Original Contribution

CRITICAL ANALYSES OF BDS 17365 - 94

L. Dospatliev*

Department of Pharmacology, Animal Physiology and Physiological Chemistry,
Trakia University, Stara Zagora, Bulgaria.

ABSTRACT

A method allowing the simultaneous determination of the most essential for tobacco culture growth macro and trace elements (K, Ca, Mg, P, Mn, Fe, Cu, Zn, Pb and Cd) was developed by Inductively Coupled Plasma (ICP). H_2SO_4 and $HClO_4$ were not applied as they provoked losses of the basic macro elements as a result of sludge deposition of hardly dissolved compounds. Acid consumption was significantly decreases and the analysis time was shortened.

Key words: BDS 17365-94, tobacco, new method, ICP, X-ray analyses

INTRODUCTION

The method applied in Bulgaria for the preliminary preparation of tobacco and tobacco products in order to determine the content of As, Mn, Cu, Cd, Ni, Pb and Zn is regulated by BDS 17365-94 (1). The procedure is based on sample mineralization with a mixture of sulfuric, nitric and perchloric acid.

1. Tobacco or tobacco product sample (2.00 - 2.50 g) dried to constant weight and cut to 1.0 mm particle fractions is placed in a 100 - 150 cm^3 kheldahl flask. 20 cm^3 nitric acid and 5 cm^3 sulfuric acid are added and the mixture is left to stay 2 - 4 h. The flask is heated carefully on a sand bath for 30 - 40 min at 80 - 90°C. The temperature is gradually increased up to the appearance of dense white sulfur trioxide vapours. The solution colour is observed. The flask is immediately removed and cooled as soon as dark yellow or brown colouring of the solution appears.
2. 10 cm^3 nitric acid and 2.0 cm^3 perchloric acid are added. The heating proceeds till the elimination of greater acids portion. 10 cm^3 double distilled water is added twice and the mixture is heated till most of the solution is evaporated. The residuum is treated twice with 4 cm^3 1M HCl after cooling, then it is

poured into a graduated test tube or 10 cm^3 cylinder. The latter is filled to the 10 cm^3 mark with 1M hydrochloric acid.

Mn, Cu and Zn contents in the solution are determined by atomic absorption spectrometry by injection of the solution in oxidation acetylene-air flame and after consecutive atomization of the sample in a graphite furnace for the determination of Cd, Ni and Pb.

Arsenic determination is conducted after reduction with sodium borohydride in sulfuric acid medium, production of gaseous arsenic and atomization in electrothermally or flame heated quartz tube.

Disadvantages of the method:
1. The method does not include determination of basic macro and trace elements as phosphorus, potassium, calcium, magnesium, iron, etc.
2. The analyses requires significant consumption of acids (30.0 cm^3 nitric acid, 5.0 cm^3 sulfuric acid and 2.0 cm^3 perchloric acid), energy (4.0 - 5.0 kW/sample) and is time consuming (total duration of one analyses - 6 - 8 h).
3. The use of perchloric acid.

The goal of the present study was to eliminate the disadvantages stated by the elaboration of an innovative method which would allow the simultaneous determination of all essential for the tobacco plant macro and trace elements at lower materials and time consumption.

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*Correspondence to: *L. Dospatliev*. *Department of Pharmacology, Animal Physiology and Physiological Chemistry, Trakia University – Stara Zagora, Bulgaria, lkd@abv.bg*

MATERIALS AND METHODS

Apparatuses

The measurements of macro and trace elements content in plant samples were accomplished on atomic absorption spectrometer (ICP) Spectro. **Fig. 1** presents the scheme of the apparatuses used for the preliminary samples preparation. It consists of a heating block with 24 sockets allowing precise temperature regulation, borosilicate glass test tubes, Teflon collector for gases discharge, vacuum meter and a water pump.

Figure 1. A scheme of the apparatuses for sample preparation.

Reagents

Double distilled water was used for all experimental procedures. All reagents applied were of analytical grade (p.a. Merck , Fluka). The initial standard solutions for K, Ca, Mg, P, Mn, Fe, Cu, Zn, Pb, Cd determination by ICP with a concentration 1 000 mg dm^{-3} were supplied by Merck, Darmstadt, Germany.

Samples

Reference material CTA-VTL-2 (Virginia tobacco leaves) was used for examination of the method accuracy.

Sample preparation for ICP by the new method

Samples mineralization with a mixture of nitric acid and hydrogen peroxide included the following stages:

1. 0.5 g from the sample dried to a constant weight were initially placed in borosilicate test tubes. Then the Teflon collector for removal of the discharged gases was fixed. 6 cm^3 concentrated HNO_3 were added to each test tube through the openings of the collector, and the heating block was heated to 40^oC for 30 min. Consequently, the temperature was increased to 100^oC. Temperature was kept constant till the reaction abatement.

2. 1.0 cm^3 30% H_2O_2 was added through the collector openings dropwise after cooling, then the thermo block was heated again to 12^0C for 30 min. In case of need, H_2O_2 addition was added till the solution was clarified. Consequently, the solution was quantitatively placed in a measuring flask, which was filled with distilled water to the 25 cm^3 mark. The content of K, Ca, Mg, P, Mn, Fe, Cu, Zn, Pb and Cd was then determined.

RESULTS AND DISCUSSION

The results obtained from the ICP determination of K, Ca, Mg, P, Mn, Fe, Cu, Zn, Pb and Cd in a referent material from Virginia tobacco leaves (CTA-VTL-2) are presented in **Table 1**. **R** criterion, representing the extent of element uptake in percents from the certified value, was used for accuracy estimation (2). X_{CRM} - certified value; X - measured value; σ - standard deviation of the certified value (n = 3).

The method reproducibility was estimated by means of the relative standard deviation **(RSD)** derived by the analyses of three parallel samples.

The results obtained by the method currently described were compared according to the content of K, Ca, Mg, P, Mn, Fe, Cu, Zn, Pb and Cd with the corresponding consensus values. The determined extent of uptake was in the range 87.7% - 100.0%, i.e. complete uptake was achieved.

The comparative analyses of the results obtained during the current study by the new method and those stated by Sabina et al. (3, 4, 5) for the mineralization of plant samples with HNO_3 and $HClO_4$ giving lower uptake values, namely Fe

(52%) and Mg (14%), imposed the conclusion that sample preparation is a critical stage for the determination of macro and trace elements in plant material. Sabina et al. explained that the results obtained were due to incomplete mineralization of the silicate minerals in the samples, which corresponds to the results of some other authors (6, 7, 8).

Table 1. *Results from the ICP determination of K, Ca, Mg, P, Mn, Fe, Cu, Zn, Pb and Cd in referent material from Virginia tobacco leaves (CTA-VTL-2).*

Element	$X_{CRM} \pm \sigma$	$X \pm \sigma$	RSD	R
K	1.03 ± 0.04	1.00 ± 0.03	3.0	97.1
Ca	3.60 ± 0.15	3.56 ± 0.07	2.0	98.9
Mg	0.510 ± 0.023	0.506 ± 0.01	2.0	99.2
P	2204 ± 78	2200 ± 160	7.3	99.8
Mn	79.7 ± 2.6	77.8 ± 1.1	1.4	97.6
Fe	1083 ± 33	950 ± 35	3.7	87.7
Cu	18.2 ± 0.9	18.3 ± 0.5	2.7	100.5
Zn	43.3 ± 2.1	43.1 ± 1.7	3.9	99.5
Pb	22.1 ± 1.2	22.1 ± 0.4	1.8	100.0
Cd	1.52 ± 0.17	1.49 ± 0.06	4.0	98.0

Poykio et al. (9, 10, 11) applied methods by means of a microwave-assisted system with acid mixtures of ($HNO_3 + H_2SO_4$), ($HNO_3 + HClO_4$) and ($HNO_3 + HClO_4 + HF$) for the determination of 14 macro and trace elements in certified samples from various plant materials. Complete uptake of the macro and trace elements was achieved by the applied in the recent study method, while when acid mixtures of ($HNO_3 + H_2SO_4$), ($HNO_3 + HClO_4$) and ($HNO_3 + HClO_4 + HF$) were applied the determined values were lower for most of the macro (especially K and Ca) and for some of the trace elements. The authors supposed that the reason is the formation of insoluble $KClO_4$ and $CaSO_4$ precipitate.

To ascertain the latter assumption, X-ray analyses of the precipitates formed after the tobacco samples decomposition as a result of the application of the ($HNO_3 + HClO_4$) and ($HNO_3 + H_2SO_4$) acid mixtures, was performed. On the defractogram presented on **Figure 2** the presence of high-intensity reflections that represent the formation of phases with well developed crystallites was established. The spectrum analyses proved the presence of only one crystal phase - $KClO_4$ (PDF - 70-0488).
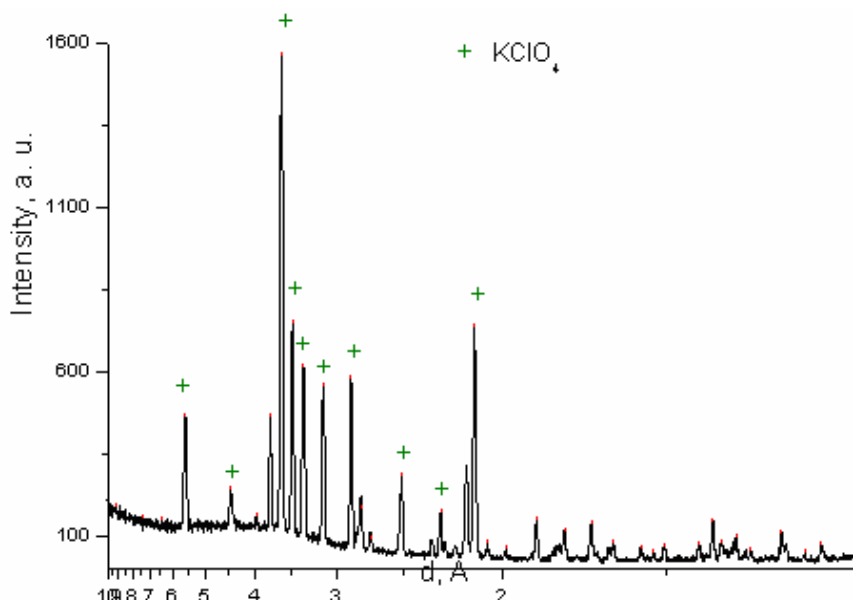

Figure 2. X-raygram of insoluble precipitate formed during tobacco sample mineralization by a method applying ($HNO_3 + HClO_4$) acid mixture

It was established that the use of an acid mixture of HNO_3 and H_2SO_4 also lead to the formation of a crystal $CaSO_4$ precipitate.

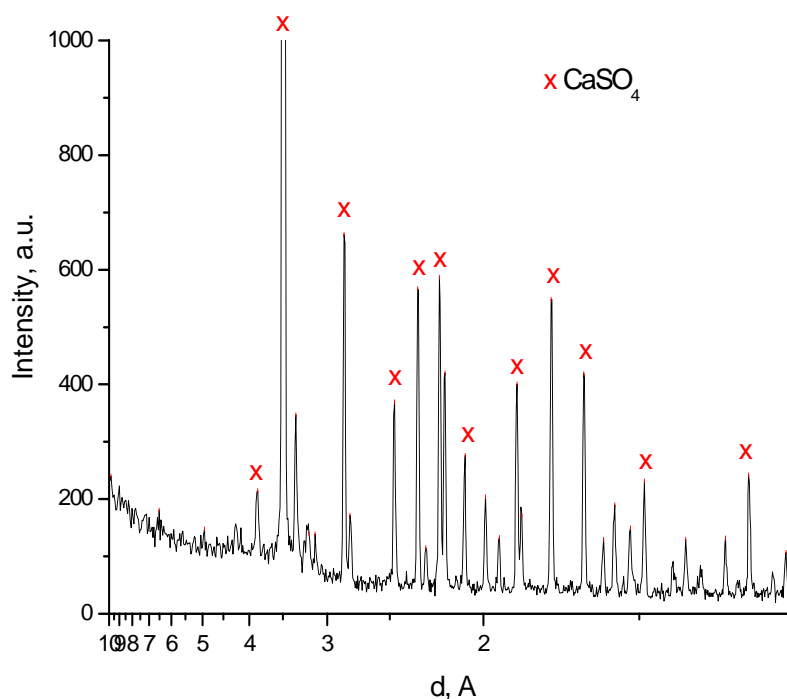**Figure 3** displays the X-raygram of the precipitate after the procedure finalization.

Figure 3. X-raygram of insoluble precipitate formed during tobacco sample mineralization by a method applying $(HNO_3 + H_2SO_4)$ acid mixture.

The results obtained in the current study stated confirmed the assumption derived, that the application of $HClO_4$ and H_2SO_4 leads to partial blockage of potassium and calcium in the form of insoluble $KClO_4$ and $CaSO_4$ and that both acids are not suitable oxidative agents for K and Ca determination in plant material.

CONCLUSIONS

1. The method currently developed applied in this study allowed the determination of the most essential for tobacco culture growth macro and trace elements.
2. The application of H_2SO_4 and $HClO_4$ was not appropriate as it lead to losses of basic macro elements due to the formation of hardly soluble compounds.
3. Acid consumption was significantly decreased and the analyses time was shortened.

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