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Spectrophotometric determination of ascorbic acid in foods with the use of vortex-assisted liquid-liquid microextraction

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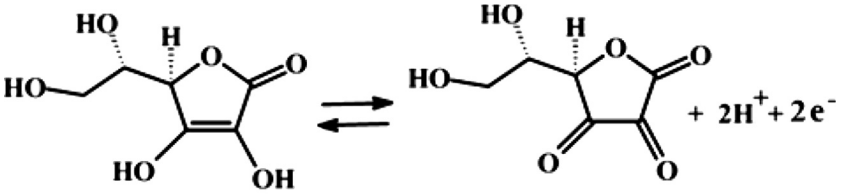
ABSTRACT

A novel, simple, sensitive and selective method for the spectrophotometric determination of ascorbic acid (Asc) has been developed. The developed method is based on the redox-reaction between Asc and triiodide ions (I_3^-) and vortex-assisted liquid-liquid microextraction (VALLME) of excess I_3^- as ion associate (IA) with polymethine dye Astra Phloxine FF (AP), with subsequent determination of IA spectrophotometrically at 560 nm. In the presence of Asc, discoloration of the extract of IA occurs. The influence of different factors was studied, and optimized conditions for the spectrophotometric determination of Asc were found: $4.0 \cdot 10^{-6}$ mol L^{-1} I_3^-, $4.0 \cdot 10^{-4}$ mol L^{-1} KI, $4.0 \cdot 10^{-5}$ mol L^{-1} AP, 500 μL of carbon tetrachloride (ratio of volumes of aqueous and organic phases 10:1), vortexing for 15 s at 3000 rpm and centrifugation for 2 min at 2000 rpm. The determination of Asc is possible in the presence of many interferants: 20,000-fold amounts of tartaric, citric and malic acids and 10,000-fold amount of oxalic acid, over 2000-fold amounts of HCO_3^-, CH_3COO^-, HPO_4^{2-}, SO_4^{2-}, Cl^-, F^-, Na^+, K^+, Li^+, Ca^{2+}, Sr^{2+}, Ni^{2+}, Co^{2+} and Zn^{2+}; and 5-fold amounts of Fe^{3+} and Cu^{2+}. The organic acids (oxalic, tartaric, citric and malic acids) eliminate the effect of > 100-fold amounts of Fe^{3+} and Cu^{2+}. The calibration curve was linear in the range from 0.003–0.53 mg L^{-1} of Asc ($R^2 = 0.997$); the limit of detection ($n = 10$, $P = 0.95$) was 0.89 μg L^{-1} of Asc, and the limit of determination ($n = 10$, $P = 0.95$) was 2.94 μg L^{-1} of Asc. The suggested procedure was successfully applied for the determination of Asc in food samples and model mixtures (RSD 2.1–3.9%, recovery 98.0–102.3%). The accuracy of the procedure was confirmed by analysis with a reference method.

1. Introduction

Ascorbic acid (Asc) belongs to the group of essential water-soluble vitamins, and it participates in redox processes. The absence of the enzyme L-gulonolactone oxidase does not allow vitamin C to be synthesized in the human body. Therefore, ascorbic acid enters the human body with fruits, vegetables and various pharmaceuticals [1]. Ascorbic acid – γ-lactone 2-oxo-L-gulonic acid – is a reducing agent, and it is easily oxidized to dehydroascorbic acid [2] according to the scheme:



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Considering the degradation of Asc during storage and heating, attention to the measurement of trace levels of Asc in food samples is still important [3]. The reduction properties of Asc are often at the heart of methods used for its determination [4]. A variety of analytical methods, including electrochemical [5–12], chromatography [13–19] and others, have been previously used for determination of Asc at trace levels in foods and other samples, but spectrophotometry is the most commonly employed method [20].

Different oxidation-reduction systems for spectrophotometric determination of Asc in various modifications have been proposed: 2,6-dichlorophenolindophenol [21,22], Cu(II) and alizarin red S [23], reagent Folin–Ciocalteu [24], 2,4-dinitrophenylhydrazine [25–27], Potassium Iodate [28], Cu(II) and Neucoproine [29–31], Potassium Peroxymonosulfate and Hydrogen Peroxide [32], Permanganate or ammonia [33], Silver nanoparticles [34], Prussian Blue [35], Bromocresol Purple [36], interference with peroxidase activity, which caused a decrease of the rate of the coupling reaction of *p*-chlorophenol with 4-aminoantipyrine [37], tris(1,10-phenanthroline) and iron(III) complex (ferritin, $[Fe(phen)_3]^{3+}$) [38], Orange G – bromate system [39], Iron(II)/Iron(III) – 2,4,6-tripyridyl-s-triazine [40], 11-Molybdobismuthophosphate [41], Wells-Dawson Type Molybdophosphate [42,43], etc. Spectrophotometric methods for determining Asc are detailed in a review article [20]. Therefore, there is no unified system; the search for new and effective analytical forms of spectrophotometric determination of Asc is topical today.

In some objects, the content of Asc is lower than the limit of its determination for spectrophotometric methods; therefore, it is necessary to pre-concentrate Asc with the use of environmentally friendly techniques. Due to the efficiency and relative ease of execution, these include liquid-liquid microextraction, which allows minimizing the amount of toxic organic solvents [44–46]. Among the liquid-liquid microextraction techniques, the Vortex-agitator [47,48] is well proven and is effective at small volumes of organic solvents. This approach has not been used before for the spectrophotometric determination of Asc.

In this study, after optimizing conditions of the redox-reaction between Asc with triiodide anion (I_3^-) in the presence of polymethine dye Astra Phloxine FF, a vortex-assisted liquid-liquid microextraction procedure for Asc was suggested for spectrophotometric determination. In the interaction of ascorbic acid with the ionic associate (IA) of the triiodide anion and Astra Phloxine FF (AP), its destruction causes a decrease in the intensity of absorption of the extract of the ionic associate, and based on this, a spectrophotometric method for determining Asc in some food samples was developed.

2. Experimental

2.1. Reagent and solution

All chemicals used were of analytical grade, and double-distilled water was used throughout the work. The 0.01 mol L^{-1} triiodide anion stock solution was prepared by dissolving an exact amount of purified crystalline iodine (Sigma-Aldrich, Germany) in an 18% solution of KI (Sigma-Aldrich, Germany). The stock solution was stored in the dark. The working solutions of triiodide anion (0.001–0.0001 mol L^{-1}) were made daily by successive dilutions of the stock solution with water. The 0.001 mol L^{-1} standard solution of Astra Phloxine FF (Basic Red 12, 1,3,3-trimethyl-2-[3-(1,3,3-trimethyl-2-indolinylidene) propenyl]-3*H*-indolium chloride) was prepared by dissolving an exact amount of sample dye (Hangzhou Dayangchem Co. Ltd., China) in water. The 0.001 mol L^{-1} standard water solution L-ascorbic acid (Sigma-Aldrich, Germany) was prepared by dissolving an exact amount of sample. The solutions were made daily.

To create the acidity of the medium, a 5.0–1.0 mol L^{-1} solution of sulfuric acid (Sigma-Aldrich, Germany) and a 0.5 mol L^{-1} acetate buffer solution were used.

2.2. Apparatus

A Lightwave II UV–Vis spectrophotometer (Biochrom, UK) equipped with a quartz ultramicrocuvettes (Starna Scientific Ltd., UK) of 10 mm path length was used for absorbance and recording the absorption spectra and for routine measurements. A VM-3000MD vortex mixer (Medline Scientific, UK) was used to assist the extraction process. Centrifugation was performed using a MCD-2000 centrifuge (MRC ltd., China). The pH values of the solutions were measured with an InoLab pH Level 1 potentiometer (WTW, Germany) with a BlueLine 23 pH Model electrode (SI Analytics, Germany).

2.3. Procedure for ascorbic acid determination

A 4.0 mL aliquot containing from 0.003–0.53 mg L^{-1} of Asc (at a higher concentration of Asc, an appropriately smaller volume is added, which is adjusted to 4.0 mL with water) was placed into a polypropylene tube with a conical bottom. Then 0.2 mL of the solution of triiodide anion (0.0001 mol L^{-1}), 0.6 mL of acetate buffer solution (pH 3.0), 0.2 mL of aqueous solution AP (0.001 mol L^{-1}) were added and thoroughly mixed. As a result, the ascorbic acid reacts with triiodide anion to form iodide, which does not form an ionic associate with AP. Next, 500 μL of carbon tetrachloride was added, and the screw cap was closed. The mixture was shaken on a vortex agitator at 3000 rpm for 15 s. Then the tube was centrifuged at 2000 rpm for 2 min. A cloudy solution was formed, and the unreacted triiodide anion formed by the IA was extracted into an organic solvent. The organic phase was withdrawn using a micropipette and transferred to a ultramicrocuvette; the absorbance was measured at 560 nm against a blank.

The Asc was determined using a calibration plot constructed under similar conditions.

2.4. Preparation of samples

The washed and dried fruits and berries have to be thoroughly crushed. A weight of 10.0 g of the crushed sample was put into a conical flask of 250 mL and 50 mL of double-distilled water was added. The mixture was then shaken for 10 min and filtered through a paper filter. Liquid samples are analyzed directly. Carbonated drinks have to be degassed.

3. Results and discussion

3.1. Reaction chemistry

The method suggested in this paper includes spectrophotometric determination of Asc, which is based on redox-reaction between Asc with triiodide ions according to the scheme: