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Determination of tetracycline in honey by voltammetry

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Abstract

The possibility of the voltammetry use for tetracycline determination in probes of honey on a glassy carbon electrode with preliminary electrochemical treatment has been shown for the first time. The algorithm of honey samples treatment using solid phase extraction (SPE) has been developed.

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Keywords: Voltammetry, tetracycline, glassy carbon electrode;

1. Introduction

The problem of determination of antibiotics residual quantities in bee products remains relevant today. This is due to the huge market of different varieties of honey, the availability of modern antibiotics for producers and stricter requirements to their content in final products.

Tetracycline antibiotics (TC) are a group of broad-spectrum antibiotics, which are widely used in beekeeping. Unfortunately, the side effects, characteristic of tetracycline group drugs, led to the need to control their residual amount in manufacturing raw materials and food. In accordance with the Technical Regulations of the Customs Union TR CU 021/2011 "On food safety", the content of tetracyclines in honey must not exceed 0.01 mg/kg.

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To determine tetracyclines in foods, besides a conventional microbiological method of analysis, an immunoenzyme method (ELISA) with a detection limit of 0.005 mg/kg [1-2] and high pressure liquid chromatography with a mass-spectrometric detector are used. Applied physicochemical methods are high-sensitive and selective, but their implementation in a broad laboratory practice is prevented by a number of reasons. It is a high cost and short term of consumables storage (mainly imported); a high cost of equipment and personnel qualification requirements. Therefore, for screening purposes and in routine laboratory practice the development of new methods for products analysis, particularly for tetracycline content in honey, is extremely relevant.

In recent years, in foreign and domestic publications there appeared a number of publications [5-8], in which a variety of options of the voltammetry (VA) method for TC determination is proposed.

The method of voltammetry (VA) is one of the most effective methods of electrochemical analysis of drugs, which combines high information capabilities with the ease of a signal measurement process considering a low cost of the equipment.

However, in the laboratory application of the method is limited mainly to VA analysis of the TC content in drugs. This is due to the complexity of food matrices, required sample preparation and low values of MPC. The purpose of this work is to develop voltammetric methods of quantitative chemical analysis of tetracycline in honey.

2. Material and methods

The work was performed on a voltammetric analyzer STA (LLC "ITM", Tomsk), which is a device consisting of an electronic unit, a measuring unit with three electrochemical cells complete with IBM – a compatible computer with STA software package.

The measurements were carried out in a two-electrode measurement system; voltammograms were recorded at a linear potential sweep in differential mode. A silver/silver chloride electrode served as a reference electrode in a saturated solution of potassium chloride with a resistance less than 3.0 ohms. Needle glassy carbon electrodes (GCE) of glassy carbon SU-2500 with a total working surface area of about 100 mm^2 served as indicator electrodes. For electrochemical treatment of GCE potentiostat-galvanostat PI 50-1.1 (Gomel, Belarus) and a laboratory source-meter IIL (LLC "ITM", Tomsk) were used.

The tetracycline stock solutions were prepared by dissolving the sample of pharmacopoeial drugs containing the basic substance not less than 99.5% (as hydrochloride tetracycline) in ethanol.

3. Results and Discussion

The working solutions were prepared by serial dilution of the stock solutions in ethanol. The simulated solutions were prepared from the working solutions. All other reagents were analytical grade or extra-pure grade.

The mass concentration of the antibiotic was calculated by adding tetracycline into solution to be analyzed. Previously, for the determination of tetracycline antibiotics a tetracycline oxidation process on glass indicator electrodes, previously modified electrochemically, was selected as a source of analytical signal [4]. It made it possible to obtain the analytical signal linearity in the concentration range of 0.2-5.0 mg/dm^3, which was sufficient to determine the TC in pharmaceuticals (drops, tablets and capsules). The problem of determining antibiotics in food demanded a reduction in the limits of their detection at least 100 times to 0.001-0.002 mg/dm^3 and the introduction of additional stages of surface preparation of the indicator electrode. For this purpose, in addition to a primary electrochemical treatment of GCE in 0.5 M NaOH solution at a potential of 4.0 V for 60-120 seconds, carried out once, they were kept in 0.1 M NaOH solution at a potential of 1.8 V for 15-30 seconds before carrying out each series of voltammetric measurements of the TC analytical signal in the range of concentrations allowing determining low contents of antibiotic in the analyzed samples (less than 0.01 mg/kg) of 1 g. Trisodium citrate solutions ($Na_3C_6H_5O_7$), previously used to determine tetracycline in pharmaceuticals, as well as mixed aqueous-organic backgrounds, including part-organic solvents-eluents, used in the stage of the sample preparation - ethanol (EtOH) and ethyl acetate (EtOAc) were considered as a supporting electrolyte.

It has been found that reproducible analytical signals of TC are recorded in 0.05 mol/dm^3 of $Na_3C_6H_5O_7$ solution at electrolysis potential E_e = 0.05 V and the linear dependence of the calibration curve remains in the concentration

range from 0.001 to 0.2 mg/dm³. At higher concentrations, there is a deviation from linearity of the calibration curve, which may be due to the saturation of the indicator electrode surface.

The resulting tetracycline analytical signal is shown in Figure1.

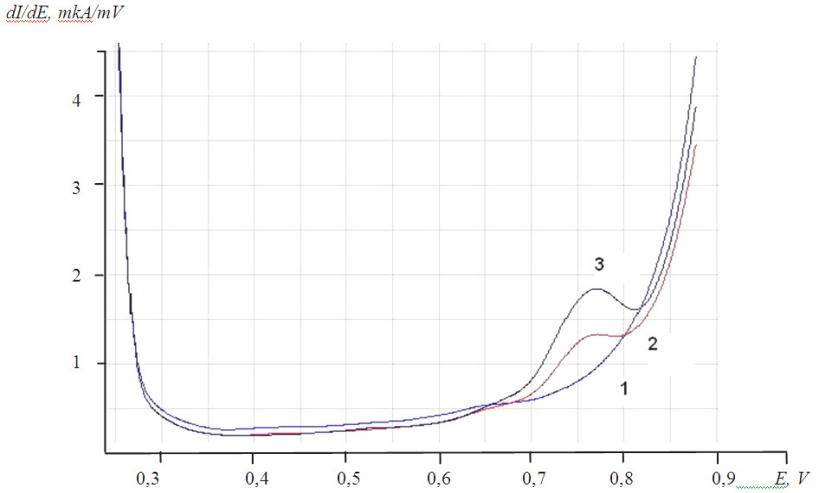

Fig.1. Voltammograms of tetracycline:
1 - Background: 0.05 mol/dm³ $Na_3C_6H_5O_7$; 2 - C (TC) = 0.01 mg/dm³; 3 - C (TC) = 0.02mg/dm³

As can be seen from Figure 1, on voltammograms (Graphs 2,3), at a potential of 0.70 ± 0.05 there occurs a well-defined peak, the height of which increases in proportion to the introduced TC concentration, indicating the possibility of a quantitative assessment of the antibiotic content.

It is generally known that the chemical composition of natural honey includes sugars (up to 75 %), dextrin, water, amino acids, non-protein nitrogen, vitamins, pigments and other components of honey obtained from the nectar of various kinds. Most of them (sugars, amino acids, water-soluble vitamins) are electroactive and able to oxidize on glass indicator electrodes, thus impeding the direct voltammetric determination of tetracycline without extracting it from the samples and purification of obtained extracts. The problem of sample preparation when analyzing food products, particularly honey, a considerable number of works of Russian and foreign researchers is devoted to the TC content [9-11].

Considering the complexity of the matrix and previous research, a method of solid-phase extraction was selected for sample preparation.

The TC sorption on polymer sorbents of hypercrosslinked polystyrene, belonging to the class of so-called styrosorbs, was studied on the simulated solutions.

These sorbents allow quantitative extracting organic substances from the aqueous solutions in small and ultra-small concentrations (10^{-10} - 10^{-8} M) and are now commercially available.

Based on the results, the method of the TC determination is as follows:

The sample of honey with mass of 1 g was dissolved in 20 ml of bidistilled water and, after stirring, centrifuged at a rate of 6000 rotation/min for 20 min. Further, the resulting solution was subjected to a solid phase extraction procedure. For this purpose, using a syringe, it was passed through a solid phase extraction cartridge containing 2 ml of a hypercrosslinked polystyrene sorbent Purosep P (a standard concentrating cartridge DIAPAK-P was used). The cartridge was washed with 10 ml of double distilled water and then concentrated TC was eluted with 2 ml of ethyl acetate. 2 ml of ethyl alcohol was added to the eluate, then its volume was adjusted to 10 ml with the original background electrolyte (sodium citrate solution concentration of 0.05 mol/l) to obtain a mixed aqueous organic pattern, suitable for VA measurements. Prepared sample or its aliquot was analyzed by anodic voltammetry. The scheme of the sample preparation is shown in Figure 2.

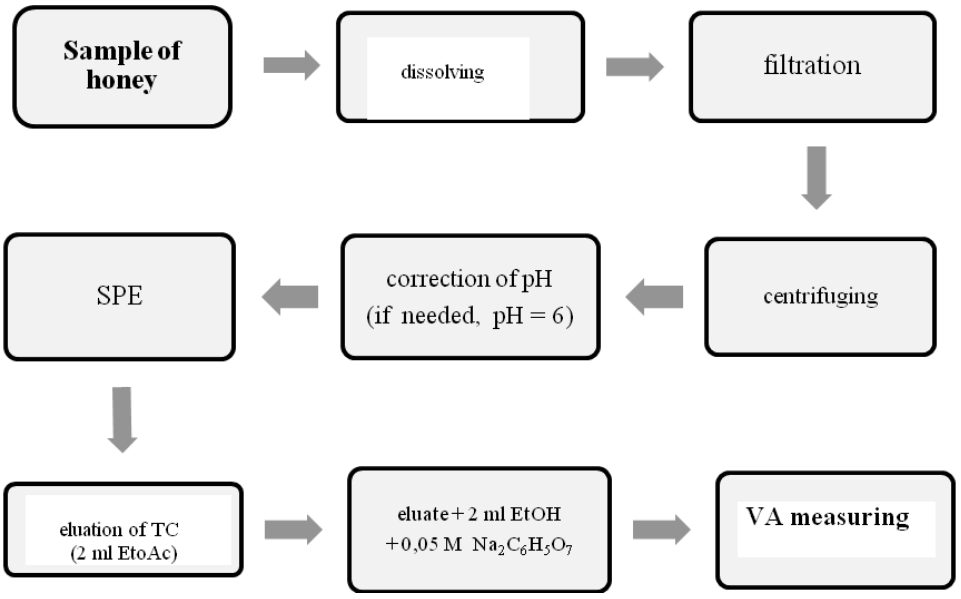

Fig.2. Algorithm of sample preparation in the analysis of honey samples for the tetracycline content

Using the proposed method algorithm, the analysis of simulated mixtures and real objects was carried out. The obtained results are presented in Table 1.

Table 1. Content of tetracycline in honey samples and validation by method of "added-found" (P = 0.95, n = 3)

Object	Contents of TC, mg / kg			
	Sample	Added	Found	Δ, %
“White” Honey	Less than 0.005	0.01	0.0096	18
“Altai“ Honey	Less than 0.005	0.05	0.054	16
“Motley grass “ Honey	Less than 0.005	0.1	0.98	13

As seen from Table 1, the results are consistent with each other.

4. Conclusion

Based on the studies the method for the voltammetric determination of tetracycline in honey has been developed. The total analysis error is less than 20% at the lower boundary of the measurement of 0.005 mg/kg. The analysis time (with sample preparation) is not more than 1 hour. The method allows reliable detecting small contents both of the traditional TC antibiotics (tetracycline, oxytetracycline) and the current ones (doxycycline).

Thus, the proposed method has features comparable to HPLC-MS, but does not require expensive equipment and consumables.

Acknowledgments

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