

BLACKCURRANT EXTRACT

Prepared at the 57th JECFA (2001) and published in FNP 52 Add 9 (2001), superseding tentative specifications prepared at the 55th JECFA (2000), published in FNP 52 Add 8 (2000). No ADI was allocated at the 30th JECFA (1986)

SYNONYMS

INS No. 163 (iii)

DEFINITION

Blackcurrant extract is obtained from blackcurrant pomace by aqueous extraction. The main colouring principles are four anthocyanins (cyanidin 3-rutinoside, delphinidin 3-rutinoside, cyanidin 3-glucoside, delphinidin 3-glucoside). Most of the extracted sugars are fermented to alcohol and practically all the alcohol is removed during the concentration of fermented extract by vacuum evaporation. During the extraction process, sulfur dioxide is used and residual sulfur dioxide may be present. Commercial products may be in the form of a concentrated liquid, a paste, or a spray-dried powder. The spray-dried powder may contain an added carrier such as maltodextrin or glucose syrup.

Chemical names

- | | |
|------------------------------|-----------------------------|
| I. Cyanidin 3-rutinoside | III. Cyanidin 3-glucoside |
| II. Delphinidin 3-rutinoside | IV. Delphinidin 3-glucoside |

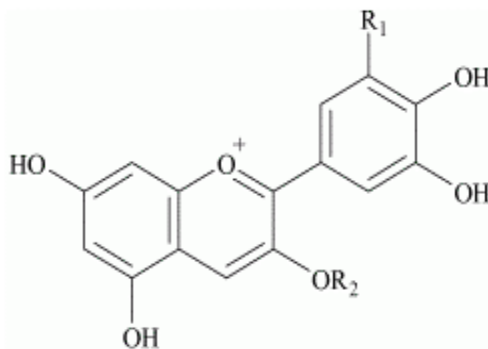
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|----------------|----------------|
| I. 18719-76-1 | III. 7084-24-4 |
| II. 15674-58-5 | IV. 6906-38-3 |

Chemical formula

- | | |
|----------------------------------|-----------------------------------|
| I. $[C_{27}H_{31}O_{15}]^+ X^-$ | III. $[C_{21}H_{21}O_{11}]^+ X^-$ |
| II. $[C_{27}H_{31}O_{16}]^+ X^-$ | IV. $[C_{21}H_{21}O_{12}]^+ X^-$ |
- Where X^- = counter ion

Structural formula



- | | |
|------|-----------------------------------|
| I. | $R_1 = H, R_2 = \text{rutinose}$ |
| II. | $R_1 = OH, R_2 = \text{rutinose}$ |
| III. | $R_1 = H, R_2 = \text{glucose}$ |
| IV. | $R_1 = OH, R_2 = \text{glucose}$ |

Assay

The colour intensity is not less than declared.

DESCRIPTION

Purplish-red liquid, paste or powder having a slight characteristic odour.

FUNCTIONAL USES

Colour

CHARACTERISTICS

IDENTIFICATION

<u>Solubility</u> (Vol. 4)	Soluble in water and ethanol.
<u>Spectrometry</u> (Vol. 4)	At pH 3 the absorbance maximum is about 520 nm.
<u>Colour reaction</u>	Add 0.1 g of the sample to 50 ml of water and shake thoroughly. Filter if necessary. The solution shows red to purplish-red colour and it turns to blue or dark green on the addition of sodium hydroxide TS.
<u>Chromatography</u>	The retention times for the major two peaks in the chromatogram of sample solution correspond to those for cyanidin 3-rutinoside and delphinidin 3-rutinoside in the chromatogram of reference using the conditions described under TESTS.

PURITY

<u>Sulfur dioxide</u>	Not more than 50 mg/kg for each unit of colour intensity See description under TESTS and METHOD OF ASSAY.
<u>Basic colouring matter</u>	Add 1 g of the sample to 100 ml of 1% sodium hydroxide solution and shake well. Extract 30 ml of this solution with 15 ml ether. Extract the ether solution twice with 5 ml dilute acetic acid TS. The acetic acid extract is colourless.
<u>Other acidic colouring matters</u>	Add 1 ml of ammonia TS and 10 ml of water to 1 g of the sample. Following the directions in Chromatography (<u>FNP 5</u>) place 2 µl of the solution on the chromatographic sheet and dry it. Use a mixture of pyridine and ammonia TS (2:1 by volume) as developing solvent and stop the development when the solvent front reaches about 15 cm height from the point where the sample solution was placed. No spot is observed in daylight at the solvent front after drying. If any spot is observed, it should be decolorized when sprayed with a solution of 40% stannous chloride in hydrochloric acid.
<u>Lead</u> (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described on Volume 4, "Instrumental methods".

TESTS

IDENTIFICATION TESTS

<u>Chromatography</u>	Determined by liquid chromatography (Volume 4) using the following procedure: <u>Preparation of sample solution</u> Dissolve 500 mg of sample into 25 ml of 0.3% HCl and wash with three volumes of ethyl acetate. Filter through a 0.45 µm filter. <u>Preparation of reference solution</u>
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Dissolve 2 mg of cyanidin 3-rutinoside and delphinidin 3-rutinoside into 10 ml of 0.3% HCl and filter through a 0.45 µm filter.

Apparatus

Liquid chromatograph equipped with a UV or diode array detector and an integrator.

Conditions

Column: Lichrosorb RP18 (length 25 cm, diameter 4.6 mm, particle size 5 µm) or equivalent

Column temperature: 35°

Mobile phase: Linear gradient from 40% B to 100% B during 20 min

A: Formic acid - water (10:90)

B: Formic acid - water - acetonitrile (10:75:15)

Flow rate: 0.8 ml/min

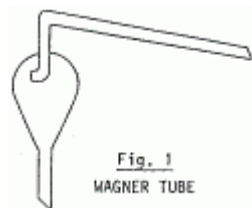
Injection volume: 10 µl

Wave length: 520 nm

PURITY TESTS

Sulfur dioxide

Distil 10 g of the sample with 100 ml of water and 25 ml of 30% phosphoric acid solution in a distilling flask with the Wagner tube. In an absorption flask, place 25 ml of 2% lead acetate solution previously prepared. Insert the lower end of the condenser into the lead acetate solution in the absorption flask. Distil until the liquid in the absorption flask reaches about 100 ml and rinse the end of the condenser with a little amount of water. To the distilled solution add 5 ml of hydrochloric acid and 1 ml of starch TS, and titrate with 0.01 N iodine. Each ml of 0.01 N iodine is equivalent to 0.3203 mg of SO₂.



METHOD OF ASSAY

Prepare approximately 200 ml of pH 3.0 citric acid - dibasic sodium phosphate buffer solution: Mix 159 volumes of 2.1% citric acid solution and 41 volumes of 0.16% dibasic sodium phosphate solution, and adjust the pH to 3.0, using the citric acid solution or dibasic sodium phosphate solution.

Weigh accurately an adequate amount of the sample so that the measured absorbance is between 0.2 and 0.7, and add pH 3.0 citric acid - dibasic sodium phosphate buffer solution to make up a 100-ml solution. Measure the absorbance A of this solution in a 1-cm cell at the wavelength of maximum absorption around 520 nm, using pH 3.0 citric acid - dibasic sodium phosphate buffer solution as the blank.

Colour intensity = $[A \times 10]/[\text{weight of sample (g)}]$