ASCORBIC ACID DETERMINATION FROM ALCOHOLIC AND SOFT DRINKS BY DIFFERENT TECHNIQUES

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Abstract: The present study consists in a comparative evaluation of the L-ascorbic acid (AA) concentration from some commercial Romanian juices and wines. The samples was analyzed by high-performance liquid chromatography (HPLC) on an Alltima C(18) column with isocratic elution of methanol and water with formic acid (pH 3) (65:35, v/v) at a flow rate of 0.3 mL/min, a column temperature of 26°C, and UV detection at 243 nm. The amperometric measurements were carried out under magnetic stirring using 0.1 M phosphate buffer solution as supporting electrolyte and pH 7 with a new biosensor, consisting in ascorbate oxidase (AOx) and bovine serum albumine immobilization on the membrane of an oxygen electrode, followed by cross-linking with glutaraldehyde (GA). Our study showed that the biosensor works well in synthetic samples of AA and may be recommended for testing in real samples (wine and fruit juices).

Keywords: L-ascorbic acid, HPLC, electrochemical method, drinks.

Introduction

Ascorbic acid (vitamin C) is essential for the body health and helps to avoid diseases, being a very efficient antioxidant [1]. Oxidation of AA and avoiding its loss after processing represents a real interest for nutritionists, processors and consumers.

In order to quantify the AA in different matrices, a variety of methods are used, as follows: titrimetry [2-4] high-performance liquid chromatography (HPLC) [5, 6], spectrophotometry [7, 8] and electrochemical methods [9, 10]. Besides these methods, those exploiting enzymatic biosensors appear particularly attractive, because enzymes are known to be versatile, highly sensitive and selective [11].

In the recent years, HPLC was one of the most used techniques for the analysis of AA from different samples, because it provides good accuracy, repeatability and reproducibility, relatively short time analysis and precise identification [12]. Comparative to HPLC, electrochemical methods were considered lately for the AA detection, due to its simplicity, high sensitivity and its speed. In this aim it has been proposed to use a variety of chemically modified electrodes. Electrodes modified with redox mediators have attracted the most notice because redox mediators facilitate the oxidation of AA at low electrode potential [13].

The amperometric detection of AA is based on the catalytic oxidation of this turning it into dehydroascorbic acid. On platinum electrode or vitreous carbon the process occurs at high potential (+500 mV), but it is possible to minimize the over potential by using modified electrodes with various mediators.

Compared to the conventional electrodes, the chemically modified electrodes provides well known advantages, especially in cases when the sample requires a great over potential like in electrocatalysts [14]. Thus, a number of chemically modified

electrodes were used for the determination of AA from different samples. Besides the modified electrodes, the methods that exploit enzyme biosensors seem particularly attractive, because they are known to be versatile, highly sensitive and selective [11].

In the literature are described for AA biosensors based on ascorbate oxidase. This enzyme catalyzes the oxidation of AA in the presence of molecular oxygen. Enzymatic methods have the advantage of higher selectivity, the disadvantage being the short-term stability. Moreover, the electrodes manufacture for routine quantitative determination of AA present difficulties such as low selectivity and difficult preparation [15, 16]. For this reason, the obtaining of new sensors / biosensors for the AA detection from different samples remains in the attention of researchers.

The aim of the present work it was to assess the content of AA from some Romanian commercial juices and wines with a new biosensor. In order to verify the efficiency of the biosensor, the results were compared with those obtained by the HPLC method.

Experimental data

Materials

The juice and wine samples were acquired from supermarkets. L-ascorbic acid (AA), ascorbate oxidase (EC 1.10.3.3; 162 U / mg solid AOX,), bovine serum albumin (BSA) and glutaraldehyde (GA) were purchased from Sigma (St. Louis, MO, USA). Monopotasium phosphate ($KH_2PO_4 \cdot H_2O$), dipotassium phosphate ($K_2HPO_4 \cdot 2H_2O$) and methanol of HPLC grade were obtained from Merck (Darmstadt, Germany). All chemicals were analytical reagent grade. Supporting electrolyte used was a solution of 0.1 M phosphate buffer concentration and pH 7. Water of high purity used in all experiments was prepared using a Milli-Q Ultrapure water purification system (Millipore, USA).

Electrochemical method

All amperometric measurements were carried out in an electrochemical cell under magnetic stirring using a computer controlled electrochemical analyzer (AUTOLAB-PGSTE 10 EcoChemie, Utrecht, Netherlands). The applied potential was -650 mV vs. Ag/AgCl/KCl 1 M. Amperometric biosensors, specific for AA determination were realized by immobilization of ascorbate-oxidase (AOx) on the membrane of Clark oxygen electrode, using glutaraldehide (GA) and bovin serum albumine (BSA) [17].

HPLC method

The chromatographic analysis was carried out with a Shimadzu LC-MS 2010 system consisting of a binary pump, degasser, autosampler, thermostat set at 26°C, PDA and MS detectors. Separation was carried out on a Grace Alltima C18 column (100 x 3 mm, 3 μm) with a mobile phase formed from methanol (A) and water containing formic acid (pH 3) (B). The mobile phase was delivered at a flow rate of 0.3 mL/min in the isocratic mode A:B (65:35, v/v). The samples were filtered through nylon syringe filters (0.45 μm) before HPLC-MS analysis. The injection of the juice and wine samples (20 μL) into HPLC system was performed three times and the detection of AA was carried out at 243 nm.

Results and discussions

Electrochemical method

In order to check the functioning of obtained biosensor experimental measurements were performed to determine the concentrations of the AA from known synthetic samples, followed by the determination of AA from real samples.

The AA from the known concentrations of synthetic samples was determined by the standard addition method.

To determine the concentration of AA from the real samples by the standard addition method amperometric response of the biosensor was recorded, obtained as a result of the addition in the electrochemical cell (containing 10 mL of phosphate buffer, pH 7), of known volumes of the juice or wine. After this are carried out 3-5 successive additions of 10-20 µL from a known concentration of synthetic AA (0.01- 0.1 M) sample. Obtained data corresponding dependence ICAT = f ([AA]) were plotted and from the ordinate origin were calculated the concentration of analyte from the electrochemical cell. Taking into account the dilution degree of the real sample (if any) the value of the AA concentration from the sample is calculate.

The linear range of the biosensor (Fig. 1) it was determined also, and the sensitivity (µA/M) was calculated by means of parameters obtained by fitting the Michaelis-Menten (MM).

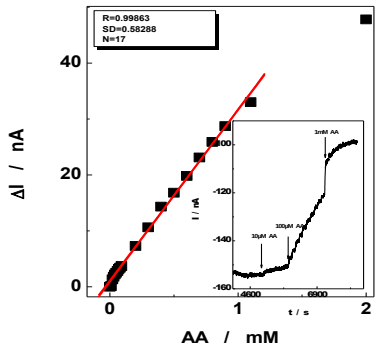

Fig. 1. The amperometric response and linear range of biosensor.

The biosensors based on ascorbate oxidase present a good stability and reproducibility, sensitivity for AA (328.36 µA/M), detection limit (5·10-5M) and a small response time, in phosphate solution, pH 7, at an applied potential of - 650 mV vs. Ag/AgCl.

HPLC method

The chromatographic method was developed for identification and quantification of AA from juice and wine samples. AA from analyzed samples was quantified by external standard method. Standard solutions in the concentration range 0.3-1 µg/mL were prepared by serial dilutions with ultrapure water starting from the 1 mg/mL stock solution. The calibration curve was drawn (Fig. 2). It has also calculated the coefficient of correlation (R^2).

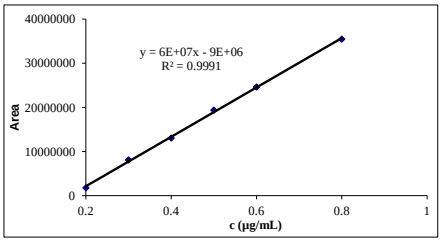

Fig. 2. The calibration curve of AA.

The limit of detection (LOD) and the limit of the quantification (LOQ) were determined for the proposed chromatographic method as being 0.2 µg/mL and 0.22 µg/mL.

L-Ascorbic acid quantification from alcoholic and soft drinks

Our study included a comparative quantitative evaluation of the AA from alcoholic and soft drinks by electrochemical and chromatographic methods. In the case of the wine samples, the obtained values by the HPLC method (reference) differs significantly from the results obtained with amperometric biosensor. One possible explanation consists in the complexity of the samples matrix, in which there are numerous interferents that may alter the biosensor response. In conclusion, AA determination with amperometric method (biosensor) requires a study of the interferents influence. A summary of all the results is shown in Table 1.

Table 1. The AA content determined by HPLC method and with amperometric biosensor.

Juice or wine sample	AA (mg/100 mL)	
	HPLC	Biosensor
Frutti fresh juice	20	28
Tedy juice	50	27
Recas wine “Burgund”	140.8	2.2
Jidvei wine "Royal Feteasca"	15.8	25
Recas Wine "Cabernet"	140.8	12.25

Comparing the results obtained with amperometric biosensor and HPLC method it was observed that the biosensor works well in AA synthetic samples and can be recommended for testing in real samples (wine and fruit juices).

The determination of AA from real samples using biosensor requires future studies of interferences that can influence the response of biosensor in the fruit juices and wines samples.

Conclusions

Our experimental results have demonstrated that the biosensor can be used for quantification of AA in wines and fruit juices samples. For improving the results achieved by amperometric method are required new studies concerning the interferences from wines and juices that may influence the biosensor response. Comparing with HPLC, the amperometric method requires a small response time, but obtaining of the biosensor is difficult and present low selectivity.

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