Journal of Pharmaceutical and Biomedical Analysis 56 (2011) 846–850

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

# Elemental and structural analysis of silicon forms in herbal drugs using silicon-29 MAS NMR and WD-XRF spectroscopic methods

L. Pajchel*, P. Nykiel, W. Kolodziejski

*Medical University of Warsaw, Department of Inorganic and Analytical Chemistry, ul. Banacha 1, 02-091 Warsaw, Poland*

ARTICLE INFO

*Article history:*
Received 13 June 2011
Accepted 27 June 2011
Available online 2 July 2011

*Keywords:*
*Equisetum arvense*
*Urtica dioica*
Silicon
$^{29}$Si MAS NMR
X-ray fluorescence

ABSTRACT

The objective of this work was to study concentration of silicon and its structural forms present in herbal drugs. *Equisetum arvense* and *Urtica dioica* L. from teapot bags, dietary supplements (tablets and capsules) containing those herbs, dry extract obtained from a teapot bag of *E. arvense*, and samples of the latter herb harvested in wild habitat over four months were studied using wavelength dispersive X-ray spectroscopy (WD-XRF) and high-resolution solid-state $^{29}$Si NMR. The highest concentration of Si, ca. 27 mg/g, was found in the herbal material from the teapot bags containing *E. arvense*. The Si content in natural *E. arvense* (whole plants) increased from May to August by ca. 7 mg/g, reaching value 26 mg/g. Three different silicon forms were detected in the studied herbal samples: Si(OSi≡)4 ($Q_4$), Si(OH)(OSi≡)3 ($Q_3$) and Si(OH)2(OSi≡)2 ($Q_2$). Those sites were populated in *E. arvense* in the following order: $Q_4 \gg Q_3 > Q_2$. A dramatic, ca. 50-fold decrease of the Si concentration during the infusion process was observed. The infusion process and the subsequent drying procedure augmented population of the $Q_4$ sites at the cost of the $Q_2$ sites. The WD-XRF and $^{29}$Si NMR methods occurred useful and complementary in the study of herbal materials.



## 1. Introduction

In Polish traditional medicine, medical plants are often used in primary healthcare. Common Polish herbs, *Equisetum arvense* and *Urtica dioica* L., are natural sources of easily available Si forms. Although Si is the second most abundant element on Earth, most of its compounds cannot be consumed by plants and animals. The occurrence of Si in plants is a result of an uptake of its soluble forms: orthosilicic acid $Si(OH)_4$ and/or its ionized form $Si(OH)_3O^-$ [1].

Silicon is a necessary microelement for human health. The largest concentration of Si in the human body has been found in hair and nails [2–5]. The symptoms of a possible Si deficiency are cardiovascular and arterial problems, fragile bones, weakened gums and teeth, joint deterioration and digestive disorders. Silicon does not have a specified recommended daily allowance (RDA). According to the FDA suggestions [6], the total daily intake for men and women should contain 40 and 19 mg of Si, respectively. A safe upper limit is thought to be 50 mg a day [6]. It is essential to keep proper Si levels during growth periods, while the daily uptake should be increased for the elderly. Silicon is known to lessen or eliminate many body ailments associated with aging.

On the Polish market, there are many products containing *E. arvense* and *U. dioica* L. The great majority of them are dietary supplements which, according to the state regulations, can be marketed without any registration or control. It is of great importance to know Si concentrations and forms in such products. A typical method to analyze Si requires long sample preparation. Silicon has to be incorporated into color complexes to achieve UV/vis absorption [7]. This type of analysis is time-consuming and expensive. That's why our intention was to find a faster and cheaper method of elemental and structural analysis of Si species in herbal drugs.

The aim of this work was to test the wavelength dispersive X-ray spectroscopy (WD-XRF) in the quantitative analysis of silicon and the solid-state $^{29}$Si NMR spectroscopy in the characterization of structural forms of silicon in *E. arvense* and *U. dioica* L., and in various products containing those herbs.

## 2. Materials and methods

### 2.1. Materials

Herbs of *E. arvense* and *U. dioica* L. from herbal teapot bags (designated EA and UD, respectively) and four herb dietary supplements (Table 1), designated A–D, offered in tablets (A and B) and capsules (C and D), were received from retail pharmacies located in Warsaw. Samples of *E. arvense* from natural habitat were harvested from May to August. Whole plants were obtained from a wild habitat

* Corresponding author at: Medical University of Warsaw, Faculty of Pharmacy, Department of Inorganic and Analytical Chemistry, ul. Banacha 1, 02-097 Warsaw, Poland. Tel.: +48 22 5720784; fax: +48 22 5720784.
*E-mail address:* lukasz.pajchel@wum.edu.pl (L. Pajchel).

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doi:10.1016/j.jpba.2011.06.024

**Table 1**
Ingredients of the dietary supplements studied in this work.

| Supplement | Composition |
|---|---|
| A | - Dry extract of *Equisetum arvense* 60 wt.%<br>- Excipients |
| B | - Dry extract of *Urtica dioica* L. 60 wt.%<br>- Excipients |
| C | - Dry extract of *Equisetum arvense* 14.5 wt.%<br>- Dry extract of *Urtica dioica* L. 14.5 wt.%<br>- Silica (content not specified)<br>- Excipients |
| D | - Dry extract of *Equisetum arvense* 21.2 wt.%<br>- Herb of *Equisetum arvense* 21.2 wt.%<br>- Excipients |

situated in a rural area about 30 km from major roads (near the city of Wrocław in south-western Poland). Soil was not fertilized. All the samples were collected from the same 20 m × 20 m area and at the same time of the day. The plants were dried in open air in a shaded place for 2 weeks. The friable herb materials from the pharmacies, the samples from the natural habitat and the dietary supplements were first pulverized and homogenized by grinding them for 10 min in stainless steel vessels using a vibratory laboratory grinder LMW-S (Testchem, Pszów, Poland). Then, they were distributed into 5 g samples by weighing and prepared in the form of analytical tablets (30 mm in diameter, 5 mm thick). We used a hydraulic press with a 30 mm pellet die (Specac, London, United Kingdom), pressure of 15 tonnes applied during 20 min. In addition, dried residues from *E. arvense* infusions (EAinf) were studied. Each infusion was done by inserting one herbal teapot bag into 250 mL of boiling, distilled water for 15 min. The extracts were dried in a rotary evaporator, then prepared and analyzed in the same way as other samples.

### 2.2. WD-XRF experiments

The elemental analysis of the herb tablets was carried out on the Si $K_\alpha$ line using an Advant'XP WD-XRF spectrometer (THERMO ARL, En Vallaire, Switzerland) with its proprietary WinXRF software.

Each analysis was repeated 5 times. We employed the calibration curve method, for which seven standards were prepared by mixing talcum with cellulose and pressed into tablets as described above. Furthermore, the analyses were positively verified using three certified standards of powdered herbs: Virginia Tobacco Leaves (CTA-VTL-2; Institute of Nuclear Chemistry and Technology, Warsaw, Poland), Spinach (NCS ZC73013; China National Analysis Center for Iron and Steel, Beijing, China) and Cabbage (NCS ZC73012; China National Analysis Center for Iron and Steel, Beijing, China). They were pressed into tablets without former grinding.

### 2.3. NMR experiments

High-resolution solid-state NMR spectra were acquired at 298 K on a Avance 400WB spectrometer (Bruker, Rheinstetten, Germany) with a Bruker 4 mm CP/MAS probe. The acronym CP stands for cross-polarization [8,9], a signal enhancement method applied in this work for silicon-29, the isotope which has only 4% natural abundance. In such experiment, the $^{29}$Si signal is generated with the aid of protons located in the vicinity of the observed silicon nucleus. Our $^{29}$Si CP NMR experiments were set on natural kaolinite from Greenland (obtained from Faculty of Geology, University of Warsaw, Warsaw, Poland). The acronym MAS stands for magic-angle spinning [8], which is a method for narrowing signals in the solid-state NMR, that is for achieving high resolution spectra. The NMR measurements were carried out on powdered herbs at the $^{29}$Si and $^{1}$H resonance frequencies of 79 and 400 MHz, respectively. The samples were spun by dry air in 4 mm $ZrO_2$ rotors at MAS of 5 kHz. Two different $^{29}$Si MAS NMR experiments were done with high-power proton decoupling during signal acquisition [8]: the conventional pulse-acquire sequence (called Bloch-decay, hereafter designated BD) and the single-contact $^{1}H \rightarrow {}^{29}Si$ CP pulse sequence with reversal of spin temperature in the rotating frame. For BD, the silicon $\pi/2$ pulse of 2.55 μs and the recycle delay of 40 s were used. For CP, the proton $\pi/2$ pulse of 2.75 ms, the optimized contact time of 2 ms and the optimized recycle delay of 1 s were used. The $^{29}$Si chemical shifts were externally referenced against TMS, using kaolinite as a secondary standard with a signal at 91.5 ppm. Peak fittings were done using a NutsPro NMR computer program (Acorn 2007). Mixed Lorentzian/Gaussian line shapes were applied to fit experimental spectra and obtain chemical shifts together with approximate peak areas.

## 3. WD-XRF method optimization

During method optimization several factors were tested namely: grinding time, amount of sample in the tablet and resulting thickness.

### 3.1. Grinding times

Grinding times from 5 to 20 min with 5 min interval were tested. Homogenization was assessed visually. Homogeneous powder was achieved after 10 min whereas longer grinding time did not produced better results.

### 3.2. Amount of sample in the tablet

Tablets containing were prepared from 2 to 7 g of sample, which resulted in different tabled thickness. Increase of Si signal was observed in as sample amount increased from 2 g to 4 g. No increase was observed for samples amount increasing from 4 g to 7 g. Eventually 5 g tablets were chosen for subsequent experiments.

## 4. WD-XRF method validation

The WD-XRF method for determination of silicone was validated before it has been used to test the actual sample. The validation was performed in accordance to ICH\Q2R1 [10]. Following parameters were included in the validation: specificity, accuracy, precision, linearity detection limit and quantization limit.

### 4.1. Specificity

XRF spectra of 40 mg/g reference mixture were compared with spectra recorded for natural samples (herb of *E. arvense*). All spectra were recorded in the range from 95° to 115°. Comparison shows that regardless of sample there is no interference of signal Ka for Si. Fig. 1 depicts comparison of example spectra.

### 4.2. Accuracy

A determination of standardized samples containing known amount of Si was performed using a calibration curve established in linearity study. Standardized Virginia Tobacco Leaves and Spinach was used for the study. Each measurement of was repeated 5 times. The recovery was found to be 82.61% and 76.19% for Virginia Tobacco Leaves and Spinach respectively (RSD $n = 5$ was 4.00% for Virginia Tobacco Leaves and 3.71 for Spinach).

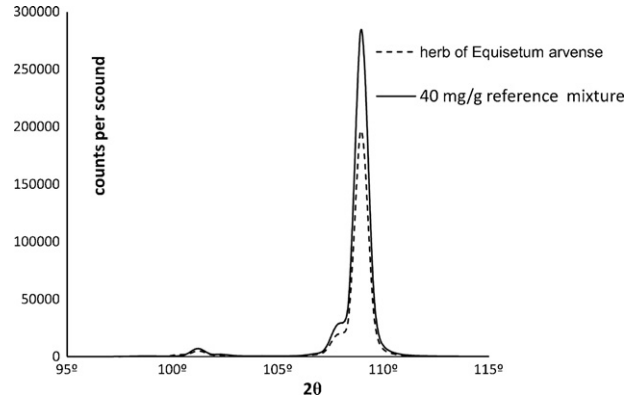



**Fig. 1.** The WD-XRF spectra of 40 mg/g reference mixture and herb of *Equisetum arvense*.

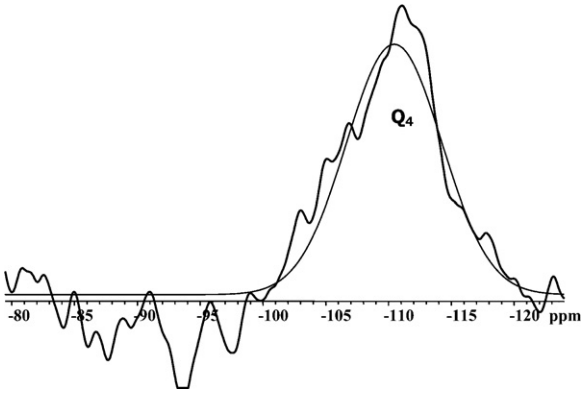



**Fig. 2.** The $^{29}$Si BD/MAS NMR spectrum of *Equisetum arvense* from a herbal teapot bag.

### 4.3. Precision

Precision was assessed by making 5 determinations of 2 mg/g reference mixture (mixture of talcum with cellulose). Standard deviation was 1440.18 and relative standard deviation was 3.44%.

To assess method repeatability 5 different samples (tablets) of *E. arvense* were prepared and measured. It was found that the RSD of those determinations was 1.53%.

The above results shows that the method is precise.

### 4.4. Linearity

A calibration curve was prepared by measuring reference mixtures at following concentrations: 2 mg/g; 5 mg/g; 10 mg/g; 20 mg/g; 30 mg/g; 40 mg/g; 50 mg/g. Each concentration was measured 5 times. Regression coefficient was $R^2 = 0.9980$, and the resulting regression curve expressed as $y = (a \pm S_a)x \pm (b \pm S_b)$, where $S_b$, $S_a$ are standard deviation of slope and intercept, is as follows: $y = (1855.11 \pm 37.40)x + (2588.78 \pm 1051.20)$. *T*-tests were performed for both slope and intercept coefficients. For slope $t = a/S_a$ ($t = 49.60$ $t_{tab} = 2.57$ $(n - 2 = 5)$) which means that there is a proportionality between concentration and signal. For intercept $t = b/S_b$ $t = 2.46$ $t_{tab} = 2.57$ which means that intercept coefficient value in negligible and it can be 0. In result a calibration curve was found: $y = ax$ ($y = (1928.62 \pm 30.61)x$). With $r^2 = 0.9985$. This calibration curve was used in subsequent determinations of natural samples.

### 4.5. Detection and quantitation limits

Detection and quantitation limits were established by measuring now low concentration sample Cabbage. The sample with Si concentration of 0.24 mg/g registered signal of 224.5 cps and with the noise signal of 58.5 cps hence this concentration is considered to be LOD. LOQ was calculated by multiplying LOD 3 times ($3 \times 0.24$ mg/g = 0.72 mg/g).

## 5. Results and discussion

Silicon assay results are presented in Table 2. We found the highest concentration of Si in EA, ca. 26 mg/g. However, there was a dramatic loss of Si during the infusion process. Out of 48.8 mg of Si from EA teabag only 1.1 mg was transferred to EA inf. The silicon levels found in EA and UD were similar to those reported in the literature [11,12]. Dietary supplements had much lower contents of Si than natural herbs (Table 2).

The determined concentrations of silicone in natural *E. arvense* from the wild habitat collected between may and august were: $19.8 \pm 0.9, 20.3 \pm 0.9, 21.1 \pm 1.1, 26.5 \pm 1.4$, respectively. This shows increase of the Si concentration as the material was harvested in successive, spring and summer months. The concentration of Si in herbs collected in August was quite close to that determined in the commercial product from herbal teapot bags, i.e. 27.1 mg/g (Table 2). This coincidence may indicate that pharmaceutical producers used herbs harvested in the same month.

Silicon forms were identified using high-resolution solid-state $^{29}$Si NMR. Because of the low Si contents, the spectra were very noisy despite long accumulation times. Therefore, we had to resort to peak fittings. Possible silicon sites are listed in Table 3 [1,13–16]. In the studied samples, we have only detected $Q_4$, $Q_3$ and $Q_2$ forms. In the BD spectrum of EA (Fig. 2) there was only one signal from a $Q_4$ site. On the other hand, CP exposed also peaks from $Q_2$ and $Q_3$ sites (Fig. 3). When discussing the CP enhancement, one has to remember that it is more effective for silicons bearing more hydroxyl groups, that is increases in the order: $Q_4 < Q_3 < Q_2$. It follows that in all the studied samples there is more $Q_3$ than $Q_2$ sites (larger $Q_3$ signal despite worse CP, see Table 4). The comparison of the BD and CP spectra of EA (Figs. 2 and 3) clearly indicates that this sample contains much more $Q_4$ than $Q_2$ and $Q_3$ sites. Then, considering the $Q_2$ and $Q_3$ peak areas (Fig. 3 and Table 4) and larger enhancement of $Q_2$ signals than $Q_3$ signals (two hydroxyl groups vs. one hydroxyl group, respectively), one can conclude that in EA there is at least 5 times more $Q_3$ sites than $Q_2$ sites. Hence, in EA the site populations obey the following series: $Q_4 \gg Q_3 > Q_2$.

Unsuccessfully, the BD experiment for UD gave no signal, although we used 4 times more scans than for EA. This failure can be explained by two reasons. The first reason is that the BD experiment is inherently less sensitive than the CP experiment. The second

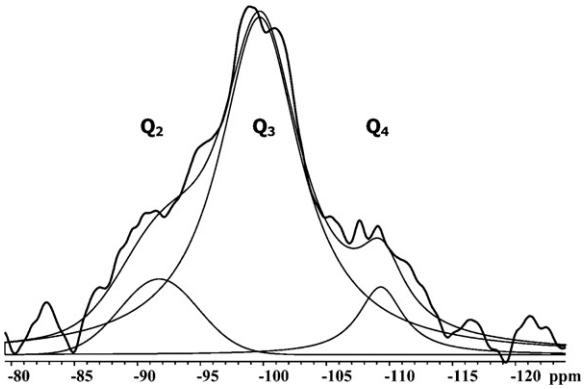



**Fig. 3.** The $^{29}$Si CP/MAS NMR spectrum of *Equisetum arvense* from a herbal teapot bag.

**Table 2**
Concentration of Si in the studied samples.

| Sample | Concentration of Si (mg/g) | Dosage form | Si in one dose (mg) |
|---|---|---|---|
| *Equisetum arvense* (EA) | 27.1 ± 1.3 | Dry herb from a herbal teapot bag[a] | 48.8 |
| *Urtica dioica* L. (UD) | 6.4 ± 0.3 | Dry herb from a herbal teapot bag | 11.5 |
| *Equisetum arvense* (EAinf) | 2.5 ± 0.3 | Dry residue from infusion (one herbal teapot bag)[b] | 1.1 |
| Dietary supplement A | 4.2 ± 0.2 | Tablet | 2.1 |
| Dietary supplement B | 2.4 ± 0.1 | Tablet | 0.6 |
| Dietary supplement C | 15.6 ± 0.2 | Capsule | 10.7 |
| Dietary supplement D | 14.2 ± 0.5 | Capsule | 6.1 |

[a] 1.8 g of herb.
[b] 425 mg of dry residue.

reason is that the silicon content in UD was ca. 4 times lower than that in EA, according to our WD-XRF estimates (Table 2). For the same reasons, the BD experiments on other than EA samples occurred inutile. However, considering analogous relative peak intensities in the CP spectra of EA and UD (Fig. 4), we may expect analogous silicon forms and their relative populations in those samples.

The CP signals from the $Q_2$, $Q_3$ and $Q_4$ sites were found for all the studied samples with the $Q_3$ signal being predominant (Table 4). Better water-soluble forms, neither $Q_1$ nor $Q_0$ (orthosilicic acid $Si(OH)_4$) have been detected. In the supplement B, two different $Q_3$ forms were found, giving signals with a ca. 1:2 area ratio. In the supplements A and C, two $Q_4$ signals were present (Fig. 5): regular at −111.8 and −110.6 ppm, respectively, and an extra one at −116.5 and −114.6 ppm, respectively. The latter peak was relatively strong and we assign it to silica present in the supplement C (Table 1). In the supplement A, silica was not declared by the producer but a small amount of it was observed there. The comparison of the CP spectra of EA and EA inf. (Table 4) indicates that the infusion process and the subsequent drying procedure increased

**Table 3**
Silicon forms found in plants and ranges of their chemical shifts [1,13–16].

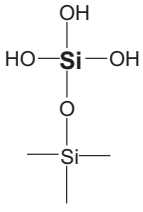
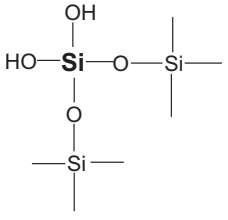
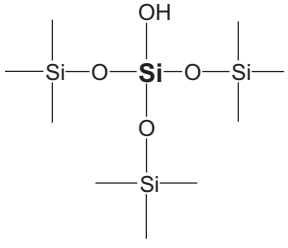
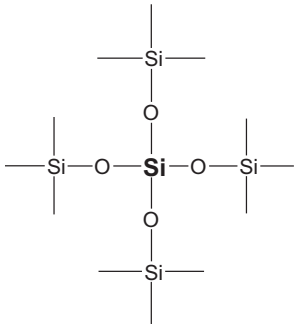
| Symbol | Chemical shift [ppm] | Silicon site | Structural formula |
|---|---|---|---|
| $Q_1$ | From −78 to −86 | $Si(OH)_3(OSi\equiv)$ |  |
| $Q_2$ | From −86 to −94 | $Si(OH)_2(OSi\equiv)_2$ |  |
| $Q_3$ | From −95 to −107 | $Si(OH)(OSi\equiv)_3$ |  |
| $Q_4$ | From −107 to −120 | $Si(OSi\equiv)_4$ |  |

**Table 4**
Silicon forms in the studied samples and their chemical shifts. Percent contributions of the signals to the total spectrum area are given in brackets.

| Sample | NMR technique | $Q_2$ | $Q_3$ | $Q_4$ |
|---|---|---|---|---|
| *Equisetum arvense* (EA) | CP | −91.7 (16.0) | −99.7 (74.6) | −109.3 (8.4) |
| *Equisetum arvense* (EA) | BD | – | – | −110.5 (100.0) |
| *Equisetum arvense* (EAinf) | CP | −92.0 (4.0) | −101.6 (74.4) | −111.2 (21.6) |
| *Urtica dioica* L. (UD) | CP | −91.1 (19.2) | −101.8 (55.7) | −111.5 (25.1) |
| Dietary supplement A | CP | −92.8 (16.0) | −101.5 (61.3) | −111.8 (15.7) |
| | | | | −116.5 (7.0) |
| Dietary supplement B | CP | −89.3 (4.0) | −97.4 (23.9) | −112.3 (18.6) |
| | | | −104.1 (53.5) | |
| Dietary supplement C | CP | −91.6 (2.5) | −101.1(60.0) | −110.1 (17.1) |
| | | | | −114.6 (20.4) |
| Dietary supplement D | CP | −92.0 (20.1) | −100.6(69.5) | −109.9 (10.4) |

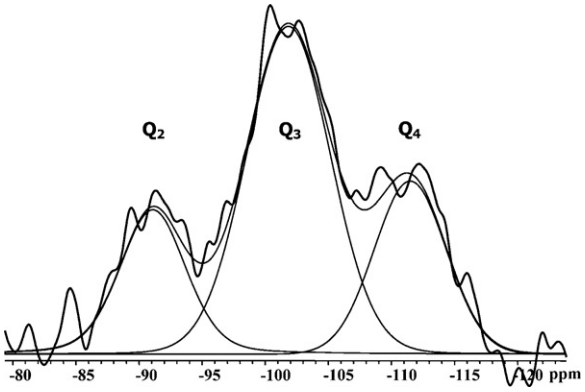



**Fig. 4.** The $^{29}$Si CP/MAS NMR spectrum of *Urtica dioica* L. from a herbal teapot bag.

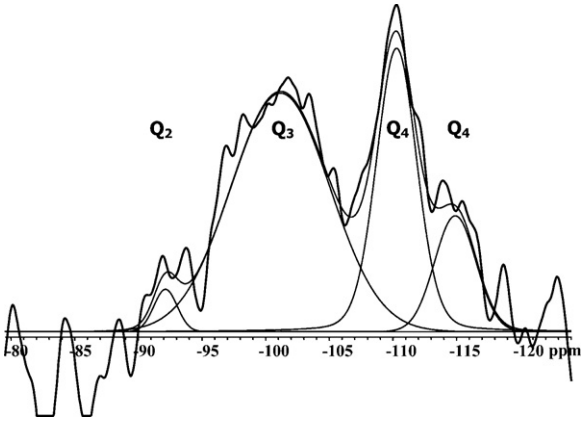



**Fig. 5.** The $^{29}$Si CP/MAS NMR spectrum of the herbal material from the dietary supplement C.

population of the $Q_4$ sites at the cost of the $Q_2$ sites.

## 6. Conclusions

The major conclusions can be summarized as follows:

(1) The Si concentration in all studied samples was in the range 2.4–27.1 mg/g (2.4–14.2 mg/g in the dietary supplements). The highest value was found in *E. arvense* from a herbal teapot bag, while for *U. dioica* L. from the same dosage form the Si content was ca. 4 times lower.
(2) A low recovery in spinach was observed. A likely explanation for this phenomenon is that in case of spinach a sample preparation and especially drying and grinding may not be sufficient enough to achieve better results. A further study is necessary to fully explain low recovery results. Nevertheless a successful assay can be performed with satisfying results.
(3) The Si concentration in *E. arvense* (whole plants), harvested in the wild habitat from May to August, increased from 19.8 to 26.5 mg/g.
(4) Three different silicon forms were found in the herbal materials: $Si(OSi\equiv)_4$ ($Q_4$), $Si(OH)(OSi\equiv)_3$ ($Q_3$) and $Si(OH)_2(OSi\equiv)_2$ ($Q_2$). Their concentrations in *E. arvense* complied with the series: $Q_4 \gg Q_3 > Q_2$.
(5) There was spectacular loss of Si during the infusion process, its concentration decreased ca. 50 times and the population of the $Q_4$ sites increased at the cost of the $Q_2$ sites.
(6) We proved that the WD-XRF spectroscopy can be used to measure the Si content in medical herbs, while the solid-state $^{29}$Si NMR can be successfully applied to determine the Si forms in those materials. Thus, both methods are complementary in the analysis of herbs.

## Acknowledgment

The Medical University of Warsaw is gratefully acknowledged for financial support (grants FW-23/N/2010 and FW-23/W1/2010).

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