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COMPARISON OF DIGESTION METHODS FOR ICP DETERMINATION OF TOTAL PHOSPHOROUS IN PLANT MATERIALS

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ABSTRACT

A comparative study of the most commonly used methods for sample preparation for ICP determination of the content of total phosphorus and sulfur in plant materials was performed on the basis of reference material CTA-VTL-2 (Virginia tobacco leaves). The methods used in the study were evaluated according to the recovery of total phosphorus and sulfur, ease of application and rapidity of performance. It was found out that microwave digestion is the most suitable method for sample preparation for simultaneous determination of phosphorus and sulfur by ICP in plant material. Dry ashing is not suitable because of the considerable losses of sulfur during thermal processing of the material in open vessels. The investigation revealed high correlation between colorimetric and ICP methods for total phosphorus determination, with results generally differing within 5 to 10 %.

Key words: tobacco, digestion methods, total phosphorus and sulfur, ICP, colorimetric methods

СПОРЕДУВАЊЕ НА ДИГЕСТИВНИТЕ МЕТОДИ ЗА ОДРЕДУВАЊЕ НА ВКУПНИОТ ФОСФОР КАЈ РАСТИТЕЛНИОТ МАТЕРИЈАЛ СО ICP

Направено е компаративно проучување на вообичаените методи за подготвување на примероци за одредување ICP-одредување на содржината на вкупниот фосфор и сулфур во растителниот материјал врз база на референтен материјал CTA-VTL-2 (листови од вирџиниски тутун). Методите применти во проучувањето се проценети според враќањето на вкупниот фосфор и сулфур, сложеноста на апликацијата и брзината на изведувањето. Утврдено е дека микробрановата дигестија е најпогоден метод за подготовка на примероците за симултано одредување на фосфорот и сулфурот со ICP во растителен материјал. Сувото спалување не е погоден метод поради значителните загуби на сулфур за време на термалната обработка на материјалот во отворени садови. Направена е споредба помеѓу колориметрискиот и ICP-методот за одредување на вкупниот фосфор и утврдена е висока корелација помеѓу нив, со резултати што се разликуваат за 5% до 10%.

Клучни зборови: тутун, дигестивни методи, вкупен фосфор и сулфур, ICP, колориметриски методи

INTRODUCTION

The determination of total phosphorus and sulfur in soils and plants is very important for agricultural and environmental studies. Phosphorus participates in a number of processes determining the growth, development and the productivity of the plant: formation of cell nucleus and cell multiplication, synthesis of lipids and specific proteins, transmission of hereditary properties, breathing and photosynthesis, energy transmission from richer to poorer energetic compounds etc. The multifarious role of P in plant metabolism is related to its participation in many biologically important organic compounds – nucleid acids, nucleoproteides, enzymes, vitamins, hormones, etc.

The determination of the total P in plant materials requires initial mineralization of the sample by digestion with mixtures of acids or ashing and phosphorous determination by different techniques, mainly colorimetry and inductively coupled plasma optical emission spectrometry (ICP-OES) (1, 2**)**. The problems related to its precise determination by ICP-OES are mainly due to the decrease of the signal of phosphorus in the content of calcium and other slightly ionizing elements in test samples. This repression of the signal could be overcome by using scandium as internal standard (3).

As a compound of the amino acids cysteine, cystin and meteonin, tripeptide glutation, of different proteins and inorganic sulphates, sulfur has an important role for oxidation-reduction processes, the energy balance of the plant, the functioning of the phytohormones, the enzyme activation, chlorophill formation etc. Nitrogen and sulfur ratios (N : S) are often used as a diagnostic tool (4).

While the problem with phosphorus is well known and its content in the soil and plants is subject to constant control, in the case of sulfur this issue has been underestimated. During the last years, however, decreasing sulfur input from atmospheric deposition and fertilizers has led to increasingly widespread S deficiencies in the UK (5). Due to the strengthening of pollution control measures, similar trends have been observed in other West European and North American countries.

The problem of increased S deficiency has led to a greater need for plant tissue and soil testing in order to diagnose whether applications of S fertilizers are necessary. Compared to other important elements, testing for sulfur is relatively new. The most widely used method for determination of total S in plant tissues involves the initial destruction of the organic matter by digestion with mixtures of nitric and perchloric acid, or ashing in the presence of magnesium nitrate in a muffle furnace, followed by dissolution of the ash in diluted acid (6). The total S in solution may then be analysed by colorimetry with the methylene blue method (7), by turbidimetry with barium sulphate formation (6, 8), X-ray fluorescence analysis (9, 10) or ion chromatography (IC) (11). In the recent years, ICP has been accepted as a basic technique for measuring sulfur, because of its capacity for measuring in a UV spectral range, the relative non-presence of spectral interferences and the possibility of multi-element analysis (12, 13).

In spite of the significant number of publications regarding determination of the sulfur content in plant materials, plant total S analysis shows greater variations than those shown for other elements. Examination of data from a bimonthly report of the International Plant Analytical Exchange Programme (IPE) shows that the coefficient of variation (CV) for plant total S is typically about 22 %, whereas for P, K and N the CVs ranges from 10 to 15 % (14). The higher CV for S supports the view that S analysis is more difficult for laboratories. This problem forces more efforts to be focused on the two main stages of analysis – sample preparation and appropriate methods for sulfur determination. This necessity defines the objective of this study - to compare different digestion methods for determination of total phosphorus and sulfur in plant material.

MATERIAL AND METHODS

***1.* Plant material**

A Polish reference material CTA-VTL-2 (**Virginia tobacco leaves**), containing 2204 ± 78 *ppm* phosphorous was used in the study. For the sulfur, a single information value of 0.669 wt % is given.

2. Digestion methods

The inductively coupled plasma (ICP) emission spectrometry allows the determination of both metals and non-metals. This is why it is most suitable as a chief method for complete analysis of the tested material. A main consideration in the selection of digestion methods was the possibility for simultaneous determination of the most important macro- and micronutrients in the plants by a single digestion procedure. The most common methods for total or nearly total decomposition of the sample were used as follows:

2.1. Dry ashing:

Procedure for dry ashing at 400 ^{o}C, 450 ^{o}C, 500 ^{o}C and 550 ^{o}C in a muffle furnace, following BDS 11708-93, was used. Samples (0.5 g) were weighed in 50 mLglass beakers, charged on a hot plate with stepwise increasing temperature up to 350^{o}C for 4 hours and finally ashed in a muffle furnace at 400, 450, 500 and 550 ^{o}C for 1 hour. After cooling, ashes were dissolved in 20 mLof 1.5 % HNO_3, or in a composition 3:1 v/v of HCl: HNO_3.

2.2. Wet mineralization:

a) $HNO_3 + H_2O_2$ digestion
One gram of oven-dried sample was transferred to a teflon beaker to which 10 mL of concentrated nitric acid were added. The sample was then warmed on a hot plate to about 85-95 ^{o}C until the initial reaction had subsided. After cooling 0.5 mLof 30% hydrogen peroxide was added dropwise, and the sample was then reheated. Stepwise additions of peroxide were repeated until the sample solution had clarified and no fats were visible. The sample was then diluted to 50 ml.

b) Procedure for acid digestion
One gram of oven-dried sample was weighed into a Kjeldahl flask and 5 mLof 65% HNO_3 were added. The flask was placed on a preheated hot plate and heated until its content was evaporated to dryness. 14 mLof 72% $HCIO_4$ were added to the sample and it was heated for 60 min. The digest was cooled, filtrated and diluted to 100 mL in a calibrated flask.

c) Procedure for acid digestion, following ISO 5515-1979
A tobacco sample of 2.0 to 2.5 g, dried to constant weight, was put in a Kjeldahl flask of 100 to 150 mL 30 mL HNO_3 and 4.0 mL H_2SO_4 as well as a few glass pearls were added. The mixture was left to settle for 12 hours and after that the flask was carefully heated in order to avoid the formation of foam. The heating of the liquid continued until the boiling point was reached and the process of boiling continued until the liquid acquired brown color. After that portions of 1.0 to 2.0 mL HNO_3 were added until the release of nitric oxides stopped and the release of white fumes began. The solution was cooled down, 15 mL were added and it was heated up again until boiling with release of white fumes began. The cooled solution was transferred into a measurement flask of 50 or 100 mL and was filled up to the marking.

The same standard was applied in the digestion procedure, including the use of $HCIO_4$ 6.0 to 8.0 mL HNO_3 were added to the sample, it was left to settle for 12 hours and was brought to boiling without being evaporated to dryness. After cooling an additional portion of 6.0 to 8.0 mL HNO_3 was added and the liquid was brought to boiling as the procedure was repeated once or twice more. After cooling, 6.0 to 8.0 mL HNO_3 and 4.0 mL H_2SO_4 were added. The decomposition began vigorously without heating. After the vigorous reaction stopped, the sample was cooled and 4.0 to 6.0 mL HNO_3 and 3.0 to 5.0 mL $HCIO_4$ were added.

The boiling continued until the solution became colorless. Then it was cooled and brought to volume of 50 or 100 cm^3.

d) Procedure for acid digestion, following BDS 17365-94 for determination of heavy metals in tobacco and tobacco products:
A tobacco sample of 2.0 to 2.5 g dried to constant weight was put into a Kjeldahl flask of 100 to 150 mL 20 mL HNO_3 and 5.0 mL H_2SO_4 were added and the mixture was left to settle for 2 to 4 hours. After that the flask was heated in a sand bath for 30-40 minutes at 80-90°C. At the appearance of a dark yellow or brown coloring of the solution the flask was cooled and 10 mL HNO_3 and 2 mL $HClO_4$ were added. The heating continued until the elimination of most of the acids. 10 mL of distilled water were added twice and the solution was heated until most of it evaporated. After cooling, the residue was treated twice by 4 mL 1M HCl and after that it was filled up to 10 mL with 1M HCl.

2.3. Microwave digestion (MW):
A procedure for microwave digestion with different acid mixtures, following EPA METHOD 3051, suitable for flame AAS determination of heavy metals, was used. A microwave digestion system (Milestone 1200 MEGA, Italy) with 10 MRD 300 rotor with 10 positions, max. pressure of 30 bars and max. power 1000 W was used. A homogenized sample of 0.5 g dry substance was weighed on assay balance into a Teflon bomb and 10 mLof concentrated nitric acid were added. The microwave mineralization programme comprised three stages: (i) 5 min. non-pulsed 250 W microwave irradiation; (ii) 5 min. 400 W pulsed microwave irradiation and (iii) 5 min. 600 W pulsed microwave irradiation. After a one minute ventilation, the sample was cooled and diluted to 50 ml. Several additional alternative acid combinations including H_2O_2 and HF were used in accordance with EPA METHOD 3052.

3. Phosphorus and sulfur determination

An ICP-AES spectrometer Spectroflame MODULA (Spectro Analytical Instruments, Kleve, Germany), equipped with two monohromators: (i) spectral range 160 – 460 nm with nitrogen purged optics and (ii) spectral range 240 – 790 nm with air purged optics, was used. The analytical operational parameters were optimized with the aim to achieve the lowest possible limit of detection for phosphorus and sulfur (Table 1).

Table 1. Instrument settings and measurement conditions

Parameter	Index
Nebulizer	Mainhard TR 30 A3
Rate of sample delivery	1.2 $mL min^{-1}$
Argon torch gas flow (cooling gas)	14 l min^{-1} Ar
Argon auxiliary gas flow	0.5 l min^{-1} Ar
Argon nebulizer gas flow	1.4 l min^{-1} Ar

4. Statistical analysis

For evaluation of the correctness of results for phosphorous, three generally accepted criteria were used as follows:

1. $D = X - X_{CRM}$, where X is the measured value and X_{CRM} is the certified value. When D is within the borders of $\pm 2\sigma$, where σ is the standard deviation from the certified value, the result is considered to be good, when it is $-3\sigma \leq D \leq 3\sigma$ - satisfactory, and beyond these limits the result is unsatisfactory.
2. $D\ \% = D / X_{CRM} .100$ – percentage difference. When the values of D % are in the limits $\pm 200\sigma / X_{CRM}$ the result is considered to be good, when the value is

in the limits ± 200σ/ X_{CRM} and ± 300σ/ X_{CRM} - satisfactory, and when it is out of the limits ± 300σ/X_{CRM} the result is unsatisfactory.

3. $Z = X - X_{CRM} / \sigma$. When $Z \leq 2$ the result is considered to be good, when $2 \leq Z \leq 3$ - satisfactory, when $Z > 3$ - unsatisfactory.

For easier evaluation of the effectiveness of different methods for sample preparation we have used R criterion showing the extent of extraction of the element in percents from the certified value. When the measured value X is within the borders of $X_{CRM} \pm U_{CRM,}$ where U_{CRM} is the indefiniteness of the certified value, we accept the extent of extraction to be 100%. In all remaining cases the extent of extraction is equal to X/ X_{CRM}.100.

Due to the lack of data for standard deviation, when determining sulfur, there was no determined Z – criterion. In this case, when the certified value X_{CRM} is within the limits of $X \pm \sigma$, where σ is the standard deviation of the measured value, we accept the extent of extraction to be 100%.

RESULTS AND DISCUSSION

The emission lines at ICP-AES determination of phosphorous and sulfur, estimated detection limits and interferences are presented in Table 2.

Table 2. Emission lines upon ICP determination of phosphorous and sulfur, detection limits and interferences

Element	Technique / Line	Estimated D.L.	Optics	Interferences
P	ICP-OES 178.287 nm	0.015 μg ml^{-1}	V	I, Mo,Mn
	ICP-OES 177.495 nm	0.020 μg ml^{-1}		Cu, Hf
	ICP-OES 213.618 nm	0.024 μg ml^{-1}		Cu,Fe
S	ICP-OES 180.734 nm	0.010 μg ml^{-1}	V	Ca, Al
	ICP-OES 182.034 nm	0.030 μg ml^{-1}		

According to data from literary sources, in the case of ICP determination of phosphorus at 177.495 nm, there is spectral interference of copper. The concentration of copper in tobacco leaves, according to the certificate, is 18.2 ppm, and its concentration in the tested solutions after dilution was less than 0.05 ppm. The pulverization of 0.10 ppm mono standard of Cu does not give intensity different from the background at 177.495 nm. Therefore, at this concentration of copper, no off peak correction is necessary. However, the content of phosphorus in all test samples was determined at two different wavelengths -177.495 and 178.287. The results obtained are statistically indistinguishable, and the table presents the average values.

The content of sulfur was determined at wavelength of 182.034 nm. For both elements background correction was performed. For P determination we used the internal standard method by adding scandium to the samples and standard solutions. The calibration was performed using three standard solutions in 2 % v/v HNO_3. A commercial multielement standard solution with concentration 100 μg/l was used as a stock solution. The calibration standard solutions have the following concentrations: 0,0; 5.0 and 10.0 ppm.

Thirteen samples of the tested material (Virginia tobacco leaves) were prepared for analysis for total phosphorous and sulfur content. The results of the ICP analyses of phosphorous are presented in Table 3.

The results obtained show that the extraction of phosphorus is complete in all variants of dry ashing and microwave digestion. However, closer to the certified values are the results obtained during microwave mineralization, as the values of the Z – criterion do not exceed 2.0, and of D % - 7.0. It is observed that all results obtained by dry ashing are higher than the certified value, which excludes lack of

phosphorus at these temperatures. In four of the variants, however, the values of Z-criterion are greater than 2.0, and those of D % reach 10.25.

The results in the case of wet digestion are different. $HNO_3 + H_2O_2$ and $HNO_3 + HClO_4$ wet digestion methods are efficient and are estimated as "good". The efficiency of the $HNO_3 + H_2SO_4 + HClO_4$ digestion depends on the acid ratio and can be "good", while $HNO_3 + H_2SO_4$ digestion is unsuitable.

Table 3. Effectiveness of different digestion methods upon ICP-AES determination of phosphorous contents in tobacco leaves. $X_{CRM} = 2204$ *ppm*, $\sigma_{CRM} = 78$ ppm

Method	X *ppm*	σ_x *ppm*	D	D, %	Z	R
Dry ashing, 550°C	2420	200	216*	9.80*	2.77*	100
Dry ashing, 500°C	2430	220	226*	10.25*	2.90*	100
Dry ashing, 450°C	2390	180	186*	8.44*	2.38*	100
Dry ashing, 400°C	2280	150	76**	3.45**	0.97**	100
Dry ashing + ($HCl + HNO_3$)	2400	250	196*	8.89*	2.51*	100
$HNO_3 + H_2O_2$ digestion	2240	180	36**	1.63**	0.46**	100
$HNO_3 + HClO_4$ digestion	2300	100	96**	4.36**	1.23**	100
$HNO_3 + H_2SO_4 + HClO_4$ digestion	2130	160	-74**	-3.36**	0.95**	100
$HNO_3 + H_2SO_4 + HClO_4$ digestion	1930	280	-274	-12.43	3.51	87.6
$HNO_3 + H_2SO_4$ digestion	1490	260	-714	-32.40	9.15	67.6
MW, HNO_3	2230	120	26**	1.18**	0.33**	100
MW, $HNO_3 + H_2O_2$	2270	190	66**	2.99**	0.85**	100
MW, $HNO_3 + H_2O_2 + HF$	2355	110	151**	7.08**	2.00**	100

* - "satisfactory" results
** - "good" results

During the last year soil and plant laboratories have moved from colorimetry to inductively coupled plasma (ICP) spectrometry to quantify phosphorous in soil and plants. The main reason is that ICP has the advantage of being quicker and provides the possibility to quantify phosphorus and other plant nutrients in a single analytical process (2). However, we have to take into account that the P value with ICP is not always comparable with the colorimetric P value, which usually has been used to set up fertilizer P recommendations. On the other hand, colorimetric procedures offer some advantages, such as increased sensitivity and lower instrumentation cost, and it is unlikely that ICP will completely displace colorimetric procedures. With the aim to define the magnitude of the difference between ICP and colorimetric P in the plant material investigated, we analysed the same solutions, obtained by the digestion methods described above, following the colorimetric procedure for P determination, described by M.K. John (15). The results are presented in Table 4.

The results obtained give us ground to recommend the use of the microwave digestion method during sample preparation for ICP determination of the content of phosphorus in plant material. In addition to providing full extraction and preventing loss during the digestion, this method is quick and easy for application. In case of lack of the necessary equipment, dry ashing could be used.

The results of the ICP analyses for sulfur are presented in Table 5, as in this case the results of wet digestion methods, using H_2SO_4 are not included.

Table 4. Effectiveness of different digestion methods upon colorimetric determination of phosphorous contents in tobacco leaves. X_{CRM} = 2204 *ppm*, σ_{CRM} = 78 *ppm*

Method	X *ppm*	σ_x *ppm*	D	D, %	Z	R
Dry ashing, 550^{o}C	2400	95	196*	8.89*	2.51*	100
Dry ashing, 500^{o}C	2260	112	56**	2.54**	0.72**	100
Dry ashing, 450^{o}C	2350	94	146**	6.62**	1.87**	100
Dry ashing, 400^{o}C	2260	85	56**	2.54**	0.72**	100
Dry ashing + (HCl + HNO_3)	2400	102	196*	8,89*	2.51*	100
HNO_3 + H_2O_2 digestion	2360	87	156**	7.08**	2.00**	100
HNO_3 + $HClO_4$ digestion	2400	83	196*	8.89*	2.51*	100
HNO_3 + H_2SO_4 + $HClO_4$ digestion	2200	74	-4	-0.18**	-0.05**	100
HNO_3 + H_2SO_4 + $HClO_4$ digestion	2000	99	-204*	-9.26*	-2.62*	90.7
HNO_3 + H_2SO_4 digestion	1740	102	-464	-21.05	-5.95	78,9
MW, HNO_3	2320	82	116**	5.26**	1.49**	100
MW, HNO_3 + H_2O_2	2300	77	96**	4.36**	1.23**	100
MW, HNO_3 + H_2O_2 + HF	2430	95	226*	10.25*	2.90*	100

* - "satisfactory" results

** - "good" results

Table 5**.** Effectiveness of different digestion methods upon determination of sulfur contents in tobacco leaves

Method	X, %	σ_x, %	X_{CRM}, %	D	D, %	R
Dry ashing, 550^{o}C	0.564	0.060	0.669	-0.105	-15.70	84.3
Dry ashing, 500^{o}C	0.596	0.045	0.669	-0.073	-10.91	89.1
Dry ashing, 450^{o}C	0.558	0.052	0.669	-0.111	-16.59	83.4
Dry ashing, 400^{o}C	0.605	0.050	0.669	-0.064	-9.57	90,4
Dry ashing + (HCl + HNO_3)	0.581	0.061	0.669	-0.088	-13.15	86.8
HNO_3 + H_2O_2 digestion	0.598	0.061	0.669	-0.071	-10.61	89.4
HNO_3 + $HClO_4$ digestion	0.742	0.068	0.669	0.073	10.91	100
MV, HNO_3	0.744	0.057	0.669	0.075	11.21	100
MW, HNO_3 + H_2O_2	0.620	0.056	0.669	-0.049	-7.32	100
MW, HNO_3 + H_2O_2 + HF	0.653	0.052	0.669	-0.016	-2.39	100

In this case, too, the best results are obtained by microwave digestion of the samples, as the three variants provide for 100 % recovery. In spite of that, however, the results vary broadly – from 0.620 to 0.744 %, and the values of σ_x in all measurements exceed 0.05.

None of the dry ashing variants is suitable for determining sulfur in plant material. Obviously, a great part of the sulfur is lost during thermal processing of the samples in open vessels. When using HNO_3 + H_2O_2 wet digestion method an extraction of about 90% is reached. Poykio et al. (12) have also obtained results close to these when determining the content of sulfur in a certified sample of beech leaves. According to the same authors, the HNO_3 + $HClO_4$ digestion procedure gave lower results, which is in contradiction with the results that we have obtained.

Because one and the same method ICP-AES was used in all quantitative determinations, the comparative study carried out lead us to the conclusion that the sample preparation is a critical stage in determining sulfur in plant material, which requires thorough investigations in this direction. This conclusion is also supported by the inter-laboratory comparison of sulfur analysis in plant materials, summarized by Crosland et al. (5).

CONCLUSION

A comparative study of the most commonly used methods for sample preparation for ICP determination of the total phosphorus and sulfur contents in plant materials was held on the basis of reference material CTA-VTL-2 (Virginia tobacco leaves). The methods used in the study were evaluated according to the recovery of total phosphorus and sulfur, ease of application and rapidity of performance. It was found out that:

1. Microwave digestion and dry ashing methods, as well as wet methods, including the use of HNO_3 + $HClO_4$ quantitatively extract phosphorous from the studied plant samples. Microwave digestion gives the best results and could be recommended, taking into consideration its rapidity and ease of determination. HNO_3 + H_2SO_4 digestion, which gives low recoveries probably due to incomplete oxidation, and HNO_3 + H_2SO_4 + $HClO_4$ acid mixture have to be used very carefully.
2. Microwave digestion and wet HNO_3 + $HClO_4$ procedure quantitatively extract sulfur from the plant material, while dry ashing is not suitable because of considerable losses during thermal processing of the material in open vessels.
3. Microwave digestion is the most suitable method for sample preparation for simultaneous determination of phosphorus and sulfur by ICP-AES in plant material.

REFERENCES

1. Gasparatos D., Haidouti C., 2001. A comparison of wet oxidation methods for determination of total phosphorus in soils. J. Plant Nutr. Soil Sci., 164, 435.
2. Sicora F.J., Howe P.S., Hill L.E., Reid D.C., Harover D.E., 2005. Phosphorus Determination in Waters and Extracts of Soils and By-Products: Inductively Coupled Plasma Spectrometry versus Colorimetric Procedures. Commun.Soil Sci. Plant Anal., 36, 875.
3. Kola H., Peramaki P., Valimaki I.J., 2002. Correction of spectral interference of calcium in sulfur determination by inductively coupled plasma optical emission. spectrometry using multiple linear regression. Anal. At. Spectrom., 2,104.
4. Randall P.J., Wrigley C.W., 1986. Effects of sulfur supply on the yield, composition, and quality of grain from cereals, oilseeds, and legumes. Adv. Cereal Sci. Tech., 8 (1), 171.
5. Crosland A.R., Zhao F., McGrath S.P., 2001. Inter-laboratory comparison of sulphur and nitrogen analysis in plants and soils. Commun. Soil Sci. Plant Anal., 32, 685.
6. Blancharm R.W., 1986. Measurement of sulfur in soils and plants. In: Sulfur in Agriculture, (Ed. M.A. Tabatai). American Society of Agronomy, Madison, WI 455-490.
7. Johnson C.M., Nishita H., 1952. Microestimation of sulphur in plant materials, soils and irrigation waters. Anal. Chem., 24, 736.
8. Morante C., 1991. Determination of plant sulphur and sulphate-sulphur by flow-injection analysis using a two-line manifold. Analytica Chimica Acta, 249, 479.
9. Norrish K., Hutton J.T.ED(P)XRF, 1997. Screening analysis and quantitative analysis with polarized X-rais. X-ray Spectrom., 6,12.
10. Sprta V., Knob B., Janos P., 1999. Fresenius' Journal of Analytical Chemistry, 364 (8), 705.
11. Artiola J.F., Ali A.S., 1990. Determination of Sulfur in soil and plant samples using sodium bicarbonate/silver oxide dry ashing and ion chromatography. Commun. Soil Sci. Plant Anal., 21, 941
12. Poykio R., Torvela H., Peramaki P., Koukkanen T., Ronkkomaki T., 2000. Comparison of dissolution methods for multi-element analysis of some plant materials used as bioindicator of sulphur and heavy metal deposition determined. Analusis, 28, 850.
13. Hoenig M., 2001. Preparation steps in environmental trace element analysis - facts and traps. Talanta, 54, 1021.
14. Sterrett S.B., Smith C.B., Mascianica M.P., Demchak K.T., 1987. Comparison of analytical results from plant analysis laboratories. Commun. Soil Sci. Plant Anal., 18 (3), 287.
15. John M.K.,1970. Colorimetric Determination of phosphorus in soil and plant materials with ascorbic acid. Soil Science, 109 (4), 214.