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Perspective of Use of MALDI/TOF/MS for Identification of Some Natural Bioactive Compounds.

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ABSTRACT

This work represents the results of the method of plant polyphenols complexes and triacylglycerides identification consisting in use of the matrix-assisted laser desorbtion ionization (*MALDI/TOF/MS)* method allowing exclusion of the stage of pre-separation of the compound complexes being analyzed. Adequacy of the received results was confirmed by means of the chromatographic analysis methods, namely a reversed-phase high-efficiency liquid chromatography and a gas-liquid chromatography. In consequence of the carried out investigations practicability of *MALDI/TOF/MS* method use for combined plant complexes identification was ascertained.
Keywords: matrix-assisted laser desorbtion ionization, high-efficiency liquid chromatography, gas-liquid chromatography, flavonoids, triacylglycerides, fatty acids.

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INTRODUCTION

The native pharmacy gives preference to the chromatographic analysis methods, i.e. the reversed-phase high-efficiency liquid chromatography (RF HELC) and the gas-liquid chromatography (GLC). The mentioned methods have the main advantage consisting in multi-channel nature of the obtained information. In other words they provide simultaneous realization of the parameters which characterize separation, identification and quantification of components [1]. The stated methods ensure the essential part of drug preparation quality control at pharmaceutical enterprises. However it's worth noting that the current analytical material and technical base of the national pharmacy requires constant methodological and instrumental improvement. Replenishment of the instrumental base is possible due to the progressive methods which include the matrix-assisted laser desorbtion ionization (*MALDI/TOF/MS*).

MALDI/TOF/MS should be regarded as one of the soft ionization methods [2]. The method principle lies in action of laser radiation impulses on a matrix with substance being under analysis [3,4]. This method is generally applied for analysis of high-molecular compounds (peptides and proteins), therefore it became widely used in proteomic analysis [5,6]. Nevertheless *MALDI/TOF/MS* may be used for analysis of structures with any molecular weight and structure, for example flavonoids [7].

A device design for *MALDI/TOF/MS* is distinguished among others due to a built-in time-of-flight mass analyzer (*TOF/MS*) which separates the resultant ions by a period of time which is necessary for them to reach a detector [8]. That's why it has an opportunity to identify substances without need of their division into individual components.

Relevancy of this investigation is determined by experimental evidences of use of the present method for identification of bioactive compounds in comparison with the chromatographic methods.

In order to demonstrate analytical potential of *MALDI/TOF/MS* method the following plant bioactive compounds were selected as an investigation target: flavonoids and triacylglycerides. The traditional chromatographic methods, namely RF HELC and GLC were used for comparison of the obtained results.

METHODOLOGY

A liquid chromatograph made by "*Agilent Technologies 1200 Infinity"* company, USA, with an automatic sampler *Agilent 1200*, a vacuum micro degasifier, a gradient pump and a thermostat of the same series was used for analysis. Electronic absorption spectra were registered by means of a spectrophotometric detector with a diode matrix of *Agilent 1200* series, the scanning pitch - 2 nm.

Separation was made with use of a steel chromatographic column *Ascentis express* C_{18} *2.7µm x 100 mm x 4.6 mm*, in the gradient elution mode. The introduced sample volume – 1 µl. Spectral data registration and processing was performed with the aid of "*Agilent Chem Station*" software.

Chromatography by GLC method was carried out by means of a gas chromatograph *GC-2014* made by "*Zhimadzu"* company, Japan. Separation was made with use of a silica column 30 m x 0.25 mm with an immovable polar phase – 50%-propylnitrylmethylpolysiloxane with the layer thickness of 0.25 µm, the column temperature: 170°C (5 minutes) - 225°C (7 minutes), the rate of rise - 3°C/min. Gas carrier – helium with the flow rate of 0.75 ml/min, the flow pressure of 1:50, the temperature of an injector– 240°C. A flame ionization detector *FID – 40*, the detector temperature– 250°C. The introduced sample volume - 1 µl.

Registration of mass spectra was carried out by means of a mass-spectrometer "*Autoflex II*" (*Microflex* modification) "*MALDI TOF/TOF*" (instrumental bias not exceeding 0.5 *Da*) made by *Bruker Daltonics GmbH* company, Germany. The device consists of an ion source, a vacuum chamber, a mass analyzer, a personal computer and a nitrogen pulsed laser with the wavelength of 337 nm and the pulse rate of 0.5 ns.

The samples under analysis in the amount of 0.5 ml were applied to a target, dried out and then coated with a drop of matrix. α-cyanocinnamic acid was used as a matrix, spectra registration was carried out by "*Flex Control*" program, data processing was performed by means of "*Flex Analis*" program in a reversed mode and under positive polarity conditions (*Reflex Positive)*.

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MAIN PART

The possibility to use *MALDI/TOF/MS* method for identification of phytogenic polyphenolic components being a part of a combined complex was demonstrated.

The analysis of polyphenols of common agrimony herb (*Agrimonia eupatoria* L.) may be given as an example. Polyphenolic complex was extracted from *A. eupatoria* L. herb by the method of maceration with the aid of 75% ethyl alcohol.

In the course of study of polyphenolic complex of *A. eupatoria* L. herb by *MALDI/TOF/MS* method there were observed intense peaks of molecular ions with *m/z* = 433.299; 449.248 and 487.221 belonging to glycosidic forms of flavonoids in the form of monosides, which was evidenced by specific fragmentation accompanied by formation of molecular peaks of aglycon ions with *m/z* = 271.350; 287.320 and 303.291 belonging to apigenin, luteolin / kaempferol and quercetin (Figure 1).

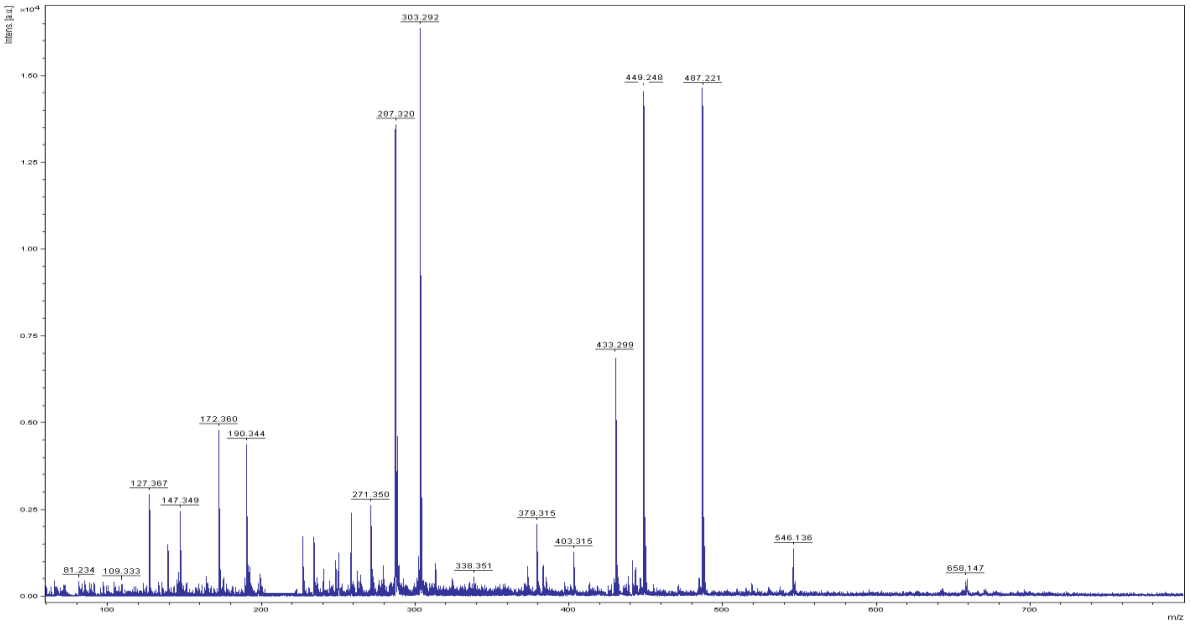

Figure 1: Mass spectrometric profile of polyphenolic complex of *A. eupatoria* L. herb

Chromatography of polyphenolic complex of *A. eupatoria* L. herb by RF HELC method established that the complex included such basic components as flavonoid glycosides and ellagic acid (Figure 2).

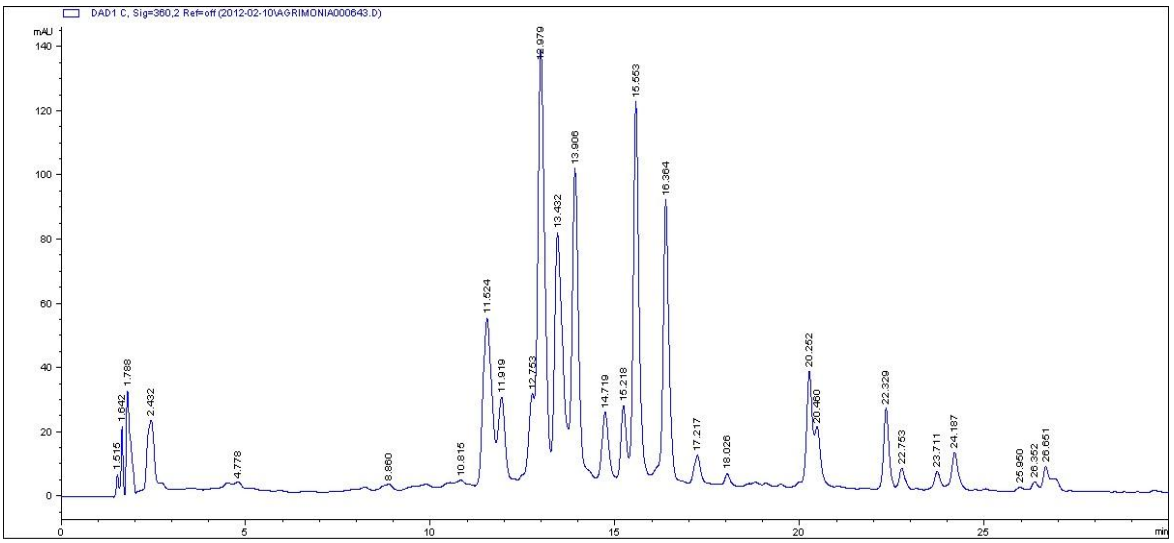

Figure 2: Chromatogramm of split-up of A. *eupatoria* L. herb polyphenols

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As the chromatogram shows the polyphenolic complex of the mentioned plant includes 6 dominant compounds belonging to glycosides of quercetin (11.52 and 13.468 min), luteolin (13.928 min), kaempferol (15.53 min), apigenin (16.36 min), as well as ellagic acid (12.7 min).

The obtained results evidence that *MALDI/TOF/MS* method appeared to be suitable for polyphenolic compounds identification.

In order to establish a capability for identification triacylglycerides (TAG) by *MALDI/TOF/MS* method there was used a sample of fatty oil of Circassian walnut (*Juglans regia* L.).

Search for TAG was performed in an extract made from the studied raw materials by means of pentane extraction in a device of *Soxlet* type in the range of 860 – 940 *Da*. The obtained spectrum is shown on Figure 3.

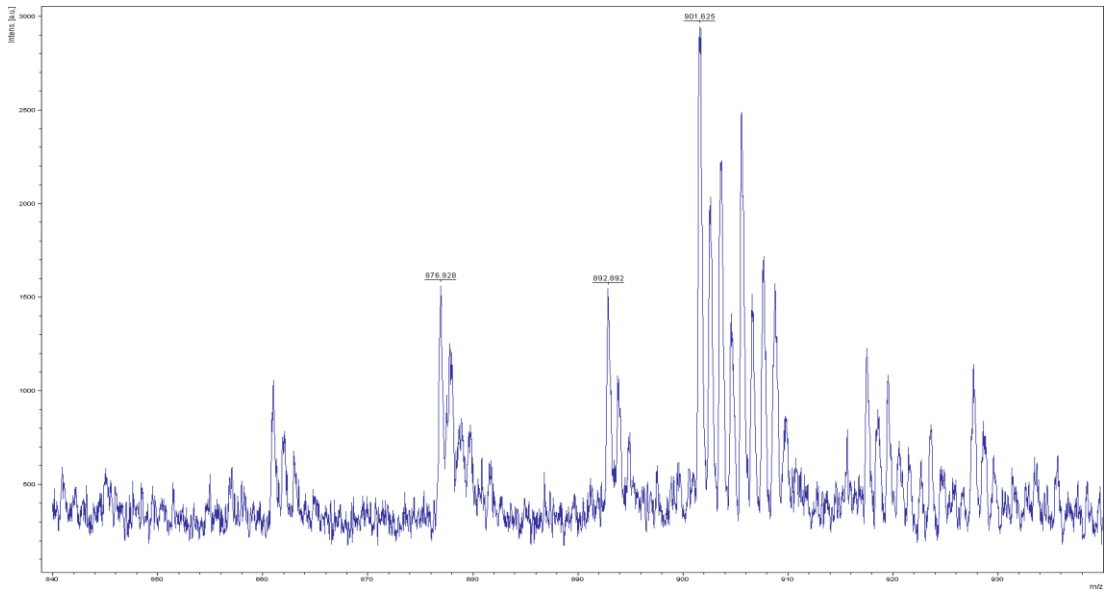

Figure 3: Mass spectra of TAG of *J. regia* L. seeds

The displayed figure demonstrates peaks of molecular ions in the range of m/z = from 901 to 907 *Da*, however molecular weigh of TAG are within 878 – 882 *Da*. This points to the fact that the analyzed TAG are presented in the form of adducts with sodion.

TAG identification was carried out based on the obtained ionic masses. The identification results are given in Table 1.

Table 1: Results of triacylglycerides identification in fatty oil of *J. regia* L. seeds by *MALDI/TOF/MS* method

m/z	Triacylglyceride code*	Fatty acids in triacylglyceride composition	Value of carbon number and olefinic links
877	LPL	Linoleic, palmic	55:5
901	LLL	Linoleic	57:6
903	LOL	Linoleic, oleinic	57:5
905	OOL	Oleinic, linoleic	57:4
907	OOO	Oleinic	57:3

*L – linoleic acid (18:2), O – oleinic acid (18:1), P - palmic acid (16:0)

Alkaline hydrolysis of triacylglycerides was carried out in order to determine the content of fatty oils in the extracts under analysis. The hydrolyzates were subject to mass spectrometry. The analysis results evidence that the analyzed sample contained 5 fatty acids: palmic, eicosoic, oleinic, linoleic, linolenic.

Acknowledgement of the results obtained from *MALDI/TOF/MS* analysis was performed by GLC method [9]. Fatty acids were previously converted into methyl ethers according to GOST 30418-96 methodology [10].

The results of identification of methyl ethers of *J. regia* L. by GLC method appeared to be comparable to those obtained by *MALDI/TOF/MS* method.

FINDINGS

The carried-out experiments give ground for affirmation that use of *MALDI/TOF/MS* method significantly complements RF HELC and GLC methods. *MALDI/TOF/MS* can be used for prompt characterization of compounds being a part of multi-component systems.

CONCLUSION

The obtained experimental data demonstrated objective analytical advantages of *MALDI/TOF/MS* method over RF HELC. *MALDI/TOF/MS* appeared to be more preferable for group characteristic of flavonoid, allowed to determine the structure of aglycone and sugar attached to it, their number and number of carbon atoms forming sugar. *MALDI/TOF/MS* demonstrated significantly higher rapidity, did not require selection of special conditions for carrying out of the experiment and availability of standard samples as compared to RF HELC.

The advantage of *MALDI/TOF/*MS over the traditional method of fatty oils analysis (gas-liquid chromatography) was approved. At that *MALDI/TOF/MS* method is characterized by considerably higher rapidity and the obtained extent of information allows to make an objective conclusion on fatty acids content.

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