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Soils, sludges and treated biowaste — Determination of pharmaceutical products

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Foreword

This document (BT/TF151 WI CSS99043:2007) has been prepared by Technical Committee CEN/TC BT/TF 151 “Horizontal”, the secretariat of which is held by DS.

This document is a working document.

This document has been developed in the framework of the project 'Horizontal-ORG' based on the results of a desk study. Numerous established test methods published in literature, available CEN and ISO standards and various guidelines from official bodies have been reviewed, compiled and described in this study. After discussion with the project partners and other interested parties the draft-standard has been developed as an modular horizontal method.

This European standard is applicable for the determination of pharmaceuticals in soil, sludge and treated biowaste.

Introduction

This standard is developed in the European project 'HORIZONTAL'. It is the result of a desk study " Selected drugs in solid matrices: A review of environmental occurrence, determination and properties of principal substances"(26), which aimed at evaluating the latest developments in assessing pharmaceuticals in sludge, soil, treated biowaste and neighbouring fields. After an evaluation study, in which e.g. the ruggedness of the method was studies, a European wide validation of the draft standard has taken place. The results of the desk studies as well as the evaluation and validation studies have been subject to discussions with all parties concerned in CEN. The standard is part of a modular horizontal approach in which the standard belongs to the analytical step.

Until now test methods determining properties of materials were often prepared in Technical Committees (TCs) working on specific products or specific sectors. In those test methods often steps as sampling, extraction, release or other processing, analyses, etc were included. In this approach it was necessary to develop, edit and validate similar procedural steps over and over again for every material or product. Consequently this has resulted in duplication of work. To avoid such duplication of work for parts of a testing procedure references to parts of test methods from other TCs were introduced. However the following problems are often encountered while using references in this way: 1) The referenced parts are often not edited in a way that they could easily be referred to, 2) the referenced parts are often not validated for the other type of material and 3) the updates of such test standards on products might lead to inadequate references.

In the growing amount of product and sector oriented test methods it was recognised that many steps in test procedures are or could be used in test procedures for many products, materials and sectors. It was supposed that, by careful determination of these steps and selection of specific questions within these steps, elements of the test procedure could be described in a way that can be used for all materials and products or for all materials and products with certain specifications.

Based on this hypothesis a horizontal modular approach is being investigated and developed in the project 'Horizontal'. 'Horizontal' means that the methods can be used for a wide range of materials and products with certain properties. 'Modular' means that a test standard developed in this approach concerns a specific step in assessing a property and not the whole ” chain of measurement” (from sampling to analyses). A beneficial feature of this approach is that “modules” can be replaced by better ones without jeopardizing the standard “chain”.

The use of modular horizontal standards implies the drawing of test schemes as well. Before executing a test on a certain material or product to determine certain characteristics it is necessary to draw up a protocol in which the adequate modules are selected and together form the basis for the test procedure.

The modules that relates to this standard are specified in section XX Normative references.

An overview of modules and the manner, in which modules are selected will be worked out later, at which time proper reference in this standard will be provided.

Drugs absorbed by the organism after intake are subject to metabolic reactions, such 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 wasted 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. So it is necessary to analyse these molecules and this is why an analytical method by SPELC-MS/MS is presented here allowing to search 12 molecules belonging to the four predominant

therapeutic classes in France which are analgesics/anti-inflammatories, lipid regulators, betablockers and anti-epileptics.

1 Scope

The purpose of this report is to present the development of an analytical method to analyse pharmaceutical compounds in solid matrices. Pharmaceuticals analysis has been carried out on a LC/MS-MS quantum. The main difficulty in this project is the lack of sample certified in researched analytes. Even with spiked solid matrices it is still delicate to verify correctly the impact of extraction step because it does not reproduce a real sample.

What is proposed here is an analytical method on pharmaceuticals products on sludge, soils and sediments.

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

2 Principle

After pre-treatment the sample (freeze-dried) is extracted by ultrasonication with an appropriated solvent. Then the extract is purified on a suitable cartridge. The extract is analyzed by high performance liquid chromatography (HPLC) on a C18 column and detected by mass spectrometry.

The identification is based on the retention times of the analytes and on the MS-detection. The detection is made with the mode MS/MS in order to avoid interferences and the problem of overquantification.

3 Normative references

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text, and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies (including amendments).

CSS99031 Sludge, treated biowaste, and soils in the landscape – Sampling – Framework for the preparation and application of a sampling plan

CSS99058 Sludge, treated biowaste, and soils in the landscape – Sampling – Part 1: Guidance on selection and application of criteria for sampling under various conditions

CSS99057 Sludge, treated biowaste, and soils in the landscape – Sampling – Part 2: Guidance on sampling techniques

CSS99032 Sludge, treated biowaste, and soils in the landscape – Sampling - Part 3: Guidance on sub-sampling in the field

CSS99059 Sludge, treated biowaste, and soils in the landscape – Sampling – Part 4: Guidance on procedures for sample packaging, storage, preservation, transport and delivery

CSS99060 Sludge, treated biowaste, and soils in the landscape – Sampling – Part 5: Guidance on the process of defining the sampling plan

CSS99035 Soil, sludge and treated biowaste – Pre-treatment for organic characterisation

4 Reagents

4.1 Chemicals

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

3.1.1 Water

3.1.2 Methanol

3.1.3 Acetonitrile

4.2 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 1.

Table 1 — Proposed pharmaceuticals for use as internal standards

Therapeutic group	**Compound**	**CAS-RN**
Analgesics/antiinflammatories	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
Betablockers	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. Yet if it is not possible the recovery should be checked.

4.3 Preparation of stock solutions

4.3.1 Solution 1

Prepare 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 for one month at maximum.

4.3.2 Solution 2

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

4.4 Working solutions preparation

Prepare the working solutions by dilution of stock solutions (3.3) each time that extractions have to be done.

NOTE Some pharmaceutical compounds can be deteriorated in solution in less than 24 h [1].

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

Prepare a mix of the internal standards at 100 µg/l (solution 4) by dilution of solution 2 (3.3.2) in a water (3.1.1)/methanol (3.1.2) mixture (95/5).

Prepare calibration standards with appropriate amounts of the working solutions. For example concentrations for the calibration can be between 5 ng/ml and 500 ng/ml and concentrations for internal standards can 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 That allows to compare 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.

5 Apparatus

4.1 Ultrasonication tank

4.2 Centrifuge

4.3 Visiprep

4.4 Evaporator under nitrogen

4.5 Chromatographic separation

High performance liquid chromatograph (HPLC) consisting of an autosampler, LC pump and a column oven. Preferably work with a C18 column with a C18 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 C18 column are 150 mm * 2,1 mm, 5 µm and the dimensions of the C18 guard column are 10 mm * 2,1 mm, 5 µm.

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

4.6 Mass spectrometry detection

The detection is carried out using a tandem mass spectrometer.

The mode ESI 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).

6 Sampling and sample pre-treatment

6.1 Sampling

Sampling should be carried out in accordance with EN yyyy.

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.

6.2 Sample pre-treatment

Before extraction, the sludge is freezed, lyophilised and grinded at 0,2 mm. Then the dry material is kept at room temperature in amber bottle until analysis.

7 Extraction and clean-up

7.1 Extraction of a dry sample

All types of solid matrices (sludge, soil, sediments and composts) are extracted following the same protocol.

— Take 1 g of sample.

— Add 20 ml of acetonitrile (3.1.3) with 0,1 % NH_3.

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

— Put the flask in the sonication cuve and extract for 15 min.

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

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

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

7.2 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.

7.3 Clean-up

Add 100 ml of water (3.1.1) to the extract (see 6.2). Then filter the mixture and adjust to acidic pH 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 is 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.

8 Analysis

8.1 Blanks

8.1.1 Injection blank

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.

8.1.2 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.

8.2 Calibration

All the working solutions have to be prepared each time. They have prepared by dilution of stock solutions. Indeed it was observed that some of the molecules can be deteriorated in solution in less than 24 h so the calibration shall be done each time [2].

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.

8.3 Control solution

The control solution (solution 5) is prepared by dilution of stocked 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.

8.4 Analysis

The background noise shall be verified. Control samples by calculating the control ratio which is defined as follows :

Control ratio R = area of internal standard in the sample/area of internal standard in the point of control.

The targeted value is 1 ± 0,4.

If the control ratio is not in the defined range the operator shall verify areas of internal standards in the sample or in points of control in order to examine the origin of the problem.

9 Calculation and expression of results

9.1 Calibration

From the chromatograms of the calibration standards obtain a calibration curve by plotting the ratio of the mass concentrations against the ratio of the peak areas using equation (1):

$$\frac{A_c}{A_{is,c}} = s \times \frac{\rho_c}{\rho_{is,c}} + b \quad (1)$$

Where:

A_c is the response of analyte in the calibration standard;

$A_{is,\,c}$ is the response of internal standard in the calibration standard;

s is the slope of the calibration function;

ρ_c is the mass concentration of analyte in the calibration standard solution in micrograms per millilitre (µg/ml);

$\rho_{is,\,c}$ is the mass concentration of internal standard in the calibration standard solution in micrograms per millilitre (µg/ml);

b is the intercept of the calibration curve with the ordinate.

9.2 Calculation

From the chromatograms of the samples calculate the mass concentrations of the analytes from the calibration curve using equation (2):

$$\omega_s = \frac{\left(A_s / A_{is,\,s}\right) - b}{s \times m} \quad (2)$$

Where:

ω_s is the concentration of analyte found in the pre-treated sample in milligrams per kilogram (mg/kg) of freeze-dried sample;

A_s is the response of analyte in the sample;

$A_{is,\,s}$ is the response of internal standard in the sample;

b is the intercept of the calibration curve with the ordinate;

m is the mass of test sample used for extraction in grams (g).

10 Precision data

Precision data see Annex D.

11 Test report

The test report shall contain the following information:

a) reference to this European Technical Specification including its date of publication;

b) sampling report including precise identification of the sample;

c) pre-treatment report;

d) analytical results;

e) recoveries of the individual internal standards;

f) any deviation from this document, and any facts which may have influenced the result. Where the test is not carried out in accordance with this document, reference may only be made to CEN/TS xxxx in the report in case all deviations from the procedures prescribed in this document are indicated in the report stating the reason for deviation.

Annex A
(informative)

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Bibliography

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