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Development of assay method for the determination of iodine-containing organic compounds by high-performance liquid chromatography

***I.N.Vladymyrova*[1], *V.A.Georgiyants*[1], *V.I.Husarov*[2], *T.S.Tishakova*[3], *D.S.Kharchenko*[4]**

[1]National University of Pharmacy, 4 Pushkinska Str., 61003 Kharkiv, Ukraine
[2]Pharmtechnology LLC, 22 Korzhenevskogo Str., 220024 Minsk, Belarus
[3]Kharkiv National Medical University, 4 Nauky Ave., 61022 Kharkiv, Ukraine
[4]Ukrainian Military Medical Academy, 24 Mel'nykova Str., 04655 Kyiv, Ukraine

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Scientific data on the availability and application of methods for the determination of iodine-containing compounds in herbal raw materials, food products and medicinal products has been analyzed. It has been established that in most methods of iodine detection, an organic component of food interferes analysis. Dry ashing procedure (dry digestion) in muffle furnace at 400–500°C or treatment with concentrated acids (wet digestion) at the presence of oxidizing agents is used to eliminate the potential interference. Inversion voltammetry is used for the iodine determination in food products but it has a number of disadvantages, in particular, toxicity of mercury, its relative easy oxidizability and relative complexity of used equipment. The content of total iodine in herbal raw material is determined by the titrimetric method. The technique has been developed for the determination of tyrosine and its iodinated derivatives by high performance liquid chromatography. The article presents the parameters of chromatographic separation of tyrosine and iodinated derivatives.

Keywords: iodine-containing organic compounds, laminaria, high performance liquid chromatography.

Проанализированы данные научной литературы относительно наличия и аспектов применения методов определения соединений йода в лекарственном растительном сырье (ЛРС), пищевых продуктах и лекарственных средствах. Установлено, что в большинстве способов детектирования йода органическая составляющая растительного сырья (пищевого продукта) мешает проведению анализа. Для устранения этого влияния используется техника щелочного сухого сжигания ("сухое" озоление) в муфельной печи при температуре от 400 до 500°C или обработка сильными кислотами в присутствии окислителей ("мокрое" озоление). Для определения йода в объектах биологического происхождения, в частности в продуктах питания, применяется и метод инверсионной вольтамперометрии, однако он характеризуется рядом недостатков аппаратуры. Для ЛРС содержание общего йода определяется титриметрическим методом. Разработана методика определения тирозина и его йодированных производных методом высокоэффективной жидкостной хроматографии. Приведены параметры хроматографического разделения тирозина и йодированных производных.

Розробка методики кількісного визначення йодорганічних сполук методом високоефективної рідинної хроматографії. *І.М.Владимирова, В.А.Георгіянц, В.І.Гусаров, Т.С.Тишакова, Д.С.Харченко.*

Проаналізовано дані наукової літератури щодо наявності та аспектів застосування методів визначення сполук йоду у лікарській рослинній сировині (ЛРС), харчових продуктах та лікарських засобах. Визначено, що у більшості способів детектування йоду органічна складова рослинної сировини (харчового продукту) заважає проведенню аналізу. Для усунення цього впливу використовується техніка лужного сухого спалювання ("сухе" озолення) у муфельній печі при температурі від 400 до 500°С або обробка сильними кислотами у присутності окислювачів ("мокре" озолення). Для визначення йоду в об'єктах біологічного походження, зокрема у продуктах харчування, застосовується і метод інверсійної вольтамперометрії. Для ЛРС вміст загального йоду визначається титриметричним методом. Розроблено методику визначення тирозину і його йодованих похідних методом високоефективної рідинної хроматографії. Наведено параметри хроматографічного поділу тирозину і йодованих похідних.

1. Introduction

Iodine-containing compounds of natural and synthetic origin are essential for prophylaxis and treatment of thyroid function abnormalities. Species of brown algae are absolute leaders among the natural sources of iodine-containing compounds, the most common of which is laminaria used as food and medicinal products [6–8]. Iodine compounds in the thallus of laminaria include different inorganic and organic compounds [1, 9].

Iodine content varies depending on the place of growth of algae, season, the method of iodine analysis in plants, and also on the part of the plant taken for the iodine analysis.

Methods of identification and quantitative determination of iodine-containing compounds depend on the chemical forms of iodine in the investigated objects, as well as in herbal substances. In natural water, especially seawater, iodine mainly exists as iodide and iodate. Iodine in herbal objects can be found in organic and inorganic forms. Algae and marine products are rich sources of iodine, which contain mainly organic compounds of iodine among other halogen-containing compounds. Organic compounds of iodine in biological specimens can be determined by classical chemical methods after their pre-isolation or by more advanced spectroscopic methods. Gas chromatography and high performance liquid chromatography are ones of express methods for the determination of these compounds. The catalytic, electrochemical, titrimetric, and spectrometric methods are used for analysis of the inorganic iodine species [4, 8, 10, 12].

Practically all methods of iodine analysis need preliminary sample preparation; it is a critical step in iodine analysis in food, food ingredients and herbal raw material [4, 11, 13]. An organic component of food interferes with the analysis in most methods of iodine detection. Dry ashing procedure (dry digestion) in muffle furnace at 400–500°C or treatment with concentrated acids (wet digestion) at the presence of oxidizing agents is used to eliminate potential interference [2, 9].

Many analytical methods have been developed for the analysis of iodine in different biological samples. In particular, inversion voltammetry (IVA) is used for iodine determination in food products [3]. IVA has a high sensitivity and it is a very accurate electrochemical method. Voltammetric determination of iodine is based on the ability of iodide-ions to deposit on the surface of a mercury electrode in the form of insoluble salt of mercury (Hg_2I_2) at the potential of electrodissolution of metallic mercury; further, cathodic reduction of the precipitate takes place when the potential changes. The analytical signal is a value of the cathodic peak of iodide proportional to its concentration in optimal conditions. But this method has a number of disadvantages, in particular, toxicity of mercury, its relative easy oxidizability and relative complexity of used equipment.

The determination of total iodine in the thallus of laminaria as herbal raw material is regulated by the requirements of the State Pharmacopoeia of Ukraine (SPhU). Prior to the SPhU's publication, a general monograph of State Pharmacopoeia XI, ed. 2 "Thallus of laminaria" was used; according to its recommendations, the iodine content in laminaria was determined by burning the raw material in a closed oxygen-filled flask [5]. By analogy with the monograph "Kelp" of the European Pharmacopoeia [2], SPhU recommends using a more advanced method for the determination of iodine; the procedure

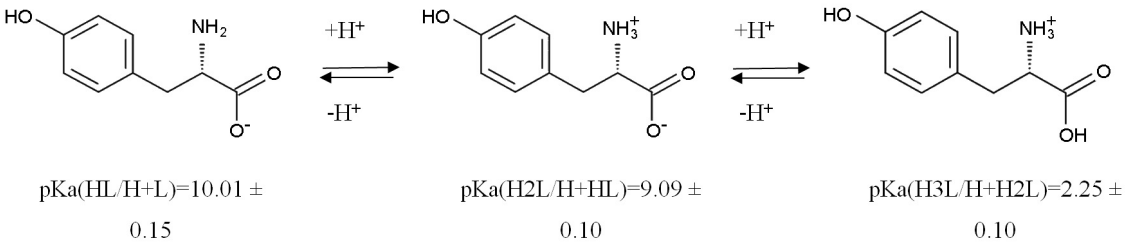

Fig. 1. Possible ionic forms of tyrosine.

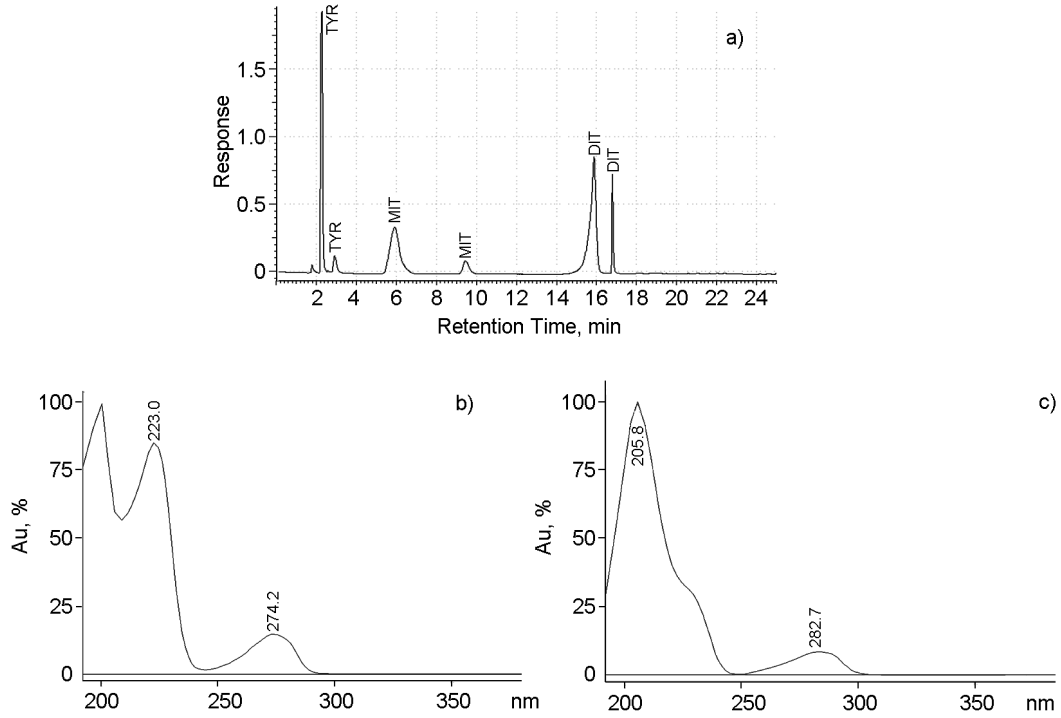

Fig. 2. Chromatograms of the total reference solution, pH of buffer is 2.5.

includes alkaline mineralization of raw material with further determination of iodine by the titrimetric method (titrant is a 0.01 M sodium thiosulfate solution, indicator is a starch) [13].

Taking into consideration the sufficiently high content of organic compounds which play a crucial role in the therapeutic action of laminaria and laminaria-based remedies, the aim of our research was to develop a method for the determination of iodine-containing organic compounds by high-performance liquid chromatography (HPLC), that is a modern, highly-sensitive and accurate method for determination of biologically active compounds.

2. Experimental

The analysis was performed on a Varian ProStar liquid chromatograph (Varian, USA) consisting of a high-pressure gradient system ProStar 210; a spectrophotometric photodiode array detector ProStar 330; an autosampler ProStar 400 with the volume of 20 μl; a column heating oven ProStar 500 with a Waters XbridgeTM column C18 (150 mm, 4.6 mm, 3.5 μm) with a pre-column.

The following reagents were used: acetonitrile "gradient grade" (Sigma-Aldrich), methanol "gradient grade" (Sigma-Aldrich), trifluoroethanoic acid (Fluka), water for chromatography (Millipore Direct-Q5).

The following standards were used: D,L-tyrosine (SigmaAldrich, 145726), 3-iodo-L-tyrosine (SigmaAldrich, I8250), 3.5-diiodo-L-tyrosine (SigmaAldrich, D0754).

Preparation of standard solutions.

D,L-tyrosine, ~1 mg/ml. Dissolve approximately 25 mg of D,L-tyrosine in 25-mL volu-

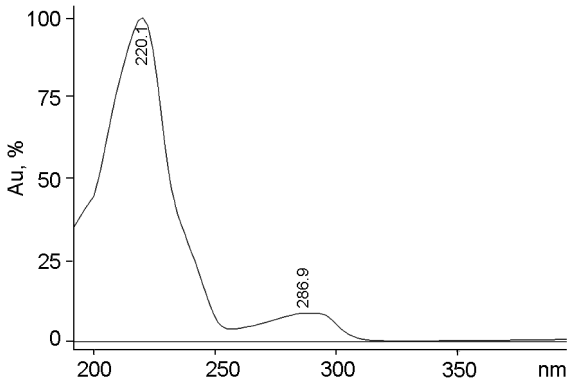

Fig. 3. Identity of peak-pairs composition for every component by UV-diode array for every peak. Absorbance spectrum of peaks with the retention time 2.27 min and 2.92 min (tyrosine). Absorbance spectrum of peaks with the retention time 5.92 min and 9.46 min (monoiodtyrosine).

metric flask containing 10 mL of water, 5 mL of methanol and 200 μl of 300 g/l solution of potassium hydroxide and dilute to volume with water.

3-Iodo-L-tyrosine, ~1 mg/mL was prepared similarly to the solution of D,L-tyrosine.

3.5-Diiodo-L-tyrosine, ~1 mg/mL was prepared similarly to the solution of D,L-tyrosine.

Total tyrosine solution (contains 0.33 mg/mL of tyrosine and its derivatives) was prepared by the mixing solutions of tyrosine and its derivatives in the ratio 1:1:1 (v/v/v).

3. Results

The first stage of developing the method for the determination of tyrosine and its iodinated derivatives in herbal raw material — monoiodtyrosine (3-iodo-L-tyrosine, MIT) and diiodotyrosine (3.5-diiodo-L-tyrosine, DIT) — was the selection of the chromatographic conditions under which it would be possible to determine all the components simultaneously. Tyrosine, MIT, and DIT are inner salts. Depending on the pH of a mobile phase, several retention mechanisms can be simultaneously realized for ionogenic compounds during the analysis. As a result, the compounds elute, and two peaks are observed, each of them corresponds to the molecular or protonated (deprotonated) forms. Acidic or basic eluents, inhibiting (or enhancing) hydrolysis and transferring the compound into one of the possible ionic forms, are used to avoid this phenomenon.

Table 1

Column	Waters XBridge™ C18 3.5 μm, 150×4.6
Column temperature	25°C
Mobile phase A	0.1 % CF_3COOH in 5 % acetonitrile
Mobile phase B	0.1 % CF_3COOH in acetonitrile
Flow rate	1.0 ml/min;
Detector	Spectrophotometric
Detection wavelength	223 nm
Injection volume	20 μl

The pKa value of tyrosine was determined in order to estimate the pH value at which amino acid is fully protonated (deprotonated). It was found that the presence of the two forms is possible in the range of pH from 2.25±0.10 to 10.01±0.15. That's why it is meaningful to use mobile phases with pH less than 2.15 or higher than 10.26 at the chromatographic separation (Fig. 1).

The abovementioned statement was confirmed experimentally. The chromatograms were obtained using a 50 mM perchlorate buffer with pH 2.5 (mobile phase A) and acetonitrile (mobile phase B) as a mobile phase (gradient elution, content of mobile phase A:

0 min → 10 min — 90 %;
10 min → 18 min — 90 % → 30 %;
18 min → 22 min — 30 %;
22 min → 23 min — 30 % → 90 %;
23 min → 25 min — 90 %),

where every component of the total reference solution appeared in the form of two well separated peaks (Fig. 2, the order of the peak-pairs appearance: tyrosine, monoiodtyrosine, and diiodotyrosine (in ionized and molecular forms or in the form

Table 2. Gradient timetable

Time	Mobile phase A, %	Mobile phase B, %
0	95	5
2	95	5
2 → 12	95 → 40	5 → 60
12 → 13	40 → 10	60 → 90
18	10	90
18 → 20	10 → 95	90 → 5
30	95	5

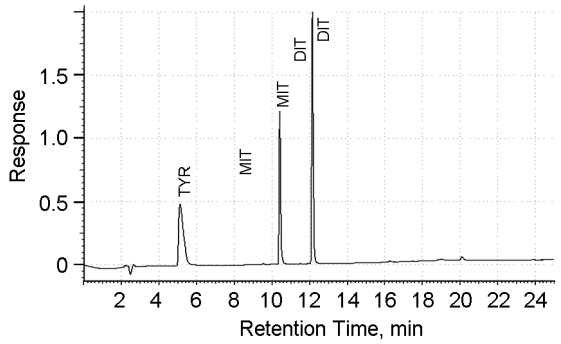

Fig. 4. Chromatogram of the resultant reference solution with the use of 0.1 % trifluoroacetic acid.

of inner salt)). The identity of the peak-pairs to the composition of every component was confirmed by the comparison of electronic absorption spectra obtained with the use of an UV-diode array for every peak (Fig. 3). Simultaneous detection of the compounds is possible at the tyrosine absorption maximum taking into account the compounds' absorption maxima (at the wavelength of 223 nm).

When the mixtures of 50 mM perchlorate buffer (pH 2.0) with addition of 5 % acetonitrile as a mobile phase A and 50 mM perchlorate buffer (pH 2.0) with addition of 60 % acetonitrile as mobile phase B (gradient eluation, content of mobile phase A:

0 min → 5 min — 100 % — 90 %;
5 min → 15 min — 90 % → 0 %;
15 min → 22 min — 0 %;
22 min → 23 min — 0 % — 100 %;
23 min → 25 min — 100 %)

were used, the chromatograms (after complete separation of the components) showed the iodinated derivatives as individual peaks, and the split peak of tyrosine. (Fig. 4, the order of the peak appearance: tyrosine, monoiodtyrosine, and diiodtyrosine).

A rapid analytical method capable to determine tyrosine and its derivatives was developed in the present research.

The chromatographic conditions proposed for the determination of tyrosine and its derivatives in the herbal raw material are the following.

As can be seen from the above, we have proposed a procedure for the simultaneous determination of tyrosine and its iodinated derivatives by the HPLC.

4. Conclusions

A highly-sensitive method for the determination of tyrosine and its iodinated derivatives in the raw of laminaria saccharina by the high-performance liquid chromatography has been developed. Conditions for the analysis and sample preparation procedure have been chosen. This method can be applied for the determination of iodine-containing compounds in herbal raw materials, dietary supplements, medicinal products or food. The proposed method is modern, comprehensible and characterized by a variety of advantages during analysis, high repeatability; control of analysis, information processing and presentation of final results is completely automatic.

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