

**Loomasööt. Semduramitsiini sisalduse määramine  
vedelikkromatograafilise meetodiga kasutades „puu“  
analüütelist lähenemisviisi**

**Animal feeding stuffs - Determination of semduramicin  
content - Liquid chromatographic method using a "tree"  
analytical approach**

## EESTI STANDARDI EESSÕNA

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ICS 65.120

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EUROPEAN STANDARD

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Animal feeding stuffs - Determination of semduramicin content -  
Liquid chromatographic method using a "tree" analytical  
approach

Aliments pour animaux - Dosage de la semduramicine -  
Chromatographie liquide utilisant une approche analytique  
en arbre

Futtermittel - Bestimmung des Semduramicingehalts -  
Flüssigkeitschromatographisches Verfahren mit  
verzweigter analytischer Vorgehensweise

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## Foreword

This document (EN 16158:2012) has been prepared by Technical Committee CEN/TC 327 "Animal feeding stuffs", 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of an identical text or by endorsement, at the latest by August 2012, and conflicting national standards shall be withdrawn at the latest by August 2012.

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## 1 Scope

This European standard specifies a high-performance liquid chromatographic (HPLC) method for the determination of the semduramicin content at authorized level in animal feeding stuffs [2], using mass spectrometry detection or post-column derivatization and (UV)-VIS detection (hereinafter UV detection). This method is applicable to poultry feed. The limit of quantitation is 1,0 mg/kg when mass spectrometry is used for detection and 3,0 mg/kg when the detection is performed by UV with post-column derivatization. Lower limits of quantitation are achievable but this is to be validated by the user.

The method allows the discrimination of semduramicin from monensin, salinomycin, narasin, maduramicin and lasalocid.

## 2 Normative references

The following referenced documents are indispensable for the application of this protocol. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

prEN ISO 6498, *Animal feeding stuffs — Guidelines for sample preparation (ISO/DIS 6498)*

## 3 Principle

Semduramicin is extracted using acetonitrile with mechanical shaking during 30 min. The extracts are filtered through 0,2 µm Nylon filters. Semduramicin is determined by reverse-phase liquid chromatography using electrospray (ESI) single quadrupole mass spectrometry detection in single ion monitoring (SIM) mode (LC-MS) [4] or using post-column derivatization with dimethylaminobenzaldehyde (DMAB) and spectrophotometric detection at 598 nm (LC-PCD-UV) [5]. If the detection used is ESI-MS the quantitation is performed through a standard addition approach. When LC-PCD-UV is used the quantitation is performed through external standard calibration.

## 4 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

### 4.1 LC-MS.

4.1.1 **Water**, HPLC grade, or equivalent (e.g. Milli-Q purified water).

4.1.2 **Acetonitrile**, HPLC gradient grade, minimum 99,9 % purity.

4.1.3 **Methanol**, HPLC grade or hypergrade LC-MS.

4.1.4 **Ammonium formate**, HPLC grade.

4.1.5 **Mobile phase**.

4.1.5.1 **Ammonium formate solution**, c = 20 mmol/l.

Accurately weigh 1,25 g to the nearest 0,01 g of ammonium formate (4.1.4) into a 1 000 ml volumetric flask. Dissolve in water (4.1.1) and make up to 1 000 ml of volume with water. Prepare fresh solutions monthly.