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Organic Chemistry

Elixir Org. Chem. 79 (2015) 30508-30510

ISSN: 2229-712X

Determination of ascorbic acid in different citrus fruits under reversed phase conditions with UPLC

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ARTICLE INFO

Article history:

Received: 26 September 2013;

Received in revised form:

29 January 2015;

Accepted: 19 February 2015;

Keywords

Ascorbic acid,

Citrus fruits,

Reverse phase,

UPLC.

ABSTRACT

This study compared the concentration of ascorbic acid between citrus fruits collected from farm of Kerman in Iran. Determination of ascorbic acid was carried out using a liquid chromatograph coupled to a diode array detector, with reverse phase and isocratic elution. The validation parameters showed efficiency, adequate linearity, relative standard deviation values 0.02 % (n=10) for repeatability and 0.5 % (n=15) for reproducibility, limit of detection (LD) was 0.2 mg L^{-1} recovery was between 97.3 % and 103.6%. Ascorbic acid 10 species of citrus: sour and sweet orange, umbilical orange, novel orange ,lime, lemon, pink and white grapefruit, aegle marmelos, bergamot, sour and sweet tangerines and clementine was determined. The average ascorbic acid was the highest in Shahdad's citrus fruits.

Introduction

The nature and concentration of the organic acids in fruits are of interest because of their important influence on the organoleptic properties and stability of fruit juices. The organic acid profile and concentration in fruits and vegetables depends on factors such as species, soil and stress conditions to which the fruit was submitted (1). Citrus juices are highly appreciated and consumed because of their taste and high vitamin C content. The shelf life evaluation is based on the evolution of vitamin C during storage, although there are other quality parameters such as color and flavor. Vitamin C is a very important vitamin for human nutrition that is supplied by fruits and vegetables (2). L-ascorbic acid is the main biologically active form of vitamin C. As a potent antioxidant, it has the capacity to eliminate several different free radicals (3).

Citrus fruits, one of the important fruit crop groups, are consumed mostly as fresh or as juice because of their nutritional value and special flavor. Consumption of citrus juice is found to be beneficial in preventing coronary diseases and chronic asthma (4). Citrus fruit extracts are also found to have antioxidant, anti-inflammatory, anti-tumor, anti-fungal and blood clot inhibition activities (5). These health benefits of citrus fruit have mainly been attributed to the presence of bioactive compounds, such as ferrulic acid, hydrocinnamic acid, cyanidin glucoside, hisperidine, vitamin C, carotenoid and naringin content (5; 6). Citrus fruits are classified as acid fruits, since their soluble solids are composed mainly of organic acids and sugars, which are used as the main index of maturity and one of the major analytical measures of flavor quality.

Several analytical methods have been reported for the determination of ascorbic acid using titrimetry (7), spectrometry (8) and amperometry (9). The preferred choice for ascorbic acid determination is separation techniques: capillary electrophoresis (10), gas chromatography (11) and liquid chromatography (12-14). In this field, high performance liquid chromatography (HPLC) is one of more promising and more used techniques, either by direct determination or by the analysis of derivatized products. Most of procedures developed until now for food and beverage analysis utilize either reverse phase partition chromatography (15) or ion exchange chromatography (16, 17) with a refractive index (RI), UV spectrophotometric, conductimetric or electrochemical detection (18).

In this study , ascorbic acid from different species of citrus fruits from Kerman in Iran such as: sour and sweet orange, umbilical orange, novel orange ,lime, lemon, pink and white grapefruit, aegle marmelos, bergamot, sour and sweet tangerines and clemantine, to separate and was determined with using ultra-performance liquid chromatography (UPLC) with photodiode array detection (DAD).

Experimental

Apparatus

The UPLC–DAD system consisted of an Agilent 1200 series (Agilent, Santa Clara, CA) (quaternary pump, vacuum degasser and diode array detector) connected to a computer loaded with Agilent ChemStation Software. For the detection of ascorbic acid, the detector was set at λ=254 nm. This setting was chosen since ascorbic acid has its maximum optical absorbance close to 254 nm. The ascorbic acid in the sample test solution was separated by reversed phase chromatography on a 150 mm×4.6 mm i.d., 5 µm particle ZORBAX Eclipse XDB-C_{18} (Agilent, USA) analytical column, of which was detected by absorbance and quantified with external calibration graph. A corning pH meter (Massachusetts, USA) was employed for pH measurements.

Reagent

All reagents were of analytical grade. The stock solution of ascorbic acid was prepared by dissolving an appropriate amount ascorbic acid (Merck, Darmstadt, Germany) in deionized water and stored in dark places between the experiments, at low temperature (+4°C).

Grade HPLC acetonitrile (Merck, Darmstadt, Germany), potassium dihydrogen orthophosphate and phosphoric acid were of analytical purity or for chromatographic use. The water used was deionized.

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Preparation of juice sample

Fresh fruits of sour and sweet orange, umbilical orange, novel orange ,lime, lemon, pink and white grapefruit, aegle marmelos, bergamot, sour and sweet tangerines and clemantine were purchased from the local markets. Healthy fruits were selected randomly for uniformity of shape and color. The citrus fruit juice was extracted by cutting the fruit in half and careful hand-squeezing to obtain the juice. The juice was passed through a strainer to remove pulp and seeds. The freshly squeezed juice was centrifuged at 3000 rpm for 10 min and twice, the supernatant was diluted 1:5. The dilutions were membrane filtered (0.20 µm) before injection. Two samples were analyzed in duplicate.

General procedure

The determinations were made in isocratic conditions, at ambient temperature, using a mobile phase made of 0.2 % acetonitrile and 50 mM phosphate solution (dissolve 6.8 g potassium dihydrogen phosphate in 900 ml water; the pH value should be adjusted to pH =2.8 with phosphoric acid and then filled to 1000 ml with water) filtered through a polyamide membrane (0.2 µm) and degassed in a vacuum. The flow rate of the mobile phase was 1.2 ml/min for all the chromatographic separations. The separation column was balanced with mobile phase until the baseline was stabilized. Sample injections were made at this point. The volume injected was 5 µl for either prepared sample or standard solution.

Table. 1. Wavelength, retention time, concentration range of lineal response, correlation coefficient and detection limit for ascorbic acid

analyte	λ_{nm}	$RT_{(min)}$	Concentration rang (mg L^{-1})	Correlation coefficient (r^2)	Detection limit (mg L^{-1})
Ascorbic acid	254	3.2	0.5-200	0.999	0.20

Results and discussion

In Fig. 1 the chromatogram of ascorbic acid in the standard solution and real samples was given. The linearity of the method was evaluated according to area response. Selected wavelength for ascorbic acid, retention time, concentration ranges of linear response, correlation coefficients and detection of limit were summarized in Table. 1. The detection limit (LOD) could be defined as the smallest peak detected with a signal height three times that of the baseline, while the limit of quantification (LOQ) referred to the lowest level of analyte which could be determined with an acceptable degree of confidence. In the present work, detection limits were estimated according to the hypothesis that a peak, to be detected, should have a signal-to-noise ratio >3. Precision was tested on ten replicated analyses of independent preparations of sweet orange juice. The RSD value was % indicating that the method was precise with a high degree of repeatability. The recovery of ascorbic acid from citrus fruits ranged from 97.2 to 103.6% . The amount ascorbic acid found in citrus juices was shown in Table. 2. The ascorbic acid content of orange was higher than that of the other citrus fruits while the lemon has the lowest of content of ascorbic acid. Shahdah's fruits have higher amounts of ascorbic acid among in citrus juices.

Conclusions

This work is a contribution to the development of a rapid and precise uPLC procedure for quantitative determination of ascorbic acid in citrus fruits. Ascorbic acid has been eluted from the column within 4 minutes. The method could successfully used to quantify ascorbic acids in natural citrus juices. Sample preparation is simple, and mobile phase consisted of a simple buffer It was observed that the ascorbic acid present in citrus juices were species, cultivar and horticultural practice dependent and could be considered as an active parameter for authenticity determination.

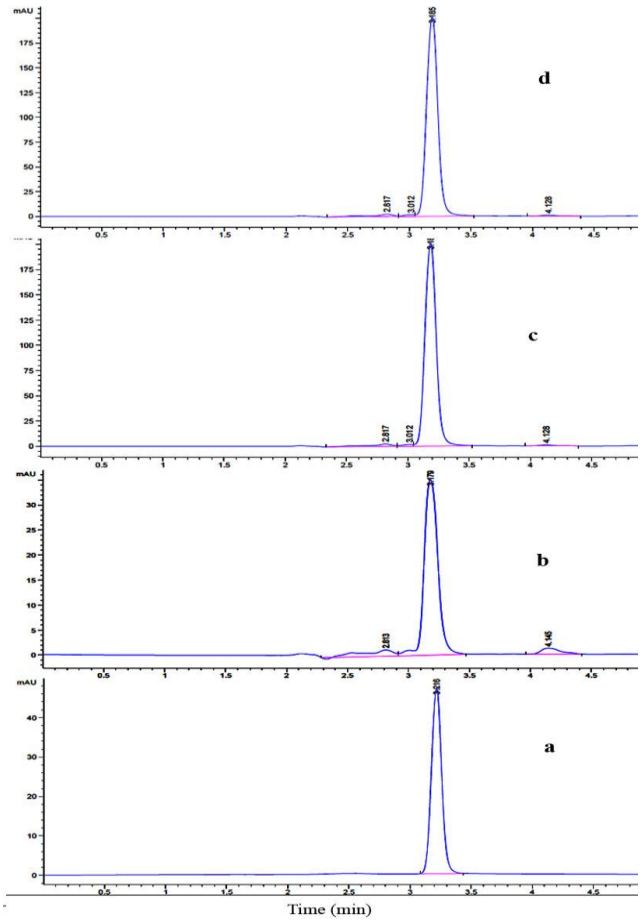

Fig. 1. Chromatogram of the (a) standard solution of ascorbic acid (30 ppm), (b) Sour tangerines of Bam, (c) Sweet orange of Jiroft and (d) Sweet orange of Shahdad

Table. 2. Ascorbic acid content of citrus fruits (mg L^{-1})

Fruit	Shahdad Mean±SD *	Jiroft Mean±SD *	Bam Mean±SD *
Sour orange	118.51±0.11	73.75±1.0	113.63±0.54
Sweet orange	159.72±0.14	132.62±0.34	93.30±1.1
Umbilical orange	94.60±0.71	92.57±0.86	99.10±0.75
Novel orange	92.70±0.8	72.47±0.77	-
Lime	52.38±1.1	43.24±0.90	-
Lemon	96.90±0.53	88.32±1.4	49.27±1.0
Pink grapefruit	-	73.12±0.88	-
White grapefruit	84.11±1.5	80.41±1.2	88.97±0.80
Aegle marmelos	106.61±1.06	-	-
Bergamot	-	64.18±0.51	-
Sour tangerines	55.39±0.75	53.38±0.71	41.70±0.47
Sweet tangerines	69.15±0.86	94.58±0.32	49.55±0.83
Clementine	-	36.93±1.2	-

*Results are expressed as mean±SD (standard deviation) (n=3)

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