SLOVENSKI STANDARD
SIST EN 17299:2019

01-oktober-2019

Krma: metode vzorčenja in analize - Pregled in določevanje dovoljenih kokcidiostatikov v koncentracijah dodatkov in njihovih nosilcih v območju od 1 do 3 % v krmnih mešanicah s tekočinsko kromatografijo visoke ločljivosti - Tandemska masna spektrometrija (LC-MS/MS)

Animal feeding stuffs: Methods of sampling and analysis - Screening and determination of authorized coccidiostats at additive and 1 % and 3 % cross-contamination levels, and of non-registered coccidiostats and of one antibiotic at sub-additive levels, in compound feed with High Performance Liquid Chromatography - Tandem Mass Spectrometry detection (LC-MS/MS)

Futtermittel - Probenahme- und Untersuchungsverfahren - Bestimmung zugelassener Kokzidiostatika in Konzentrationen von Zusatzstoffen und deren Verschleppungen im Bereich von 1 % bis 3 % in Mischfuttermitteln mittels Hochleistungs-LC-MS

Aliments des animaux: Méthodes d'échantillonnage et d'analyse - Recherche et dosage dans des aliments composés pour animaux des coccidiostatiques autorisés au taux d'additif et de contamination croisée à 1 % et 3 %, de coccidiostatiques non enregistrés et d'un antibiotique aux taux sub-additifs, par chromatographie en phase liquide à haute performance couplée à une détection par spectrométrie de masse en tandem (CL-SM/SM)

Ta slovenski standard je istoveten z: **EN 17299:2019**

ICS:

65.120	Krmila	Animal feeding stuffs
71.040.50	Fizikalnokemijske analitske metode	Physicochemical methods of analysis

SIST EN 17299:2019 **en,fr,de**

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Animal feeding stuffs: Methods of sampling and analysis - Screening and determination of authorized coccidiostats at additive and 1 % and 3 % cross-contamination level, and of non-registered coccidiostats and of one antibiotic at sub-additive levels, in compound feed with High Performance Liquid Chromatography - Tandem Mass Spectrometry detection (LC-MS/MS)

Aliments des animaux: Méthodes d'échantillonnage et d'analyse - Recherche et dosage dans des aliments composés pour animaux des coccidiostatiques autorisés au taux d'additif et de contamination croisée à 1 % et 3 %, de coccidiostatiques non enregistrés et d'un antibiotique aux taux sub-additifs, par chromatographie en phase liquide à haute performance couplée à une détection par spectrométrie de masse en tandem (CL-SM/SM)

Futtermittel - Probenahme- und Untersuchungsverfahren - Bestimmung zugelassener Kokzidiostatika in Konzentrationen von Zusatzstoffen und deren Verschleppungen im Bereich von 1 % bis 3 % in Mischfuttermitteln mittels Hochleistungs-LC-MS

This European Standard was approved by CEN on 17 June 2019.

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

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

Contents

Page

European foreword

This document (EN 17299:2019) 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 2020, and conflicting national standards shall be withdrawn at the latest by February 2020.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document has been prepared under a standardization request given to CEN by the European Commission and the European Free Trade Association.

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1 Scope

This document specifies a high performance liquid chromatographic – tandem mass spectrometry (HPLC-MS/MS) method for the simultaneous screening and/or determination of the eleven authorized coccidiostats (halofuginone hydrobromide, robenidine hydrochloride, nicarbazin, diclazuril, decoquinate, monensin sodium, salinomycin sodium, narasin, lasalocid A sodium, semduramicin sodium and maduramicin ammonium alpha) contents in compound feeds and feed materials of plant origin at additive and cross-contamination levels and of the five non-registered coccidiostats (ethopabate, clopidol, ronidazole, dimetridazole and amprolium) at sub-additive levels and for the screening of the prohibited furazolidone antibiotic at sub-additive level, in the same matrices. It has been fully validated only for poultry, cattle and pig compound feeds.

The range of application of the method is fit for the purpose of the screening and determination of all eleven registered coccidiostats at the values set by European legislation, of the non-registered coccidiostats and of the screening of the banned antibiotic.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. 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.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at http://www.iso.org/obp

3.1
Internal Standard
I.S.
compound of known concentration added to a sample to facilitate the qualitative identification and/or quantitative determination of the sample components

[SOURCE: ISO 20752:2014, 3.2]

3.2
target feed
feed for target animal species or categories for which the additives are intended

[SOURCE: Commission Regulation (EC) No 124/2009]

3.3
non-target feed
feed for which the use of coccidiostats or histomonostats are not authorised, such as feed intended for animal species or categories not provided for in the additive authorisation

[SOURCE: Commission Regulation (EC) No 124/2009]

4 Principle

All 11 authorized coccidiostats, non-registered coccidiostats and the banned antibiotic are extracted using solid-liquid extraction. The extracts are centrifuged and supernatants filtered. After a first screening analysis, the analytes are determined by reverse phase HPLC using electrospray ionization with further tandem mass spectrometry detection. The quantitation of the detected target analytes is performed using a multi-level standard additions approach.

5 Reagents and materials

WARNING — Avoid inhalation of and exposure to the toxic standard materials and solutions thereof. Work in a fume-hood when handling the solvents and solutions.

Use only reagents recognized as analytical grade at least unless otherwise stated.

5.1 Water, HPLC grade or equivalent (e.g. milli-Q purified water).

5.2 Acetonitrile, HPLC gradient grade or hypergrade LC-MS, minimum 99,9 % purity.

5.3 Methanol, HPLC grade or hypergrade LC-MS.

5.4 Formic acid, HPLC grade or hypergrade LC-MS, minimum purity 98 %.

5.5 Ethanol, absolute, minimum purity 99,9 %.

5.6 N, N-Dimethylformamide, minimum purity 99 %.

5.7 Dimethylsulfoxide, minimum purity 99,5 %.

5.8 Calcium chloride, anhydrous, minimum purity 93 %.

5.9 Acetonitrile solution in water 1:1, *V:V.*

Take 50 ml of acetonitrile (5.2) into a 100 ml graduated cylinder (6.11) and add 50 ml water (5.1). Prepare freshly every 3 months.

5.10 1 % Calcium chloride solution in methanol

Accurately weigh to the nearest 0,1 g, 1,0 g of calcium chloride anhydrous (5.8) into a 100 ml volumetric flask (6.9). Note down the exact weight of calcium chloride anhydrous (5.8). Dissolve in methanol (5.3) and make up to 100 ml volume with methanol (5.3). Store at 4 °C in amber glassware (6.13). Prepare freshly every 3 months.

5.11 Solvent mixture for feed extraction acetonitrile: methanol: water 80:10:10 *V:V:V.*

Take 800 ml of acetonitrile (5.2) into a 1 000 ml volumetric flask (6.9) or a 1 000 ml graduated cylinder (6.11) and add 100 ml of methanol (5.3) and 100 ml of water (5.1). Mix thoroughly. Prepare freshly before extraction and leave at room temperature.

5.12 Mobile phase for HPLC

5.12.1 Water, containing 0,5 % (*V:V*) formic acid – Phase A

Take 5 ml of formic acid (5.4) into a 1 000 ml volumetric flask (6.9) and make up to 1 000 ml of volume with water (5.1). Prepare fresh solutions monthly.

5.12.2 Methanol, containing 0,5 % (*V:V*) formic acid – Phase B

Take 5 ml of formic acid (5.4) into a 1 000 ml volumetric flask (6.9) and make up to 1 000 ml of volume with methanol (5.3). Prepare fresh solutions monthly.

5.13 Reference standards

Purity required for each lot of reference and internal standards.

NOTE In the event of non-availability of the standards described below, at the given purities, standard substances with other purities can be used provided that the purity is duly taken into account in the calculation and that the chromatographic behaviour e.g. peak shape, is not affected.

5.13.1 Halofuginone hydrobromide, CAS N° 64924-67-0, minimum purity 99 % expressed as **halofuginone hydrobromide.**

5.13.2 Robenidine hydrochloride, CAS N° 25875-50-7, minimum purity 97 %.

5.13.3 Nicarbazin, CAS N° 330-95-0, minimum purity 97 %.

5.13.4 Diclazuril, CAS N° 101831-37-2, minimum purity 98 %.

5.13.5 Decoquinate, CAS N° 18507-89-6, minimum purity 98 %.

5.13.6 Semduramicin sodium, CAS N° 113378-31-7, minimum purity 93 % expressed as semduramicin.

5.13.7 Monensin sodium, CAS N° 22373-78-0, minimum purity 86 % expressed as **monensin A sodium.**

5.13.8 Maduramicin ammonium, CAS N° 84878-61-5, minimum purity 97 % expressed as **maduramicin ammonium alpha.**

5.13.9 Salinomycin sodium, CAS N° 55721-31-8, minimum purity 93 %.

5.13.10 Lasalocid sodium, CAS N° 25999-20-6, minimum purity 95 % expressed as **lasalocid A sodium.**

5.13.11 Narasin, CAS N° 55134-13-9, minimum purity 97 % expressed as narasin A.

5.13.12 Ethopabate, CAS N° 59-06-3, minimum purity 99 %.

5.13.13 Clopidol, CAS N° 2971-90-6, minimum purity 99 %.

5.13.14 Amprolium hydrochloride, CAS N° 137-88-2, minimum purity 99 %.

5.13.15 Furazolidone, CAS N° 67-45-8, minimum purity 99 %.

5.13.16 Ronidazole, CAS N° 7681-76-7, minimum purity 99 %.

5.13.17 Dimetridazole, CAS N° 551-92-8, minimum purity 99 %.

5.13.18 Robenidine-D_8, hydrochloride, CAS N° 1173097-77-2, minimum purity 98 %, to be used as internal standard (I.S.) for robenidine (5.13.2).

5.13.19 Dinitrocarbanilide-D_8, CAS N° 1156508-87-0, minimum purity 99 %, to be used as internal standard (I.S.) for dinitrocarbanilide (nicarbazin (5.13.3) marker).

5.13.20 Bis-Diclazuril, CAS N° 103337-71-9, minimum purity 97 %, to be used as internal standard (I.S.) for diclazuril (5.13.4).

5.13.21 Decoquinate-D_5, CAS N° 1228182-55-5, minimum purity 98 %, to be used as internal standard (I.S.) for decoquinate (5.13.5).

5.13.22 Nigericin sodium, CAS N° 28643-80-3, minimum purity 98 % to be used as internal standard (I.S.) for ionophore coccidiostats (5.13.6; 5.13.7; 5.13.8; 5.13.9; 5.13.10; 5.13.11).

5.13.23 Dimetridazole-D_3, CAS N° 64678-69-9, minimum purity 99 % to be used as internal standard (I.S.) for dimetridazole (5.13.17).

5.13.24 Ethopabate-D_5, CAS N° 59-06-03 - unlabelled, minimum purity 99 % to be used as internal standard (I.S.) for ethopabate (5.13.12).

5.13.25 Furazolidone-D_4, CAS N° 1217222-76-8, minimum purity 99 % to be used as internal standard (I.S.) for furazolidone (5.13.15).

5.13.26 Ronidazole-D_3, CAS N° 1015855-87-4, minimum purity 99 % to be used as internal standard (I.S.) for ronidazole (5.13.16) and clopidol (5.13.13).

5.14 Standard solutions

5.14.1 Stock standard solutions, ca. 1 mg/ml.

5.14.1.1 General

5.14.1.2 Halofuginone hydrobromide

Accurately weigh to the nearest 0,1 mg 10 mg of halofuginone hydrobromide standard (5.13.1) into a 10 ml volumetric flask (6.9). Note down the exact weight of halofuginone hydrobromide. Dissolve in the acetonitrile solution in water (5.9) and make up to 10 ml volume with the acetonitrile solution in water (5.9). Store at −20 °C in amber vials (6.13). Prepare freshly every 3 months.

Determine the accurate concentration of the halofuginone hydrobromide stock standard solution using the reference standard purity value provided by the supplier using Formula (1).

$$C_{HAL} = \frac{m}{10} \times P \quad (1)$$

where

C_{HAL} is the concentration of halofuginone hydrobromide in the stock standard solution, in mg/ml;

P is the purity of the halofuginone hydrobromide standard given by the supplier, in %;
NOTE For example 0,99.

m is the weighed mass of halofuginone hydrobromide standard (5.13.1), in mg.

5.14.1.3 Robenidine hydrochloride

Accurately weigh to the nearest 0,1 mg 10 mg of robenidine hydrochloride standard (5.13.2) into a 10 ml volumetric flask (6.9). Note down the exact weight of robenidine hydrochloride. Dissolve in ethanol (5.5) and make up to 10 ml volume with ethanol (5.5). Store at −20 °C in amber vials (6.13). Prepare freshly every 3 months.

Determine the accurate concentration of the robenidine hydrochloride stock standard solution using the reference standard purity value provided by the supplier using Formula (2).

$$C_{\mathrm{ROB}} = \frac{m}{10} \times P \qquad (2)$$

where

C_{ROB} is the concentration of robenidine hydrochloride in the stock standard solution, in mg/ml;

P is the purity of the robenidine hydrochloride standard given by the supplier, in %;
NOTE For example 0,97.

m is the weighed mass of robenidine hydrochloride standard (5.13.2), in mg.

5.14.1.4 Nicarbazin

Accurately weigh to the nearest 0,1 mg 10 mg of nicarbazin standard (5.13.3) into a 10 ml volumetric flask (6.9). Note down the exact weight of nicarbazin. Dissolve in dimethylsulfoxide (5.7) and make up to 10 ml volume with dimethylsulfoxide (5.7). Store at −20 °C in amber vials (6.13). Prepare freshly every 3 months.

Determine the accurate concentration of the nicarbazin stock standard solution using the reference standard purity value provided by the supplier using Formula (3).

$$C_{\mathrm{NIC}} = \frac{m}{10} \times P \qquad (3)$$

where

C_{NIC} is the concentration of nicarbazin in the stock standard solution, in mg/ml;

P is the purity of the nicarbazin standard given by the supplier, in %;
NOTE For example 0,97.

m is the weighed mass of nicarbazin standard (5.13.3), in mg.

5.14.1.5 Diclazuril

Accurately weigh to the nearest 0,1 mg 10 mg of diclazuril standard (5.13.4) into a 10 ml volumetric flask (6.9). Note down the exact weight of diclazuril. Dissolve in N,N-dimethylformamide (5.6) and make up to 10 ml volume with N,N-dimethylformamide (5.6). Store at −20 °C in amber vials (6.13). Prepare freshly every 3 months.

Determine the accurate concentration of the diclazuril stock standard solution using the reference standard purity value provided by the supplier using Formula (4).

$$C_{\mathrm{DICL}} = \frac{m}{10} \times P \quad (4)$$

where

C_{DICL} is the concentration of diclazuril in the stock standard solution, in mg/ml;

P is the purity of the diclazuril standard given by the supplier, in %;

NOTE For example 0,98.

m is the weighed mass of diclazuril standard (5.13.4), in mg.

5.14.1.6 Semduramicin sodium

Accurately weigh to the nearest 0,1 mg 10 mg of semduramicin sodium standard (5.13.6) into a 10 ml volumetric flask (6.9). Note down the exact weight of semduramicin sodium. Dissolve in 3 ml methanol (5.3) and make up to 10 ml volume with acetonitrile (5.2). Store at −20 °C in amber vials (6.13). Prepare freshly every 3 months.

Determine the accurate concentration of the semduramicin sodium stock standard solution using the reference standard purity value provided by the supplier using Formula (5).

$$C_{\mathrm{SEM}} = \frac{m}{10} \times P \quad (5)$$

where

C_{SEM} is the concentration of semduramicin sodium in the stock standard solution, in mg/ml;

P is the purity of the semduramicin sodium standard given by the supplier, in %;

NOTE For example 0,93.

m is the weighed mass of semduramicin sodium standard (5.13.6), in mg.

5.14.1.7 Monensin sodium

Accurately weigh to the nearest 0,1 mg 10 mg of monensin sodium standard (5.13.7) into a 10 ml volumetric flask (6.9). Note down the exact weight of monensin sodium. Dissolve in 3 ml methanol (5.3) and make up to 10 ml volume with acetonitrile (5.2). Store at −20 °C in amber vials (6.13). Prepare freshly every 3 months.

Determine the accurate concentration of the monensin sodium stock standard solution using the reference standard purity value provided by the supplier using Formula (6).

$$C_{\mathrm{MON}} = \frac{m}{10} \times P \quad (6)$$

where

C_{MON} is the concentration of monensin sodium in the stock standard solution, in mg/ml;

P is the purity of the monensin sodium standard given by the supplier, in %;

NOTE For example 0,86.

m is the weighed mass of monensin sodium standard (5.13.7), in mg.

5.14.1.8 Maduramicin ammonium

Accurately weigh to the nearest 0,1 mg 10 mg of maduramicin ammonium standard (5.13.8) into a 10 ml volumetric flask (6.9). Note down the exact weight of maduramicin ammonium. Dissolve in 3 ml methanol (5.3) and make up to 10 ml volume with acetonitrile (5.2). Store at −20 °C in amber vials (6.13). Prepare freshly every 3 months.

Determine the accurate concentration of the maduramicin ammonium stock standard solution using the reference standard purity value provided by the supplier using Formula (7).

$$C_{\text{MAD}} = \frac{m}{10} \times P \qquad (7)$$

where

C_{MAD} is the concentration of maduramicin ammonium in the stock standard solution, in mg/ml;

P is the purity of the maduramicin ammonium alpha standard given by the supplier, in %;

NOTE For example 0,97.

m is the weighed mass of maduramicin ammonium alpha standard (5.13.8), in mg.

5.14.1.9 Salinomycin sodium

Accurately weigh to the nearest 0,1 mg 10 mg of salinomycin sodium standard (5.13.9) into a 10 ml volumetric flask (6.9). Note down the exact weight of salinomycin sodium. Dissolve in 3 ml methanol (5.3) and make up to 10 ml volume with acetonitrile (5.2). Store at −20 °C in amber vials (6.13). Prepare freshly every 3 months.

Determine the accurate concentration of the salinomycin sodium stock standard solution using the reference standard purity value provided by the supplier using Formula (8).

$$C_{\text{SAL}} = \frac{m}{10} \times P \qquad (8)$$

where

C_{SAL} is the concentration of salinomycin sodium in the stock standard solution, in mg/ml;

P is the purity of the salinomycin sodium standard given by the supplier, in %;

NOTE For example 0,93.

m is the weighed mass of salinomycin sodium standard (5.13.9), in mg.

5.14.1.10 Lasalocid A sodium

Accurately weigh to the nearest 0,1 mg 10 mg of lasalocid sodium standard (5.13.10) into a 10 ml volumetric flask (6.9). Note down the exact weight of lasalocid sodium. Dissolve in 3 ml methanol (5.3) and make up to 10 ml volume with acetonitrile (5.2). Store at −20 °C in amber vials (6.13). Prepare freshly every 3 months.

Determine the accurate concentration of the lasalocid A sodium stock standard solution using the reference standard purity value provided by the supplier using Formula (9).

$$C_{\mathrm{LAS}} = \frac{m}{10} \times P \quad (9)$$

where

C_{LAS} is the concentration of lasalocid A sodium in the stock standard solution, in mg/ml;

P is the purity of the lasalocid sodium standard given by the supplier, in %;

NOTE For example 0,95.

m is the weighed mass of lasalocid sodium standard (5.13.10), in mg.

5.14.1.11 Narasin

Accurately weigh to the nearest 0,1 mg 10 mg of narasin standard (5.13.11) into a 10 ml volumetric flask (6.9). Note down the exact weight of narasin. Dissolve in 3 ml methanol (5.3) and make up to 10 ml volume with acetonitrile (5.2). Store at −20 °C in amber vials (6.13). Prepare freshly every 3 months.

Determine the accurate concentration of the narasin stock standard solution using the reference standard purity value provided by the supplier using Formula (10).

$$C_{\mathrm{NAR}} = \frac{m}{10} \times P \quad (10)$$

where

C_{NAR} is the concentration of narasin in the stock standard solution, in mg/ml;

P is the purity of the narasin standard given by the supplier, in %;

NOTE For example 0,97.

m is the weighed mass of narasin standard (5.13.11), in mg.

5.14.1.12 Amprolium

Accurately weigh to the nearest 0,1 mg 10 mg of amprolium hydrochloride standard (5.13.14) into a 10 ml volumetric flask (6.9). Note down the exact weight of amprolium. Dissolve in 5 ml methanol (5.3) and make up to 10 ml volume with methanol (5.2). Store at −20 °C in amber vials (6.13). Prepare freshly every 3 months.

Determine the accurate concentration of the amprolium stock standard solution using the reference standard purity value provided by the supplier using Formula (11).

$$C_{\mathrm{AMP}} = \frac{m}{10} \times P \quad (11)$$

where

C_{AMP} is the concentration of amprolium in the stock standard solution, in mg/ml;

P is the purity of the amprolium standard given by the supplier, in %;

NOTE For example 0,99.

m is the weighed mass of amprolium standard (5.13.14), in mg.

5.14.1.13 Clopidol

Accurately weigh to the nearest 0,1 mg 10 mg of clopidol standard (5.13.13) into a 10 ml volumetric flask (6.9). Note down the exact weight of clopidol. Dissolve in 5 ml dimethylsulfoxide (5.7) and make up to 10 ml volume with dimethylsulfoxide (5.7). Store at −20 °C in amber vials (6.13). Prepare freshly every 3 months.

Determine the accurate concentration of the clopidol stock standard solution using the reference standard purity value provided by the supplier using Formula (12).

$$C_{\mathrm{CLOP}} = \frac{m}{10} \times P \qquad (12)$$

where

C_{CLOP} is the concentration of clopidol in the stock standard solution, in mg/ml;

P is the purity of the clopidol standard given by the supplier, in %;

NOTE For example 0,99.

m is the weighed mass of clopidol standard (5.13.13), in mg.

5.14.1.14 Dimetridazole

Accurately weigh to the nearest 0,1 mg 10 mg of dimetridazole standard (5.13.17) into a 10 ml volumetric flask (6.9). Note down the exact weight of dimetridazole. Dissolve in 5 ml ethanol (5.5) and make up to 10 ml volume with ethanol (5.5). Store at −20 °C in amber vials (6.13). Prepare freshly every 3 months.

Determine the accurate concentration of the dimetridazole stock standard solution using the reference standard purity value provided by the supplier using Formula (13).

$$C_{\mathrm{DIM}} = \frac{m}{10} \times P \qquad (13)$$

where

C_{DIM} is the concentration of dimetridazole in the stock standard solution, in mg/ml;

P is the purity of the dimetridazole standard given by the supplier, in %;

NOTE For example 0,98.

m is the weighed mass of dimetridazole standard (5.13.17), in mg.

5.14.1.15 Ethopabate

Accurately weigh to the nearest 0,1 mg 10 mg of ethopabate standard (5.13.12) into a 10 ml volumetric flask (6.9). Note down the exact weight of ethopabate. Dissolve in 5 ml ethanol (5.5) and make up to 10 ml volume with ethanol (5.5). Store at −20 °C in amber vials (6.13). Prepare freshly every 3 months.

Determine the accurate concentration of the ethopabate stock standard solution using the reference standard purity value provided by the supplier using Formula (14).

$$C_{\mathrm{ETHO}} = \frac{m}{10} \times P \quad (14)$$

where

C_{ETHO} is the concentration of ethopabate in the stock standard solution, in mg/ml;

P is the purity of the ethopabate standard given by the supplier, in %;
NOTE For example 0,99.

m is the weighed mass of ethopabate standard (5.13.12), in mg.

5.14.1.16 Furazolidone

Accurately weigh to the nearest 0,1 mg 10 mg of furazolidone standard (5.13.15) into a 10 ml volumetric flask (6.9). Note down the exact weight of furazolidone. Dissolve in 5 ml dimethylsulfoxide (5.7) and make up to 10 ml volume with dimethylsulfoxide (5.7). Store at −20 °C in amber vials (6.13). Prepare freshly every 3 months.

Determine the accurate concentration of the furazolidone stock standard solution using the reference standard purity value provided by the supplier using Formula (15).

$$C_{\mathrm{FURA}} = \frac{m}{10} \times P \quad (15)$$

where

C_{FURA} is the concentration of furazolidone in the stock standard solution, in mg/ml;

P is the purity of the furazolidone standard given by the supplier, in %;
NOTE For example 0,99.

m is the weighed mass of furazolidone standard (5.13.15), in mg.

5.14.1.17 Ronidazole

Accurately weigh to the nearest 0,1 mg 10 mg of ronidazole standard (5.13.16) into a 10 ml volumetric flask (6.9). Note down the exact weight of ronidazole. Dissolve in 5 ml ethanol (5.5) and make up to 10 ml volume with ethanol (5.5). Store at −20 °C in amber vials (6.13). Prepare freshly every 3 months.

Determine the accurate concentration of the ronidazole stock standard solution using the reference standard purity value provided by the supplier using Formula (16).

$$C_{\mathrm{RON}} = \frac{m}{10} \times P \quad (16)$$

where

C_{RON} is the concentration of ronidazole in the stock standard solution, in mg/ml;

P is the purity of the ronidazole standard given by the supplier, in %;
NOTE For example 0,99.

m is the weighed mass of ronidazole standard (5.13.16), in mg.