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Eggs and egg products — Determination of fipronil and metabolites residues — Liquid chromatography tandem mass spectrometry method

Œufs et ovoproduits — Dosage des résidus de fipronil et de ses métabolites — Méthode de chromatographie en phase liquide couplée à la spectrométrie de masse en tandem

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

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Contents

Page

Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 6, *Meat, poultry, fish, eggs and their products*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Eggs and egg products — Determination of fipronil and metabolites residues — Liquid chromatography tandem mass spectrometry method

1 Scope

This document specifies a liquid chromatography tandem mass spectrometry method (LC-MS/MS) for the determination of fipronil and metabolites (including fipronil-desulfinyl, fipronil-sulfide and fipronil-sulfone) residues in eggs and egg products.

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. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
fipronil and metabolites content
ω
mass fraction of fipronil and metabolites residues in egg and egg products

Note 1 to entry: The fipronil and metabolites content is determined according to the procedure specified in this document.

Note 2 to entry: The fipronil and metabolites content is expressed in micrograms per kilogram.

4 Principle

The test portion is extracted with acetonitrile. The extract is cleaned up with dispersive solid phase extraction. The fipronil and metabolites are determined and confirmed by LC-MS/MS, and are quantified with an internal standard method.

5 Sampling

Sampling is not part of the method specified in this document. A recommended sampling method is given in CAC/GL 50-2004.

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Start from a representative sample of at least 1 kg.

Store the sample in such a way that deterioration and change in composition are prevented.

6 Preparing and storing the test sample

6.1 Preparing

6.1.1 Fresh eggs and egg products with shell

Wash approximately 15 to 20 fresh eggs (with a mass of about 1 kg) and remove the shells. Smash and homogenize the egg samples with the appropriate equipment (7.6.6). Place the sample in a polyethylene bottle.

Weigh about 1 kg of egg products (with shells) and then remove the shells. Smash and homogenize the egg samples with the appropriate equipment (7.6.6). Place the sample in a polyethylene bottle.

6.1.2 Other egg products

Homogenize the egg product samples. Place each sample in a polyethylene bottle.

6.2 Storing

Store the test sample and a standby sample at a temperature of −20 °C to −16 °C, respectively.

7 Liquid chromatography tandem mass spectrometry method

7.1 Reagents

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

7.1.1 Water, conforming to grade 1 of ISO 3696.

7.1.2 Acetonitrile, HPLC grade.

7.1.3 Methanol, HPLC grade.

7.1.4 Formic acid, HPLC grade.

7.1.5 Ammonium acetate, HPLC grade.

7.1.6 Anhydrous magnesium sulfate.

7.1.7 Sodium chloride.

7.1.8 Anhydrous sodium sulfate.

7.2 Preparation of solutions

7.2.1 Formic acid solution, 1 ml/l.

Pipette 1 ml formic acid (7.1.4) to a 1 000 ml one-mark volumetric flask. Dilute to the mark with water and mix.

7.2.2 Ammonium acetate-formic acid solution, c(CH_3COONH_4) = 5 mmol/l.

Dissolve 0,385 4 g ammonium acetate in 1 ml/l formic acid solution (7.2.1), dilute to 1 000 ml and mix.

7.3 Standard materials

7.3.1 Fipronil and its metabolites, purity ≥ 95 %, see Annex A.

7.3.2 Fipronil isotope internal: standard stock solution of fipronil-^{13}C6, 100 mg/l.

7.4 Preparation of standard solutions

7.4.1 Standard stock solution, 100 mg/l.

Weigh, to the nearest 0,1 mg, 10 mg of fipronil, fipronil-desulfinyl, fipronil-sulfide and fipronil-sulfone into four individual 100 ml one-mark volumetric flasks. Dilute to the mark with acetonitrile and mix. Prepare the standard stock solution with the concentration of 100 mg/l.

These prepared stock solutions are stable for one year when stored in the dark at −18 °C.

7.4.2 Mixed standard intermediate solution, 1 mg/l.

Pipette 1,0 ml each of the standard stock solutions (7.4.1) into a 100 ml one-mark volumetric flask. Dilute to the mark with acetonitrile and mix.

This solution is stable for one month when stored in the dark at a temperature of 0 °C to 4 °C.

7.4.3 Standard intermediate solution of isotope internal (fipronil-^{13}C6), 1 mg/l.

Pipette 1,0 ml of the standard stock solution of fipronil isotope internal (7.3.2) into a 100 ml one-mark volumetric flask. Dilute to the mark with acetonitrile and mix.

This solution is stable for one month when stored in the dark at a temperature of 0 °C to 4 °C.

7.4.4 Mixed standard working solution.

Pipette 0,025 ml, 0,1 ml, 0,5 ml, 1,0 ml and 5,0 ml of the mixed standard intermediate solution (7.4.2) and 0,1 ml of the standard intermediate solution of isotope internal (fipronil-^{13}C6) (7.4.3) to five 100 ml volumetric flasks. Dilute with acetonitrile to obtain mixed standard solutions with a target content of 0,25 µg/l, 1,0 µg/l, 5,0 µg/l, 10,0 µg/l and 50,0 µg/l. The content of fipronil-^{13}C6 is 1,0 µg/l.

7.5 Materials

7.5.1 Primary secondary amine (PSA), of particle size 40 µm to 60 µm.

7.5.2 Octadecylsilane (C_{18}), of particle size 40 µm to 60 µm.

7.5.3 Membrane filter, of low dead volume and pore size 0,22 µm.

7.6 Apparatus

The usual laboratory apparatus and, in particular, the following shall be used.

7.6.1 LC-MS/MS equipment.

Liquid chromatography combined with electrospray ionization mass spectrometry.

7.6.2 Analytical balance, capable of weighing to the nearest 0,1 mg, 0,01 g.

7.6.3 Centrifuge, operating at a radial acceleration of about 2 000*g*.

7.6.4 Vortex mixer.

7.6.5 Oscillator.

7.6.6 Tissue homogenizer.

7.7 Procedure

7.7.1 General

If it is required to check whether the repeatability limit is met, carry out two single determinations in accordance with 7.7.2 to 7.7.4.

7.7.2 Extraction

Weigh, to the nearest 0,01 g, 5 g of the prepared test sample (see Clause 6) into a 50 ml centrifuge tube. (If the sample is in powder form, weigh 2 g of the powder and add 4 g of water.) Add 20 µl of standard intermediate solution of isotope internal (fipronil-^{13}C6) (7.4.3). Add 20 ml acetonitrile. Mix for 1 min using the vortex mixer (7.6.4). Shake for 5 min using the oscillator (7.6.5). Add 2 g sodium chloride (7.1.7) and 6 g anhydrous sodium sulfate (7.1.8). Mix for 1 min using the vortex mixer. Centrifuge for 5 min at 5 000 r/min. Collect the supernatant to purify.

7.7.3 Purification

Pipette 1,0 ml supernatant into a 2 ml polypropylene centrifuge tube. Add 50 mg PSA (7.5.1), 50 mg C_{18} (7.5.2) and 150 mg anhydrous magnesium sulfate (7.1.6). Mix for 30 s using the vortex mixer (7.6.4). Centrifuge for 5 min at 5 000 r/min. Pass the supernatant through a 0,22 µm membrane filter (7.5.3) for LC-MS/MS (7.6.1) determination.

7.7.4 LC-MS/MS analysis

7.7.4.1 HPLC conditions

Mobile phase A:	Ammonium acetate-formic acid solution (7.2.2).
Mobile phase B:	Methanol (7.1.3).
Column:	Phenyl, 2,1 mm × 100 mm, 1,7 µm, or the equivalent.
Column temperature:	35 °C.
Flow rate:	0,4 ml/min.
Injection volume:	2 µl.
Gradient:	LC gradient as described in Table 1.

Table 1 — LC gradient used for the analysis of fipronil and metabolites

Time min	Mobile phase A %	Mobile phase B %
0	40	60
3,0	30	70
3,5	2	98
4,5	2	98
6,0	40	60

7.7.4.2 MS parameters

Ionization type: Electrospray (ESI).

Polarity: Negative ionization.

Mode: Multiple reaction monitoring (MRM). For monitoring conditions, see Table 2.

Capillary voltage: 4 500 V.

Ion source temperature: 250 °C.

Curtain gas flow: 7 l/min.

Atomizing gas: 240 kPa.

Sheath gas temperature: 325 °C.

Sheath gas (N_2) flow: 11 l/min.

Spray voltage: 400 V.

Table 2 — MRM conditions

No.	Compound	Retention time min	Quantification ion pair m/z	Collision energy eV	Qualitative ion pair m/z	Collision energy eV
1	Fipronil	4,05	434,9/329,9	−21	434,9/249,9	−40
2	Fipronil-desulfinyl	3,95	387,0/351,0	−20	387,0/281,8	−45
3	Fipronil-sulfone	4,33	450,9/281,9	−38	450,9/243,8	−65
4	Fipronil-sulfide	4,25	419,0/382,9	−21	419,0/261,7	−45
5	Fipronil-^{13}C6	4,05	440,6/336,0	−24	440,6/256,1	−36

7.8 Identification criteria

Under the same test conditions, the standard working solution and sample solution are detected, and the retention times of sample chromatogram peaks shall correspond to those of the standard solution at a tolerance of ±2,5 %. The relative intensities of the detected mass transitions (based on peak area), expressed as a percentage of the intensity of the most intense mass transition, shall correspond to those of the standard solution for confirmation. The concentration of the standard solution shall correspond to that of the sample solution. The permitted tolerances are listed in Table 3, and then the corresponding analyte can be present in the sample.

Table 3 — Maximum permitted tolerances for relative ion intensities during identification

Relative intensity	Permitted tolerances
> 50 %	±20 %
> 20 % to 50 %	±25 %
> 10 % to 20 %	±30 %
≤ 10 %	±50 %

7.9 Determination

A series of mixed standard working solutions (7.4.4) and sample solutions (7.7.2) are injected for LC-MS/MS (7.6.1) determination. The retention time and mass transitions are used for identification. Draw a standard curve by plotting the ratios of the quantifier transitions peak area of pesticides to the quantifier transition peak area of fipronil-^{13}C6 (on the ordinate (y)) versus the corresponding mass concentrations of the pesticide standard solution (on the abscissa (x, µg/l)). The response value of the target analyte in the sample solution to be tested shall be within the linear range of quantitative determination of the instrument. If it exceeds the linear range, it shall be diluted appropriately before analysis.

7.10 Blank test

The operation of the blank test is the same as the description in the method of determination (see 7.7 to 7.9), but without sample addition.

7.11 Calculation

The fipronil and metabolites content is calculated using Formula (1):

$$\omega = \rho \times \frac{(A / A_{IS} - b)}{a} \times \frac{V}{m} \quad (1)$$

where

ω is the fipronil and metabolites content, in micrograms per kilogram, of the test sample (µg/kg);

ρ is the analyte mass concentration of the internal standard solution in the unknown sample, in micrograms per litre (ρ = 1,0 µg/l);

A is the peak area of the fipronil and metabolites in the sample solution;

A_{IS} is the peak area of the internal standard in the sample solution;

a is the slope of the regression line of peak area ratio versus concentration ratio;

b is the intercept of the regression line of peak area ratio versus concentration ratio;

V is the final diluted volume of the sample solution, in millilitres (ml);

m is the sample mass, in grams (g).

Express the calculation result as the arithmetic average of the two single test results obtained under the repetitive conditions. Express the results to two significant figures.

7.12 Limit of quantification

The limit of quantification (LOQ) for fipronil, fipronil-desulfinyl, fipronil-sulfide, fipronil-sulfone is 1,0 µg/kg.