STATUTORY INSTRUMENTS

1996 No. 1261

MEDICINES

The Medicines (Animal Feeding Stuffs) (Enforcement) (Amendment) Regulations 1996

Made - - - -	*1st May 1996*
Laid before Parliament	*10th May 1996*
Coming into force - -	*1st June 1996*

The Minister of Agriculture, Fisheries and Food, the Secretaries of State respectively concerned with agriculture in Scotland and in Wales and the Department of Agriculture for Northern Ireland, acting jointly, in exercise of the powers conferred on them by section 117(2) and (3) of the Medicines Act 1968(**1**), and now vested in them(**2**), and of all other powers enabling them in that behalf, after consulting such organisations as appear to them to be representative of interests likely to be substantially affected by these Regulations in accordance with section 129(6) of the said Act, hereby make the following Regulations:

Title and commencement

1. These Regulations may be cited as the Medicines (Animal Feeding Stuffs) (Enforcement) (Amendment) Regulations 1996 and shall come into force on 1st June 1996.

Amendment of the Medicines (Animal Feeding Stuffs) (Enforcement) Regulations 1985

2.—(1) The Medicines (Animal Feeding Stuffs) (Enforcement) Regulations 1985(**3**) shall be amended in accordance with the following paragraphs of this regulation.

(2) In regulation 4 (Methods of analysis)—

(a) in paragraph (1), after "Paragraphs (2)" there shall be inserted "(2A)";

(b) for paragraph (2) the following paragraphs shall be substituted—

"(2) For the purpose of determining what quantity or proportion (if any) of a substance of a description or class listed in column 1 of Table A in division 3, or in divisions 4 and 5, of Schedule 3 to these Regulations has been incorporated in a sample of an animal feeding

(**1**) 1968 c. 67.

(**2**) In the case of the Secretary of State concerned with agriculture in Wales, by virtue of S.I.1978/272, and, in the case of the Department of Agriculture for Northern Ireland, by virtue of the Northern Ireland Constitution Act 1973 (c. 36), section 40 and Schedule 5, and the Northern Ireland Act 1974 (c. 28), section 1(3) and Schedule 1, paragraph 2(1)(b).

(**3**) S.I. 1985/273 as amended by S.I. 1989/2324.

stuff to be analysed pursuant to sections 112, 113 or 115 of the Act, the provisions of divisions 1 and 2 of the said Schedule 3 shall apply, and—

(a) in relation to a substance of a description or class listed in column 1 of Table A in division 3, the method of analysis set out in the Community provision specified in the corresponding entry of column 2 of that Table shall be used; and—

(b) in relation to substances respectively specified in divisions 4 and 5 of the said Schedule, the methods of analysis respectively set out in those divisions shall be used.

(2A) Where a reference to a Directive is made in column 2 of Table A of division 3 of Schedule 3 to these Regulations for which there is an entry in column 1 of Table B of that division, the reference shall be construed as being a reference to the Commission Directive establishing or relating to Community methods of analysis for the official control of feeding stuffs described in the corresponding entries in columns 2 and 3 of Table B.; and"

(c) in paragraph (3), after "paragraph (2)", there shall be inserted "or (2A)".

(3) In Schedule 3 (Methods of analysis)—

(a) for items 3 to 28 inclusive of the list of main divisions the following items shall be substituted—

"**3.** Community methods of analysis

4. Determination of meticlorpindol

5. Determination of nifursol"; and

(b) for divisions 3 to 28 inclusive the following divisions shall be substituted—

"3.

COMMUNITY METHODS OF ANALYSIS

Table A–

Methods of Analysis

Column (l) *Substance*	Column (2) *Community provision*
Antibiotics of the tetracycline group	Part 1 of Annex II to Directive 72/199/EEC
Amprolium	Part 1 of Annex II to Directive 74/203/EEC
Avoparcin	Part 1 of the Annex to Directive 81/715/EEC
Buquinolate	Part 1 of the Annex to Directive 75/84/EEC
Chlortetracycline oxytetracycline and tetracycline	Part 2 of Annex II to Directive 72/199/EEC
Copper	Part 3 of the Annex to Directive 78/633/EEC
Dinitolmide (DOT)	Part 3 of Annex II to Directive 74/203/EEC
Ethopabate	Part 2 of Annex II to Directive 74/203/EEC
Flavophospholipol	Part 2 of the Annex to Directive 78/633/EEC

Column (l) *Substance*	Column (2) *Community provision*
Furazolidone	Part 3 of the Annex to Directive 75/84/EEC
Halofuginone	Annex to Directive 93/70/EEC
Methyl benzoquate	Part 2 of the Annex to Directive 93/117/EC
Monensin sodium	Part 2 of the Annex to Directive 81/715/EEC
Nicarbazin	Part 4 of Annex II to Directive 74/203/EEC
Oleandomycin	Part 3 of Annex II to Directive 72/199/EEC
Robenidine	Part 1 of the Annex to Directive 93/117/EC
Spiramycin	Annex to Directive 84/425/EEC
Sulphaquinoxaline	Part 2 of the Annex to Directive 75/84/EEC
Tylosin	Part 4 of Annex II to Directive 72/199/EEC
Virginiamycin	Part 5 of Annex II of Directive 72/199/EEC as replaced by Article 2 of,, and Annex II to,, Directive 84/4/EEC
Zinc bacitracin	Part 1 of the Annex to Directive 78/633/EEC as replaced by Article 3 of,, and Annex III to,, Directive 84/4/EEC

Table B–

Interpretation

Column (1) *Directive reference*	Column (2) *Commission Directive*	Column (3) *Reference*
Directive 72/199/EEC	Third Commission Directive 72/199/EEC	OJNo. L 123,, 29.5.72,, p.6 (OJ/SE 1966-72 p.74)
Directive 74/203/EEC	Fifth Commission Directive 74/203/EEC	OJ No. L 108,, 22.4.74,, p.7
Directive 75/84/EEC	Sixth Commission Directive 75/84/EEC	OJ No. L 32,, 5.2.75,, p.26
Directive 78/633/EEC	Eight Commission Directive 78/633/EEC	OJ No. L 206,, 29.7.78,, p.43
Directive 81/715/EEC	Ninth Commission Directive 81/715/EEC	OJ No. L 257,, 10.9.81,, p.38
Directive 84/4/EEC	Commission Directive 84/4/EEC	OJ No. L 15,, 18.1.84,, p.28
Directive 84/425/EEC	Tenth Commission Directive 84/425/EEC	OJ No. L 238,, 6.9.84,, p.34
Directive 93/70/EEC	Eleventh Commission Directive 93/70/EEC	OJ No. L 234,, 17.9.93,, p.l7

Column (1) *Directive reference*	Column (2) *Commission Directive*	Column (3) *Reference*
Directive 93/117/EC	Twelfth Commission Directive 93/117/EC	OJ No. L 329,, 30.12.93,, p.54

4.

DETERMINATION OF METICLORPINDOL

(3,5-dichloro-2,6-dimethylpyridine-4-ol)

SCOPE AND FIELD OF APPLICATION

1. The method is for the determination of the quantity of meticlorpindol in complete feeding stuffs, protein concentrates and feed supplements. The lower limit of the determination is 60mg/kg.

PRINCIPLE

2. Meticlorpindol is extracted from the feed with methanolic ammonia solution, and a portion of the extract is passed through a column of aluminium oxide onto a column of ion-exchange resin. The meticlorpindol is retained on the resin and interfering substances are removed by washing with 80% methanol. The meticlorpindol is eluted from the resin with 40% acetic acid and the absorbance is measured at 267nm.

REAGENTS

3. Note: The suitability of a batch of aluminium oxide and of the other reagents should be tested before use by analysing a blank feed to which a known amount of meticlorpindol has been added.

(3.1) Aluminium oxide for column chromatography, 100 to 250 mesh, alkaline, Brockman activity 1.

(3.2) Ammonia (density 0.88g per ml).

(3.3) Anion exchange resin, AG1-X8 or Dowex 1-X8, 100 to 200 mesh — To convert Dowex resin in the chloride form to the acetate form add 1 litre of 6M hydrochloric acid to 350g of resin in a 3 litre beaker, and heat the mixture on a steam bath for 2 to 3 hours. Pour the slurry into a glass Buchner funnel, and wash the resin with water until the washings are free from chloride (about 6 litres of water are required). Transfer the resin to a 50mm diameter glass column having a coarse sintered-glass disc at the bottom end, and wash with sodium acetate solution (5g sodium acetate, anhydrous, dissolved in water and diluted to 100ml) until the column effluent gives only a cloudy solution on addition of silver nitrate solution. Return the resin to the glass Buchner funnel, and wash with water. Transfer the resin to a 3 litre beaker, add 1 litre of 40% v/v acetic acid solution (3.4) and heat on a steam bath for 3 hours or longer. Filter, and wash the resin again with water until the washings are free from chloride. Store the resin in water.

(3.4) Acetic acid solution 40% v/v.

(3.5) Methanol.

(3.6) Methanol solution 80% v/v.

(3.7) Ammoniacal methanol solution: dilute 1 volume of ammonia (3.2) with 19 volumes of methanol (3.5).

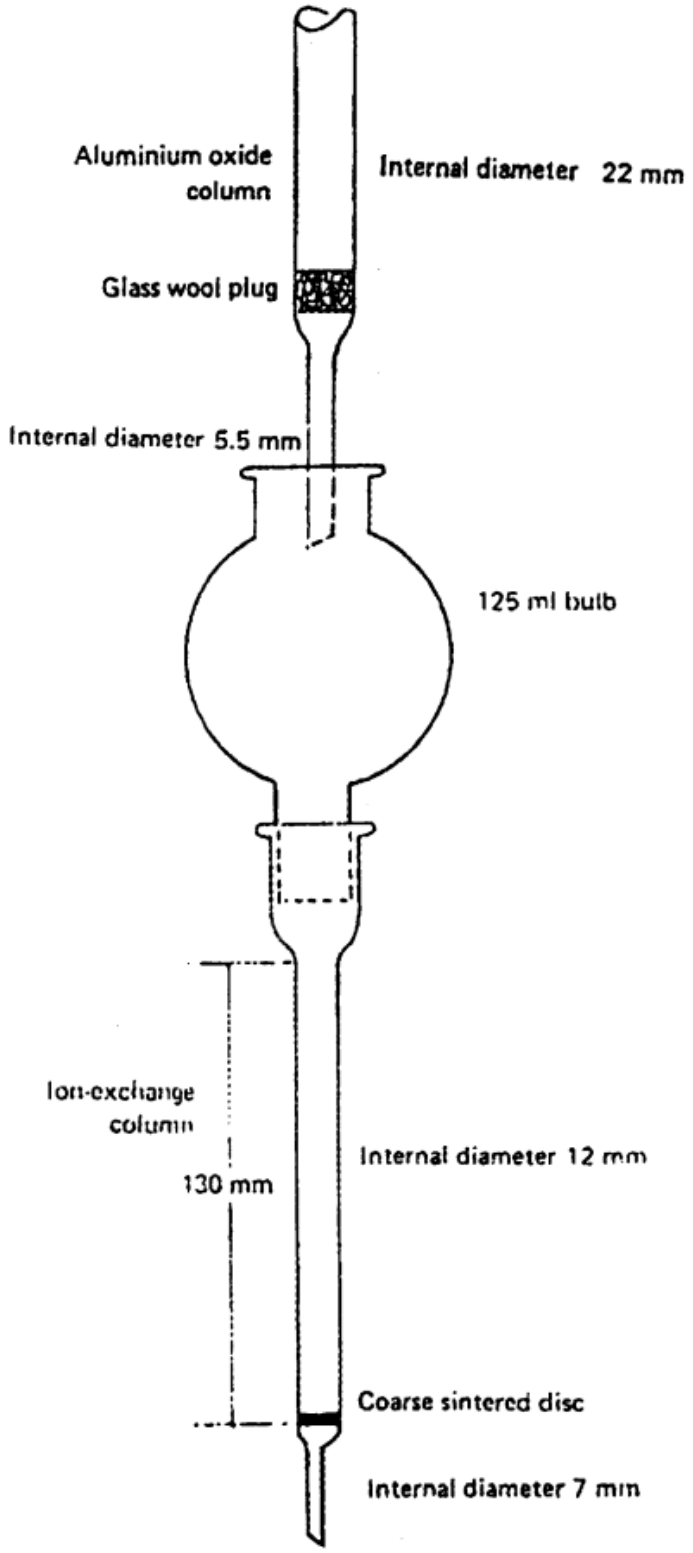
(3.8) Meticlorpindol standard solution: weigh, to the nearest 0.lmg, 125mg of meticlorpindol into a beaker, add 25ml of sodium hydroxide solution (2g sodium hydroxide dissolved in water and diluted to 100ml) to dissolve the meticlorpindol, transfer the solution to a 500ml graduated flask, and dilute to the mark with water. This solution contains 250mg per ml meticlorpindol.

APPARATUS

4.—(4.1) Aluminium oxide column: constructed as indicated in the diagram overleaf.

(4.2) Ion exchange column: constructed as indicated in the diagram overleaf.

(4.3) Spectrophotometer, recording, with 10mm silica cells.


Aluminium oxide column
Internal diameter 22 mm
Glass wool plug
Internal diameter 5.5 mm
125 ml bulb
Ion-exchange column
130 mm
Internal diameter 12 mm
Coarse sintered disc
Internal diameter 7 mm

PROCEDURE

Extraction of meticlorpindol

Extraction of meticlorpindol

5.—(5.1) Weigh, to the nearest 0.001g, approximately 50g of the finely divided and mixed sample, or a suitable amount expected to contain about 12mg of meticlorpindol, transfer to a 500ml graduated flask, and add 400ml of ammoniacal methanol solution (3.7). Place a magnetic stirring bar in the flask and stir the mixture on a magnetic stirrer for 20 minutes. Remove the stirring bar from the flask, dilute to the mark with ammoniacal methanol solution (3.7), mix the contents well, and set aside for 20 to 30 minutes.

Purification

(5.2.1) (5.2) Aluminium oxide column: For each column required weigh approximately 25g of aluminium oxide (3.1) into an aluminium foil dish and place in an oven at 105°C for 1 hour. Remove the dish from the oven and cool to room temperature in a desiccator. Make a slurry of the aluminium oxide with 25ml of ammoniacal methanol solution (3.7) and filter on a Buchner funnel. Wash the aluminium oxide with methanol (3.5) until the washings are neutral. Form a slurry of the aluminium oxide with 50ml of methanol (3.5) and pour the slurry into the column (4.1). Allow the methanol to drip through the column. Place a plug of glass wool lightly on top of the aluminium oxide and then wash with 25ml of methanol (3.5). Do not allow the liquid in the column to fall below the top of the aluminium oxide. Discard the eluate.

(5.2.2) Anion exchange column: Form a slurry in acetic acid (3.4) of sufficient resin (3.3) to fill the columns required. Filter on a Buchner funnel, wash the resin with twice its own volume of acetic acid (3.4) and then with aqueous methanol (3.6) until the washings are neutral. Form a slurry of the resin with aqueous methanol (3.6) and add sufficient to a column (4.2) to give a resin bed 20 to 30 mm deep after settling. Place a small plug of glass wool on top of the resin and wash the column with two 13ml portions of aqueous methanol (3.6). Do not allow the liquid level in the column to fall below the top of the resin. Discard the eluate.

(5.2.3) Chromatographic procedure: By pipette transfer 10.0ml of the extract of the feed sample (5.1) directly onto an aluminium oxide column and at the same time transfer the same volume of ammoniacal methanol solution (3.7) directly onto a second aluminium oxide column (reagent blank). Allow the solutions to drain to the top of the aluminium oxide and then wash each column with three 12ml portions of aqueous methanol (3.6), allowing the liquid to drain to the top of the aluminium oxide each time. Let all the eluate from each column drain directly into separate ion-exchange columns, and then remove the aluminium oxide columns. Allow the liquid to drain to the top of the ion-exchange resin and then wash each column with four 13ml portions of aqueous methanol (3.6). Discard the eluates.

Elute each column with two 10ml and then one 4ml portions of acetic acid (3.4). Collect the eluates from each column in separate 25ml graduated flasks and dilute the contents of each to the mark with acetic acid (3.4).

Determination

(5.3) Record the absorption spectrum of the sample extract between 350 and 245nm in 10mm silica cells with the reagent blank solution (5.2.3) as reference. Measure the absorbance of the sample extract at 267nm above a baseline obtained by drawing a line through the absorbance at 327 and 297nm and extending it through 267nm.

(Note: Background absorption due to the feed approaches a linear function that can be described by the points on the curve at 296 and 327nm. Occasionally this is not the case, as can be detected by absorption peaks in the region between 350 and 297nm).

Determine the concentration of meticlorpindol in the sample by reference to the calibration curve (5.4).

Calibration curve

(5.4) By pipette transfer 1, 5, 7.5, 10, 12.5 and 15ml portions of meticlorpindol standard solution (3.8) to separate 250ml graduated flasks. Dilute the contents of each flask to the mark with acetic acid (3.4). Record the absorption spectra of these solutions in 10 mm silica cells between 350 and 245nm with acetic acid (3.4) as reference. Construct a calibration curve using the absorbances at 267nm as ordinates and the corresponding concentrations of meticlorpindol in mg per ml as abscissae.

CALCULATION OF RESULTS

6. The meticlorpindol content in mg/kg of sample is given by the formula:

$$\frac{23.23 \times C \times 50}{W}$$

in which:

C = concentration of meticlorpindol, in mg per ml, read from the calibration curve equivalent to the absorbance of the test solution;

23.~~23~~ a factor that makes allowance for the volume of the feed sample in the flask; and

W = weight of test portion in g.

Absorbance at 327 and 297nm should not differ by more than 0.05 units and both points should be below 0.2. Results should be satisfactory as long as these criteria are kept in mind along with any obvious distortion in the appearance of the curve. No maximum other than that of meticlorpindol should be present.

5.

DETERMINATION OF NIFURSOL

[3,5-dinitro-2-(5-nitrofurfurylidene)salicylohydrazide]

SCOPE AND FIELD OF APPLICATION

1. The method is for the determination of the quantity of nifursol in complete feeding stuffs, protein concentrates and feed supplements. Other substances that will provide a nitro group under the conditions of the method, eg nitrofurazone and furazolidone, will interfere. The lower limit of the determination is 20mg/kg.

PRINCIPLE

2. The sample is extracted with dimethylformamide and the extract is purified on a column of aluminium oxide. A portion of the purified extract containing the nifursol is treated with phenylhydrazine hydrochloride and the resulting phenylhydrazone extracted into toluene. The addition of methylbenzethonium hydroxide to the toluene solution produces a blue colour, the absorbance of which is measured as 515nm.

REAGENTS

3.—(3.1) Toluene.

(3.2) Aluminium oxide for column chromatography, 80 to 200 mesh, alkaline, Brockman activity 1. To 100 parts of the aluminium oxide add 6 parts of powdered magnesium hydroxide. Shake in a screw-cap bottle to mix, add 8 parts of water, and mix until free from lumps.

(3.3) Sand; acid washed.

(3.4) Dimethylformamide solution, 95% v/v.

(3.5) Dimethylformamide solution, 50% v/v.

(3.6) Phenylhydrazine, hydrochloride solution: shake 0.25 0.005g of phenylhydrazine hydrochloride in 25ml of water, add 25ml of concentrated hydrochloric acid, and shake to dissolve the solid, filtering if necessary. Prepare this reagent immediately before use.

(3.7) Methylbenzethonium hydroxide solution: about 10% in methanol.

(3.8) Nifursol standard solution: weigh, to the nearest 0.1 mg, 25mg of pure nifursol into a 100ml graduated flask, add 5ml of 95% v/v dimethylformamide solution (3.4), and mix until all the solid has dissolved. Dilute to the mark with methanol. Prepare this solution freshly each day.

APPARATUS

4.—(4.1) Chromatographic column— A glass column, internal diameter: 20 to 25mm; length: 100 to 150mm plugged at the lower end with glass wool.

(4.2) Spectrophotometer, with 10mm cells.

PROCEDURE

Extraction

Extraction

5.—(5.1) Weigh to the nearest 0.001g, approximately 5g of the finely divided and mixed sample, or a suitable amount expected to contain about 350 mg of nifursol and transfer to a 125ml conical flask. Add 50.0ml of 95% v/v dimethylformamide solution (3.4), insert a stopper loosely, and place the flask in a water-bath at 60°C for 30 minutes. Swirl the contents of the flask occasionally during this period. Shake the flask on a mechanical shaker for 30 minutes and then filter the contents through a rapid filter-paper, preferably under reduced pressure on a Buchner funnel. Transfer 40.0ml of the filtrate to a beaker, add 40.0ml of water, and stir. Set the beaker aside, protected from light, for 30 minutes.

Purification

(5.2) Pack the chromatographic column (4.1) to a depth of 70mm with the prepared aluminium oxide (3.2) and on top of the aluminium oxide add a layer of sand (3.3) 15mm deep. Wash the column with 50ml of 50% v/v dimethylformamide solution (3.5) and then pass the dimethylformamide extract of the test sample through the column; reject the first 45ml of eluate and collect the next 17ml.

Determination

(5.3) Pipette 5.0ml of the eluate to a 20ml centrifuge tube, add 5ml of phenylhydrazine hydrochloride solution (3.6), mix, and place the tube in a water-bath at 40°C for 20 minutes. Remove the tube from the water-bath and cool it in running water for 5 minutes. Add 5.0ml of toluene (3.1) to the contents of the tube, insert a glass or plastic stopper (a rubber stopper must not be used), and shake vigorously 40 times. Centrifuge for 5 minutes to clear the toluene layer, and transfer 3.0ml of the toluene layer to a 10mm spectrophotometer cell. Add 0.2ml of methylbenzethonium hydroxide solution (3.7), mix immediately, and measure the absorbance of the solution within one minute at 515nm with toluene as reference. Determine the quantity of nifursol by reference to the calibration curve (5.4).

Calibration curve

(5.4) Pipette 5.0ml of nifursol standard solution (3.8) to a 200ml graduated flask, add 100ml of 95% v/v dimethylformamide solution (3.4), dilute to the mark with water and mix. Into separate 20ml centrifuge tubes transfer by pipette 1, 2, 3, 4 and 5 ml portions of this solution and dilute the contents of each tube to 5ml with 50% v/v dimethylformamide solution (3.5).

Treat the contents of each tube as described under “Determination” (5.3) beginning at “…. add 5ml of phenylhydrazine hydrochloride solution (3.6)…”. Plot the calibration curve using the absorbances as the ordinates and the corresponding quantities of nifursol in mg as abscissae.

CALCULATION OF RESULTS

6. The nifursol content in mg/kg of sample is given by the formula:

$$\frac{20 \times A}{W}$$

in which:

A = µg of nifursol read from the calibration curve; and
W = weight of the test portion in g.”

Angela Browning
Parliamentary Secretary, Ministry of Agriculture, Fisheries and Food

1st May 1996

Lindsay
Parliamentary Under Secretary of State, Scottish Office

30th April 1996

Signed by authority of the Secretary of State for Wales

Gwilym Jones
Parliamentary Under Secretary of State, Welsh Office

1st May 1996

Sealed with the Official Seal of the Department of Agriculture for Northern Ireland this

L.S.

J. Murray
Permanent Secretary

1st day of May 1996

EXPLANATORY NOTE

(This note is not part of the Regulations)

These Regulations, which come into force on 1st June 1996, further amend the Medicines (Animal Feeding Stuffs) (Enforcement) Regulations 1985 ("the principal Regulations"), and implement:

(a) Eleventh Commission Directive 93/70/EEC establishing Community analysis methods for official control of feeding stuffs (OJ No. L 234, 17.9.93, p.17) which establishes a Community method of analysis to be used in the course of official checks on animal feeding stuffs to identify their halofuginone content (regulation 2(2) and (3)(b)); and

(b) Twelfth Commission Directive 93/117/EC establishing Community analysis methods for official control of feeding stuffs (OJ No. L 329, 30.12.93, p.54) to be used in the course of official checks on animal feeding stuffs to identify their robenidine and methyl benzoquate content (regulation 2(2) and (3)(b)).

The Regulations also revise the format of, and make other minor amendments to, Schedule 3 to the principal Regulations (regulation 2(3)), including increasing the amount of concentrated hydrochloric acid which is used as a reagent in the method of analysis for the determination of nifursol from 24ml to 25ml.

The methods of analysis which must be used for the specified substances are unchanged save that:

(a) the methods of analysis previously specified for acinitrazole, nitrofurazone and nitrovin have been omitted from Schedule 3 to the principal Regulations because the incorporation of such substances in animal feeding stuffs is no longer permitted; and

(b) methods of analysis are now prescribed for halofuginone, robenidine and methyl benzoquate.

The Regulations also make other consequential amendments to the principal Regulations to take into account the new revised format of Schedule 3 (regulation 2(2)).