Irish Standard
I.S. EN 16939:2017

Animal feeding stuffs: Methods of sampling and analysis - Detection of tylosin, spiramycin and virginiamycin - Thin Layer Chromatography and bioautography

I.S. EN 16939:2017

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National Foreword

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Animal feeding stuffs: Methods of sampling and analysis - Detection of tylosin, spiramycin and virginiamycin - Thin Layer Chromatography and bioautography

Aliments pour animaux : Méthodes d'échantillonnage et d'analyse - Détection de tylosine, spiramycine et virginiamycine - Chromatographie sur couche mince et bioautographie

Futtermittel - Probenahme- und Untersuchungsverfahren - Nachweis von Tylosin, Spiramycin und Virginiamycin - Dünnschichtchromatographie und Bioautographie

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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European foreword

This document (EN 16939:2017) has been prepared by Technical Committee CEN/TC 327 “Animal feeding stuffs - Methods of sampling and analysis”, the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by February 2018, and conflicting national standards shall be withdrawn at the latest by February 2018.

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1 Scope

The method makes it possible to detect and identify spiramycin, tylosin and virginiamycin in animal feeding stuffs (feed raw materials of mainly plant origin and compound feeds) excluding mineral feeds and premixtures. The limit of detection is about 2 mg/kg for spiramycin, 1 mg/kg for tylosin and 1 mg/kg for virginiamycin. In some milk replacers, it can be slightly higher than 1 mg/kg for virginiamycin.

Reported limits of detection are probably little overestimated but were fully validated during the collaborative study (see Annex B). In each laboratory, each day of analysis, spiked blank samples at 1 mg/kg for spiramycin and virginiamycin and at 0,5 mg/kg for tylosin are analysed for checking lower detection limits (see 9.2 and 9.3). These lower limits of detection are achievable, but should be established with an in-house validation first.

Some other antibiotics can interfere in the detection of these 3 specific macrolide antibiotics. The known interferences are specified in Annex A of the method.

That method should be used as a qualitative screening and/or a post-screening method (after microbiological plate test, for example). The follow-up of the antibiotics presence may be done by other analytical technics (LC and/or LC-MS technics) ([4], [10]). For confirmatory purposes, LCMS is required.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN ISO 6498, *Animal feeding stuffs - Guidelines for sample preparation (ISO 6498)*

3 Terms and definitions

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

3.1
microbiological activity of antibiotics
ratio of the dose that inhibits the growth of a suitable susceptible microorganism to the dose of an International Chemical Reference Substance/Antibiotic that produces similar inhibition

Note 1 to entry Microbiological activity is a property measured by a microbiological assay. The activity (potency) of an antibiotic product is expressed as the ratio of the dose that inhibits the growth of a suitable susceptible microorganism to the dose of an International Chemical Reference Substance/Antibiotic that produces similar inhibition.

Note 2 to entry The microbiological activity is expressed as International Unit/mg or µg/mg with possibility to have microbiological activities higher or lower than 1 000 µg/mg.

3.2
retardation factor
Rf
ratio of the distance which the product travelled by the distance which the solvent front travelled using the initial spotting site as reference

Note 1 to entry These values depend on the solvent used and the type of TLC plate and are not physical constants, see Figure 1.