Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed (Text with EEA relevance)

[F1ANNEX VI

METHODS OF ANALYSIS FOR THE DETERMINATION OF CONSTITUENTS OF ANIMAL ORIGIN FOR THE OFFICIAL CONTROL OF FEED

Textual Amendments

F1 Substituted by Commission Regulation (EU) No 51/2013 of 16 January 2013 amending Regulation (EC) No 152/2009 as regards the methods of analysis for the determination of constituents of animal origin for the official control of feed (Text with EEA relevance).

2. METHODS

2.1. **Light microscopy**

2.1.2. *Reagents and equipment*

2.1.2.1. Reagents

2.1.2.1.1. Concentrating agent

2.1.2.1.1.1. Tetrachloroethylene (specific gravity 1,62)

2.1.2.1.2. Staining reagent

2.1.2.1.2.1. Alizarin Red solution (dilute 2,5 ml 1M hydrochloric acid in 100 ml water and add 200 mg Alizarin Red to this solution)

2.1.2.1.3. Mounting media

2.1.2.1.3.1. Lye (NaOH 2,5 % w/v or KOH 2,5 % w/v)

2.1.2.1.3.2. [F2Glycerol (undiluted, viscosity: 1 490 cP) or a mounting medium with equivalent properties for non-permanent slide preparation]

Textual Amendments

F2 Substituted by Commission Implementing Regulation (EU) 2020/1560 of 26 October 2020 amending Annex VI to Regulation (EC) No 152/2009 laying down the methods of analysis for the determination of constituents of animal origin for the official control of feed (Text with EEA relevance).

2.1.2.1.3.3. Norland ® Optical Adhesive 65 (viscosity: 1 200 cP) or a resin with equivalent properties for permanent slide preparation

2.1.2.1.4. Mounting media with staining properties

2.1.2.1.4.1. Lugol solution (dissolve 2 g potassium iodide in 100 ml water and add 1 g iodine while frequently shaking)

2.1.2.1.4.2. Cystine reagent (2 g lead acetate, 10 g NaOH/100 ml water)

2.1.2.1.4.3. Fehling's reagent (prepared before use from equals parts (1/1) of two stock solutions A and B. Solution A: dissolve 6,9 g copper (II) sulphate pentahydrate in 100 ml water. Solution B: dissolve 34,6 g potassium sodium tartrate tetrahydrate and 12 g NaOH in 100 ml water)

2.1.2.1.4. Tetramethylbenzidine/Hydrogen peroxide. (dissolve 1 g 3,3',5,5' tetramethylbenzidine (TMB) in 100 ml glacial acetic acid and 150 ml water. Before use, mix 4 parts of this TMB solution with 1 part 3 % hydrogen peroxide)

2.1.2.1.5. Rinsing agents

2.1.2.1.5.1. Ethanol ≥ 96 % (technical grade)

2.1.2.1.5.2. Acetone (technical grade)

2.1.2.1.6. Bleaching reagent

2.1.2.1.6.1. Commercial sodium hypochlorite solution (9 - 14 % active chlorine)

2.1.2.2. Equipment

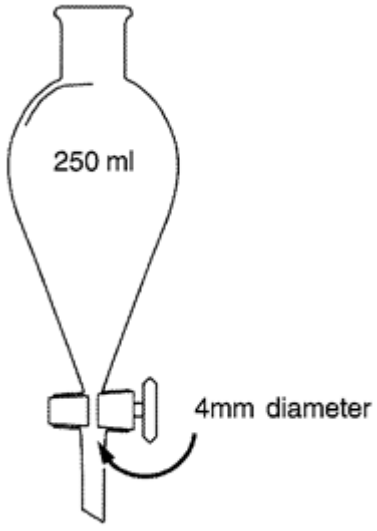
2.1.2.2.1. Analytical balance with an accuracy of 0,001 g

2.1.2.2.2. **[**[F2] Grinding equipment: knife or rotor mill. If a rotor mill is used, mill sieves ≤ 0,5 mm shall be prohibited**]**

2.1.2.2.3. **[**[F2] Sieves with square meshes of 0,25 mm and 1 mm width. With the exception of sample pre-sieving, the diameter of the sieves should not exceed 10 cm to avoid loss of materials. Calibration of sieves is not required**]**

2.1.2.2.4. Conical glass separation funnel with a content of 250 ml with Teflon or ground glass stopcock at the base of the cone. Stopcock opening diameter shall be ≥ 4mm. Alternatively, a conical bottomed settling beaker may be used provided the laboratory has demonstrated that detection levels are equivalent to that obtained using the conical glass separation funnel.

Separation funnel



2.1.2.2.5. Stereomicroscope covering at least a 6,5× to 40× final magnification range

2.1.2.2.6. Compound microscope covering at least a 100× to 400× final magnification range with transmitted light bright field. Polarised light and differential interferential contrast can additionally be used

2.1.2.2.7. Standard laboratory glassware

2.1.2.2.8. Equipment for slide preparation: classical microscope slides, hollow slides, coverslips (20 × 20 mm), tweezers, fine spatula

[F3 2.1.2.2.9. Laboratory oven

Textual Amendments

F3 Inserted by Commission Implementing Regulation (EU) 2020/1560 of 26 October 2020 amending Annex VI to Regulation (EC) No 152/2009 laying down the methods of analysis for the determination of constituents of animal origin for the official control of feed (Text with EEA relevance).

2.1.2.2.10. Centrifuge

2.1.2.2.11. Filter paper: qualitative cellulose filter (pore size 4-11 µm)]

Changes to legislation:
There are currently no known outstanding effects for the Commission Regulation (EC) No 152/2009, Division 2.1.2..