INSTITUTE OF CHEMICAL TECHNOLOGY, PRAGUE
Faculty of Food and Biochemical Technology

Department of Food Analysis and Nutrition

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ANALYSIS OF FOOD AND NATURAL PRODUCTS

LABORATORY EXERCISE

Determination of vitamins, caffeine and preservatives
(method: liquid chromatography with UV detection)

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Exercise guarantor: Assoc.Prof. Ing.Věra Schulzová, Ph.D.

CONTENT

Required knowledge

1. Principle of sample preparation for unstable compounds determination (isolation and extraction methods)
2. Principle of liquid chromatography (stationary phases, types of columns, mobile phases, detectors)
3. Principle of spectrophotometry (Lambert-Beer´s law) structure and properties of vitamin C (stability, occurrence in foodstuffs)

Evaluation criteria

- proved theoretical knowledge
- quality of practical work execution
- compliance with laboratory work rules (safety, order)
- protocol – integrity of experimental data, results calculation and discussion

Laboratory exercise content

A. Determination of vitamin C in beverages, fruits and vegetables by HPLC/UV method (accredited SZPI method A/11, ČSN EN 14130)

- **Monitoring of vitamin C stability during sample preparation, storage and thermal processing**

B. Determination of caffeine, vitamin C and preservatives in beverages by HPLC/UV method (ČSN EN 12856)

A. Working procedure for the determination of vitamin C in beverages, fruits and vegetables

(according to accredited method SZPI Praha A/11 and ČSN EN 14130)

1. USEFULNESS OF THE METHOD

The method is used to determine the content of ascorbic acid in beverages, fruit, vegetables, vegetable products, and in vitamin supplements.

2. THE PRINCIPLE OF THE METHOD

Ascorbic acid is extracted from a homogenized sample by the metaphosphoric acid solution. The extract is filtered and then the concentration of vitamin C is determined by the method of high performance liquid chromatography with UV detection. Identification and quantification is done by the method of external standard – calibration curve.

3. INSTRUMENTS AND EQUIPMENTS

- Liquid chromatograph – Hewlett Packard Series 1100 (UV detector $\lambda = 254$ nm)
- Analytical column RP C_{18} or RP C_8 (250 x 4,6 mm, 5 μm; 125 × 4 mm, 5 μm)
- HP Chemstation, Hewlett Packard, USA
- Analytical scales
- Ultrasonic bath
- Laboratory mixer or homogenizer, glass or plastic grater
- Cooker, stainless pot, kettle
- Vacuum filtration device

4. STANDARD AND CALIBRATION SOLUTIONS

- L-ascorbic acid p.a. (PENTA)

Preparation of standard solutions:

Prepare a stock solution of L-ascorbic acid of the concentration of 1 mg/ml in extraction solvent (preparation of extraction solvent per point 6.1.)

For completion of calibration curve prepare standard solutions of concentration of 10, 30, 60, 90, and 120 mg/l in extraction solution (preparation of extraction solvent per point 6.1)

Note: Maximal time for usefulness of the standard solution is one day.

5. CHEMICALS, SOLUTIONS, AND MATERIALS

- methanol (for HPLC)
- demineralised and distilled water
- metaphosphoric acid
- unless specified otherwise, use chemical of cleanliness class p.a.
- membrane filter of porosity 5 μm
- common laboratory glass

6. WORK PROCEDURE

6.1. Preparation of the laboratory sample

Preparation of extraction agent

3% solution of metaphosphoric acid (dissolve 15 g of solid metaphosphoric acid in 500 ml of distilled water).

Solid Samples

10 - 25 g of solid sample is homogenized with 60 – 80 ml of extraction agent for the duration of one minute. The obtained extract is filtered through filtration paper, which is washed through few times; it is also possible to use vacuum filtration. The filtrate is quantitatively transferred into a 100 ml volumetric flask and an extraction agent is added up to the 100 ml mark. All sample solutions are filtered before injection into a liquid chromatograph using a membrane filter.

Liquid Samples

Liquid materials after throughout mixing are diluted by the extraction agent (the amount is chosen according to the expected vitamin C content, in such a way that concentration of analyzed solution is corresponding to the range of calibration of 10 – 120 mg/l). Before introduction into a liquid chromatograph, it is necessary to filtrate it through membrane filter.

Tablet

Declaration – 100 mg / tablet.
Dissolve the tablet in 100 ml volumetric flask by extraction agent and dilute 20x (0.5 ml to 10 ml) – resulting concentration is about 50 mg/l.

Note: In case of need (the result is outside the calibration range), it is possible to dilute the sample by extraction agent (metaphosphoric acid)

Monitoring of stability of vitamin C during sample preparation, storage and thermal processing

Solid samples

Samples of fruits and vegetables are heated in water and in steam in stainless vessel – conditions will be specified by assistant

Liquid samples

Tea with vitamin content is poured by hot water or stored under defined conditions

6.2. Analysis by liquid chromatography

Analytical column:	Analytical column RP C_{18} or RP C_8 (250 x 4,6 mm, 5 μm; 125 ×4,6 mm, 5 μm;) with precolumn
Mobile phase:	methanol : water (5:95, *v/v*) pH= 3 (H_3PO_4), flow 1 ml/min

Injection volume	20 μl
Detection:	UV 254 nm
Temperature:	Laboratory temperature

6.3. Identification and quantification

Identification is performed by the comparison of retention time of analyte in analyzed sample with the retention time of the calibration standard. Quantitative analysis is performed using the external standard method by the calculating of the concentration in analytical sample from the calibration curve equation (the dependence of the area or height of the peak on the concentration of standard in mg/l; concentration in the analytical sample must be in interval between the lowest and highest point of calibration curve) and by recalculation on the original sample by the following formula:

$$c_a = \frac{x \cdot V_a}{m}$$

The student compares the calculated content of the ascorbic acid with the declared content of vitamin C on the label of the sample (if it is mentioned), or with a common concentration of vitamin C in the given sample.

B. Working procedure for the determination of caffeine, vitamin C and preservatives in beverages *(ČSN EN 12856)*

1. USEFULNESS OF THE METHOD

The method is used to determine the content of caffeine, ascorbic acid and preservatives in beverages (tea, coffee, energetic drinks etc.)

2. THE PRINCIPLE OF THE METHOD

Target compounds are extracted from a homogenized beverage sample by water. The extract is filtered and then the concentration of analytes is determined by the method of high performance liquid chromatography with UV detection. Identification and quantification is done by the method of external standard – calibration curve.

3. INSTRUMENTS AND EQUIPMENTS
- Liquid chromatograph – Hewlett Packard Series 1100 (UV detector)
- Analytical column RP C_{18} or RP C_8 (250 x 4,6 mm, 5 µm; 125 × 4 mm, 5 µm)
- HP Chemstation, Hewlett Packard, USA
- Analytical scales
- Ultrasonic bath
- Vacuum filtration device

4. STANDARD AND CALIBRATION SOLUTIONS
- L-ascorbic acid p.a. (PENTA)
    Preparation of standard solutions as in point A.
- Sorbic acid, benzoic acid, caffeine (Sigma Aldrich)
    Standards will be provided by assistant as solid substance or as solution
    For completion of calibration curve prepare standard solutions of concentration of 50, 100, 200, and 400 µg/ml (from stock solution c = 1000 µg/ml).

5. CHEMICALS, SOLUTIONS, AND MATERIALS
- methanol (for HPLC)
- demineralised and distilled water
- acetate buffer (preparation of 500 ml: 0.8204 g of sodium acetate trihydrate and 0.575 ml of acetic acid put into a 500 ml volumetric flask and fill it with demineralised water)
- unless specified otherwise, use chemical of cleanliness class p.a.
- membrane filter of porosity 5 µm
- common laboratory glass

6. WORK PROCEDURE

6.1. Preparation of the laboratory sample

Liquid Samples

Liquid materials, after throughout mixing and degassing in an ultrasonic bath, are diluted by water agent. Before introduction into liquid chromatograph, it is necessary to filtrate it through membrane filter.

6.2. Analysis by liquid chromatography

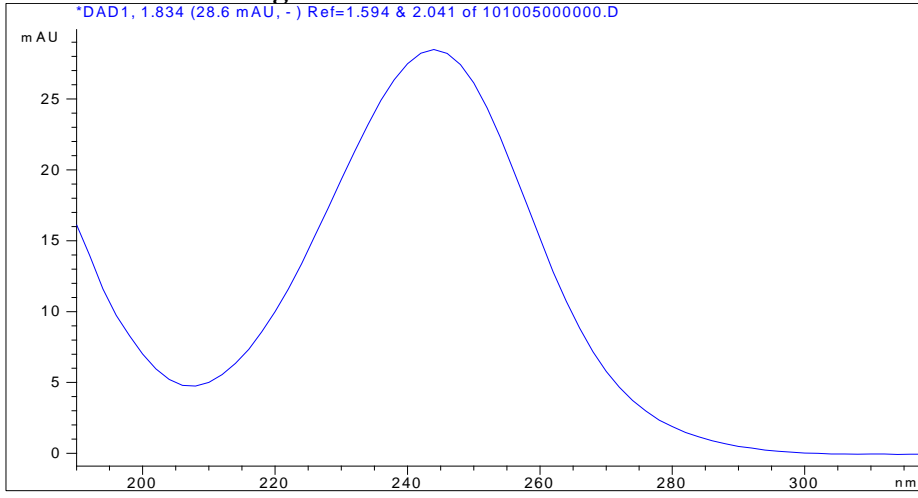|	
Analytical column:	Analytical column RP C_{18} or RP C_8 (250 x 4,6 mm, 5 μm; 125 × 4,6 mm, 5 μm;) with precolumn
Mobile phase:	MeOH/acetate buffer (3/7, v/v), flow 1 ml/min (prepared mobile phases is filtered and degassed in ultrasonic bath for duration of 10 minutes)
Injection volume:	10 or 20 μl
Detection:	UV 273 nm for caffeine, 254 nm for vitamin C, 228 nm for benzoic acid, 254 nm for sorbic acid
Temperature:	Laboratory temperature

6.3. Identification and quantification

The identification of the analyte is performed by the comparison of its retention time in analyzed sample with the retention time of the calibration standard. Quantitative analysis is performed using the external standard method by the calculating of the concentration in analytical sample from the calibration curve equation (the dependence of the area or height of the peak on the concentration of standard in mg/l; concentration in the analytical sample must be in interval between the lowest and highest point of calibration curve) and by recalculation on the original sample by the following formula:
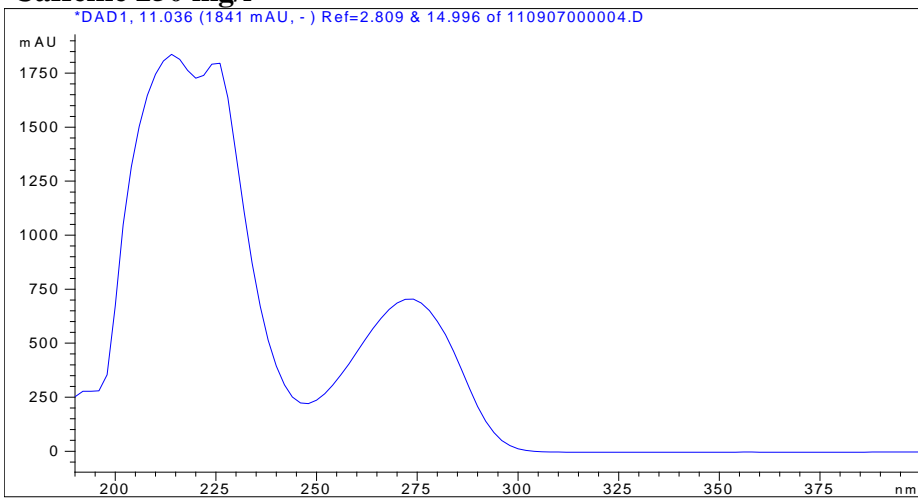
$$c_a = \frac{x \cdot V_a}{m}$$

The calculated content of the ascorbic acid, caffeine and preservatives in beverages express in mg/l, content in tea and coffee in mg/kg.
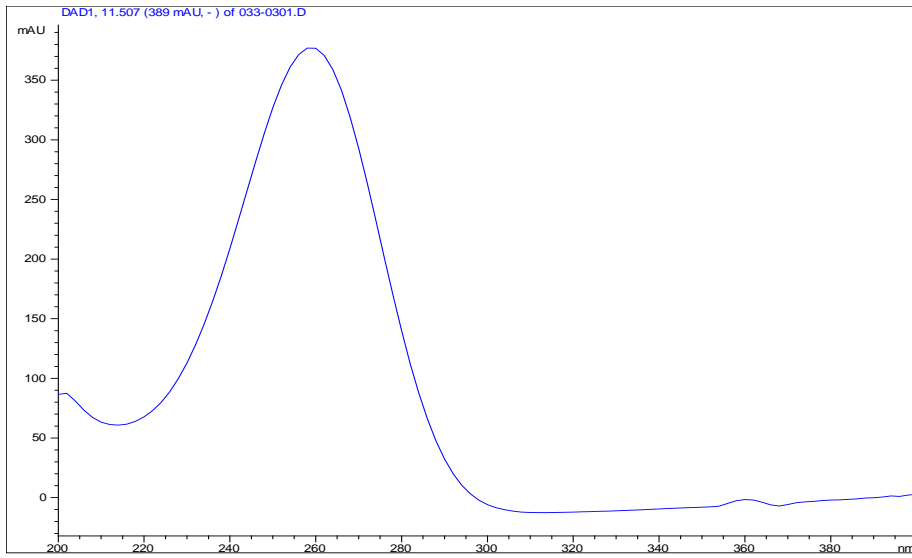
UV spectra of analytes

Ascorbic acid 12 mg/l

*DAD1, 1.834 (28.6 mAU, -) Ref=1.594 & 2.041 of 101005000000.D

Caffeine 250 mg/l

*DAD1, 11.036 (1841 mAU, -) Ref=2.809 & 14.996 of 110907000004.D

Sorbic acid 50 mg/ml

mAU

350

300

250

200

150

100

50

0

200 220 240 260 280 300 320 340 360 380 nm

Benzoic acid 100 mg/ml

m AU

140

120

100

80

60

40

20

0

220 240 260 280 300 n m