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Soil quality — Determination of selected explosives and related compounds —

Part 3: Method using liquid chromatography- tandem mass spectrometry (LC-MS/ MS)

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*Qualité du sol — Dosage d'une sélection d'explosifs et de composés
apparentés* — 11916-3

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*Partie 3: Méthode utilisant la chromatographie en phase liquide
couplée à la spectrométrie de masse en tandem (CL-SM/SM)*

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 3, *Chemical and physical characterization*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Currently two ISO standards exist for the analysis of explosives and related compounds in soil: ISO 11916-1 (HPLC with UV detection method), ISO 11916-2 (GC-ECD or MS). According to the results of inter-laboratory trial with ISO 11916-1, it showed some problematic aspects to analyze PETN, 1,3,5-TNB and tetryl. In case of ISO 11916-2, it also gave poor inter-laboratory trial results for 1,3,5-TNB. Therefore, it is necessary to develop new method effectively applicable to the determination of PETN, 1,3,5-TNB and tetryl. In addition to this, lower risk-based PRGs (Preliminary Remediation Goal), new regulatory concerns, and change of land use have created the atmosphere to apply more sensitive and selective instruments to determine explosive and related compounds. From the view of these aspects, liquid chromatography–tandem mass spectrometry (LC-MS/MS) is one of alternative methods for these purposes. LC-MS/MS method provides 10-20 times or more lower detection limit than that of HPLC/UV method. In this document, LC-MS/MS method is intended for the trace analysis of explosives and related compounds and applicable to 12 compounds (1,3-DNB, 1,3,5-TNB, 2,4-DNT, 2,6-DNT, 2,4,6-TNT, 4-A-2,6-DNT, 2-A-4,6-DNT, Tetryl, Hexyl, RDX, HMX, PETN) listed in ISO 11916-1 (soil, HPLC with UV detection method) except for nitrobenzene, 2-nitrotoluene, 3-nitrotoluene and 4-nitrotoluene. In case of nitrobenzene and nitrotoluenes, they have the low sensitivity in LC-MS/MS measurement than using HPLC with UV detection method. In particular LC-MS/MS measurement is effective for the analysis of PETN, 1,3,5-TNB and tetryl when comparing with the method using HPLC with UV detection method. Also LC-MS/MS method is getting more familiar in ISO standard development (e.g. ISO 22104 Water quality-Microcystins, ISO/NP 21677 Water quality-HBCD, ISO 21675 Water quality-PFAS).

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Soil quality — Determination of selected explosives and related compounds —

Part 3: Method using liquid chromatography-tandem mass spectrometry (LC-MS/MS)

1 Scope

This document specifies the measurement of explosives and related nitrocompounds (as given in [Table 1](#)) in soil and soil materials. This document is intended for the trace analysis of explosives and related compounds by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Generally, LC-MS/MS measurement shows the lower LOQ (limit of quantification) for each compound in [Table 1](#) than using high-performance liquid chromatography (HPLC) with UV-detection.

Under the conditions specified in this document, concentrations as low as 0,005 mg/kg to 0,014 mg/kg-dry matter can be determined, depending on the substance. Similar compounds, in particular various nitroaromatics, by-products and degradation products of explosive compounds may be analysed using this method. However, the applicability should be checked on a case-by-case basis.

Table 1 — Explosive and related nitrocompounds for analysis

Compound	ISO/FDIS 11916-3	Abbreviation	CAS-RN ^a
1,3-Dinitrobenzene	https://standards.iteh.ai/catalog/standards/sist/93141265-3608-4334-9d7d-8662614311c5/iso-fdis-11916-3	1,3-DNB	99-65-0
1,3,5-Trinitrobenzene		1,3,5-TNB	99-35-4
2,4-Dinitrotoluene		2,4-DNT	121-14-2
2,6-Dinitrotoluene		2,6-DNT	606-20-2
2,4,6-Trinitrotoluene		2,4,6-TNT	118-96-7
4-Amino-2,6-dinitrotoluene		4-A-2,6-DNT	19406-51-6
2-Amino-4,6-dinitrotoluene		2-A-4,6-DNT	35572-78-2
<i>N</i> -Methyl- <i>N</i> -2,4,6-tetranitroaniline		Tetryl	479-45-8
2,4,6-Trinitro- <i>N</i> -(2,4,6-trinitrophenyl)aniline		Hexyl	131-73-7
1,3,5-Trinitrohexahydro-1,3,5-triazine		RDX	121-82-4
1,3,5,7-Tetranitro-octahydro-1,3,5,7-tetrazocine		HMX	2691-41-0
Pentaerythrityl tetranitrate		PETN	78-11-5

^a CAS-RN: Chemical Abstract Service-Registry Number

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.

ISO 11465, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

3 Terms and definitions

No terms and definitions are listed in this document.

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

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>

4 Principle

Explosive materials in soils are extracted with acetonitrile by using one of the following techniques:

- ultrasonic bath with ultrasonic waves as medium (USE);
- horizontal mechanical shaker at room temperature (MSE).

There are two further extraction procedures such as pressurized liquid extraction (PLE) and soxhlet apparatus that works isothermally at boiling temperature (SOX). However, they might not be suitable for PETN, tetryl and 1,3,5-TNB.

The extract containing the analytes is either injected directly, or if necessary diluted prior to injection, into a reversed-phase high-performance liquid chromatograph–tandem mass spectrometer (LC-MS/MS).

WARNING — Take care when transporting, storing or treating explosive materials. High temperature, high pressure and static electricity shall be prevented when storing explosive materials. Small amounts of explosive materials should be kept moist in a cool, dark place. Soil samples containing explosives with a mass fraction of less than 1 % do not have a risk of explosion.

5 Interferences

Solvents, reagents, glassware, and other hardware used for sample processing can yield artifacts and/or elevated baselines, causing misinterpretation of the chromatograms. All of these materials shall therefore be demonstrated to be free of contaminants and interferences through the analysis of method blanks.

Samples containing 2,4,6-trinitrobenzoic acid should not be extracted with acetonitrile as it can result in the overestimation of 1,3,5-TNB due to decarboxylation. To avoid this interference, methanol extraction can be an alternative method for 1,3,5-TNB.

When comparing with acetonitrile, methanol has showed serious problem in the recovery test for HMX, RDX and tetryl with LC-MS/MS detection (refer to [Annex D](#)). Also, it should be avoided to extract tetryl at temperatures above room temperature.

6 Reagents

6.1 General

All reagents shall be blank-free and of recognized analytical grade.

6.2 Chemicals

6.2.1 Water, with an electrical conductivity of $\leq 0,01$ mS/m (25 °C).

6.2.2 Acetonitrile, CH₃CN, HPLC grade or equivalent.

6.2.3 Methanol, CH₃OH, HPLC grade or equivalent.

6.2.4 Ammonium acetate in water, 2,5 mmol/l.

For the preparation, weigh 96,3 mg of ammonium acetate(C₂H₇NO₂) into 500 ml measuring flasks (scale: mg/ml), fill up to the mark with water (6.2.1). Prepare the reagent just before it is used. Before using as a mobile phase, filter the reagent using filter paper (7.1.6). After filtration, degas the filtrate using ultrasonic bath or other methods.

6.2.5 Ammonium acetate in methanol, 2,5 mmol/l.

For the preparation, weigh 96,3 mg of ammonium acetate(C₂H₇NO₂) into 500 ml measuring flasks (scale: mg/ml), fill up to the mark with methanol (6.2.3). Prepare the reagent just before it is used. Before using as a mobile phase, filter the reagent using filter paper (7.1.6). After filtration, degas the filtrate using ultrasonic bath or other methods.

6.3 Standard substances and solutions

6.3.1 Standard substances

6.3.1.1 Reference substances

Reference substances are listed in [Table 1](#).

6.3.1.2 Method-checking standards

Suitable compound(s) not found in the sample (i.e. 2,5-dinitrotoluene or 1,2-dinitrobenzene) can be used as method-checking standards. It is recommended that the concentration of method-checking standards in the final extract is ranged from 0,04 mg/l to 0,1 mg/l. Before selecting the method-checking standards, confirm the applicability of those standards according to the analytical conditions of each laboratory.

6.3.2 Standard solutions

6.3.2.1 General

All standard solutions used in this method shall be prepared as described below.

If commercially available certified standard stock solutions are used, calibration solutions are prepared in volumetric flasks by diluting the stock solutions with acetonitrile (6.2.2).

All dilution steps shall not exceed the factor 100.

6.3.2.2 Single-substance stock solutions

For the preparation, weigh 50 mg ± 0,1 mg of the reference substances into 50 ml measuring flasks (scale: mg/ml), fill up to the mark with acetonitrile (6.2.2) and let the reference substances dissolve completely.

Transfer the stock solutions to amber-glass flasks and seal with polytetrafluoroethylene (PTFE)-coated screw caps. The stock solutions can be kept in the refrigerator at 2 °C to 6 °C in the dark for up to 6 months.

6.3.2.3 Multi-component stock solutions

Prepare multi-component stock solutions of different concentrations from the various single-substance stock solutions (6.3.2.2) by mixing and diluting with acetonitrile (6.2.2). At concentrations below 1 mg/ml, solutions should be checked after one week as reference substances can decompose.

6.3.2.4 Calibration standard solutions

Calibration standard solutions are prepared by the dilution of multi-component stock solutions. The working range of 0,01 mg/l to 0,2 mg/l is recommendable. A minimum of 5 concentration levels is needed for the calibration.

7 Apparatus

7.1 General

Usual laboratory apparatus and the followings are used for this standard.

7.1.1 Amber glass containers with caps containing PTFE coated lining.

7.1.2 Amber glass vials with caps containing septa with PTFE coated lining.

7.1.3 Amber glass conical bottles with ground-in stopper.

7.1.4 Analytical balance, with a precision of at least 0,1 mg.

7.1.5 Laboratory centrifuge, capable of producing an acceleration of at least 1 000 *g*.

7.1.6 Membrane filter, 0,45 µm pore size.

Any adsorption of the target compounds shall be avoided. No interfering material shall be eluted. PTFE, polyamide or an equivalent material is recommended.

7.2 Equipment for extraction

7.2.1 Temperature-controlled ultrasonic bath, 35 Hz, effective HF-power of at least 140 W.

Water bath capable of maintaining the temperature at (30 ± 5) °C or at (50 ± 5) °C during ultrasonic extraction.

7.2.2 Horizontal mechanical shaker.

The shaker shall maintain a frequency of 100 cycles/min and offer a shaking width of about 10 cm.

7.3 Liquid chromatograph-tandem mass spectrometer (LC-MS/MS)

7.3.1 LC system, consisting of a pump that supports a pressure of at least 40 MPa (400 bar) and an injection system with an appropriate loop capacity depending on injection volume.

7.3.1.1 LC-Column

Temperature-controlled columns packed with reversed phase material. The stainless column (25 cm x 2,1 mm~4,6 mm internal diameter) filled with silica gel (particle diameter, 5 µm) chemically

bonded with octadecylsilyl (ODS) group or those with an equivalent separating ability should be used. If the applicability is verified, other types of column can be used.

For verification purposes, where applicable, repeat the chromatographic separation using a column of different selectivity; CN reversed-phase column or phenyl-hexyl reversed-phase column are recommended.

7.3.1.2 Mobile phase

A solution made by mixing 2,5 mmol/l ammonium acetate in water (6.2.4) with 2,5 mmol/l ammonium acetate in methanol (6.2.5) can be used as a mobile phase. The ratio of mixture depends on the LC system to be applied (refer to Annex A).

7.3.2 Tandem mass spectrometer

The LC-MS/MS system should be capable of negative ion atmospheric pressure chemical Ionization (APCI). Use a triple quadrupole tandem mass analyser (MS/MS) consisting of two successive quadrupole mass analysers or a system with at least equivalent performance as a mass spectrometer. Also, multiple reaction monitoring (MRM) mode should be available for mass analysis.

8 Procedure

8.1 Sample pre-treatment, sample storage and determination of water content

While taking a field-moist sample, remove coarse impurities, e.g. plant residues and stones. Put the sample in an amber glass flask and store immediately in a cool, dark transport container.

Soil samples shall be analysed as soon as possible. Before analysing the sample, homogenize the sample through a sieve with an aperture of 2 mm.

Soil samples shall be stored in a dark place at (4 ± 2) °C. Samples that are stored for longer periods (i.e. > than 1 week) prior to analysis, shall be stored at -20 °C.

In order to calculate the dry matter based content of explosive compounds, determine the dry matter content of the field-moist soil in accordance with ISO 11465. Be aware of potential evaporation of volatile toxic contaminants.

8.2 Extraction

8.2.1 General

For extraction, the following two methods can be applied:

- extraction using ultrasonic waves;
- extraction using mechanical shaking.

The use of a method-checking standard is recommended. Method-checking standards have to be added prior to extraction. For the selection of suitable method-checking standards, refer to 6.3.1.2.

8.2.2 Extraction using ultrasonic waves

Take approximately 20 g of the field-moist sample and weigh it into the extraction container (7.1.1) with a precision of $\pm 0,1$ g and add the method-checking standard (6.3.1.2), if used, with a concentration range of 0,04 mg/l to 0,1 mg/l in the final extract. Add 40 ml \pm 0,1 ml of acetonitrile (6.2.2) and seal with a cap containing a PTFE coated lining. Shake the vial briefly by hand, and then apply ultrasonic extraction in the bath (7.2.1) for 16 h at (30 ± 5) °C or 4 h at (50 ± 5) °C. During extraction, the water

level in the bath should be at least 1 cm above the level of the solvent inside the extraction flasks. This extraction method is not be proper for tetryl.

After applying ultrasonic extraction, allow the soil particles to settle for 30 min. Do not open the vial before it has cooled down to room temperature. If necessary, filter an aliquot of the supernatant using a 0,45 µm PTFE or polyamide filter (7.1.6) into the vial (7.1.2) or centrifuge at 1 000 *g* for 20 min.

It is recommended to lightly moisten the filter with acetonitrile prior to filtration.

The total volume of the extract corresponds to the volume of solvent used for extraction plus the water content of the soil sample.

8.2.3 Extraction using mechanical shaking

Take approximately 20 g of the field-moist sample and weigh it into the extraction container (7.1.1) with a precision of ±0,1 g and add the method-checking standard (6.3.1.2), if used, with a concentration range of 0,04 mg/l to 0,1 mg/l in the final extract. Add 40 ml ± 0,1 ml of acetonitrile (6.2.2) and seal with a cap containing a PTFE coated lining. Shake the vial briefly by hand, then place the extraction vial in a horizontal mechanical shaker (7.2.2) and shake it for 16 h. After shaking, allow the soil particles to settle in the container for 30 min. If necessary, filter an aliquot of the supernatant using a 0,45 µm PTFE or polyamide filter (7.1.6) into the vial (7.1.2) or centrifuge at 1 000 *g* for 20 min.

It is recommended to lightly moisten the syringe filter with solvent prior to filtration.

The total volume of the extract corresponds to the volume of solvent used for extraction plus the water content of the soil sample.

8.3 Storage of extract

If the acetonitrilic extract cannot be analysed immediately, it shall be stored in a refrigerator at (4 ± 2) °C in the dark. In case of precipitation, ensure that the precipitate is re-dissolved before analysis, i.e. through ultrasonication.

9 Liquid chromatography tandem mass spectrometry (LC-MS/MS)

9.1 General

The separation of analytes is performed by means of high-performance liquid chromatography with a suitable reversed-phase column. For detection a tandem mass spectrometer is used.

Atmospheric pressure chemical ionization (APCI) in negative mode should be used as the ionizing method. The ionization is induced outside the heated tube by a corona discharge needle, in the orbit from the heated tube to the entrance of the mass spectrometer (MS). The tandem quadrupole type of MS/MS or a system with at least equivalent performance as a mass spectrometer is applicable to this method. The MRM for quantitative analysis is desirable. HPLC and mass spectrometer conditions are shown in [Annex A](#).

9.2 Identification and quantification

The compounds are identified by MRM transition. Full scan analysis of the different explosives and related compounds are acquired in the first quadrupole (Q1) scanning mode in the mass range of 100 to 600 amu in order to find precursor ions for MS–MS experiments.

Precursor ions are selected in the first quadrupole (Q1) and fragmented in the second quadrupole (Q2), which plays a role as a collision cell using nitrogen gas. In the third quadrupole (Q3) the product ions are detected using the MRM mode.