COMMISSION

TENTH COMMISSION DIRECTIVE

of 25 July 1984

establishing Community methods of analysis for the official control of feedingstuffs

(84/425/EEC)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Directive 70/373/EEC of 20 July 1970 on the introduction of Community methods of sampling and analysis for the official control of feedingstuffs (1), as last amended by the Act of Accession of Greece, and in particular Article 2 thereof,

Whereas that Directive requires that official control of feedingstuffs for the purpose of checking compliance with requirements under the provisions laid down by law, regulation or administrative action concerning the quality and composition of feedingstuffs be carried out using Community methods of sampling and analysis ;

Whereas Commission Directives 71/250/EEC (2), 73/46/EEC (3), 74/203/EEC (4), 75/84/EEC (5), 76/372/EEC (6), as last amended by Directive 81/680/EEC (7), Directives 71/393/EEC (8), 72/199/EEC (9), 78/633/EEC (10), as last amended by Directive 84/4/EEC (11), and Directive 81/715/EEC (12) have already established a number of Community methods of analysis ; whereas the progress of work since then makes it advisable to adopt a new method ;

Whereas the measures provided for in this Directive are in accordance with the opinion of the Standing Committee for Feedingstuffs,

HAS ADOPTED THIS DIRECTIVE :

Article 1

Member States shall require that analyses for official controls of feedingstuffs, as regards their content of spiramycin be carried out in accordance with the method described in the Annex.

Article 2

Member States shall bring into force the laws, regulations or administrative provisions necessary to comply with this Directive by 30 June 1985 at the latest and shall forthwith inform the Community thereof.

Article 3

This Directive is addressed to the Member States.

Done at Brussels, 25 July 1984.

For the Commission

Poul DALSAGER

Member of the Commission

(1) OJ No L 170, 3. 8. 1970, p. 2.
(2) OJ No L 155, 12. 7. 1971, p. 13.
(3) OJ No L 83, 30. 3. 1973, p. 21.
(4) OJ No L 108, 22. 4. 1974, p. 7.
(5) OJ No L 32, 5. 2. 1975, p. 26.
(6) OJ No L 102, 15. 4. 1976, p. 8.
(7) OJ No L 246, 29. 8. 1981, p. 32.
(8) OJ No L 279, 20. 12. 1971, p. 7.
(9) OJ No L 123, 29. 5. 1972, p. 6.
(10) OJ No L 206, 29. 7. 1978, p. 43.
(11) OJ No L 15, 18. 1. 1984, p. 28.
(12) OJ No L 257, 10. 9. 1981, p. 38.

ANNEX

DETERMINATION OF SPIRAMYCIN BY DIFFUSION IN AN AGAR MEDIUM

1. Purpose and scope

The method is for the determination of spiramycin in feedingstuffs and premixes. The lower limit of determination is 1 mg/kg (1 ppm) (1)

2. Principle

The sample is extracted with a mixture of methanol/phosphate-bicarbonate buffer at pH 8. The extract is decanted or centrifuged and diluted. Its antibiotic activity is determined by measuring the diffusion of spiramycin in an agar medium inoculated with *Micrococcus luteus*. Diffusion is shown by the formation of zones of inhibition of the micro-organism. The diameter of these zones is taken to be in direct proportion to the logarithm of the antibiotic concentration over the range of antibiotic concentrations employed.

3. Micro-organism : *Micrococcus luteus* ATCC 9341 (NCTC 8340, NCIB 8553)

3.1. *Maintenance of stock culture*

Inoculate tubes containing slopes of culture medium (4.1) with *Micrococcus luteus* and incubate for 24 hours at 30 °C. Store the culture in a refrigerator at about 4 °C. Reinoculate every two weeks.

3.2. *Preparation of the bacterial suspension* (a)

Harvest the growth from a recently prepared agar slope (3.1) with 2 to 3 ml of sodium chloride solution (4.3). Use this suspension to inoculate 250 ml of culture medium (4.1) contained in a Roux flask and incubate for 18 to 20 hours at 30 °C. Harvest the growth in 25 ml of sodium chloride solution (4.3) and mix. Dilute the suspension to 1/10 with sodium chloride solution (4.3). The light transmission of the suspension must be about 75 %, measured at 650 nm in a 1 cm cell against sodium chloride solution (4.3). This suspension may be kept for one week at about 4 °C.

4. Culture media and reagents

4.1. *Culture medium* (b)

Meat peptone	6,0 g
Tryptone	4,0 g
Yeast extract	3,0 g
Meat extract	1,5 g
Glucose	1,0 g
Agar	10,0 to 20,0 g
Water	1 000 ml

pH 6,5 to 6,6 (after sterilization).

4.2. *Assay medium* (b)

Tryptone	5,0 g
Yeast extract	4,0 g
Meat extract	3,0 g
Agar	10,0 to 20,0 g
Water	1 000 ml

pH 8,0 (after sterilization).

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(1) 1 mg spiramycin base is equivalent to 3 200 international units (IU).
(a) Other methods may be used provided that it has been established that they give similar bacterial suspensions.
(b) Any commercial culture medium of similar composition and giving the same results may be used.