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Determination of neohesperidin dihydrochalcone in feeds - High performance liquid chromatography

饲料中新甲基橙皮苷二氢查耳酮的测定 高效液相色谱法

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1 Scope

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

This document applies to the determination of neohesperidin dihydrochalcone in compound feeds, concentrate feeds, concentrate supplements and compound premixes.

For compound feeds, concentrate feeds and concentrate supplements the method has a detection limit of 0.5 mg/kg and a quantification limit of 1.0 mg/kg; for compound premixes the detection limit is 2.5 mg/kg and the quantification limit is 5.0 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

Neohesperidin dihydrochalcone in the test sample is extracted with a methanol and formic acid acetonitrile solution, cleaned up on a solid phase extraction cartridge, 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 Methanol: chromatographic grade. 5.3 Formic acid: chromatographic grade. 5.4 Acetonitrile: chromatographic grade.

5.5 Methanol solution, 90 % by volume: measure 90 mL of methanol (5.2), dilute to 100 mL with water and mix.

5.6 Formic acid acetonitrile solution, 2 % by volume: pipette 2.0 mL of formic acid (5.3), dilute to 100 mL with acetonitrile (5.4) and mix.

5.7 Extraction solution: measure 400 mL of methanol (5.2) and 100 mL of formic acid acetonitrile solution (5.6) and mix.

5.8 Formic acid solution, 0.1 % by volume: pipette 1.0 mL of formic acid (5.3), dilute to 1000 mL with water and mix.

5.9 Standard stock solution, 1 mg/mL: accurately weigh 10 mg, to the nearest 0.01 mg, of neohesperidin dihydrochalcone reference substance ($C_{28}H_{36}O_{15}$, CAS number 20702-77-6, purity not less than 98.0 %) into a 10 mL volumetric flask, dissolve in methanol (5.2), make up to the mark and mix. Store below minus 18 degrees C; valid for 6 months.

5.10 Intermediate standard solution, 100 micrograms per millilitre: accurately transfer 1 mL of the standard stock solution (5.9) into a 10 mL volumetric flask, dilute to the mark with methanol (5.2) and mix. Store below minus 18 degrees C; valid for 1 month.

5.11 Series of standard solutions: accurately transfer suitable volumes of the intermediate standard solution (5.10) into volumetric flasks and dilute to the mark with the methanol solution (5.5) to give standard solutions at mass concentrations of 0.5, 1, 5, 10, 20 and 50 micrograms per millilitre. Prepare fresh before use.

5.12 Solid phase extraction cartridge: packing of N-propylethylenediamine, trifunctional octadecyl and synthetic graphitized carbon black, 598 mg in 6 mL, or equivalent performance.

5.13 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 0.1 mg and to 0.01 mg. 6.3 Vortex shaker. 6.4 Nitrogen evaporator with heating, temperature control to plus or minus 2 degrees C. 6.5 Centrifuge, speed not lower than 5000 r/min. 6.6 Solid phase extraction manifold. 6.7 Vortex mixer.

7 Samples

Prepare the test sample in accordance with GB/T 20195. Take at least 200 g of sample, grind it so that it all passes a 0.425 mm analytical sieve, mix thoroughly and place in a closed container ready for use.

8 Test procedure

8.1 Extraction. Carry out two determinations in parallel. Weigh 2.5 g of the test sample, to the nearest 0.1 mg, into a 50 mL centrifuge tube, add exactly 10 mL of the extraction solution (5.7), vortex to mix, shake to extract for 20 min and centrifuge at 5000 r/min for 5 min. Retain the supernatant.

8.2 Clean-up. For compound feeds, concentrate feeds and concentrate supplements, accurately transfer 4 mL of the supernatant (8.1) through the solid phase extraction cartridge (5.12) at a flow rate below 1 mL/min, collect the eluate, pass a further 3 mL of methanol (5.2) through the cartridge, draw it dry, collect all the eluate, mix, evaporate to dryness under nitrogen at 40 degrees C, add exactly 1 mL of the methanol solution (5.5) to dissolve the residue, vortex to mix and filter through the membrane filter (5.13) ready for determination.

For compound premixes, accurately transfer 4 mL of the supernatant (8.1) through the solid phase extraction cartridge (5.12) at a flow rate below 1 mL/min, collect the eluate, pass a further 3 mL of methanol (5.2) through the cartridge, draw it dry, collect all the eluate in a 10 mL volumetric flask, make up to the mark with methanol (5.2), mix and filter through the membrane filter (5.13) ready for determination.

8.3.1 Reference liquid chromatographic conditions: a) column, C18, 250 mm long, 4.6 mm internal diameter, 5 micrometre particle size, or equivalent performance; b) mobile phase, A acetonitrile (5.4) and B the 0.1 % formic acid solution (5.8), with the gradient programme of Table 1; c) flow rate 1.0 mL/min; d) column temperature 35 degrees C; e) injection volume 10 microlitres; f) detection wavelength 281 nm.

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 0.0 min and at 0.5 min, 20 % A and 80 % B; at 9.0 min, 40 % A and 60 % B; at 9.5 min and at 11.5 min, 60 % A and 40 % B; at

12.0 min and at 16.0 min, back to 20 % A and 80 % B.

8.3.2 Determination of the standard series and the sample solutions. Under the optimum instrument conditions, inject the series of standard solutions (5.11) and the sample solutions (8.2) in turn. The chromatogram of the neohesperidin dihydrochalcone standard solution is given in Annex A.

8.3.3 Identification. Identification is by retention time. The retention time of neohesperidin dihydrochalcone in the sample solution is to agree with that of neohesperidin dihydrochalcone in the standard solution of comparable mass concentration, the relative deviation being within plus or minus 2.5 %.

8.3.4 Quantification. Plot the calibration curve with the mass concentration of neohesperidin dihydrochalcone 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 neohesperidin dihydrochalcone 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 methanol (5.2) and determined again. Where quantification is by single-point calibration, the mass concentration of neohesperidin dihydrochalcone in the sample solution is not to differ from that of the standard solution by more than 30 %.

9 Treatment of test data

The content of neohesperidin dihydrochalcone in the test sample is expressed as the mass fraction w, in milligrams per kilogram. Multi-point calibration is calculated by formula (1) and single-point calibration by formula (2).

In formula (1), w is the content of neohesperidin dihydrochalcone in the test sample in milligrams per kilogram; the Greek letter rho is the mass concentration of neohesperidin dihydrochalcone in the sample solution read off the calibration curve, in micrograms per millilitre; V1 is the total volume of the sample extraction solution in millilitres; V3 is the final volume of the sample solution in millilitres; f is the dilution factor used when the result fell outside the calibration range; 1000 is a conversion factor; m is the mass of the test portion in grams; and V2 is the volume of sample extraction solution taken for clean-up, in millilitres.

In formula (2), w is the content of neohesperidin dihydrochalcone in the test sample in milligrams per kilogram; A is the peak area of neohesperidin dihydrochalcone in the sample solution; rho with the subscript s is the mass concentration of neohesperidin dihydrochalcone in the standard solution, in micrograms per millilitre; V1 is the total volume of the sample extraction solution in millilitres; V3 is the final volume of the sample solution in millilitres; f is the dilution factor used when the result fell outside the calibration range; 1000 is a conversion factor; As is the peak area of neohesperidin dihydrochalcone in the standard solution; m is the mass of the test portion in grams; and V2 is the volume of sample extraction solution taken for clean-up, in millilitres.