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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)

Qualité du sol — Dosage d'une sélection d'explosifs et de composés apparentés —

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

Reference number
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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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 3, *Chemical and physical characterization*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 444, *Environmental characterization of solid matrices,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

A list of all parts in the ISO 11916 series can be found on the ISO website.

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 (see Annex E). 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).

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 (see Annex B and Annex C).

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 can be analysed using this method provided that the applicability is checked on a case-by-case basis.

Table 1 — Explosive and related nitrocompounds for analysis

Compound	Abbreviation	CAS-RN®[1]
1,3-Dinitrobenzene	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
N-Methyl-*N*-2,4,6-tetranitroaniline	Tetryl	479-45-8
2,4,6-Trinitro-*N*-(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

[1] CAS Registry Number® (CAS RN®) is a trademark of CAS corporation. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

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 terminology databases for use in standardization at the following addresses:

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

— IEC Electropedia: available at https://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 isothermically 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 (see 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 ≤ 0,01 mS/m (25 °C).

6.2.2 Acetonitrile, CH_3CN, HPLC grade or equivalent.

6.2.3 Methanol, CH_3OH, 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 ($C_2H_7NO_2$) 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 ($C_2H_7NO_2$) 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 ±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, 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 amu 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.

Only measuring signals within the working range can be quantified. If signals exceed the uppermost calibration mark, the respective extract is measured again after dilution of the sample. In this case, it is recommended not to exceed a higher dilution ratio of 1:100 per dilution step.

9.3 Calibration

The calibration is carried out by the external standard method.

The calibration solutions are prepared as described in 6.3.2. Take care to inject the same volume for calibration as for the sample measurement.

The initial calibration serves to establish the linear working range of the calibration curve. This calibration is performed when the method is used for the first time and after maintenance and/or repair of the equipment.

The validity of the calibration shall be checked at the start and at the end of every sample series by a standard solution (6.3.2.4), consisting of compounds that represent the retention range and substance polarities of all target analytes. The validity should additionally be proved after a maximum of 20 samples per series.

The recalibration has to cover the desired working range and is performed using a standard solution, whenever the validity checks show a deviation greater than 20 % from the expected concentration.

One feature of the resulting working range is the linear correlation between concentration and measured signal; this correlation shall be ensured for each analyte through at least five concentration levels. Working ranges exceeding the concentration decade are allowed as long as they are linear.

The pairs of varieties y_{ie} and c_{ie} obtained from the calibrating solutions for the reference substances i are displayed graphically and visually tested for linear dependence. If this is fulfilled, the best-fit line is calculated through linear regression using Formula (1).

$$y_{ie} = a_i \times c_{ie} + b_i \tag{1}$$

where

y_{ie} is the measuring value (signal area or height, respectively) of the substance i depending on c_{ie} (unit depends on evaluation);

c_{ie} is the mass concentration of the substance *i* in acetonitrilic solution, respectively (calibrating solution), in µg/ml;

a_i is the slope of the reference line for substance *i*, unit, for example, measuring value, in ml/µg;

b_i is the intercept on the ordinate of the reference line, same unit as measuring value, b_i can have positive and negative values.

Signals (peak area or peak height) are quantified by means of the calibrating function, see Formula (1).

10 Calculation of results

After LC-MS/MS analysis (Clause 8), calculate the concentration of the target compounds in soil sample using Formula (2).

The mass concentration c_{is} of the analyte *i* in the solid sample is

$$c_{is} = c_i \times V_T \times \frac{100}{m_f \times W_{dm}} \times f_{dil} \tag{2}$$

where

c_{is} is the mass concentration of the substance *i* in the solid sample, mg/kg dm;

c_i is the mass concentration of the substance *i* in the extract (calibrating standard or sample extract), µg/ml;

m_f is the mass of the soil used for extraction, g;

V_T is the total volume of the extract
[volume of solvent used for extraction]+[1 ml/g × m_f ×(100 – W_{dm})/100], ml;

W_{dm} is the dry matter (dm) content of the soil sample, %;

f_{dil} is the dilution factor of the extract.

11 Quality assurance/quality control (QA/QC)

To demonstrate the validity of the procedure, the use of a suitable method-checking standard (e.g. 2,5- DNT or other suitable compounds not found in the sample) is recommended. If it is not possible to choose a suitable standard, the efficiency of the extraction has to be checked frequently with the reference material, if available.

The procedures for the calculation of the method-checking standard recovery limit are as follows: first, calculate the average percent recovery (*p*) and the standard deviation of the percent recovery (*s*) for each of the method- checking standards after the analysis of 15 to 20 field samples. Then, calculate the upper and lower control limits for each of the method-checking standard according to the following formula.

Upper control limit = *p* + 3s

Lower control limit = *p* - 3s

Thus, the range of method-checking standard recovery limits is from the upper control limit to the lower control limit.

The method-checking standard recovery should be in the range of the method-checking standard recovery limit.

The recovery rate of each single compound shall be determined, for example, by spiking samples with other similar matrices parallel to the analysis of real samples or by performing the method of standard addition.

The determined extraction recoveries are not included in the calculation of the final result. Therefore, the constant control of the method recovery utilizing the entire analytical process, including all manual steps, has to be performed periodically. Reference materials are an adequate tool to carry out such experiments. The overall recovery rate shall be between 80 % and 110 % for each compound.

A mathematical correction of the measured values of the samples in the case of lower recoveries is not permitted.

To prove the absence of contaminants during the procedure, include at least one blank analysis per series. Monitor the accessibility of the lower limit of the working range by an adequate standard solution with every sample series. The signal-to-noise ratio of the resulting signals for each compound shall be greater than or equal to 10, otherwise maintenance at the LC-MS/MS system (e.g. replacing or flushing of the column) should be performed.

The peak symmetry (T = b/a; measured at 10 % of peak height) should not exceed the value of 2 at any time for all analytes in order to allow proper integration and quantification.

12 Expression of results

Due to the measurement uncertainty, no more than two digits of the analytical result are significant. However, if the analytical results are included in further calculations, the results are rounded to three digits (max.).

13 Test report

This test report shall contain at least the following information:

a) the test method used, together with a reference to this document, i.e. ISO 11916-3:2021;

b) all information necessary for complete identification of the sample;

c) the results of the determination according to Clause 9;

d) any details not specified in this document or that are optional, as well as any other factors that may have affected the result.

Annex A
(informative)

Conditions of high performance liquid chromatography tandem mass spectrometry (LC-MS/MS)

An example of LC-MS/MS conditions for the determination of selected explosives and related compounds are shown in Table A.1, Table A.2 and Table A.3. Table A.1 and Table A.2 is for HPLC conditions and MS conditions respectively. The precursor ion and multiple reaction monitoring (MRM) transition of each compound for quantitation are shown in Table A.3.

Table A.1 — Example of the conditions for HPLC analysis

LC Conditions	Column	Inertsil ODS-SP, 4,6 × 250 mm, 5 µm			
	Column temperature	20 °C			
	Injection volume	10 µl			
	Mobile phase	Eluent A: water +2,5 mmol/l ammonium acetate Eluent B: methanol +2,5 mmol/l ammonium acetate			
	Flow rate	0,8 ml/min			
	Gradient	Step	Time (min)	A (%)	B (%)
		0	0	42	58
		1	15	42	58
		2	18	0	100
		3	20	0	100
		4	20,1	42	58
		5	25	42	58
	Post time	3 min			
	Total run time	25 min			

Table A.2 — Example for tandem mass spectrometer experimental conditions

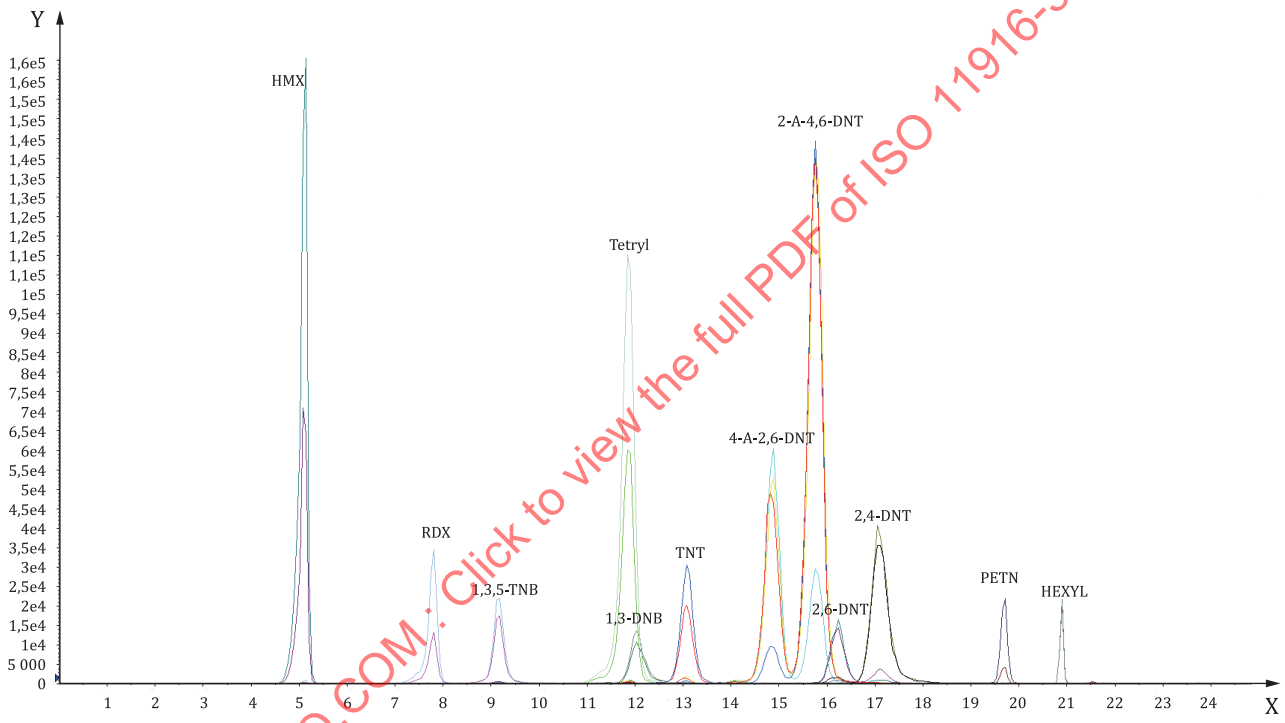
MS conditions	Ionization mode	APCI - negative
	Gas temperature	350 °C
	Pressure of curtain gas	20 psi
	Pressure of nebulizer gas	35 psi
	Pressure of drying gas	40 psi
	Ion spray voltage (IS)	5 500 V (positive) – 4 500 V (negative)

Table A.3 — Precursor ion and MRM transition selected for each compound

Compound	Precursor ion* (m/z)	Detection mode	MRM Transition
TNT	226	APCI-negative	226/196 226/46
1,3-DNB	168	APCI-negative	168/138 168/46
1,3,5-TNB	213	APCI-negative	213/183 213/95
2,6-DNT	182	APCI-negative	182/152 182/46
* Precursor ions listed above are not fixed number and could be changed according to the analysis conditions.			

Table A.3 *(continued)*

Compound	Precursor ion* (m/z)	Detection mode	MRM Transition
4-A-2,6-DNT	196	APCI-negative	196/119 196/46
Tetryl	241	APCI-negative	241/213 241/196
PETN	375	APCI-negative	375/62 375/46
2,4-DNT	181	APCI-negative	181/135 181/46
2-A-4,6-DNT	196	APCI-negative	196/136 196/46
HEXYL	438	APCI-negative	438/226 438/362
RDX	281	APCI-negative	281/59 281/46
HMX	355	APCI-negative	355/147 355/46
* Precursor ions listed above are not fixed number and could be changed according to the analysis conditions.			



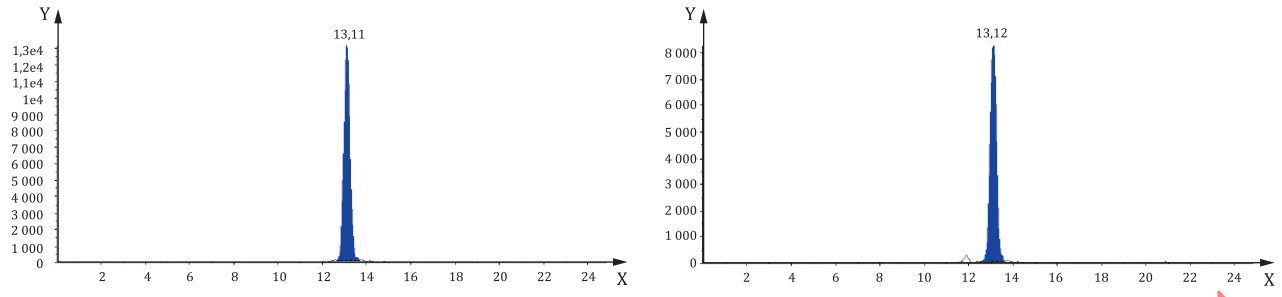
Key

X time, min

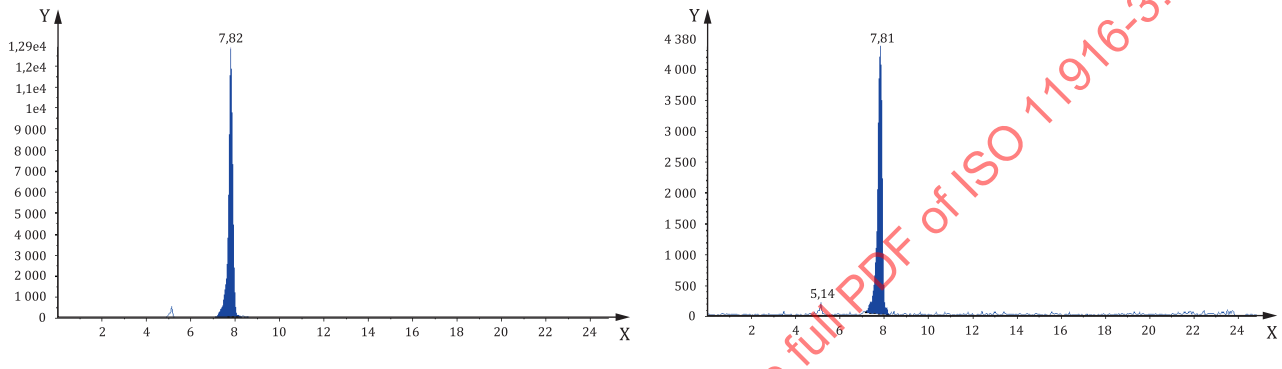
Y intensity, cps

Figure A.1 — Extracted ion chromatogram of 12 explosives using LC-MS/MS

TNT (left : m/z 226/46 right : m/z 226/196)



RDX (left : m/z 281/46 right : m/z 281/59)



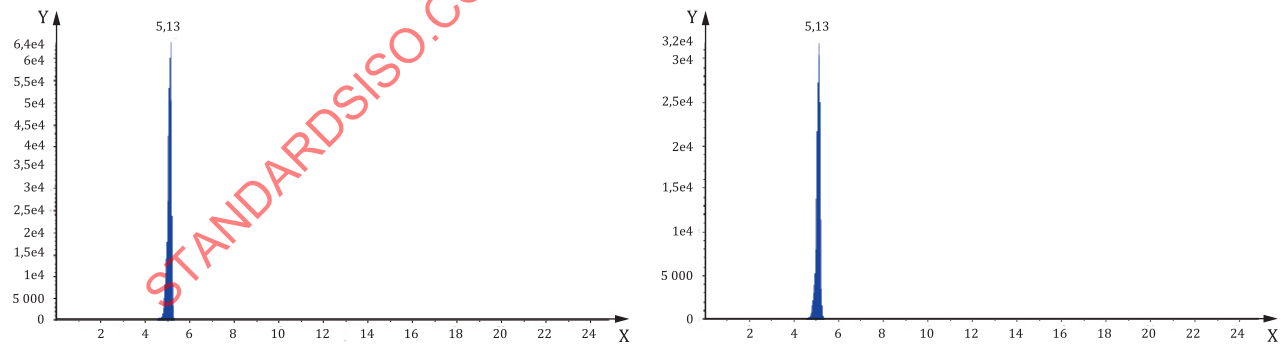
Key

X time, min

Y intensity, cps

Figure A.2 — Representative chromatograms of quantitation MRM

HMX (left : m/z 281/46 right : m/z 281/59)



Key

X time, min

Y intensity, cps

Figure A.3 — continued

Annex B
(informative)

Comparison of LC-MS and LC-MS/MS application for PETN, 1,3,5-TNB and tetryl

B.1 Descriptions of experiment

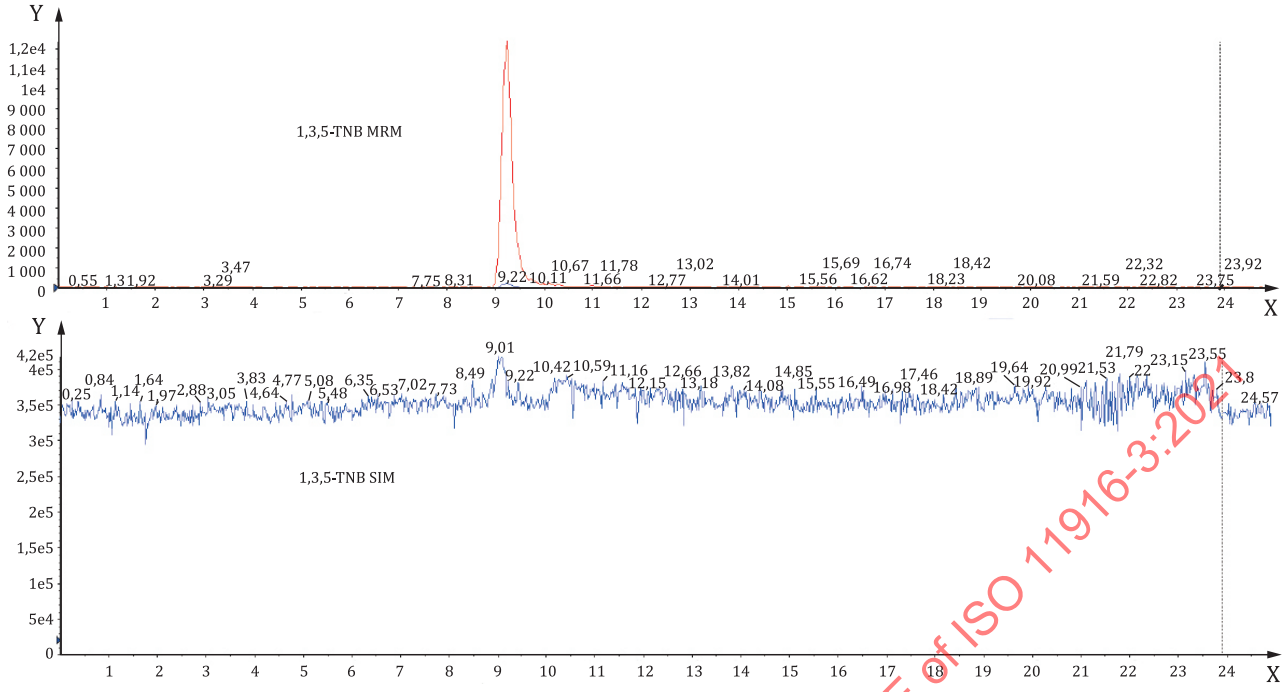
To compare the effectiveness of LC-MS and LC-MS/MS for determining PETN, 1,3,5-TNB and tetryl, 0,2 mg/l of standard solutions were measured. Analytical conditions of LC-MS/MS are the same as shown in Table A.1, Table A.2 and Table A.3. MRM (multi reaction monitoring) mode was applied in LC-MS/MS whereas SIM (selected ion monitoring) mode was applied in LC/MS (Table B.1). Except for mass analysis methods, other conditions including HPLC were the same in both methods.

Table B.1 — Conditions for mass analysis

Compound	Precursor ion (m/z)	Detection mode	MRM Transition	CE (Collision energy)
1,3,5-TNB	213	APCI-negative	213/183 213/95	-43/-44
Tetryl	241	APCI-negative	241/213 241/196	-15/-26
PETN	375	APCI-negative	375/62 375/46	-27/-20

B.2 Ion chromatogram of LC-MS (SIM) and LC-MS/MS (MRM)

Ion chromatogram of LC-MS (SIM) and LC-MS/MS (MRM) are shown as below. From the view of qualification of 1,3,5-TNB, tetryl and PETN, LC-MS/MS showed much better performance than HPLC.

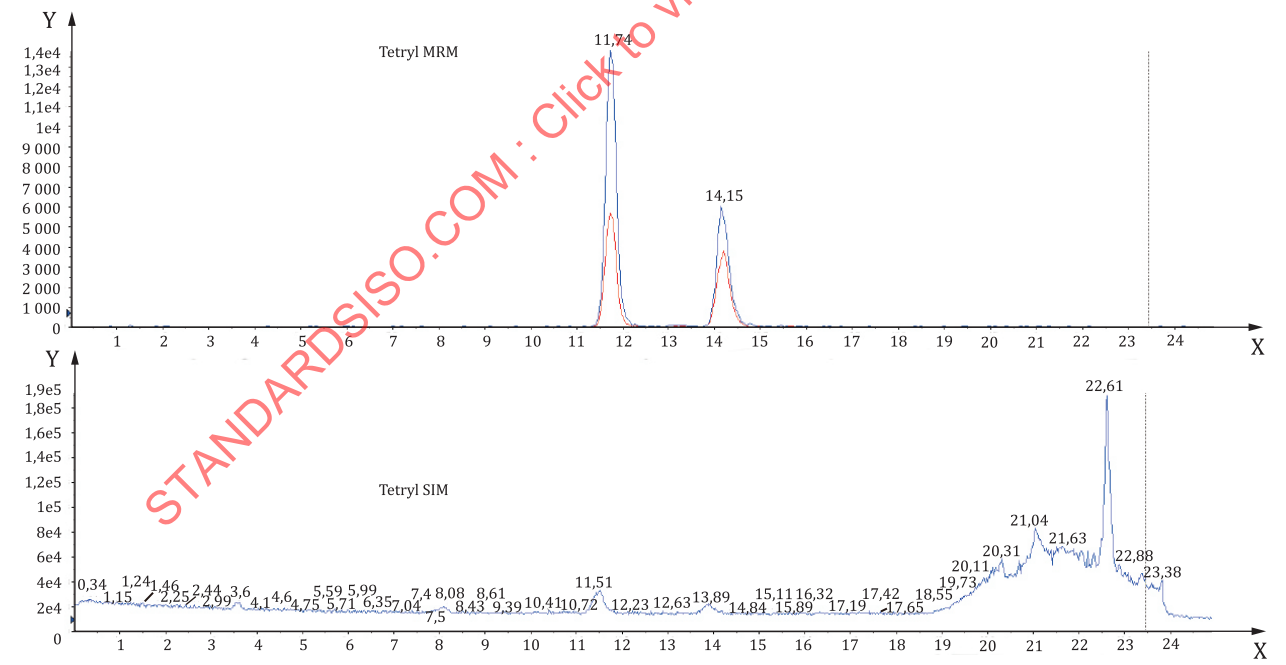


Key

X time, min

Y intensity, cps

Figure B.1 — 1,3,5-TNB (MRM 213/183 213/95, SIM 213)

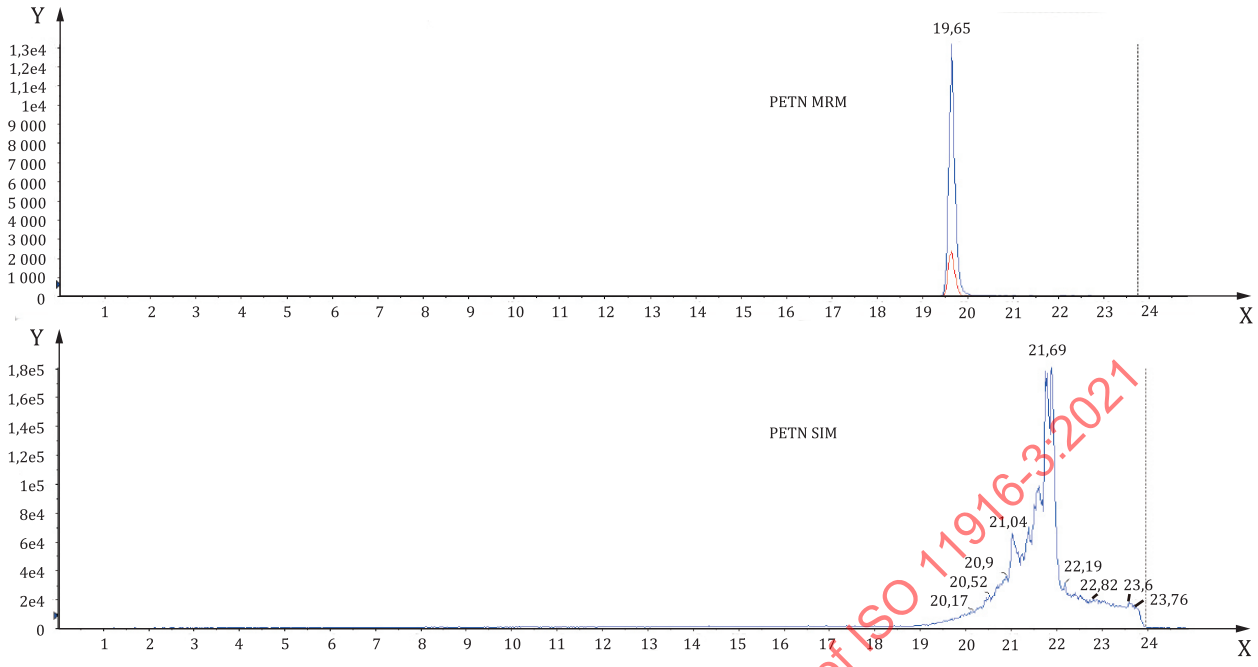


Key

X time, min

Y intensity, cps

Figure B.2 — Tetryl (MRM 241/213 241/196, SIM 241)



Key

X time, min

Y intensity, cps

Figure B.3 — PETN (MRM 375/62 375/46, SIM 375)

Annex C
(informative)

Comparison of LOD and LOQ in the measurement of HPLC and LC-MS/MS

As for HPLC method, 0,2 mg/kg of matrix spiked soil samples were prepared to determine LOD and LOQ. In case of LC-MS/MS method, 0,01 mg/kg of matrix spiked soil samples were prepared for the same purpose. The differences between the concentrations of matrix spiked soil samples for each test came from the different signal to noise (S/N) ratio of each method.

Analytical conditions of LC-MS/MS are the same as shown in Table A.1, Table A.2 and Table A.3.

Analytical conditions of HPLC are shown in Table C.1.

Table C.1 — Analytical conditions of HPLC

<table>
<tr><td rowspan="4">LC Conditions</td><td>Column</td><td colspan="5">C18 reversed phase HPLC column, 110A (150 m × 4,6 mm, 3 µm)</td></tr>
<tr><td>Column temperature</td><td colspan="5">30 °C</td></tr>
<tr><td>Injection volume</td><td colspan="5">10 µl</td></tr>
<tr><td>Mobile phase</td><td colspan="5">Eluent A: Distilled water
Eluent B: Isopropanol: Acetonitrile = 60:40</td></tr>
<tr><td>Gradient</td><td colspan="5"><table>
<tr><th>Time (min)</th><th>Flow rate (ml/min)</th><th>A (%)</th><th>B (%)</th></tr>
<tr><td>0</td><td>0,6</td><td>70</td><td>30</td></tr>
<tr><td>14</td><td>0,6</td><td>70</td><td>30</td></tr>
<tr><td>14,1</td><td>0,5</td><td>70</td><td>30</td></tr>
<tr><td>37</td><td>0,6</td><td>70</td><td>30</td></tr>
<tr><td>40</td><td>0,6</td><td>70</td><td>30</td></tr>
</table>Total run time: 40 min
Post time: 3 min</td></tr>
</table>

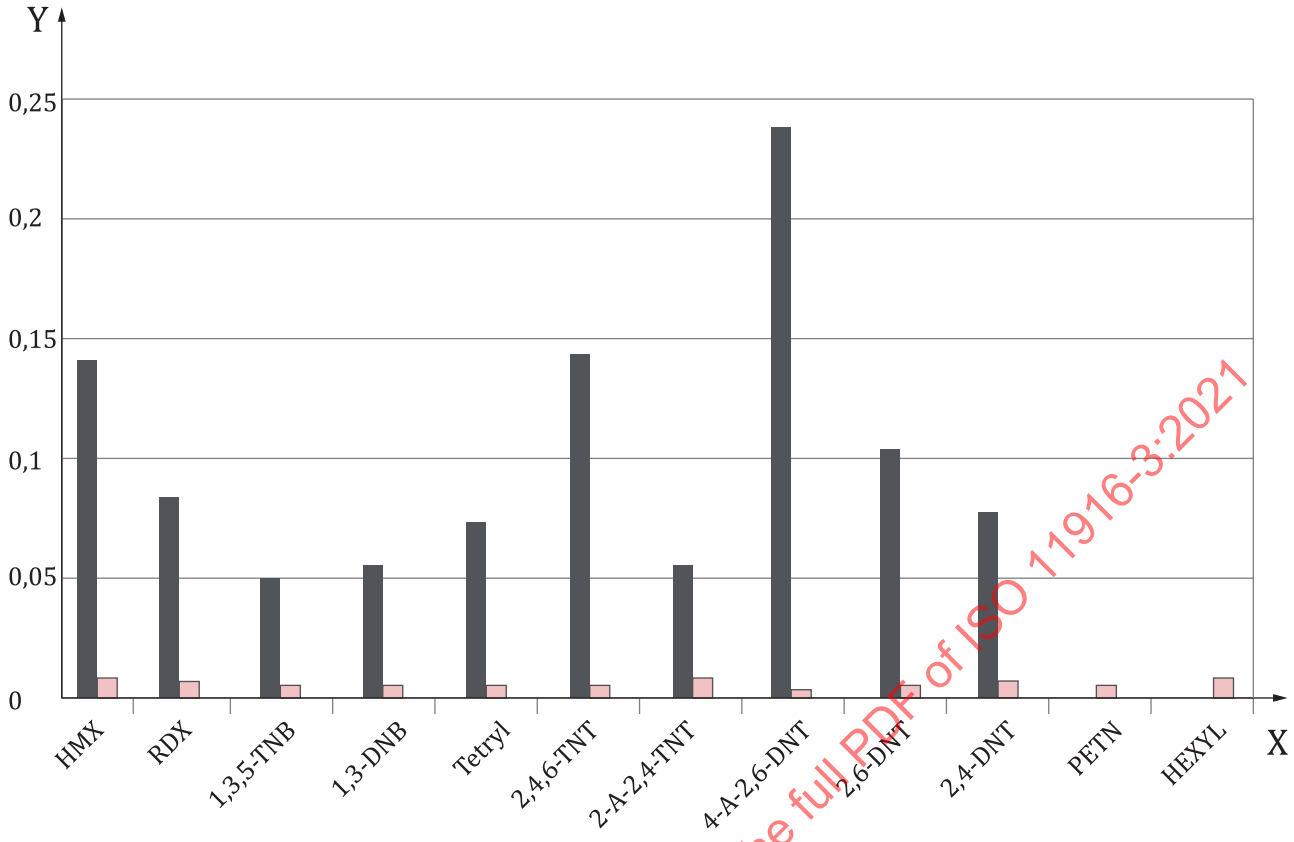
To summary the results of comparison, LOQ of the LC/MS-MS method is 6 to 47 times lower than that of the HPLC method, which depends on the compounds (Table C.2, Table C.3, Figure C.1). PETN and hexyl could not be determined in this test.

Table C.2 — LOD and LOQ according to HPLC method

Method		HMX	RDX	1,3,5-TNB	1,3-DNB	Tetryl	2,4,6-TNT	2-A-2,4-DNT	4-A-2,6-DNT	2,6-DNT	2,4-DNT
		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
HPLC	#1	0,194	0,205	0,190	0,197	0,191	0,215	0,186	0,204	0,187	0,190
	#2	0,205	0,212	0,193	0,193	0,169	0,178	0,193	0,188	0,276	0,182
	#3	0,220	0,220	0,193	0,201	0,188	0,217	0,192	0,190	0,202	0,203
	#4	0,209	0,209	0,198	0,203	0,178	0,191	0,189	0,197	0,205	0,205
	#5	0,217	0,210	0,183	0,201	0,180	0, 193	0,188	0,192	0,203	0,192
	#6	0,179	0,196	0,186	0,197	0,181	0,201	0,183	0,141	0,196	0,192
	#7	0,213	0,198	0,192	0,186	0,188	0,213	0,176	0,153	0,200	0,199
	SD	0,014	0,008	0,005	0,006	0,008	0,014	0,006	0,024	0,010	0,008
	LOD	0,043	0,025	0,015	0,018	0,023	0,043	0,018	0,071	0,031	0,024
	LOQ	0,142	0,082	0,049	0,059	0,075	0,144	0,060	0,237	0,103	0,081

Table C.3 — LOD and LOQ according to LC-MS/MS method

Method		HMX	RDX	1,3,5-TNB	1,3-DNB	Tetryl	2,4,6-TNT	2-A-2,4-DNT	4-A-2,6-DNT	2,6-DNT	2,4-DNT	PETN	HEXYL
		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
LC-MS/MS	#1	0,010	0,011	0,010	0,010	0,009	0,009	0,010	0,009	0,010	0,009	0,009	0,008
	#2	0,010	0,011	0,010	0,009	0,009	0,010	0,009	0,010	0,010	0,009	0,010	0,011
	#3	0,010	0,009	0,010	0,009	0,009	0,009	0,010	0,010	0,009	0,008	0,011	0,008
	#4	0,009	0,010	0,011	0,009	0,009	0,009	0,010	0,010	0,008	0,009	0,009	0,010
	#5	0,009	0,010	0,012	0,010	0,009	0,010	0,009	0,009	0,009	0,009	0,010	0,008
	#6	0,007	0,008	0,010	0,008	0,007	0,008	0,008	0,008	0,008	0,008	0,009	0,008
	#7	0,010	0,009	0,009	0,009	0,009	0,010	0,007	0,010	0,008	0,006	0,008	0,007
	SD	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001
	LOD	0,004	0,003	0,003	0,002	0,003	0,002	0,003	0,002	0,003	0,003	0,003	0,004
	LOQ	0,013	0,010	0,008	0,008	0,009	0,008	0,012	0,005	0,009	0,012	0,009	0,014



Key

Y	LOQ (mg/kg)
	HPLC
	LC-MS/MS

Figure C.1 — Comparison of LOQ between HPLC and LC-MS/MS method