Quantitative determination of vitamin C in fruit juice A-10.5

Key words

Instrumental HPTLC - quantitative analysis - prechromatographic derivatization - densitometry (absorbance) - food analysis - ascorbic acid - vitamin C

Scope

Quantitative determination of ascorbic acid in fruit juices and similar products is called for when their vitamin C content is declared as an advertising feature. Ascorbic acid is also used as an antioxidant in meat products to stabilize their fresh appearance. Because here ascorbic acid is an extraneous substance, its determination is a regulatory requirement.

Mild oxidation converts vitamin C into dehydroascorbic acid. As its 2.4-dinitrophenylhydrazone (DNPH) derivative this is then separated form matrix substances by TLC and quantified densitometrically by adsorbance at 510 nm.

This method was developed in the CAMAG Laboratories.

Literature

R. Strohdecker, H.M. Henning, Vitamin-Bestimmungen, Verlag Chemie, Weinheim

J.H. Roe, C.A. Kuether, Science **95,** 77 (1942)

Advantages of performing this analysis by instrumental TLC

- Easy sample preparation
- Suitable for routine analysis with large sample throughput

Reagents

Chloroform
Ethyl acetate
Acidic acid
Oxalic acid, 4% aqueous solution
Thiourea
L-Ascorbic acid, aqueous solutions = 1 mg/L (calibration standard)
2,4-Dinitrophenylhydrazine, 2% solution in 70% sulfuric acid
2,6-Dichorophenol-indophenol Na-salt (MERCK No. 3028), 0.5% in water

Sample preparation

- Pipette 2 mL fruit juice into a measuring flask
- Add 25 mL 4% oxalic acid solution
- Add 2 mL 0.5% aqueous dichlorophenol-indophenol solution. Ascorbic acid oxidation is completed within 5 min. at room temperature.
- Add 10 mg thiourea to destroy excess of oxidation reagent.
- Fill up with water to 100 mL
- Transfer 20 mL into a 50 mL centrifuge vessel and
- Add 4 mL of the 2% solution of 2,4-dinitrophenylhydrazine in 70 % sulfuric acid
- Extract the hydrazones with 3 x 6 mL ethyl acetate - acetic acid 98:2. For better phase separation centrifugate 1 min. after each extraction. Combine the extracts and fill up to 20 mL.
- Apply the orange colored extract directly onto the HPTLC layer.

Calibration Standard

L-Ascorbic acid, aqueous solution 1 mg/L

Layer

HPTLC precoated plates silica gel MERCK 60 F 254, 20 x 10 cm, prewashed by immersing for 1 hour in isopropanol followed by 30 min. drying at 120 °C

Sample application

Bandwise with CAMAG Automatic TLC Sampler III or CAMAG LINOMAT: 6 mm bands, 4 mm apart, 15 mm from the side = 17 samples per plate side; delivery speed 4 sec/µL. Apply 5 µL of the unknowns and different amounts of the standard on both sides of the plate.

Recommended application pattern:

S1 A B C S2 A B C S3 A B C S4 A B C S5

S1 = 3 µL = 60 ng absolute
S2 = 6 µL = 120 ng absolute
S3 = 9 µL = 180 ng absolute
S4 = 12 µL = 240 ng absolute
S5 = 15 µL = 300 ng absolut

S = Standard
A,B,C = Unknown 5 µL

Chromatography

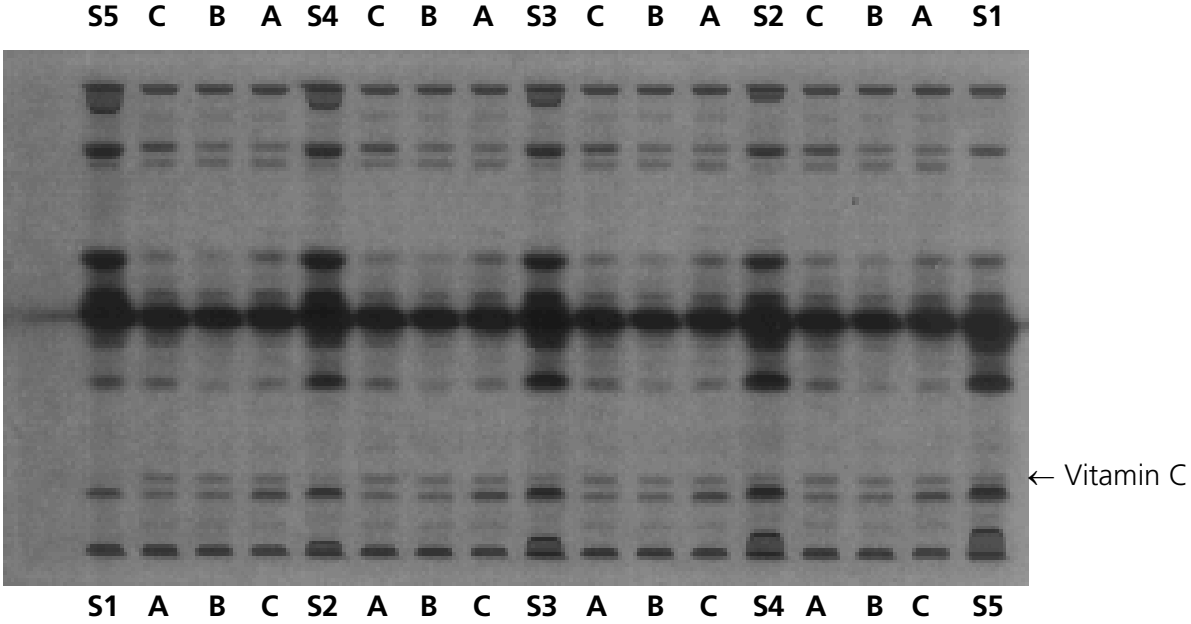
In CAMAG Horizontal Developing chamber in sandwich configuration with chloroform - ethyl acetate 1:1, two runs with gentle intermediate drying. Rf of ascorbic acid derivative is about 0.4.

Densitometric evaluation

With CAMAG TLC Scanner II with Labdata System and CATS evaluation software. Scanning by absorbance at 510 nm with tungsten lamp, monochromator bandwidth 10 nm, slit dimensions 0.2 x 5 mm; evaluation via peak area, linear regression.

Results

The reproducibility of this method is about 1.5% rel. standard deviation.



HPTLC Chromatogram of vitamin C derivatives; chromatography in CAMAG Horizontal Developing chamber; photography with CAMAG Reprostar, 254 nm UV, Mamiya camera RB 67

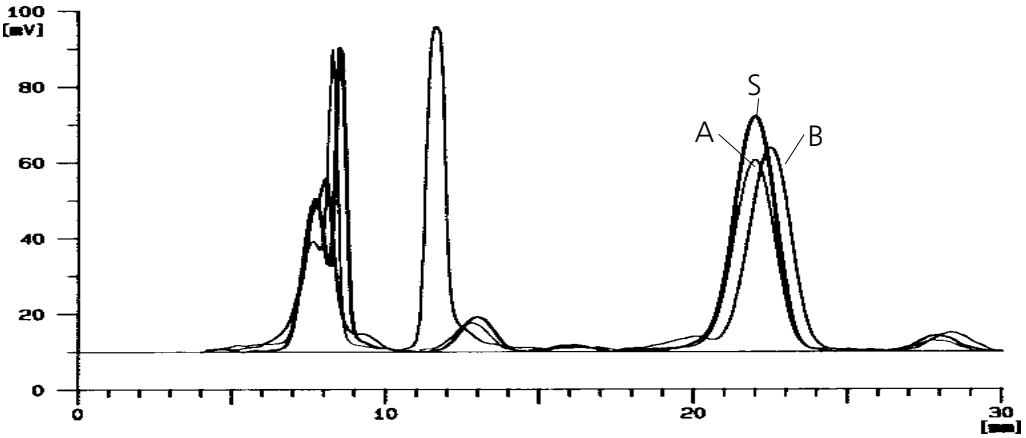


Fig. 1: Densitogram curves of two orange juice extracts (A and B) and standard (S)

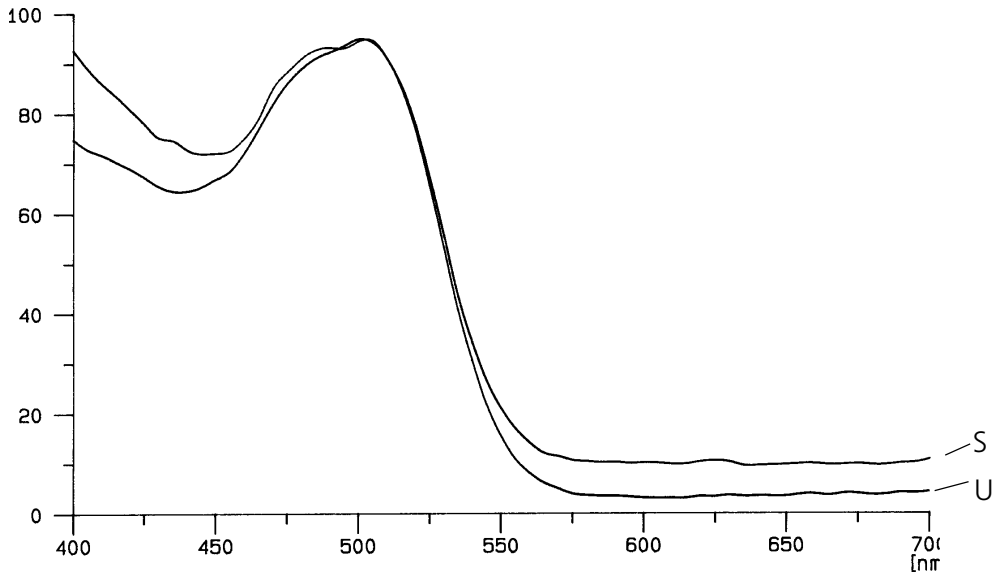


Fig. 2: In-situ absorption spectra of the DNPH-dehydroascorbic acid from standard (S) and unknown (U)

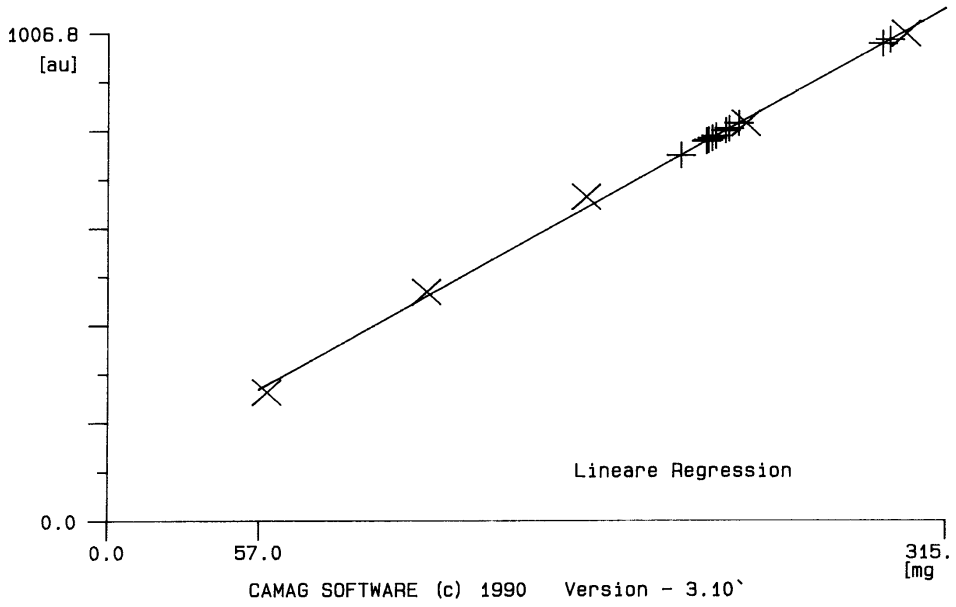


Fig. 3: Calibration function of DNPH-dehydroascorbic acid (peak area) in the range from 57 - 315 mg/L