SLOVENSKI STANDARD
SIST-TS CEN/TS 16178:2012

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Blato, obdelani biološki odpadki in tla - Določevanje ostankov farmacevtskih sredstev

Sludge, treated biowaste and soil - Determination of pharmaceutical products

Schlamm, behandelter Bioabfall und Boden - Bestimmung pharmazeutischer Produkte

Boues, bio-déchets traités et sols - Détermination des produits pharmaceutiques

Ta slovenski standard je istoveten z: **CEN/TS 16178:2012**

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13.030.20	Tekoči odpadki. Blato	Liquid wastes. Sludge
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TECHNICAL SPECIFICATION

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English Version

Sludge, treated biowaste and soil - Determination of pharmaceutical products

Boues, bio-déchets traités et sols - Détermination des produits pharmaceutiques

Schlamm, behandelter Bioabfall und Boden - Bestimmung pharmazeutischer Produkte

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Contents

Page

Foreword

This document (CEN/TS 16178:2012) has been prepared by Technical Committee CEN/TC 400 “Project Committee - Horizontal standards in the fields of sludge, biowaste and soil”, the secretariat of which is held by DIN.

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

The preparation of this document by CEN is based on a mandate by the European Commission (Mandate M/330), which assigned the development of standards on sampling and analytical methods for hygienic and biological parameters as well as inorganic and organic determinants, aiming to make these standards applicable to sludge, treated biowaste and soil as far as this is technically feasible.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to announce this Technical Specification: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Introduction

Drugs absorbed by the organism after intake are subject to metabolic reactions, such as hydroxylation or cleavage. However, a significant amount of the original or metabolised substance leaves the organism via urine or faeces. The contamination concerns ground and surface water, but also waste water and the solid matrices like sludge or soils.

Due to their polarity, persistence and water solubility, some drugs and metabolites are able to pass through the wastewater treatment plants. Their low adsorption on sludge and soils may cause the contamination of surface and ground water. It is therefore necessary to analyse these molecules through an analytical SPELC-MS/MS method as presented in this document. The method allows the identification of twelve molecules belonging to the four predominant therapeutic classes in France: analgesics/anti-inflammatories, lipid regulators, beta-blocker and anti-epileptics.

WARNING — Persons using this Technical Specification should be familiar with usual laboratory practice. This Technical Specification does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted according to this Technical Specification be carried out by suitably trained staff.

1 Scope

This Technical Specification specifies a method to analyse pharmaceutical compounds in sludge, treated biowaste and soil. Pharmaceuticals analysis has been carried out on a LC/MS-MS quantum. The main difficulty for analysis is the lack of sample certified for target analytes. Even with spiked solid matrices it is still delicate to correctly verify the impact of extraction step, because it is not commutable to a real sample.

This document provides a final protocol on the extraction and purification tested on sludge, soils and sediments spiked with the pharmaceutical compounds listed in Table 1.

Table 1 — Details to the pharmaceutical compounds of interest

Steroid hormones	CAS-RN[a]	Purity %	Temperature preservation	Formula	Molar mass g/mol	Abbreviation
Estrone	53-16-7	> 99	25°C	$C_{18}H_{22}O_2$	270,4	E1
17*β*-Estradiol	50-28-2	> 98	25°C	$C_{18}H_{24}O_2$	272,4	E2
17*α*-Estradiol	57-9	99	25°C	$C_{18}H_{24}O_2$	272,4	α-E2
Ethinylestradiol	57-63-6	> 98	25°C	$C_{20}H_{24}O_2$	296,4	EE2
Estrone d4 Estrone-2,4,16,16-d4	53866-34-5	> 95	25°C	$C_{18}H_{18}O_2D_4$	274,4	E1 d4
Estradiol d5 17b-Estradiol-2,4,16,16,17-d5	221093-45-4	> 98	25°C	$C_{18}H_{19}O_2D_5$	277,4	E2 d5
Ethinylestradiol d4 17*α*-Ethinylestradiol-2,4,16,16-d4	350820-06	> 98	25°C	$C_{20}H_{20}O_2D_4$	300,4	EE2 d4

[a] Chemical Abstracts Service Registry Number

2 Principle

After pretreatment the freeze-dried sample is extracted by ultrasonication with an appropriate solvent. Then the extract is purified on a suitable cartridge. The extract is analysed by high-performance liquid chromatography (HPLC) on a C_{18} column and detected by mass spectrometry.

The identification is based on the retention times of the analytes and on the mass-spectrometric detection. The detection is conducted in the MS/MS-mode in order to avoid interferences and over-quantification.

3 Reagents

3.1 Chemicals

Solvents used for extraction and clean-up shall be of HPLC grade or equivalent quality and checked for blanks.

3.1.1 Water, H_2O.

3.1.2 Methanol, CH_3OH.

3.1.3 Acetonitrile, C_2H_3N.

3.1.4 Ammonia, NH_3.

3.2 Pharmaceutical standards and internal standards for calibration

Pharmaceutical standards shall be of analytical grade (> 90 %).

For example, analysis can be undertaken with compounds given in Table 2.

Table 2 — Proposed pharmaceuticals for use as internal standards

Therapeutic group	Compound	CAS-RN
Analgesics/anti-inflammatories	Paracetamol	103-90-2
	Paracetamol-d4	Not available
	Diclofenac	15307-79-6
	Diclofenac-d4	Not available
	Ketoprofen	22071-15-4
	Naproxen	Not available
	Ibuprofen	15687-27-1
	Phenazone	60-80-0
	Phenazone-d3	65566-62-3
Beta-blocker	Metoprolol	56392-17-7
	Propanolol	318-98-9
	Propanolol-d7	344298-99-3
Anti-epileptic	Carbamazepine	298-46-4
	Primidone	125-33-7
Lipid regulator	Bezafibrate	49562-28-9
	Gemfibrozil	25812-30-0
	Gemfibrozil-d6	25812-30-0

NOTE It is preferable to get one internal standard per molecule. If it is not possible, the recovery should be checked.

3.3 Preparation of stock solutions

3.3.1 Solution 1

Prepare a stock solution containing all pharmaceuticals at 100 mg/l in methanol (3.1.2) (solution 1). Store this solution in dark glass bottles at –20 °C no longer than one month.

3.3.2 Solution 2

Prepare all deuterium-labelled substances at a concentration of 100 mg/l in methanol (3.1.2) (solution 2). Store this solution in dark glass bottles at –20 °C.

3.4 Preparation of working solutions

Prepare the working solutions by dilution of stock solutions (3.3). Working solutions shall be prepared on the day of analysis.

NOTE 1 Some pharmaceutical compounds deteriorate in solution in less than 24 h [3].

Prepare working solution at 1 mg/l (solution 3) by dilution of solution 1 (3.3.1) in methanol (3.1.2)

Prepare a mix of the internal standards at 100 µg/l (solution 4) by dilution of solution 2 (3.3.2) in a water:methanol mixture (95:5).

Prepare calibration standards with appropriate amounts of the working solutions. For example, concentrations for the calibration should be between 5 ng/ml and 500 ng/ml and concentrations for internal standards should be 100 ng/ml.

A point of control is necessary to follow the performance of the chromatographic system. Prepare this solution at 1 mg/l (solution 5) by dilution of solution 1 in methanol (3.1.2).

NOTE 2 That allows the comparison of the value of internal standard in the control sample with the value of internal standard in the test sample and to notice some possible losses. A low recovery of internal standard does not provide for a good calculation of the compounds in the test sample.

4 Apparatus

4.1 Sonicator.

4.2 Centrifuge.

4.3 Solid Phase Extraction Vacuum Manifolds.

4.4 Evaporator under nitrogen.

4.5 Chromatographic separation.

4.6 High performance liquid chromatograph (HPLC), consisting of an autosampler, LC pump and a column oven.

Preferably work with a C_{18} column with a C_{18} guard column. Maintain the column temperature at 25 °C.

For example, adjust the flow-rate to 0,2 ml/min and the injection volume to 35 µl.

For example, the dimensions of the C_{18} column are 150 mm (length), 2,1 mm (internal diameter), 5 µm (film thickness). The dimensions of the C_{18} guard column are: 10 mm (length), 2,1 mm (internal diameter), 5 µm (film thickness).

Optimize the chromatographic conditions in order to allow for good separation and to avoid co-elution between compounds.

4.7 Mass spectrometric detection

The detection is carried out using a tandem mass spectrometer.

The electrospray ionisation (ESI) mode is chosen to analyse the pharmaceutical compounds.

The selected reaction monitoring (SRM) mode is chosen for quantification. It allows avoiding interferences.

For example, fix the spray voltage at 3 500 V, set the temperature of the ESI heater at 350 °C and the pressure in the collision cell at 1,5 mTorr (199 983 Pa).

5 Sample pretreatment

Store the samples in a dark place at a temperature below 10 °C, if possible in a refrigerator.

NOTE Freeze-dried samples, if kept sealed, may be stored for a longer period at room temperature.

Before extraction, the sample is frozen, lyophilised and ground at 0,2 mm. Then the dry material is kept at room temperature in amber bottle until analysis.

6 Extraction and clean-up

6.1 Extraction of a dry sample

— Weigh 1 g of sample.

— Add 20 ml of acetonitrile (3.1.3) with 0,1 % ammonia (3.1.4).

— Add the internal standard and spike with the mix of pharmaceutical compounds in the medium of expected range.

— Put the flask in the sonicator and extract for 15 min.

— Put the flask in the centrifuge and centrifuge for 10 min at 3 600 *g*.

— Transfer the liquid phase into a tube and repeat the same operation twice.

If more sample material is used, adjust the quantity of solvents. This modification shall be verified and tested.

6.2 Concentration

The total volume of extract (around 60 ml) is collected in a tube and is then evaporated by applying a gentle stream of nitrogen at room temperature until around 4 ml to 5 ml remain.

6.3 Clean-up

Add 100 ml of water (3.1.1) to the extract (see 6.2). Then filter the mixture and adjust to pH < 7 before purification.

Carry out clean-up using cartridges 1 and 2 (cartridge 1 is above cartridge 2). The two cartridges are conditioned and after loading the extract, remove cartridge 1 and rinse cartridge 2 with different solvents. Then cartridge 2 is dried under nitrogen during approximately 30 min. The pharmaceutical compounds are then desorbed by adding 8 ml of methanol (3.1.2). Reduce the extract to dryness under nitrogen and reconstitute in 1 ml of water:methanol mixture (95:5) prior to LC-MS/MS analysis.

7 Procedure

7.1 Blanks

7.1.1 Injection blank

The injection blank is necessary to verify the chromatographic system. The background noise shall be controlled and it shall be less than 30 % of the limit of quantification. It allows verifying there are no interferences with calibrated compounds.

7.1.2 Extraction blank

The extraction blank is obtained by following the protocol with solvents only. The value of the extraction blank shall be less than 50 % of the limit of quantification.

7.2 Calibration

All the working solutions shall be prepared freshly by dilution of stock solutions.

NOTE It was observed that some of the molecules can deteriorate in solution in less than 24 h.

Calibration standards are prepared with appropriate amounts of the working solutions (solutions 3 and 4) to achieve correct concentrations.

For example, for the range from 5 ng/ml to 500 ng/ml the quantity of internal standard can be 100 ng.

7.3 Control solution

The control solution (solution 5) is prepared by dilution of stock solution 1 in methanol for the pharmaceuticals. The value of the points of control can be located in the 20 % and 80 % of the range.

7.4 Analysis

The background noise shall be verified. Check samples by calculating the control ratio which is defined by Formula (1):

$$R = \frac{A_{\text{is,s}}}{A_{\text{ic}}} \tag{1}$$

where

R is the control ratio;