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Determination of paprika red in feeds - High performance liquid chromatography

饲料中辣椒红的测定 高效液相色谱法

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1 Scope

Warning: those using this document should have practical experience of work in a proper laboratory. This document does not set out all the safety problems that may arise; it is the responsibility of the user to adopt appropriate safety and health measures and to make sure that the relevant national provisions are met.

This document describes a high performance liquid chromatographic method for the determination of paprika red in feeds.

This document applies to the determination of paprika red, taken as the sum of capsanthin and capsorubin, in compound feeds, concentrate feeds and vitamin premixes.

In this document the detection limit for both capsanthin and capsorubin is 0.05 mg/kg and the quantification limit for both is 0.1 mg/kg.

2 Normative references

The contents of the following documents constitute indispensable provisions of this document through normative reference in the text. For dated references, only the edition corresponding to that date applies to this document; for undated references, the latest edition (including all amendments) applies to this document.

GB/T 6682, Water for analytical laboratory use - Specification and test methods.

GB/T 20195, Animal feeding stuffs - Preparation of test samples.

3 Terms and definitions

This document contains no terms or definitions that need to be defined.

4 Principle

Capsanthin and capsorubin in the test sample are extracted with acetonitrile, determined by high performance liquid chromatography and quantified by the external standard method.

5 Reagents or materials

Unless otherwise specified, only analytical grade reagents are used.

5.1 Water: GB/T 6682, grade one. 5.2 Acetonitrile: chromatographic grade.

5.3 Phosphoric acid solution, 0.3 % by volume: pipette 1.5 mL of phosphoric acid and dilute to 500 mL with water, then mix.

5.4 Capsanthin and capsorubin standard stock solutions, 100 micrograms per millilitre: weigh 10 mg, to the nearest 0.01 mg, of each of the capsanthin reference substance (CAS number 465-42-9, purity not less than 95 %) and the capsorubin reference substance (CAS number 470-38-2, purity not less than 95 %) into separate 100 mL amber volumetric flasks, dissolve in acetonitrile (5.2), make up to the mark and mix. Store at 2 degrees C to 8 degrees C; valid for 1 month.

5.5 Mixed intermediate standard solution, 10 micrograms per millilitre: accurately transfer 1 mL of each of the capsanthin and capsorubin standard stock solutions (5.4) into a 10 mL amber volumetric flask, dilute to the mark with acetonitrile (5.2) and mix. Prepare fresh before use.

5.6 Series of mixed standard solutions: accurately transfer suitable volumes of the mixed intermediate standard solution (5.5) into 10 mL amber volumetric flasks, dilute to the mark with acetonitrile (5.2) and mix, to give mixed standard solutions at mass concentrations of 0.02, 0.1, 0.5, 1, 2 and 5 micrograms per millilitre. Prepare fresh before use.

5.7 Membrane filter: 0.45 micrometre, organic phase.

6 Apparatus and equipment

6.1 High performance liquid chromatograph, fitted with an ultraviolet detector or a diode array detector. 6.2 Analytical balance, readable to 1 mg and to 0.01 mg. 6.3 Vortex mixer. 6.4 Centrifuge, speed not lower than 5000 r/min.

7 Samples

Prepare the test sample in accordance with GB/T 20195, taking at least 200 g, grinding it so that it all passes a 0.425 mm test sieve, mixing it evenly and placing it in a closed container away from light, ready for use.

8 Test procedure

8.1 Extraction. Carry out two determinations in parallel. Weigh 5 g of the test sample, to the nearest 1 mg, into a 50 mL centrifuge tube, add exactly 25 mL of acetonitrile (5.2), vortex and shake for 10 min, adding a stirrer bar to homogenize the mixture if it is viscous, and centrifuge at 5000 r/min for 5 min. Take a suitable volume of the supernatant, filter it through the membrane filter (5.7) and keep the filtrate for determination.

8.2 Reference liquid chromatographic conditions: a) column, C8, 100 mm long, 3.0 mm internal diameter, 2.7 micrometre particle size, or equivalent performance; b) column temperature 35 degrees C; c) flow rate 0.5 mL/min; d) detection wavelength 475 nm; e) injection volume 5 microlitres; f) mobile phase, A acetonitrile (5.2) and B the 0.3 % phosphoric acid solution (5.3), with the gradient programme of Table 1.

Table 1 sets the gradient in three columns, time in minutes against the percentage of mobile phase A and the percentage of mobile phase B: at 0.00 min, 75 % A and 25 % B; at 1.00 min and at 6.00 min, 100 % A and no B; at 6.10 min and at 12.00 min, back to 75 % A and 25 % B.

8.3.1 Determination of the standard series and the sample solutions. Under the optimum instrument conditions, inject the series of mixed standard solutions (5.6) and the sample extract solutions (8.1) in turn. The chromatogram of the capsanthin and capsorubin standard solution is given in Annex A.

8.3.2 Identification. Identification is by retention time. The retention times of capsanthin and capsorubin in the sample solution are to agree with those of capsanthin and capsorubin in the standard solution of comparable mass concentration, the relative deviation being within plus or minus 2.5 %.

8.3.3 Quantification. Plot the calibration curve with the mass concentration of capsanthin and of capsorubin on the horizontal axis and the chromatographic peak area on the vertical axis; the correlation coefficient is to be not lower than 0.99. The mass concentration of the analyte in the sample solution is to lie within the linear range of the calibration curve; if it lies outside that range, the sample solution is to be diluted with acetonitrile (5.2) and determined again. Where quantification is by single-point calibration, the mass concentration of the analyte in the sample solution is not to differ from that of the standard solution by more than 30 %.

9 Treatment of test data

The contents of capsanthin and capsorubin in the test sample are expressed as mass fractions, in milligrams per kilogram. Multi-point calibration is calculated by formula (1) and single-point calibration by formula (2).

In formula (1), the Greek letter rho with the subscript i is the mass concentration of capsanthin or capsorubin in the sample solution read off the calibration curve, in micrograms per millilitre; V is the volume of the sample extraction solution in millilitres; f is the dilution factor of the sample solution; 1000 is a conversion factor; and m is the mass of the test portion in grams.

In formula (2), Ai is the peak area of capsanthin or capsorubin in the sample solution; rho with the subscripts s and i is the mass concentration of capsanthin or capsorubin in the standard solution, in micrograms per millilitre; V is the volume of the sample extraction solution in millilitres; f is the dilution factor of the sample solution; 1000 is a conversion factor; Asi is the peak area of capsanthin or capsorubin in the standard solution; and m is the mass of the test portion in grams.

The content of paprika red in the test sample is expressed as a mass fraction in milligrams per kilogram and is calculated by formula (3) as the sum of the content of capsanthin and the content of capsorubin, both in milligrams per kilogram.

The result of the determination is expressed as the arithmetic mean of the parallel determinations, retained to three significant figures.

10 Precision

Under repeatability conditions, the absolute difference between two independent determinations is not to exceed 10 % of their arithmetic mean.