PHARMACEUTICAL METHODS FOR THE ANALYSIS OF ASCORBIC ACID

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Introduction. Vitamin C (ascorbic acid, ascorbate, AA) is a water soluble organic compound involved in many biological processes. AA plays crucial roles in electron transport, hydroxylation reactions and oxidative catabolism of aromatic compounds in animal metabolism. Although all the functions of AA are not fully explained, it is likely that it is also involved in maintaining the reduced state of metal cofactors, for example at monooxygenase and dioxygenase. In cells the other role of AA is to reduce hydrogen peroxide, which preserves cells against reactive oxygen species. The details about ascorbic acid antioxidant system cooperated with glutathione was described by Meister. Besides this, primates and several other mammals are not able to synthesise ascorbic acid. The animal species, which are able to produce this molecule, biosynthesise AA from glucose catalyzed L-gulonolactonoxidase. In spite of the ability to synthesize this molecule both groups of animal species suffer from deficiency of AA.

AA can be mostly found in fruits and vegetables. The main sources of AA are citrus fruits, hips, strawberries, peppers, tomatoes, cabbage, spinach and others. If one wants to uptake AA from animal sources, liver and kidney are the tissues with highest contents of this molecule, but in comparison with plant sources the amount of AA is very low. The content of AA in food can be affected by many factors such as clime, method of harvest, storing and processing. Thus, there is a need of analytical procedures able to not only monitor AA content in agricultural and food products, but also in body liquids and tissue.

Many analytical techniques including sensors and biosensors have been suggested for a detection of ascorbic acid in very varied types of samples. Hyphenated instruments consisting of flow injection analysis, high performance liquid chromatography or capillary electrophoresis instruments and a detector are mostly utilized for the determination of AA. However, some of these methods are time-consuming, some are costly, some need special training operators, or they suffer from the insufficient sensitivity or selectivity. Limits of detection ranged from μM to nM and lower.

Electrochemical detection is an attractive alternative method for detection of electroactive species, because of its inherent advantages of simplicity, ease of miniaturization, high sensitivity and relatively low cost. Electrochemical detection typically worked in amperometric or coulometric mode can be coupled with liquid chromatography to provide high sensitivity to electroactive species. The main aim of this paper is to utilize two electrochemical detectors (amperometric – Coulouchem III and coulometric – CoulArray) coupled with flow injection analysis for detection of ascorbic acid. The more sensitive technique is further applied on analysis of real samples (pharmaceutical preparation, oranges and apples fruits, and human blood serum).

The aim. To study new methods for determining ascorbic acid in various biological objects.

Materials and methods. A literature search and analysis of new approaches to

the phaomacetic analysis of ascorbic acid was carried out.

Results and their discussion. Stationary and flow electrochemical techniques are very attractive instruments for determining various biologically important compounds such as proteins, organic compounds of plant origin, drugs, etc. Here, we aimed at utilizing two different electrochemical detectors – amperometric (Coulouchem III) or coulometric (CoulArray) coupled with high performance liquid chromatography for detection of ascorbic acid.

An electrochemical behaviour of AA at the surface of working electrodes was investigated. FIA enables us to optimise experimental conditions for analytical determination of AA easily and rapidly. Primarily, the influence of potential applied to single working electrodes on oxidation signal of AA was studied. The potential varied from 100 to 400 mV and signal of various concentration of AA (12.5, 25, 50, 100, 200, 300, 400, 500 and 1000 μM) was measured.

The concentration range of AA analysed using both coulometric and amperometric detectors was almost the same due to possibility of comparing of the sensitivity of the instruments. The tangents of the calibration curves were 0.3788 for coulometric and 0.0136 for amperometric. Based on these results the tangent of calibration curve for AA measured using coulometric detector was almost 30 times higher than the tangent measured by amperometric detector. Coulometric detector is much more sensitive to presence of AA, thus, we utilized this detector in following experiments.

Under the optimized conditions mentioned above ascorbic acid was measured using high performance liquid chromatography coupled with CoulArray electrochemical detector (HPLC-ED).

The concentration of ascorbic acid was determined in pharmaceutical preparation, two species of fruits and human blood serum under the optimized experimental conditions using HPLC coupled with CoulArray electrochemical detector. We determined the concentration of ascorbic acid as 98±2 mg per one tablet in a pharmaceutical preparation called Celaskon. The manufacturer of this preparation declares the amount of AA as 100 mg per tablet. The recovery of the amount of AA added into the sample was 105% for lower addition of AA (5 μg·mL-1) and 95% for higher addition of AA (15 μg·mL-1). Moreover we used HPLC-ED to determine AA concentration in fruits species. We found that AA amount in oranges (*Citrus aurantium*) varied in the range from 30 to 56 mg/100 g of fresh weight and in apples (Malus sp.) from 11 to 19 mg/100 g of fresh weight. The recovery of AA measured in the homogenate prepared from fruits *Citrus aurantium* was 103 % for lower addition (5 μg·mL-1) and 104% for higher addition (15 μg·mL-1). To evaluate HPLC-ED technique for analysis of human body liquids we spiked human blood serum and found out that recovery of AA varied from 102 to 98 % according to lower (5 μg·mL-1) or higher (15 μg·mL-1) content of AA. The tested blood sera contained AA within the range from 38 to 78 μM.

Conclusions. High performance liquid chromatography coupled with an eight channel electrochemical detector appears to be a very suitable analytical instrument for sensitive ascorbic acid determination. Using the optimized technique ascorbic acid was determined in pharmaceutical preparations, fruits and human blood serum samples.