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)

ANNEX I

METHODS OF SAMPLING

ANNEX II

GENERAL PROVISIONS ON METHODS OF ANALYSIS FOR FEED

ANNEX III

METHODS OF ANALYSIS TO CONTROL THE COMPOSITION OF FEED MATERIALS AND COMPOUND FEED

3. Reagents
   - 3.1. Trichloroacetic acid, solution 20 % (w/v).
   - 3.2. Magnesium oxide.
   - 3.3. Anti-foaming emulsion (e.g. silicone).
   - 3.4. Sulphuric acid 0,05 mol/litre.
   - 3.5. Sodium hydroxide solution 0,1 mol/litre.
   - 3.6. Methyl red solution 0,3 % in 95 %-96 % (v/v)...
4. Apparatus
   - 4.1. Mixer (tumbler): approximately 35 to 40 r.p.m.
   - 4.2. Distilling apparatus of the Kjeldahl type.
5. Procedure
6. Calculation of results

   Repeatability

F. DETERMINATION OF AMINO ACIDS (EXCEPT TRYPTOPHANE)

1. Purpose and scope
2. Principle
   - 2.1. Free amino acids
   - 2.2. Total amino acids
3. Reagents
   - 3.1. Hydrogen peroxide, w (w/w) = 30 %.
   - 3.2. Formic acid, w (w/w) = 98 %-100 %.
   - 3.3. Phenol.
   - 3.4. Sodium disulphite.
   - 3.5. Sodium hydroxide.
   - 3.6. 5-Sulfosalicylic acid dihydrate.
   - 3.7. Hydrochloric acid, density approximately 1,18 g/ml.
   - 3.8. tri-Sodium citrate dihydrate.
   - 3.9. 2,2'-Thiodiethanol (thiodiglycol).
   - 3.10. Sodium chloride.
   - 3.11. Ninhydrin.
   - 3.12. Light petroleum, boiling range 40-60 oC.
   - 3.13. Norleucine, or other compound suitable for use as internal standard....
   - 3.14. Nitrogen gas (< 10 ppm oxygen).
   - 3.15. 1-Octanol.
   - 3.16. Amino acids.
   - 3.16.1. Standard substances listed under paragraph 1. Pure compounds containing no...
   - 3.16.2. Cysteic acid.
   - 3.16.3. Methionine sulphone.
   - 3.17. Sodium hydroxide solution, c = 7,5 mol/l:
   - 3.18. Sodium hydroxide solution, c = 1 mol/l:
   - 3.19. Formic acid — phenol solution:
   - 3.20. Hydrolysis mixture, c = 6 mol HCl/l containing 1 g...
   - 3.21. Extraction mixture, c = 0,1 mol HCl/l containing 2 %...
   - 3.22. 5-Sulfosalicylic acid, ß = 6 %:
   - 3.23. Oxidation mixture (Performic acid — phenol):
   - 3.24. Citrate buffer, c = 0,2 mol Na+/l, pH 2,2:
   - 3.25. Elution buffers, prepared according to conditions for the analyser used...
   - 3.26. Ninhydrin reagent, prepared according to conditions for the analyser used...
   - 3.27. Standard solutions of amino acids. These solutions shall be stored...

3.3. Internal standard substance: α-methyl-tryptophan (purity/content ≥ 99 %), dried under...
3.4. Barium hydroxide octa-hydrate (care shall be taken not to expose...
3.5. Sodium hydroxide.
3.6. Ortho-phosphoric acid, w (w/w) = 85 %.
3.7. Hydrochloric acid, ρ201,19 g/ml.
3.8. Methanol, equivalent to HPLC grade.
3.9. Light petroleum, boiling range 40-60 oC.
3.10. Sodium hydroxide solution, c = 1 mol/l:
3.11. Hydrochloric acid, c = 6 mol/l:
3.12. Hydrochloric acid, c = 1 mol/l:
3.13. Hydrochloric acid, c = 0,1 mol/l:
3.14. Ortho-phosphoric acid, c = 0,5 mol/l:
3.15. Concentrated solution of tryptophan (3.2), c = 2,5 μmol/ml:
3.16. Concentrated internal standard solution, c = 2,5 μmol/ml:
3.17. Calibration standard solution of tryptophan and internal standard:
3.18. Acetic acid
3.19. 1,1,1-trichloro-2-methyl-2-propanol.
3.20. Ethanolamine w (w/w) > 98 %.
3.21. Solution of 1 g 1,1,1-trichloro-2-methyl-2-propanol (3.19) in 100 ml methanol...
3.22. Mobile phase for HPLC: 3,0 g acetic acid (3.18) +...

4. Apparatus
4.1. HPLC equipment with a spectrofluorometric detector.
4.2. Liquid chromatographic column, 125 mm x 4 mm, C18, 3...
4.3. pH-meter.
4.4. Polypropylene flask, capacity 125 ml, with wide neck and screw...
4.5. Membrane filter, 0,45 μm.
4.6. Autoclave, 110 (± 2) oC, 1,4 (±0,1) bar.
4.7. Mechanical shaker or magnetic stirrer.
4.8. Vortex mixer.

5. Procedure
5.1. Preparation of samples
5.2. Determination of free tryptophan (extract)
5.3. Determination of total tryptophan (hydrolysate)
5.4. HPLC determination

6. Calculation of results
7. Repeatability
8. Results of a collaborative study
9. Observations
9.1. Following special chromatographic conditions may give better separation between tryptophan...
9.2. The chromatography will vary according to the type of HPLC...
9.3. Barium hydroxide

H. DETERMINATION OF CRUDE OILS AND FATS

1. Purpose and scope
1.1. Procedure A — Directly extractable crude oils and fats
1.2. Procedure B — Total crude oils and fats
1.3. Interpretation of results

2. Principle
2.1. Procedure A
2.2. Procedure B

ANNEX IV

METHODS OF ANALYSIS TO CONTROL THE LEVEL OF AUTHORISED ADDITIVES IN FEED

A. DETERMINATION OF VITAMIN A

1. Purpose and Scope
2. Principle
3. Reagents
   - 3.1. Ethanol, σ = 96 %
   - 3.2. Light petroleum, boiling range 40 oC-60 oC
   - 3.3. Methanol
   - 3.4. Potassium hydroxide solution, c = 50 g/100 ml
   - 3.5. Sodium ascorbate solution, c = 10 g/100 ml (see 7.7...
   - 3.6. Sodium sulphide, Na2S · x H2O (x = 7-9)
   - 3.6.1. Sodium sulphide solution, c = 0,5 mol/l in glycerol, ß...
   - 3.7. Phenolphthalein solution, c = 2 g/100 ml in ethanol (3.1)...
   - 3.8. 2-Propanol
   - 3.9. Mobile phase for HPLC: mixture of methanol (3.3) and water,...
   - 3.10. Nitrogen, oxygen free
   - 3.11. All-trans-vitamin A acetate, extra pure, of certified activity, e.g. 2,8...
   - 3.11.1. Stock solution of all-trans-vitamin A acetate: Weigh to the nearest...
   - 3.12. All-trans-vitamin A palmitate, extra pure, of certified activity, e.g. 1,8...
   - 3.12.1. Stock solution of all-trans-vitamin A palmitate: Weigh to the nearest...
   - 3.13. 2,6-Di-tert-butyl-4-methylphenol (BHT) (see 7.5 observations)
4. Apparatus
   - 4.1. Vacuum rotary evaporator
   - 4.2. Amber glassware
   - 4.2.1. Flat bottom or conical flasks, 500 ml, with ground-glass socket...
   - 4.2.2. Graduated flasks with ground-glass stoppers, narrow-necked, 10, 25, 100 and...
   - 4.2.3. Separating funnels, conical, 1 000 ml, with ground-glass stoppers
   - 4.2.4. Pear shaped flasks, 250 ml, with ground-glass sockets
   - 4.3. Allihn condenser, jacket length 300 mm, with ground-glass joint, with...
   - 4.4. Pleated filter paper for phase separation, diameter 185 mm (e.g....
   - 4.5. HPLC equipment with injection system
   - 4.5.1. Liquid chromatographic column, 250 mm x 4 mm, C18, 5...
   - 4.5.2. UV or fluorescence detector, with variable wavelength adjustment
   - 4.6. Spectrophotometer with 10 mm quartz cells
   - 4.7. Water-bath with magnetic stirrer
   - 4.8. Extraction apparatus (see figure 1) consisting of:
   - 4.8.1. Glass cylinder of 1 l capacity fitted with a ground...
   - 4.8.2. Ground glass insert equipped with a side-arm and an adjustable...
5. Procedure
   - 5.1. Preparation of the sample
   - 5.2. Saponification
   - 5.3. Extraction
     - 5.3.1. Extraction using a separating funnel (4.2.3)
     - 5.3.2. Extraction using an extraction apparatus (4.8)
   - 5.4. Preparation of the sample solution for HPLC
   - 5.5. Determination by HPLC

     HPLC conditions

3.5. Sulphuric acid (d: 1,84 g/ml).
3.6. Hydrogen peroxide (approximately 100 volumes of oxygen (30 % by...
3.7. Standard iron solution (1 000 µg Fe/ml) prepared as follows...
3.7.1. Working standard iron solution (100 µg Fe/ml) prepared by diluting...
3.8. Standard copper solution (1 000 µg Cu/ml) prepared as follows...
3.8.1. Working standard copper solution (10 µg Cu/ml) prepared by diluting...
3.9. Standard manganese solution (1 000 µg Mn/ml) prepared as follows...
3.9.1. Working standard manganese solution (10 µg Mn/ml) prepared by diluting...
3.10. Standard zinc solution (1 000 µg Zn/ml) prepared as follows...
3.10.1. Working standard zinc solution (10 µg Zn/ml) prepared by diluting...
3.11. Lanthanum chloride solution: dissolve 12 g of lanthanum oxide in...

4. Apparatus
4.1. Muffle furnace with temperature regulation and preferably recorder.
4.2. Glassware must be of resistant borosilicate type and it is...
4.3. Atomic absorption spectrophotometer meeting the requirements of the method with...

5. Procedure
5.1. Samples containing organic matter
5.1.1. Ashing and preparation of the solution for analysis
5.1.1.1. Place 5 to 10 g of sample weighed to the...
5.1.1.2. If the residue in the filter appears black (carbon), put...
Notes:
5.1.2. Spectrophotometric determination
5.1.2.1. Preparation of calibration solutions
Iron
Copper
Manganese
Zinc
5.1.2.2. Preparation of solution for analysis
5.1.2.3. Blank experiment
5.1.2.4. Measurement of the atomic absorption
5.2. Mineral feed

6. Calculation of results
7. Repeatability
8. Observation

D. DETERMINATION OF HALOFUGINONE

1. Purpose and scope
2. Principle
3. Reagents
3.1. Acetonitrile, equivalent to HPLC grade.
3.2. Amberlite XAD-2 resin.
3.3. Ammonium acetate.
3.4. Ethyl acetate.
3.5. Acetic acid, glacial.
3.6. Halofuginone standard substance (DL-trans-7-brome-6-chloro-3-[3-hydroxy-2-piperidyl)acetonyl] quinazoline-4-(3H)-one hydrobromide, E 764).
3.6.1. Halofuginone stock standard solution, 100 µg/ml
3.6.2. Calibration solutions
3.7. Hydrochloric acid (ρ20 approximately 1,16 g/ml).
3.8. Methanol.

3.4. Molecular sieve.
3.5. Aluminium oxide acidic activity grade I for column chromatography.
3.6. Potassium dihydrogen phosphate solution, c = 0,025 mol/l.
3.7. Di-sodium hydrogen phosphate solution, c = 0,025 mol/l.
3.8. HPLC mobile phase.
3.9. Standard substance.
3.9.1. Robenidine stock standard solution: 300 µg/ml
3.9.2. Robenidine intermediate standard solution: 12 µg/ml
3.9.3. Calibration solutions
3.10. Water equivalent to HPLC grade.
4. Apparatus
4.1. Glass column.
4.2. Mechanical shaker or magnetic stirrer.
4.3. Rotary film evaporator.
4.4. HPLC equipment with variable wavelength ultraviolet detector or diode array...
4.4.1. Liquid chromatographic column: 300 mm x 4 mm, C18 10...
4.5. Glass fibre filter paper (Whatman GF/A or equivalent).
4.6. Membrane filters, 0,22 µm.
4.7. Membrane filters, 0,45 µm.
5. Procedure
5.1. General
5.1.1. A blank feed shall be analysed to check that neither...
5.1.2. A recovery test shall be carried out by analysing the...
5.2. Extraction
5.3. Purification
5.3.1. Preparation of the aluminium-oxide column
5.3.2. Sample purification
5.4. HPLC determination
5.4.1. Parameters
5.4.2. Calibration graph
5.4.3. Sample solution
6. Calculation of results
7. Validation of the results
7.1. Identity
7.1.1. Co-chromatography
7.1.2. Diode-array detection
7.2. Repeatability
7.3. Recovery
8. Results of a collaborative study

F. DETERMINATION OF DICLAZURIL
1. Purpose and scope
2. Principle
3. Reagents
3.1. Water, equivalent to HPLC-grade
3.2. Ammonium acetate
3.3. Tetrabutylammonium hydrogen sulphate (TBHS)
3.4. Acetonitrile, equivalent to HPLC grade
3.5. Methanol, equivalent to HPLC grade
3.6. N, N-dimethylformamide (DMF)
3.7. Hydrochloric acid, ρ20 = 1,19 g/ml

3.8. Standard substance: diclazuril II-24: (+)-4-chlorphenyl [2,6-dichloro-4-(2,3,4,5-tetrahydro-3,5-dioxo-1,2,4-triazin-2-yl) phenyl] acetonitrile with guaranteed...
3.8.1. Diclazuril stock standard solution, 500 µg/ml
3.8.2. Diclazuril standard solution, 50 µg/ml
3.9. Internal standard substance: 2,6 dichloro-α-(4-chlorophenyl)-4-(4,5 dihydro-3,5-dioxo-1,2,4-triazine-2 (3H) — yl) α-methylbenzene-acetonitrile...
3.9.1. Internal stock standard solution, 500 µg/ml
3.9.2. Internal standard solution, 50 µg/ml
3.9.3. Internal standard solution for premixtures, p/1 000 mg/ml
3.10. Calibration solution, 2 µg/ml.
3.11. C18 solid phase extraction cartridge, e.g. Bond Elut, size: 1...
3.12. Extraction solvent: acidified methanol.
3.13. Mobile phase for HPLC
3.13.1. Eluent A: ammonium acetate — tetrabutylammonium hydrogen sulphate solution.
3.13.2. Eluent B: acetonitrile (3.4).
3.13.3. Eluent C: methanol (3.5).

4. Apparatus
4.1. Mechanical shaker
4.2. Equipment for ternary gradient HPLC
4.2.1. Liquid chromatographic column, Hypersil ODS, 3 µm packing, 100 mm...
4.2.2. UV detector with variable wavelength adjustment or diode array detector...
4.3. Rotary film evaporator
4.4. Membrane filter, 0,45 µm
4.5. Vacuum manifold
4.6. Ultrasonic bath

5. Procedure
5.1. General
5.1.1. Blank feed
5.1.2. Recovery test
5.2. Extraction
5.2.1. Feed
5.2.2. Premixtures
5.3. HPLC determination
5.3.1. Parameters
5.3.2. Calibration solution
5.3.3. Sample solution

6. Calculation of the results
6.1. Feeds
6.2. Premixtures

7. Validation of the results
7.1. Identity
7.1.1. Co-chromatography
7.1.2. Diode-array detection
7.2. Repeatability
7.3. Recovery

8. Results of a collaborative study
9. Observations

G. DETERMINATION OF LASALOCID SODIUM

1. Purpose and scope
2. Principle
3. Reagents
   - 3.1. Potassium dihydrogen phosphate (KH2PO4).
   - 3.2. Orthophosphoric acid, w (w/w) = 85 %.
   - 3.3. Orthophosphoric acid solution, c = 20 %.
   - 3.4. 6-Methyl-2-heptylamine (1,5-dimethylhexylamine), w (w/w) = 99 %.
   - 3.5. Methanol, equivalent to HPLC grade.
   - 3.6. Hydrochloric acid, density = 1,19 g/ml.
   - 3.7. Phosphate buffer solution, c = 0,01 mol/l.
   - 3.8. Acidified methanol.
   - 3.9. HPLC mobile phase, phosphate buffer-methanol solution 5 + 95 (V...
   - 3.10. Lasalocid sodium standard substance with guaranteed purity, C34H53O8Na (sodium salt...
   - 3.10.1. Lasalocid sodium stock standard solution, 500 µg/ml
   - 3.10.2. Lasalocid sodium intermediate standard solution, 50 µg/ml
   - 3.10.3. Calibration solutions
   - 3.11. Water, equivalent to HPLC grade.
4. Apparatus
   - 4.1. Ultrasonic bath (or shaking water-bath) with temperature control.
   - 4.2. Membrane filters, 0,45 µm.
   - 4.3. HPLC equipment with injection system, suitable for injecting volumes of...
   - 4.3.1. Liquid chromatographic column 125 mm x 4 mm, reversed-phase C18,...
   - 4.3.2. Spectrofluorometer with variable wavelength adjustment of excitation and emission wavelengths....
5. Procedure
   - 5.1. General
     - 5.1.1. Blank feed
     - 5.1.2. Recovery test
   - 5.2. Extraction
     - 5.2.1. Feed
     - 5.2.2. Premixtures
   - 5.3. HPLC determination
     - 5.3.1. Parameters
     - 5.3.2. Calibration graph
     - 5.3.3. Sample solution
6. Calculation of results
   - 6.1. Feed
   - 6.2. Premixtures
7. Validation of the results
   - 7.1. Identity
     - 7.1.1. Co-chromatography
   - 7.2. Repeatability
   - 7.3. Recovery
8. Results of a collaborative study

ANNEX V

METHODS OF ANALYSIS TO CONTROL UNDESIRABLE SUBSTANCES IN FEED

CHAPTER III

Sample preparation and requirements for methods of analysis used in...

ANNEX VI

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

2.1.2.1.5. 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. Grinding equipment: knife or rotor mill. If a rotor mill...
2.1.2.2.3. Sieves with square meshes of 0,25 mm and 1 mm...
2.1.2.2.4. Conical glass separation funnel with a content of 250 ml...
Separation funnel
2.1.2.2.5. Stereomicroscope covering at least a 6,5× to 40× final magnification...
2.1.2.2.6. Compound microscope covering at least a 100× to 400× final...
2.1.2.2.7. Standard laboratory glassware
2.1.2.2.8. Equipment for slide preparation: classical microscope slides, hollow slides, coverslips...
2.1.2.2.9. Laboratory oven
2.1.2.2.10. Centrifuge
2.1.2.2.11. Filter paper: qualitative cellulose filter (pore size 4-11 µm)
2.1.3. Sampling and sample preparation
2.1.3.1. Sampling
2.1.3.2. Precautions to be taken
2.1.3.3. Preparation of samples other than fat or oil
2.1.3.3.1. Sample drying : samples with a moisture content > 14...
2.1.3.3.2. Sample pre-sieving : in order to collect information on possible...
2.1.3.3.3. Sub-sampling and grinding : at least 50 g of the...
2.1.3.3.4. Extraction and preparation of the sediment : a portion of...
2.1.3.3.5. Extraction and preparation of the flotate : after recovery of...
2.1.3.3.6. Preparation of raw material : a portion of at least...
2.1.3.4. Preparation of samples consisting of fat or oil
2.1.3.5. Use of staining reagents
2.1.4. Microscopic examination
2.1.4.1. Slide preparation
2.1.4.2. Observation flowchart for the detection of animal particles in compound...
2.1.4.3. Number of determinations
2.1.5. Expression of the results
2.2. PCR
2.2.1. Principle
2.2.2. Reagents and equipment
2.2.2.1. Reagents
2.2.2.1.1. Reagents for DNA extraction step
2.2.2.1.2. Reagents for genetic amplification step
2.2.2.1.2.1. Primers and probes
2.2.2.1.2.2. Master Mix

2.2.2.1.2. Decontamination reagents
2.2.2.1.2.1. Hydrochloric acid solution (0,1 N)
2.2.2.1.2.2. Bleach (solution of sodium hypochlorite at 0,15 % of active...
2.2.2.1.2.3. Non-corrosive reagents for decontaminating costly devices like analytical balances (e.g....
2.2.2.2. Equipment
2.2.2.2.1. Analytical balance with an accuracy of 0,001 g
2.2.2.2.2. Grinding equipment
2.2.2.2.3. Thermocycler enabling real-time PCR
2.2.2.2.4. Microcentrifuge for microfuge tubes
2.2.2.2.5. Set of micropipettes allowing to pipet from 1 µl up...
2.2.2.2.6. Standard molecular biology plastic-ware: microfuge tubes, filtered plastic tips for...
2.2.2.2.7. Freezers to store samples and reagents
2.2.3. Sampling and sample preparation
2.2.3.1. Sampling
2.2.3.2. Sample preparation
2.2.4. DNA extraction
2.2.5. Genetic amplification
2.2.6. Interpretation and expression of results
2.2.6.1. Negative result
2.2.6.2. Positive result

ANNEX VII

METHOD OF CALCULATING THE ENERGY VALUE OF POULTRYFEED

1. Method of calculation and expression of energy value

2. Tolerances applicable to declared values

3. Expression of result

4. Sampling and analysis methods

ANNEX VIII

METHODS OF ANALYSIS TO CONTROL ILLEGAL PRESENCE OF NO LONGER AUTHORISED ADDITIVES IN FEED

Important notes:

A. DETERMINATION OF METHYL BENZOQUATE
1. Purpose and scope
2. Principle
3. Reagents
3.1. Dichloromethane
3.2. Methanol, equivalent to HPLC grade
3.3. HPLC mobile phase
3.4. Methanesulfonic acid solution, c = 2 %

3.5. Hydrochloric acid solution, c = 10 %
3.6. Cation-exchange resin Amberlite CG-120 (Na), 100 to 200 mesh
3.7. Standard substance: pure methyl benzoquate (7-benzyloxy-6-butyl-3-methoxycarbonyl-4-quinolone)
3.7.1. Methyl benzoquate stock standard solution, 500 µg/ml
3.7.2. Methyl benzoquate intermediate standard solution, 50 µg/ml
3.7.3. Calibration solutions

4. Apparatus
4.1. Laboratory shaker
4.2. Rotary film evaporator
4.3. Glass column (250 mm × 15 mm) fitted with a...
4.4. HPLC equipment with variable wavelength ultraviolet detector or diode-array detector...
4.4.1. Liquid chromatographic column: 300 mm × 4 mm, C18, 10...
4.5. Membrane filters, 0,22 µm
4.6. Membrane filters, 0,45 µm

5. Procedure
5.1. General
5.1.1. A blank feed shall be analysed to check that neither...
5.1.2. A recovery test shall be carried out by analysing the...
5.2. Extraction
5.3. Liquid-liquid partition
5.4. Ion-exchange chromatography
5.4.1. Preparation of the cation-exchange column
5.4.2. Column chromatography
5.5. Liquid-liquid partition
5.6. HPLC determination
5.6.1. Parameters
5.6.2. Calibration graph
5.6.3. Sample solution

6. Calculation of results
7. Validation of the results
7.1. Identity
7.1.1. Co-chromatography
7.1.2. Diode-array detection
7.2. Repeatability
7.3. Recovery
8. Results of a collaborative study

B. DETERMINATION OF OLAQUINDOX

1. Purpose and scope
2. Principle
3. Reagents
3.1. Methanol.
3.2. Methanol, equivalent to HPLC grade.
3.3. Water, equivalent to HPLC grade.
3.4. Mobile phase for HPLC.
3.5. Standard substance: pure olaquindox 2-[N-2'-(hydroxyethyl)carbamoyl]-3-methylquinoxaline-N1,N4-dioxide, E 851.
3.5.1. Olaquindox stock standard solution, 250 µg/ml
3.5.2. Olaquindox intermediate standard solution, 25 µg/ml
3.5.3. Calibration solutions

4. Apparatus
   - 4.1. Ultrasonic bath
   - 4.2. Mechanical shaker
   - 4.3. HPLC equipment with variable wavelength ultraviolet detector or diode array...
   - 4.3.1. Liquid chromatographic column, 250 mm × 4 mm, C18, 10...
   - 4.4. Membrane filters, 0,45 μm
5. Procedure
   - 5.1. General
     - 5.1.1. A blank feed shall be analysed to check that neither...
     - 5.1.2. A recovery test shall be carried out by analysing the...
   - 5.2. Extraction
   - 5.3. HPLC determination
     - 5.3.1. Parameters:
     - 5.3.2. Calibration graph
     - 5.3.3. Sample solution
6. Calculation of the results
7. Validation of the results
   - 7.1. Identity
     - 7.1.1. Co-chromatography
     - 7.1.2. Diode array detection
   - 7.2. Repeatability
   - 7.3. Recovery
8. Results of a collaborative study
9. Observation

C. DETERMINATION OF AMPROLIUM

1. Purpose and Scope
2. Principle
3. Reagents
   - 3.1. Methanol.
   - 3.2. Acetonitrile, equivalent to HPLC grade.
   - 3.3. Water, equivalent to HPLC grade.
   - 3.4. Sodium dihydrogen phosphate solution, c = 0,1 mol/l.
   - 3.5. Sodium perchlorate solution, c = 1,6 mol/l.
   - 3.6. Mobile phase for HPLC (see observation 9.1).
   - 3.7. Standard substance: pure amprolium, 1-[(4-amino-2-propylpyrimidin-5-yl)methyl]-2-methyl-pyridinium chloride hydrochloride, E 750 (see...
   - 3.7.1. Amprolium stock standard solution, 500 μg/ml
   - 3.7.2. Amprolium intermediate standard solution, 50 μg/ml
   - 3.7.3. Calibration solutions
   - 3.8. Extraction solvent.
4. Apparatus
   - 4.1. HPLC equipment with injection system, suitable for injection volumes of...
   - 4.1.1. Liquid chromatographic column 125 mm × 4 mm, cation exchange...
   - 4.1.2. UV detector with variable wavelength adjustment or diode array detector....
   - 4.2. Membrane filter, PTFE material, 0,45 μm.
   - 4.3. Membrane filter, 0,22 μm.
   - 4.4. Ultrasonic bath.
   - 4.5. Mechanical shaker or magnetic stirrer.

5. Procedure
   - 5.1. General
     - 5.1.1. Blank feed
     - 5.1.2. Recovery test
   - 5.2. Extraction
     - 5.2.1. Premixtures (content < 1 % amprolium) and feed
     - 5.2.2. Premixtures (content ≥ 1 % amprolium)
   - 5.3. HPLC determination
     - 5.3.1. Parameters:
     - 5.3.2. Calibration graph
     - 5.3.3. Sample solution
6. Calculation of the results
7. Validation of the results
   - 7.1. Identity
     - 7.1.1. Co-chromatography
     - 7.1.2. Diode array detection
   - 7.2. Repeatability
   - 7.3. Recovery
8. Results of a collaborative study
9. Observations
   - 9.1. If the sample contains thiamine, the thiamine peak in the...
   - 9.2. According to the British Pharmacopoeia, the spectrum of an amprolium...
   - 9.3. The extract must always be diluted with the mobile phase,...

D. DETERMINATION OF CARBADOX

1. Purpose and scope
2. Principle
3. Reagents
   - 3.1. Methanol.
   - 3.2. Acetonitrile, equivalent to HPLC grade.
   - 3.3. Acetic acid, w = 100 %.
   - 3.4. Aluminium oxide: neutral, activity grade I.
   - 3.5. Methanol-acetonitrile 1 + 1 (v + v).
   - 3.6. Acetic acid, σ = 10 %.
   - 3.7. Sodium acetate.
   - 3.8. Water, equivalent to HPLC grade.
   - 3.9. Acetate buffer solution, c = 0,01 mol/l, pH = 6,0....
   - 3.10. Mobile phase for HPLC.
   - 3.11. Standard substance.
   - 3.11.1. Carbadox stock standard solution, 100 µg/ml (see Note 5. Procedure):...
   - 3.11.2. Calibration solutions
   - 3.12. Water-[methanol-acetonitrile] (3.5) mixture, 300 + 700 (v + v).
4. Apparatus
   - 4.1. Laboratory shaker or magnetic stirrer.
   - 4.2. Glass fibre filter paper (Whatman GF/A or equivalent).
   - 4.3. Glass column (length 300 to 400 mm, internal diameter approximately...
   - 4.4. HPLC equipment with injection system, suitable for injection volumes of...
   - 4.4.1. Liquid chromatographic column: 300 mm x 4 mm, C18, 10...
   - 4.4.2. UV detector with variable wavelength adjustment or diode array detector...

ANNEX IX

CORRELATION TABLES REFERRED TO IN ARTICLE 6

1. Directive 71/250/EEC

2. Directive 71/393/EEC

3. Directive 72/199/EEC

4. Directive 73/46/EEC

5. Directive 76/371/EEC

6. Directive 76/372/EEC

7. Directive 78/633/EEC

8. Directive 81/715/EEC

9. Directive 84/425/EEC

10. Directive 86/174/EEC

11. Directive 93/70/EEC

12. Directive 93/117/EC

13. Directive 98/64/EC

14. Directive 1999/27/EC

15. Directive 1999/76/EC

16. Directive 2000/45/EC

17. Directive 2002/70/EC

18. Directive 2003/126/EC

(**1**) OJ L 165, 30.4.2004, p. 1, corrected by OJ L 191, 28.5.2004, p. 1.
(**2**) OJ L 155, 12.7.1971, p. 13.
(**3**) OJ L 279, 20.12.1971, p. 7.
(**4**) OJ L 123, 29.5.1972, p. 6.
(**5**) OJ L 83, 30.3.1973, p. 21.
(**6**) OJ L 102, 15.4.1976, p. 1.
(**7**) OJ L 102, 15.4.1976, p. 8.
(**8**) OJ L 206, 29.7.1978, p. 43.
(**9**) OJ L 257, 10.9.1981, p. 38.
(**10**) OJ L 238, 6.9.1984, p. 34.
(**11**) OJ L 130, 16.5.1986, p. 53.
(**12**) OJ L 234, 17.9.1993, p. 17.
(**13**) OJ L 329, 30.12.1993, p. 54.
(**14**) OJ L 257, 19.9.1998, p. 14.
(**15**) OJ L 118, 6.5.1999, p. 36.
(**16**) OJ L 207, 6.8.1999, p. 13.
(**17**) OJ L 174, 13.7.2000, p. 32.
(**18**) OJ L 209, 6.8.2002, p. 15.
(**19**) OJ L 339, 24.12.2003, p. 78.

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