity levels. Various types of plants are used as popular indicators of contamination, from moss [1, 2, 13, 14] and lichens to tree or hovel plants [3]. They are constantly exposed to a variety of chemicals or xenobiotics of natural or man-made sources. Among inorganic chemicals, titanium, nickel, chromium, cobalt, lead, manganese beryllium, and some of their derivatives have been found as carcinogenic species under specific experimental conditions. Among these, salts of nickel and titanium appear to be most powerful. In particular, herbs are likely to suffer a greater degree of contamination from metals than from common diet as a result of their longer residence time in an increasingly polluted environment [8].

Medicinal plants are important for pharmacological research and drug development, and are widely consumed as home remedies and raw materials for the pharmaceutical

industries. There are different ways by which countries define medicinal plants, herbs or products derived from them, and they have adopted various approaches to licensing, dispensing, manufacturing and trading them to ensure their safety, quality and efficacy [7].

It should be noted that herbs are generally defined as any form of a plant or plant product, including leaves, stems, flowers, roots and seeds. Herbal products may contain a single herb or be combinations of several different herbs believed to have complementary effects. Some herbal products, including many traditional Chinese medicine formulations, also include animal products and minerals [24].

However, there are no standards for medical raw plant materials, which establish a permissible level of metals in such materials. The World Health Organization, WHO, states the maximum permissible levels in raw plant materials only for arsenic, cadmium and lead which amount to 1.0, 0.3 and 10 mg/kg, respectively [6, 17, 18]. Plants used in therapeutics should be picked in areas free of any contamination sources. The concentration of heavy metals is one of the criteria that makes raw plants admissible to the production of medicines due to the fact that the amount taken increases with concentration, elevated by constant mass of a taken dose. However, as it follows from the literature, the differentiation of medical raw plant materials, considering the content of metals, is significant and the content of nickel and molybdenum is not always included in standards recommended by WHO.

The selection of a suitable sampling strategy, in terms of species, number of sites, frequency of the collection of representative samples depends on the scope of the biomonitoring project. However, in order to control or eliminate the effects of environmental parameters that can influence the final results, some recommended rules have to be kept in mind for a correct sampling in view of trace elements determination [4, 5].

The samples have to be biosensors or part of them presenting an acceptable uniformity of botanic characteristics, degrees of exposure to atmospheric particulate, heights above the ground and must have sufficient size to allow replicate analyses. The collection cannot be done during specific meteorological events and weather conditions during the sampling period should be relatively constant for several days beforehand. The samples, after their collection, should be analyzed as soon as possible or stored and preserved so that biological activities and volatilization of elements are minimized.

Finally, in order to evaluate the presence of heavy metals associated to atmospheric particulate of antropogenic origin, particular attention should be paid to minimize the effect of soil contamination [8, 13].

Different complex matrices of the sample require prior mineralization for most analytical methods, and this step is critical in the whole analytical procedure for the determination of metal concentration. Complete digestion and less time-consuming approaches probably also provide good results of the analyses of real samples [3, 10, 16]. The two types of digestion methods which are commonly employed for determining trace elements are wet digestion [19] and dry ashing [9]. Although these techniques are important, they are generally time consuming, particularly working on refractory elements. On the other hand, it is possible to lose volatile elements during digestion. In recent years, these techniques have been replaced by the use of closed or open vessel microwave oven-assisted decomposition methods for on- or off-line analyses with the faster method of pressurized acid digestion of a variety of samples [16]. Using a closed-vessel microwave oven system, controlling the temperature, rate and pressure is easy, and such system provides faster and more efficient sample digestion. The combination of the  $\text{HNO}_3$  with  $\text{HClO}_4$  [12],  $\text{HNO}_3$  with

H<sub>2</sub>O<sub>2</sub> [1, 2, 16–18] have been shown to be useful for efficient mineralization of the herb samples.

It should be noted that these procedures have to be validated in order to ensure that no contamination and/or losses have occurred which could affect the accuracy of the final results; the validation of the whole procedure (preparation and measurement) can be made using appropriate certified reference materials.

A variety of methods are used to analyze biological samples. They include Flame atomic absorption spectrometry – FAAS [11, 16–18], Electrothermal atomic absorption spectrometry – ETAAS [9, 11, 16] and Adsorptive stripping voltammetry – AdSV [1, 2, 17]. Voltammetric techniques such as anodic stripping voltammetry [20] or cathodic stripping voltammetry should be, in principle, suitable for such determination. Their additional advantage is the fact that they require relatively inexpensive instrumentation, and are capable of determining metals accurately at trace to ultratrace levels [22].

Chromatography and spectroscopy have been also used in the last 40 years to study organic chemical compositions of medicinal herbs and plants. Many chemical compounds have been isolated and identified using gas chromatography (GC), high performance liquid chromatography (HPLC), ultra-violet spectroscopy (UV) and infra-red (IR) spectroscopy, Raman spectroscopy, nuclear magnetic resonance spectroscopy (NMR), mass spectroscopy (MS), X-ray diffraction, GC-MS and HPLC-MS. An increased sensitivity has been achieved by coupling HPLC [23] or GC with other analytical systems.

This paper describes a simple and reliable voltammetric approach for determination of nickel and molybdenum in botanical samples such as medicinal herbs. The samples were collected from different places starting from motorway shoulders, trunk roads to other roads in mountainous our regions. The accuracy of the analytical procedure was tested using oriental tobacco leaves (CTA-OTL-1, Poland). The obtained results were in good agreement with certified values.

## MATERIALS AND METHODS

### Samples

Samples of medicinal herbs were collected from various places in southwest Poland. The following herbs were collected: *Urticae folium* FP V, *Menthae piperitae folium*, *Taraxacum officinale* Web., *Rosa canina*.

The collected samples of medicinal herbs were thoroughly segregated and cleaned. The cleaned samples were placed on a filter paper and dried primarily in the air and then in a dryer at 100°C to constant mass. Next, samples of moss were pulverized for homogenization in agate mill for 10 minutes. The obtained powder was weighed on an analytical balance with the accuracy 0.0001 g. In the case of plant material containing hard particles the proper homogenization is important. For establishing the optimum time of grinding different periods (10, 15, 20 and 25 minutes) were tested. To achieve full homogenization and good analytical results for 0.1 g sample 10 min. of grinding was found to be sufficient.

### Apparatus

A commercially available Microwave Sample Preparation System, Model 1200 ML MEGA (Italy), featuring programmable mineralization procedure parameters, was used for microwave digestion of herb sample.

Adsorptive stripping voltammograms were obtained with an ECO-TRIBO Polarograph (Prague, Czech Republic). The cell was a three-electrode system with a saturated calomel electrode as the reference electrode and a hanging mercury drop electrode as the working electrode. All reagents were of analytical grade (POCH Gliwice, Poland): ammonium buffer 0.1 M, pH = 9.3; acetate buffer 0.1 M pH = 3.0; dimethylglyoxime 1% solution in ethanol, 0.1% 8-hydroxyquinoline in  $\text{CCl}_4$ . As a certified reference material Oriental tobacco leaves (CTA-OTL-1, Poland) were used.

### Analytical procedure

After mineralization of an herb sample [1, 2, 15] 0.25 cm<sup>3</sup> of the digest was transferred to an electrochemical cell and diluted to a 10 cm<sup>3</sup> with 0.1 M dimethylglyoxime and 9.3 cm<sup>3</sup> of 0.1 M ammonium buffer solution. Enrichment was continued for 140 s. In the case of molybdenum 0.2 M 8-hydroxyquinoline of 3.0 M acetate buffer were applied. Measurements were recorded at a speed of potential change 20 mV/s, impulse height 50 mV, impulse width 100 ms and sensitivity 0,1 nA.

The solutions examined were deaerated by passing purified argon for 10 min. before DPAdSV measurement.

The internal precision and the accuracy of the method, obtained during the period of measurements, can be evaluated through the measurement results of the analysis of standard CRM CTA-OTL-1 Oriental tobacco leaves (CTA-OTL-1, Poland). The standard was submitted to microwave-assisted mineralization processes.

## RESULTS AND DISCUSSION

The amounts of the studied elements (*i.e.* Mo and Ni) in herbs, which can be considered normal, are from 0.21 to 3.71 mg kg<sup>-1</sup> for nickel and from 0.74 to 4.81 mg kg<sup>-1</sup> for molybdenum (see Table 2).

Voltammetric analysis meets requirements of measuring such concentration levels, because nickel and molybdenum can be determined voltammetrically as Ni dimethylglyoximate and Mo hydroxyquinolate with high sensitivity by using the DPAdSV method.

The determination of nickel and molybdenum by the chelate adsorption voltammetric method requires a careful consideration of all experimental parameters that can influence precision and accuracy of the determination. The investigation was performed for samples containing 10<sup>-8</sup> M Ni and Mo using different buffer concentration (0.05; 0.1 M), different dimethylglyoxime and 8-hydroxyquinoline concentration (from 10<sup>-3</sup> to 10<sup>-4</sup> M). Investigation of different accumulation potential (from -0.115 mV to -0.800 mV for Ni, from -0.350 mV to -0.200 mV for Mo vs. reference electrode) and accumulation time (from 60 to 240 s) and scan rate (from 1 to 30 mV<sup>-1</sup>). The parameters selected on the basis of the measurement mentioned above were used in the analysis of CRM Oriental Tobacco Leaves (CTA-OTL-1), the results of which are given in Table 1 and a representative voltammograms are shown in Figure 1 and 2. The recommended parameters are as follows:

- (1) Ni buffer concentration 0.1 M, dimethylglyoxime concentration 4×10<sup>-4</sup> M, deposition potential -0.600 mV, deposition time 240 s, scan rate 20 mV/s.
- (2) Mo buffer concentration 0.2 M, 8-hydroxyquinoline concentration 2×10<sup>-5</sup> M, deposition potential -0,0 V, deposition time 180 s, scan rate 20 mV/s.

Table 1. The results of the determination of Ni and Mo [ppm] in Oriental Tobacco Leaves, n = 6

Element	Found value	Certificate value	Relative measurement error [%]
Nickel	6.22±0.45	6.32±0.65	2.01
Molybdenum	0.24±0.01	0.25±0.02	3.14

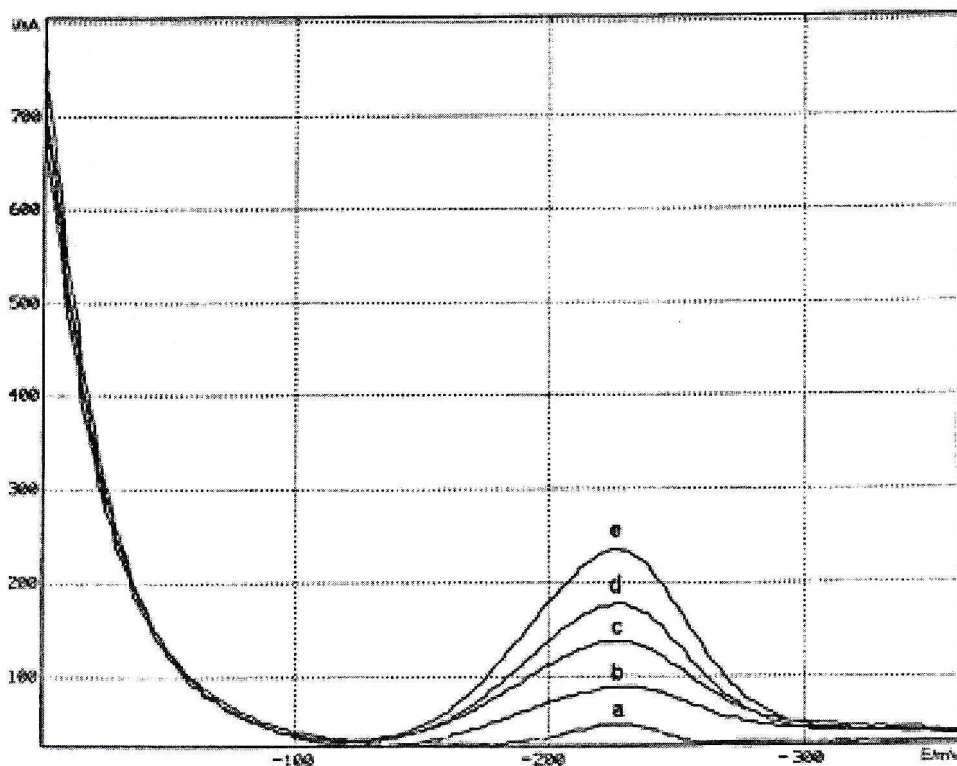


Fig. 1. Determination of molybdenum in dissolved herb sample by differential pulse adsorptive stripping voltammetry at the mercury drop electrode: (a) blank and (b) sample; c, d and e represent the response after three standard additions of 0.08 mM Mo

Results of the determination of the Ni and Mo in medicinal herbs are given in Table 2.

The results of metals determination in samples revealed expected dependence-lower level of concentration of investigated metals in herbs growing in "clean regions" than in those in polluted areas. A relatively great amount of Ni and Mo was found in herbs from vicinity of a railway station (almost equal to those obtained for samples collected near communication routes and motorway). This might be due to a factory in this area which buys up metal scraps, as is clearly seen in the case of molybdenum. Its content in samples taken from the railway station is much higher than in samples coming from the vicinity of motorways.

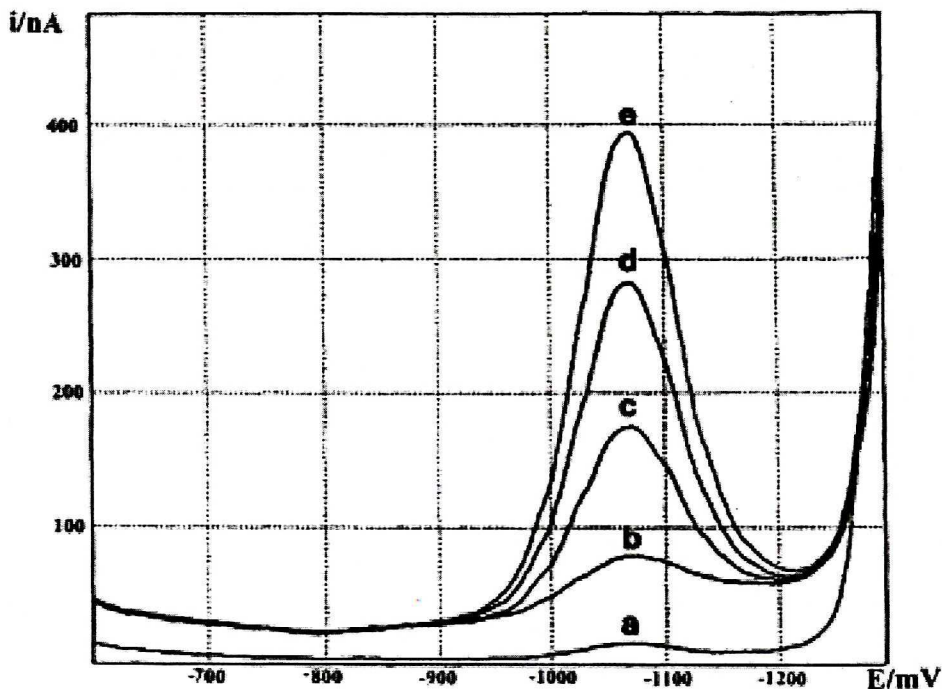


Fig. 2. Determination of nickel in dissolved herb sample by differential pulse adsorptive stripping voltammetry at the mercury drop electrode: (a) blank and (b) sample; c, d and e represent the response after three standard additions of 0.25 mM ng Ni

Table 2. The results of Ni and Mo determination in samples of medicinal herbs by DPAdSV method,  $n = 6$

Sample	Description of collecting site	Element [ppm]	
		Nickel	Molybdenum
<i>Taraxacum officinale</i> Web	Clearway	3.21	3.71
	Railway station	4.81	1.38
	Area near river	1.11	1.01
<i>Urticae folium</i> FP V	Clearway	3.04	0.21
	Area near mountains	1.59	1.18
	Railway station	3.78	2.58
<i>Menthae piperitae</i>	Area near mountains	1.37	0.21
	Urban road	4.28	0.99
	Clearway	4.63	1.01
	Allotments	0.74	0.55
<i>Rosa canina</i>	Railway station	1.12	3.22
	Urban road	2.23	2.89
	Area near mountains	3.89	0.89

The obtained results show that the examined herbs are significantly subjected to contamination of the environment by metals. Awareness of this phenomenon should be

disseminated to prevent collecting medicinal herbs near communication routes.

In conclusion, DPAdSV can be applied for the determination of Ni and Mo at low levels in a variety of botanical samples after wet digestion. Furthermore, the repeatability of the measurement is satisfactory considering statistical analysis, which is important in environmental science where different materials or treatments have to be compared.

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