Tentative Translation

JAS 0003

JAPANESE AGRICULTURAL STANDARD

Determination of the β-cryptoxanthin in Satsuma Mandarin

— High performance liquid chromatographic method

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Ministry of Agriculture, Forestry and Fisheries

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Food and Agricultural Materials Inspection Center, Incorporated Administrative Agency

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Foreword

This Japanese Agricultural Standard has been revised by the Minister of Agriculture, Forestry and Fisheries through deliberations at the Council for the Japanese Agricultural Standards as a result of proposal for the revision of Japanese Agricultural Standard submitted by Food and Agricultural Materials Inspection Center, Incorporated Administrative Agency with the original bill being attached, based on the provisions of Article 4, paragraph (1) of the Act on Japanese Agricultural Standards as applied mutatis mutandis pursuant to Article 5 of the same Act. This edition replaces the previous edition (JAS 0003:2019), which has been technically revised.

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JAPANESE AGRICULTURAL STANDARD JAS
(Tentative Translation) 0003 : 2023

Determination of the β-cryptoxanthin in Satsuma Mandarin

— High performance liquid chromatographic method

WARNING — The user of this document should be familiar with normal laboratory practice. This document does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this document to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

1 Scope

This document specifies a high performance liquid chromatographic method for the determination of β-cryptoxanthin (BCR) in the edible part of satsuma mandarin (*Citrus unshiu* Marc.) (fresh fruits).

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. The latest edition of the referenced document (including any amendments) applies.

ISO 648, *Laboratory glassware — Single-volume pipettes*

ISO 1042, *Laboratory glassware — One-mark volumetric flasks*

JIS K 0124, *General rules for high performance liquid chromatography*

JIS K 0557, *Water used for industrial water and wastewater analysis*

JIS K 8101, *Ethanol (99,5) (Reagent)*

JIS K 8150, *Sodium chloride (Reagent)*

JIS K 8355, *Acetic acid (Reagent)*

JIS K 8361, *Ethyl acetate (Reagent)*

JIS K 8574, *Potassium hydroxide (Reagent)*

JIS K 8593, *Petroleum ether (Reagent)*

JIS K 8780, *Pyrogallol (Reagent)*

JIS K 8839, *2-Propanol (Reagent)*

JIS K 8848, *Hexane (Reagent)*

JIS K 8987, *Sodium sulfate (Reagent)*

JIS K 9705, *Tetrahydrofuran (Reagent)*

3 Terms and definitions

No terms and definitions are listed in this document.

4 Principle

BCR is extracted from the test sample using ethanol. The extract is saponified with potassium hydroxide. BCR is collected from the saponified sample using hexane and ethyl acetate mixture to obtain the measurement solution. The BCR in the measurement solution is determined by high performance liquid chromatograph (HPLC) system with UV-visible absorbance detector.

5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

WARNING — It is the responsibility of users of this document to comply with legal regulations regarding the use of reagents.

5.1 Water, conforming to grade A3 or A4 of JIS K 0557.

5.2 BCR, of minimum mass fraction, 99 % (HPLC).

5.3 Ethanol, of minimum mass fraction, 99,5 %, according to JIS K 8101.

5.4 Pyrogallol, of minimum mass fraction, 99,5 %, according to JIS K 8780.

5.5 Sodium sulfate, of minimum mass fraction, 99,0 %, according to JIS K 8987.

5.6 Potassium hydroxide, of minimum mass fraction, 85,0 %, according to JIS K 8574.

5.7 Sodium chloride, of minimum mass fraction, 99,5 %, according to JIS K 8150.

5.8 Hexane, of minimum mass fraction, 96,0 %, according to JIS K 8848.

5.9 Ethyl acetate, of minimum mass fraction, 99,5 %, according to JIS K 8361.

5.10 2-Propanol, of minimum mass fraction, 99,7 %, according to JIS K8839.

5.11 Acetonitrile, HPLC grade.

5.12 Methanol, HPLC grade.

5.13 Tetrahydrofuran, with or without antioxidants, of minimum mass fraction, 99,5 %, according to JIS K 9705.

5.14 Acetic acid, of minimum mass fraction, 99,7 %, according to JIS K 8355.

5.15 *dl*-α-tocopherol, of minimum mass fraction, 97,0 %.

5.16 Petroleum ether, special grade stipulated in JIS K 8593 or those of quality equivalent or superior.

5.17 Nitrogen, of minimum volume fraction, 99,5 %.

5.18 β–carotene, of minimum mass fraction, 90 %.

5.19 Pyrogallol ethanol, dissolve 30 g of pyrogallol per 1,0 l of ethanol. Do not use if the color changes to brown.

5.20 Potassium hydroxide solution, dissolve 60 g of potassium hydroxide per 100 ml of water.

WARNING — Since irritating gas is generated, work shall be done in a place with good ventilation inside a fume cupboard etc.

5.21 Sodium chloride solution, dissolve 10 g of sodium chloride per 1,0 l of water.

5.22 Hexane/ethyl acetate mixture, mix 9 parts per volume of hexane with 1 part per volume of ethyl

acetate.

5.23 HPLC mobile phase, mix 55 parts per volume of acetonitrile with 40 parts per volume of methanol, 5 parts per volume of tetrahydrofuran and 0,1 parts per volume of acetic acid. Dissolve 0,05 g of *dl*-α-tocopherol per 1,0 l of this mixture. Degas before use.

5.24 BCR stock standard solution, prepare a BCR stock standard solution in petroleum ether containing approximately 10 µg/ml BCR. Transfer this solution into several bottles with screw cap, seal and store.

NOTE In the interlaboratory test described in Annex A, BCR stock standard solutions were stored at –30 °C to –20 °C. It has been confirmed that the BCR stock standard solutions remain stable for at least half a year when stored at –30 °C to –20 °C.

Return the BCR stock standard solution to room temperature and shake to mix before use. Remove undissolved matter with a membrane filter.

5.25 Standard solutions

5.25.1 General

A standard solution for absorbance measurement (see 5.25.2) and a series of the standard solutions prescribed in 5.25.3 shall be prepared from the same BCR stock standard solution each time. Once the BCR stock standard solution is returned to room temperature, the standard solution for absorbance measurement shall be prepared and measured the concentration of that on the same day. Any remaining BCR stock standard solution shall not be stored.

5.25.2 Standard solution for absorbance measurement, dilute the BCR stock standard solution 5 times with petroleum ether using a single volume pipette and a volumetric flask.

NOTE In the interlaboratory test described in Annex A, 2 ml of BCR stock standard solution was transferred into a 10 ml volumetric flask and filled up to the mark.

Set up and operate the spectrometer in accordance with the manufacturer's instructions. Measure the concentration of standard solution for absorbance measurement by using a spectrometer at 452 nm with petroleum ether as reference. The BCR concentration of the stock standard solution, ρ_0, is given by the formula:

$$\rho_0 = \frac{A \times V_2 \times 10\,000}{\varepsilon \times V_1}$$

where

ρ_0 is the concentration of BCR in the stock standard solution (µg/ml);

A is the absorbance of the standard solution for absorbance measurement determined at 452 nm (petroleum ether, optical path length 1 cm);

ε is the absorption coefficient of BCR in concentration 1 % and optical path length 1 cm, 2 386 [7] [8];

V_1 is the nominal volume (ml) of the single volume pipette used;

V_2 is the nominal volume (ml) of the one-mark volumetric flask used.

Perform the procedure in 5.25.3 on the same day.

5.25.3 A series of standard solutions, using a single volume pipette, transfer the BCR stock standard solution into a round-bottomed flask. Gently blow nitrogen on this solution to evaporate the petroleum ether. Dissolve the contents in the round-bottomed flask completely with ethanol. Ultrasound may be used for about 10 seconds as a means of dissolution. Rinsing this flask several times with ethanol, transfer the contents completely into a one-mark volumetric flask. Add ethanol up to the mark of the volumetric flask and shake to mix. Filter the mixed solution with a membrane filter and use as the standard solution. Use the

same procedure to prepare standard solutions with a total of 4 or more concentration levels to create a series of standard solutions. An example of the prepared series of standard solutions is given in Table 1. The BCR concentration of the dilute standard solution, ρi, is given by the formula:

$$\rho_i = \frac{\rho_0 \times V_3}{V_4}$$

where

ρ_i is the concentration of BCR in *i*th levels of standard solution (μg/ml);

ρ_0 is the concentration of BCR in the stock standard solution (μg/ml) obtained in 5.25.2;

V_3 is the nominal volume (ml) of the single volume pipette used;

V_4 is the nominal volume (ml) of the one-mark volumetric flask used.

Table 1 — An example of the prepared series of standard solutions

Standard solutions	The nominal volume of the single volume pipette (ml)	The nominal volume of the one-mark volumetric flask (ml)	The concentration of BCR in standard solutions (equivalent to μg/ml)
A	1	5	2,0
B	1,5	10	1,5
C	1	10	1,0
D	0,5	10	0,50
E	0,5	20	0,25

Perform the procedure in 8.5.2 on the day of preparation, or store the standard solutions at –30 °C to –20 °C.

A series of standard solutions stored at –30 °C to –20 °C shall be returned to room temperature before HPLC measurement (see 8.5.2). After mixing thoroughly and using ultrasound for about 10 seconds as necessary to dissolve the undissolved matter, pass through a membrane filter.

NOTE 1 Between 0,25 μg/ml and 2,0 μg/ml of the calibration curve, it has been confirmed that the coefficient of determination is more than 0,990 and the origin is within the 95 % confidence interval of the y-intercept.

NOTE 2 It has been confirmed that the standard solutions remain stable for at least a week when stored at –30 °C to –20 °C.

5.26 β-carotene solution

Dissolve β-carotene in ethanol, and prepare a solution of a certain concentration that is within the BCR concentration range of the series of standard solutions prepared in 5.25.3.

NOTE When preparing a high concentration β-carotene solution, the β-carotene may not completely dissolve in the ethanol. In such a case, dissolve the β-carotene in hexane, and take a fraction. Blow nitrogen gently to evaporate the hexane so that it can dissolve in ethanol to prepare a solution with the targeted concentration.

6 Apparatus

The usual laboratory apparatus and the following shall be used.

6.1 Electronic analytical balances, capable of weighing to an accuracy of ±0,1mg, and more than 200 g.

6.2 Centrifuge tubes, made of glass with a round-bottom and around 50 ml in capacity, with a cap, keeping the space for shaking, and suitable for centrifugation at 400×*g*. The cap shall be either a stopper or screw type that is resistant to organic solvents. Furthermore, the cap for saponification (see 8.2) shall be

resistant to strong alkaline solutions.

6.3 Shaker, capable of mixing the centrifuge tube by shaking vertically.

6.4 Centrifuge, capable of 400×*g*.

WARNING — To prevent accidents, operate the centrifuge according to the instruction manual of the equipment.

6.5 Single volume pipettes, to cover the volume range for dilution of standard solutions (see 5.25) and saponification (see 8.2), of ISO648, class A.

6.6 One-mark volumetric flasks, to cover the volume range for dilution of standard solutions (see 5.25), extraction (see 8.1) and saponification (see 8.2), of ISO1042, class A.

6.7 Water bath, capable of being maintained at (70±3) °C and size for centrifuge tube rack.

6.8 Round-bottomed flasks, of 100 ml capacity, with ground neck, usable for evaporation.

6.9 Rotary evaporator, with water bath and vacuum control, for evaporation of solvent, for example hexane, ethyl acetate and ethanol.

6.10 Membrane filters, made of polytetrafluoroethylene (PTFE), suitable for organic solvents, with a pore size of 0,2 μm or less. The filter and the housing shall be unitary, and the housing material shall be resistant to organic solvents.

6.11 Vials, suitable for HPLC to be used, made of glass, deactivated, or other glass vials which have been checked for no influence on the measurement. Septum of the cap shall be made of PTFE or coated with PTFE.

6.12 Spectrometer, capable of measuring wavelengths 452 nm and holding cells.

6.13 Cells, made of quartz glass or glass, with optical path length of 1 cm, and should have stoppers. When using multiple cells, optical characteristics shall be equivalent.

6.14 HPLC system

6.14.1 HPLC, equipped with a mobile phase delivery system, a column oven with temperature control function, a UV-visible absorbance detector able to measure absorbance at 455 nm and a data processing unit, prescribed in JIS K 0124. A mobile phase delivery system should have a degassing device.

6.14.2 Chromatographic column for HPLC, reverse-phase C18 (ODS) columns, with the following characteristics:

—length: 150 mm

—internal diameter: 4,6 mm

—spherical particle size: 3 μm to 5 μm

—β-carotene shall be eluted within 20 min. Confirm that elution time of β-carotene and its peak do not overlap with BCR peak in accordance with 8.5.

If a guard column is used, select the one matching the C18 (ODS) column.

7 Preparation of test samples

After removing only the outer peel of the sample, pulverize it using a homogenizer or the like to obtain the test sample.

Immediately perform the procedure in 8.1, or freeze the test sample to store. When storing the test sample frozen, transfer into a sealable glass container soon after pulverized. Return it to room temperature and mix well before use.

NOTE 1 It has been confirmed that the samples remain stable for 2 weeks when stored fresh and refrigerated [4].

NOTE 2 It has been confirmed that the test samples remain stable for at least 2 months when stored frozen at –20 °C or below.

8 Procedure

8.1 Extraction

8.1.1 Weigh, to the nearest 10 mg, approximately 2 g of the test sample into a centrifuge tube. Add 15 ml of pyrogallol ethanol and 10 g of sodium sulfate to the tube.

8.1.2 Shake vigorously for 5 min by shaker. Separate the contents by the centrifuge at 400×*g* for 5 min. Transfer the supernatant into a 50 ml one-mark volumetric flask.

8.1.3 Add 15 ml of pyrogallol ethanol to the residue in the tube and repeat the procedure in 8.1.2. Transfer the supernatant into the same volumetric flask as in 8.1.2.

8.1.4 Repeat the procedure in 8.1.3.

8.1.5 Add pyrogallol ethanol up to the mark of the volumetric flask containing the supernatant, shake to mix, and use as the extract.

8.2 Saponification

Using a single volume pipette, transfer 10 ml of the extract (8.1.5) into a centrifuge tube and add 1 ml of potassium hydroxide solution to the tube. Shake gently. Place the tube in a constant temperature water bath set at 70 °C, heat the tube for 30 min shaking every 5 min. Then cool the tube to room temperature and use as the saponified sample.

8.3 Collection of BCR

8.3.1 Add 20 ml of sodium chloride solution, 5 ml of 2-propanol and 12 ml of hexane/ethyl acetate mixture to the saponified sample (8.2) and shake to mix.

8.3.2 Shake the tube vigorously for 5 min by shaker. Separate the contents by the centrifuge at 400×*g* for 5 min. Transfer the supernatant into a round-bottomed flask.

8.3.3 Add 12 ml of hexane/ethyl acetate mixture to the liquid left in the tube. Repeat the procedure in 8.3.2. Transfer the supernatant into the same round-bottomed flask as in 8.3.2.

8.3.4 Repeat the procedure in 8.3.3.

8.3.5 Evaporate almost the solvent in the round-bottomed flask (8.3.4) at 40 °C or below by rotary evaporator. Then gently blow nitrogen on the residue to dryness, and use as the dry residue.

8.4 Dissolution

Dissolve the dry residue in the round-bottomed flask (8.3.5) completely with ethanol. Ultrasound may be used for about 10 seconds as a means of dissolution. Rinsing the flask several times with ethanol, and transfer the solution completely to a one-mark volumetric flask.

NOTE 1 In the interlaboratory test described in Annex A, 5 ml one-mark volumetric flasks were used.

Add ethanol up to the mark of the volumetric flask and shake to mix. Pass through a membrane filter into a vial, and use as the measurement solution.

Perform HPLC measurement (see 8.5.2) on the same day, or store the measurement solution at –30 °C to –20 °C.

NOTE 2 It has been confirmed that the measurement solution remains stable for at least one week when stored at –30 °C to –20 °C.

The measurement solution stored at –30 °C to –20 °C shall be returned to room temperature on the day of measurement. Mix using a test tube mixer and use ultrasound for about 10 seconds as necessary to dissolve the undissolved matter well, then pass through a membrane filter.

8.5 Determination

8.5.1 HPLC operating conditions

Set up the HPLC system in accordance with the manufacturer's instructions and adjust it as follows.

a) Flow rate of the mobile phase: 1,5 ml/min.

b) Column Temperature: 40 °C.

c) Detection wavelength: 455 nm.

d) Volume injected: 20 µl.

e) Measurement time: 25 min. If, by injecting β-carotene, it is confirmed that the peak of β-carotene does not affect the subsequent measurement, the measurement time may be shortened.

8.5.2 HPLC analysis

Allow the entire system to run for a while to stabilize it. Confirm that the fluctuation of the base line gives no hindrance for the determination of BCR by a blank run under the specified conditions (see 8.5.1). Then inject the series of standard solutions into the column, followed by equal volumes of the measurement solutions.

8.5.3 Identification

Identify the individual BCR peak in the sample chromatogram by comparing retention times with those obtained from the standard solutions under the same HPLC conditions (see 8.5.1).

NOTE Typical HPLC chromatogram of a satsuma mandarin extract are given in Annex B.

9 Calculation

9.1 General

Quantitative determination is performed by the external standard method with integration of the peak area, which is then related to the corresponding value for the standard substance. For the peaks of impurities, take appropriate measures according to the perpendicular or tangent method prescribed in JIS K 0124. For the shoulder peaks derived from *cis* isomers, their peak areas are summed up as the peak area of BCR.

NOTE Typical shoulder peaks derived from *cis* isomers are given in Annex C. Generally with isomers of BCR, all-*trans* isomer is the most stable, and partial isomerization to *cis* isomer has been reported. Under the HPLC operating conditions described in 8.5.1, *cis* isomers peaks are detected immediately after the all-*trans* isomer peak. (see Figure C.1 a)). Furthermore, it has been reported that the main absorption spectrum of *cis* isomer shifts at 2 nm to 5 nm towards the short wavelengths compared to the all-*trans* isomer, and an characteristic peak appears in the near-UV region that is not present in the all-*trans* isomer [9] (see Figure C.1 b)).

9.2 Quantitation

Obtain the BCR peak areas for each of the series of standard solutions. Perform a linear regression of peak areas for each standard solution against the BCR concentrations of the respective standard solutions to create a calibration curve.

Calculate the concentration of BCR in each measurement solution by using the calibration curve. The BCR content in the test sample, w_i, is given by the formula:

$$w_i = \frac{C \times V_5 \times d_1}{W \times d_2}$$

where

w_i is the content of BCR in the test sample (mg/kg);

C is the concentration of BCR in the measurement solution (μg/ml);

V_5 is the constant volume (ml) in preparation of the measurement solution (see 8.4);

d_1 is the constant volume (ml) in preparation of the extract (see 8.1), typically 50;

d_2 is the volume of fraction (ml) in saponification of the extract (see 8.2), typically 10;

W is the mass (g) of the test sample.

9.3 Expression of results

Express the results to two significant figures.

10 Precision

10.1 Interlaboratory test

An interlaboratory test was carried out to determine the precision of the test method, and the results are summarized in Annex A. The values derived from this interlaboratory test can be inapplicable to the content ranges other than the given one (4,7 mg/kg to 23 mg/kg) nor the matrices other than those given.

10.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, is expected in not more than 5 % of cases to be greater than the repeatability limit (r) values [1] given in Table A.1 as long as the specified operation is correctly done [2].

10.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, is expected in not more than 5 % of cases to be greater than the reproducibility limit (R) values [1] given in Table A.1 as long as the specified operation is correctly done [2].

11 Quality control

The laboratory shall have internal quality control procedures for tests.

12 Test report

The test report shall include at least the following information:

a) the title or the reference number of this document;

b) every detail to identify the test sample;

c) the date of the test;

d) the results of the test.

Annex A
(informative)

Results of interlaboratory test

An interlaboratory test was carried out in accordance with IUPAC protocol [3] in 2015 in Japan, and gave the statistical results given in Table A.1 [5]. Some commercially available satsuma mandarins, with the outer peel removed, were weighed 150 g to 200 g. Pyrogallol was added as an antioxidant at 10 % of the sample mass and pulverized at 12 000 r/min for 10 min by a pulverizing device.

NOTE 1 Since carotenoids can be decomposed by light, oxygen and enzymes in the sample, the antioxidant was added.

After the homogeneity [6] was confirmed, the pulverized material was used as a test sample. The experimental protocol and test samples were supplied to the participating laboratories by the Food and Agricultural Materials Inspection Center (FAMIC), the organizer of this interlaboratory test. Each laboratory tested a total of 10 test samples (5 pairs of blind duplicates) according to the experimental protocol.

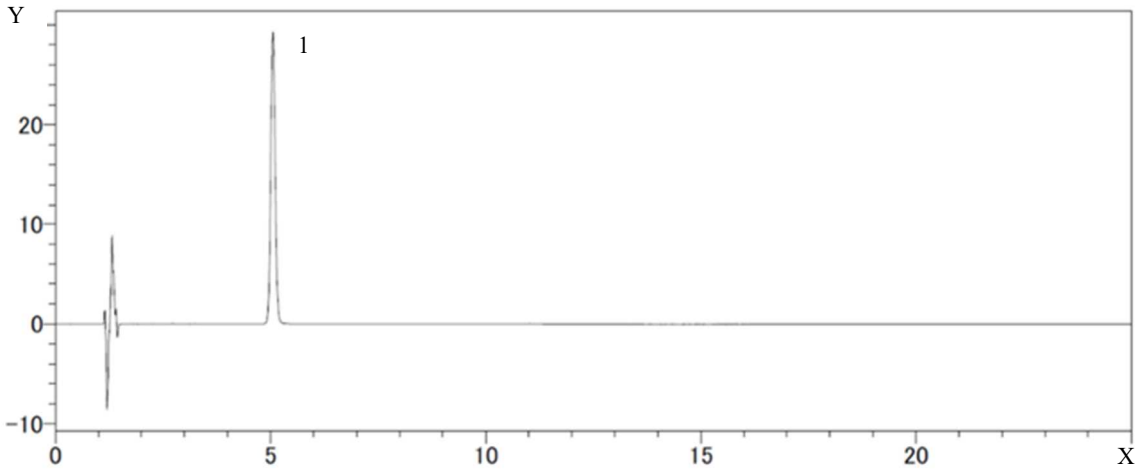
NOTE 2 In this interlaboratory test, instead of the HPLC mobile phase prescribed in 5.23, a mixture of 24 parts per volume of methanol and 1 part per volume of chloroform, containing 0.05 g per 1.0 l of ascorbyl palmitate was used. When FAMIC examined the difference in quantitative value from using these mobile phases in 2022, no significant difference was found (significance level: 5 %).

Table A.1 — Precision data

Sample identification	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**
Number of participating laboratories	11	11	11	11	11
Number of accepted test results	10	9	9	9	10
Mean BCR content, mg/kg (mass fraction),	4,73	6,75	10,2	13,7	23,4
Repeatability standard deviation s_r mg/kg	0,12	0,13	0,32	0,57	0,50
Repeatability relative standard deviation, RSD_r, %	2,6	2,0	3,1	4,2	2,1
Repeatability limit r ($r = 2{,}8\ s_r$) mg/kg	0,34	0,36	0,90	1,6	1,4
Reproducibility standard deviation s_R mg/kg	0,67	0,61	1,0	1,3	2,6
Reproducibility relative standard deviation, RSD_R, %	14	9,0	9,9	9,6	11
Reproducibility limit R ($R = 2{,}8\ s_R$) mg/kg	1,9	1,7	2,8	3,6	7,3

Annex B
(informative)

Typical HPLC chromatograms



Key

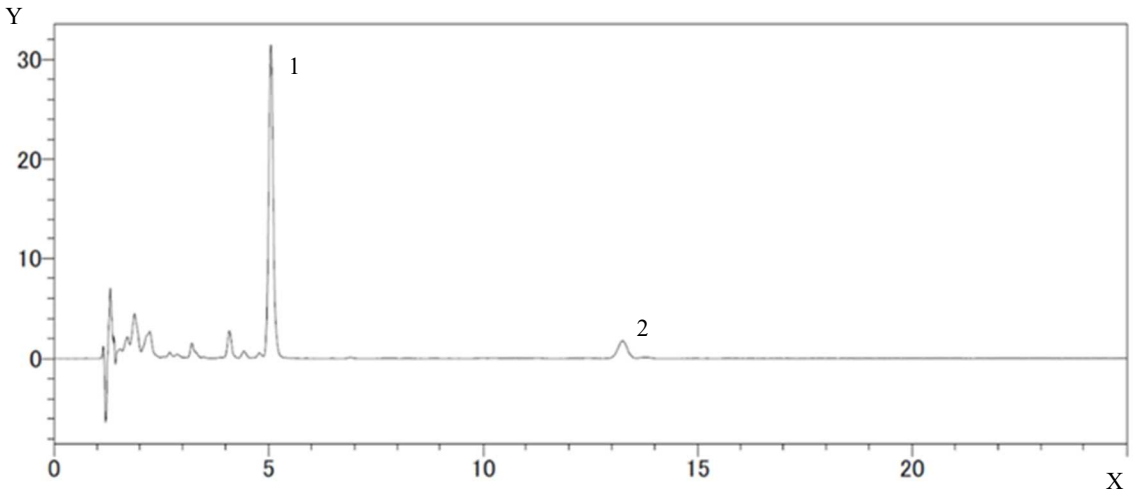
X retention time, min

Y response, mAU

1 BCR

NOTE For the HPLC operating conditions, in addition to 8.5.1, Inertsil® ODS-3 was used as the column. This information is given for the convenience of the users of this document and does not constitute an endorsement of the product.

Figure B.1 — BCR standard solution (equivalent to 1 µg/ml)



Key

X retention time, min

Y response, mAU

1 BCR

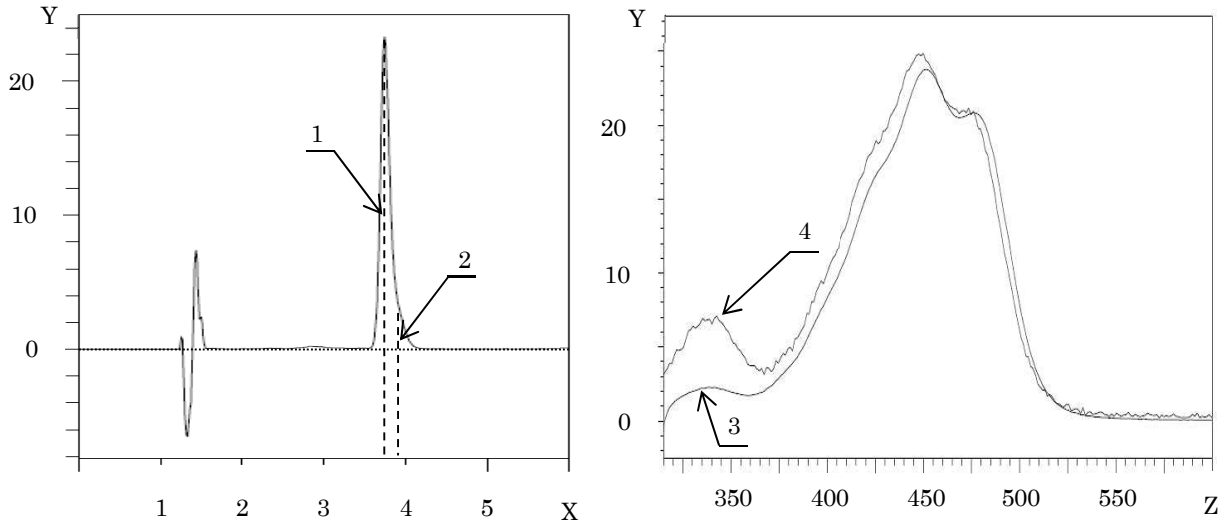
2 β-carotene

NOTE For the HPLC operating conditions, in addition to 8.5.1, Inertsil® ODS-3 was used as the column. This information is given for the convenience of the users of this document and does not constitute an endorsement of the product.

Figure B.2 — Measurement solution

Annex C
(informative)

Shoulder peaks derived from isomers of BCR



a) Chromatogram of BCR standard solution **b) Comparison of isomeric absorption spectra**

Key

X retention time, min

Y response, mAU

Z wavelength, nm

1 peak of all-*trans* isomer of BCR (X=3,7 min)

2 shoulder peak of *cis* isomers of BCR (X=3,9 min)

3 absorption spectrum of all-*trans* isomer

4 absorption spectrum of *cis* isomers (distinct at 325 nm to 350 nm)

NOTE For the HPLC operating conditions, in addition to 8.5.1, TSKgel® ODS-120A was used as the column. This information is given for the convenience of the users of this document and does not constitute an endorsement of the product.

Figure C — Examples of detected isomers

Bibliography

[1] ISO 5725-6:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 6: Use in practice of accuracy values*

NOTE Section 4 "Determination of limits" of the referenced document was referred to for the calculation of the repeatability limit and the reproducibility limit.

[2] ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

NOTE Section 7.1.5 of the referenced document was referred to for the expression of the repeatability limit and the reproducibility limit.

[3] Horwitz, W., Protocol for the design, conduct and interpretation of method-performance studies, *Pure & Appl. Chem.*, 1995, **67**(2), pp. 331-343.

[4] Matsumoto, H., et al., Effect of Postharvest Temperature and Ethylene on Carotenoid Accumulation in the Flavedo and Juice Sacs of Satsuma Mandarin (*Citrus unshiu* Marc.) Fruit, *J. Agric. Food Chem.*, 2009, **57**(11), pp. 4724-4732.

[5] Kumagai, M., et al., Validation of a Method for Determination of β-cryptoxanthin in Satsuma Mandarin (*Citrus unshiu* Marc.) by Interlaboratory Study , *Nippon Shokuhin Kagaku Kogaku Kaishi*. 2016, **63**(10), pp. 450-454.

[6] Thompson, M., et al., The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories, *Pure & Appl. Chem.*, 2006, **78**(1), pp. 145-196.

NOTE Section 3.11 "Testing for sufficient homogeneity and stability" of the referenced document was referred to for the method to confirm the homogeneity.

[7] JAPAN. *Syokuhinhyoujikijyun ni tsuite (March 30, 2015, syou syoku hyou dai 139 Consumer Affairs Agency jityou tsuuti).* betten Eiyou hyouji kankei.

[8] Bauernfeind, J. C, *Carotenoids as Colorants and Vitamin A Precursors*, ACADEMIC PRESS, United States of America, 1981, p.893.

[9] Goodwin, T. W. ed., *Chemistry and Biochemistry of Plant Pigments*, 2nd ed., ACADEMIC PRESS, London/New York, 1976, pp. 98-101.