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Water quality — Determination of certain explosives and related compounds — Method using high-performance liquid chromatography (HPLC) with UV detection

Qualité de l'eau — Dosage de certains explosifs et de composés apparentés — Méthode utilisant la chromatographie en phase liquide à haute performance (CLHP) avec détection UV

Reference number
ISO 22478:2006(E)

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Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 22478 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

Introduction

Explosives and related compounds are frequently encountered in groundwater areas near to soil sites contaminated by armaments waste and may also be found in drinking water taken from nearby catchment areas. The range of pollutants will depend on the waste concerned, but will not, as a rule, include all the compounds listed in Table 1. Instead, samples of groundwater containing such pollutants may contain numerous other substances, such as nitro- and dinitrobenzoic acid, nitrophenols and aromatic amines. The compounds listed in Table 1 are frequently used for exploratory examinations of armaments waste.

When using this International Standard, it may be necessary in some cases to determine whether and to what extent particular problems will require the specification of additional conditions.

Water quality — Determination of certain explosives and related compounds — Method using high-performance liquid chromatography (HPLC) with UV detection

WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This standard 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 in accordance with this International Standard be carried out by suitably trained staff.

1 Scope

This International Standard specifies a method for determination of certain explosives, in particular nitrotoluenes, nitroamines and nitrate esters, and related compounds (by-products and degradation products), such as those listed in Table 1, in drinking water, groundwater and surface water.

Depending on the type of sample and the compound to be analysed, the lower limit of the working range for nitroaromatics and nitramines can be assumed to be between 0,1 µg/l and 0,5 µg/l (in some cases, the lower limit may be extended down to 0,05 µg/l). The lower limit of the working range for nitrate esters may be assumed to be higher (0,5 µg/l or more).

Similar compounds, in particular other nitroaromatics, may also be determined by this method, but its applicability will have to be checked in each individual case.

2 Normative references

The following referenced documents are indispensable for the application 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 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 5667-1, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes*

ISO 5667-2, *Water quality — Sampling — Part 2: Guidance on sampling techniques*

ISO 5667-3, *Water quality — Sampling — Part 3: Guidance on the preservation and handling of water samples*

3 Principle

The substances in the water sample are concentrated by solid-phase extraction using a polystyrene/divinylbenzene-based adsorbent. After elution with a solvent mixture, the eluate is concentrated and the substances are separated by high-performance liquid chromatography (HPLC) and analysed using a UV photodiode array (UV-PDA) detector.

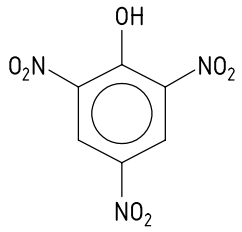
Table 1 — Explosives and related compounds determined by this method (the compounds listed here are particularly likely to be encountered in water samples containing armaments waste)

Name	Abbreviation [a]	Other name	CAS [b] No.	Molar mass	Reference No./ letter in example chromatograms in Clause			
				g/mol	A.1	A.2	A.3	B.1
2,4,6-Trinitrophenol	PA	Picric acid	88-89-1	229,1	1	2	1	a
1,3,5,7-Tetranitro-octahydro-1,3,5,7-tetrazocine	HMX	Octogen	2691-41-0	296,2	2	1	2	b
1,3,5-Trinitro-hexahydro-1,3,5-triazine	RDX	Hexogen	121-82-4	222,1	3	3	4	d
2,2',4,4',6,6'-Hexanitrodiphenylamine	—	Hexyl	131-73-7	439,2	4	19	3	c
Ethylene glycol dinitrate	EGDN	—	628-96-6	152,1	5	4	5	e
Diethylene glycol dinitrate	DEGN	—	693-21-0	196,1	6	5	6	f
1,3,5-Trinitrobenzene	1,3,5-TNB	—	99-35-4	213,1	7	6	7	g
1,3-Dinitrobenzene	1,3-DNB	—	99-65-0	168,1	8	7	9	i
N-Methyl-*N*-2,4,6-tetranitroaniline	CE	Tetryl	479-45-8	287,2	9	8	8	h
Glycerol trinitrate	NG	Nitroglycerine	55-63-0	227,1	10	9	10	j
2,4,6-Trinitrotoluene	2,4,6-TNT	TNT	118-96-7	227,1	11	10	11	k
4-Amino-2,6-dinitrotoluene	4-A-2,6-DNT	—	19406-51-6	197,1	12	11	12	l
2-Amino-4,6-dinitrotoluene	2-A-4,6-DNT	—	35572-78-2	197,1	13	12	13	m
2,6-Dinitrotoluene	2,6-DNT	—	606-20-2	182,1	14	13	14	n
2,4-Dinitrotoluene	2,4-DNT	—	121-14-2	182,1	15	14	15	o
2-Nitrotoluene	2-NT	—	88-72-2	137,1	16	15	17	q
Pentaerythritol tetranitrate	PETN	Nitropenta	78-11-5	316,2	17	18	16	p
4-Nitrotoluene	4-NT	—	99-99-0	137,1	18	16	18	r
3-Nitrotoluene	3-NT	—	99-08-1	137,1	19	17	19	s
Diphenylamine	DPA	—	122-39-4	169,24	20	—	—	—

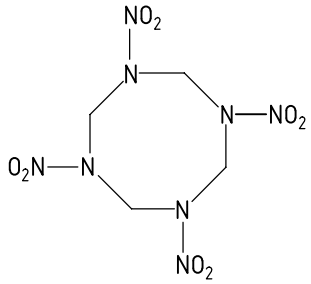
[a] Standard abbreviations, some of Anglo-Saxon origin.

[b] Chemical Abstracts Service.

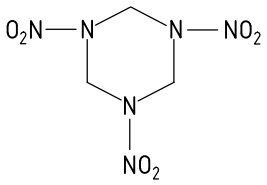
The structural formulae of selected compounds are given in Figure 1.



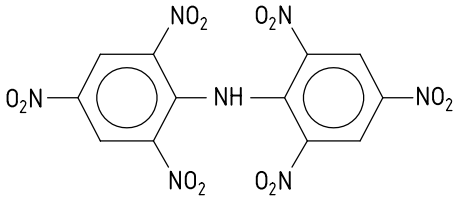
Picric acid (PA)



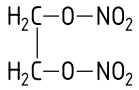
Octogen (HMX)



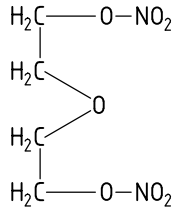
Hexogen (RDX)



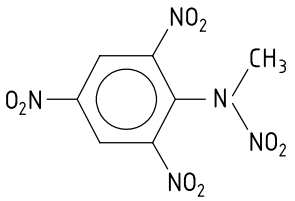
Hexyl



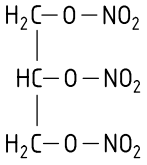
Ethylene glycol dinitrate (EGDN)



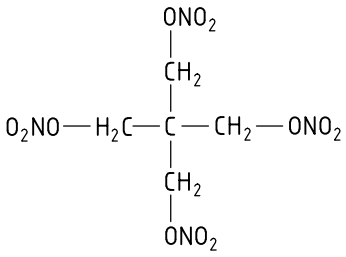
Diethylene glycol dinitrate (DEGN)



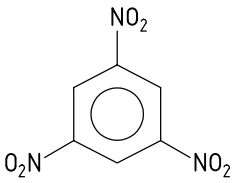
Tetryl (CE)



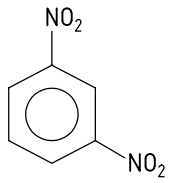
Nitroglycerine (NG)



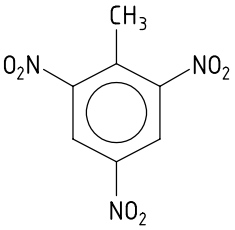
Nitropenta (PETN)



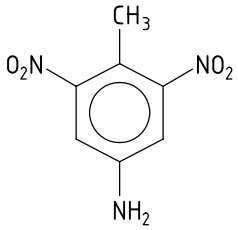
1,3,5-Trinitrobenzene



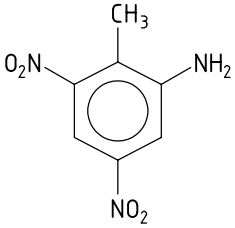
1,3-Dinitrobenzene



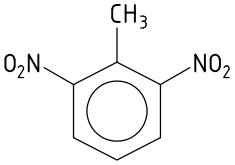
2,4,6-Trinitrotoluene (TNT)



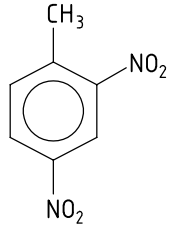
4-Amino-2,6-dinitrotoluene



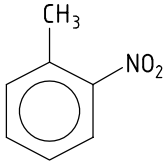
2-Amino-4,6-dinitrotoluene



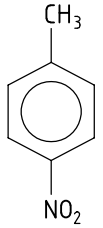
2,6-Dinitrotoluene



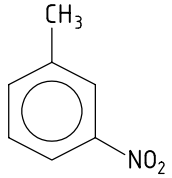
2,4-Dinitrotoluene



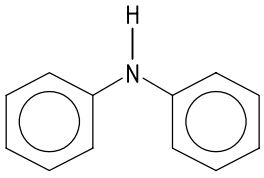
2-Nitrotoluene



4-Nitrotoluene



3-Nitrotoluene



Diphenylamine (DPA)

Figure 1 — Structural formulae of selected explosives

4 Interference

4.1 Sampling

To avoid interference, collect samples as specified in Clause 7, observing the instructions given in ISO 5667-1, ISO 5667-2 and ISO 5667-3.

4.2 Filtration

If the sample contains suspended matter, first filter it through a borosilicate glass fibre filter to prevent blockage of the solid phase concentrating cartridge and record this operation in the test report.

4.3 Concentration by evaporation

Mononitrotoluenes may be lost when the eluates are evaporated as specified in 8.2.

4.4 Chromatography

Asymmetrical peaks and peaks that are wider than normal may indicate incompletely resolved peaks or peak overlapping due to compounds with similar retention times and/or absorption at the same or at a similar wavelength to those of the compounds being determined. To detect peak overlaps, check the peak purity and compare the UV spectrum with that of a reference material.

Interference may also be caused by humic matter eluted in the same range as the analytes being determined.

4.5 Exposure to light

Reference solutions, samples and sample extracts may decompose on exposure to light. This is particularly important in the case of tetryl (see Annex C). Therefore a detailed investigation of the recovery of tetryl should be carried out.

4.6 Determination of hexogen

When determining small concentrations of hexogen, interference may be caused by high concentrations of nitroaromatics.

5 Reagents

The reagents shall not have blank values that would interfere with the HPLC analysis.

If available, use reagents of HPLC grade or residue grade. The solvents shall not contain any measurable UV-absorbing substances that interfere with the method.

5.1 Water, complying with grade 1 as defined in ISO 3696. The water shall not contain any measurable quantities of UV-absorbing substances that interfere with the determination.

5.2 Nitrogen, purity not less than 99,996 % by volume, for drying the adsorbent and, if necessary, for concentrating the eluates.

5.3 Helium, purity not less than 99,996 % by volume, for degassing the eluent if a vacuum degasser is not used.

5.4 Microporous polystyrene/divinylbenzene adsorbent, with a high specific surface area (e.g. greater than 900 m^2/g). Other adsorbents may be used provided that the recovery rate is greater than 85 % for octogen (HMX) at a concentration of 1 µg/l in a 1 l sample.

5.5 Methanol, CH_3OH.

5.6 Acetonitrile, CH_3CN.

5.7 Sodium chloride, NaCl.

5.8 Potassium dihydrogen phosphate solution, $c(KH_2PO_4)$ = 0,025 mol/l (3,40 g/l).

5.9 Orthophosphoric acid, $w(H_3PO_4)$ = 85 %.

5.10 Reference compounds, as listed in Table 1, some of which are obtainable only as commercial solutions with a specified mass concentration.

5.11 Solutions of reference compounds, prepared as follows:

Add methanol (5.5) to 100 mg of the reference compound in a 100 ml volumetric flask, dissolve the reference compound and make up to volume with methanol.

Prepare solutions of hexyl and octogen using acetonitrile (5.6) because of their poor solubility in methanol.

Store the solutions at a maximum of 6 °C, protected from light; their shelf-life is limited (six months at most).

5.12 Stock solutions, prepared as follows:

Transfer 1 ml of each of the solutions of reference compounds (5.11) to a separate 100 ml volumetric flask and make up to volume with methanol (5.5).

Store the solutions at a maximum of 6 °C, protected from light; the solutions are stable for up to six months.

5.13 Reference solutions for multipoint calibration, prepared as follows:

Prior to each calibration, prepare at least five dilutions of each of the stock solutions prepared in 5.12, preferably using a methanol/water mixture (50 + 50 parts by volume) as solvent.

Store the reference solutions at a maximum of 6 °C, protected from light; check their concentrations regularly as their stability is limited.

5.14 Buffer solution, to improve gradient elution of hexyl and picric acid, prepared by adjusting the pH of a 0,025 mol/l solution of potassium dihydrogen phosphate (5.8) to 3,2 using orthophosphoric acid (5.9).

6 Apparatus

Those parts of the apparatus that come into contact with the sample or the extract shall be free of compounds that may produce blank values. The apparatus shall be made of materials that do not adsorb the compounds under investigation and that provide protection from light, e.g. brown glass, stainless steel or perfluorinated plastics.

6.1 Brown-glass narrow-necked flat-bottomed flasks, 1 000 ml.

6.2 Vacuum or pressure equipment, for sample enrichment.

6.3 Glass fibre filter, diameter of fibres 0,75 µm to 1,5 µm, of borosilicate glass containing an inorganic binder.

6.4 Solvent-resistant micromembrane filter, e.g. polyamide or cellulose membrane, pore size of 0,2 µm to 0,45 µm, for filtration of HPLC extracts.

6.5 Glass or polypropene cartridges, packed with a polystyrene/divinylbenzene-based adsorbent (5.4).

6.6 Glass volumetric flasks, 1 ml, 10 ml and 100 ml.

6.7 Graduated cylinder, 100 ml.

6.8 pH-meter, with electrodes.

6.9 Equipment for concentrating the eluates by evaporation, e.g. a rotary evaporator, regulatable for constant vacuum and with a temperature-controlled water bath, or stripping equipment using nitrogen gas.

6.10 Glass vessels, for collecting and concentrating the eluates by evaporation (e.g. tapered flask with cylindrical graduated extension).

6.11 Glass sample bottles, with inert closure (e.g. PTFE-coated septum), for storing the extracts.

6.12 High-performance liquid chromatograph, consisting of:

a) an analytical pump system for isocratic or gradient elution;

b) a manual or automatic sample introduction device;

c) a degassing device;

d) a column thermostat;

e) a photodiode array (PDA) detector with a wavelength range of e.g. 200 nm to 400 nm;

f) a data evaluation system;

g) an analytical column (examples of suitable column types are given in Annex A).

7 Sampling

Fill carefully cleaned brown-glass flat-bottomed flasks with the water to be examined. Store the samples immediately after sampling and during transport at not more than 4 °C, protected from light (e.g. in a cool box). Extract the sample, if possible, within three days after collection since the concentration of explosives and related compounds is more stable in the organic eluates than in the samples. If storage cannot be avoided, keep the sample at about 4 °C in the dark.

Carry out the determination as soon as possible after extraction.

8 Procedure

8.1 Conditioning of adsorbent

Use not less than 0,2 g of polystyrene/divinylbenzene adsorbent (5.4) in a cartridge (6.5) for each 1 000 ml of sample. Add 3 ml of methanol (5.5) over a period of 5 min and wash with 3 ml of acetonitrile (5.6) and then with 10 ml of water. Ensure that the adsorbent does not run dry.

8.2 Concentration and elution

If necessary, filter the sample through a glass fibre filter (6.3) to remove suspended matter.

Take e.g. 1 000 ml of the sample to be examined and dissolve in it about 5 g of sodium chloride (5.7). Pass the sample through the adsorbent conditioned as in 8.1 at a flow rate of up to 1 000 ml/h and then flush with 1 ml of water (5.1). Dry the adsorbent in a stream of nitrogen (5.2) for not less than 10 min.

Elute once with 2 ml of a methanol/acetonitrile solvent mixture (50 + 50 parts by volume) and then once with 3 ml of the same solvent mixture, each time allowing 5 min for the eluent to soak in. Collect the eluate in a glass vessel (6.10) and add 0,5 ml of water as keeper. Carefully evaporate the eluate down to a final volume of about 0,8 ml, e.g. in a stream of nitrogen or on a rotary evaporator (6.9) at reduced pressure (150 hPa to 200 hPa) at 40 °C. Great care is essential to avoid losses of the readily volatile mononitrotoluenes during concentration by evaporation. Make up the concentrate to 1,0 ml with methanol/water mixture (50 + 50 parts by volume). If necessary, filter the extract through a micromembrane filter (6.4), then use an aliquot for the HPLC determination.

8.3 High-performance liquid chromatography (HPLC)

8.3.1 General

Pay due attention to the manufacturer's instructions when operating the HPLC system. Occasionally check the system against the manufacturer's specifications relating to baseline noise and baseline drift and, if they are not met, follow the instructions in the operating manual for detecting and eliminating interference.

When setting up the method and regularly analysing reference solutions, check the performance of the analytical column, observing the manufacturer´s instructions.

8.3.2 Chromatographic separation

Use thermostatted columns packed with reversed-phase materials to separate the compounds listed in Table 1.

If necessary, for validation purposes, repeat the separation using a column with a different selectivity, e.g. a cyanophase column (see example in Annex A).

To improve gradient elution of hexyl and picric acid, use an acid eluent with a pH of 3 to 4 (see 5.14).

8.3.3 Detection

Use a photodiode array (PDA) detector with a wavelength range from 200 nm to 400 nm.

8.3.4 Blank determinations

Check by regular blank tests that the state of the apparatus and the purity of the reagents are satisfactory, determining the background level for the laboratory at least once during a series of analyses. To do this, process and analyse 1 000 ml of water (5.1) in the same way as the sample. If blank values are found, determine the source of contamination by systematic investigations and eliminate it.

8.3.5 Identification of compounds

Use the retention times in the chromatograms of the sample to identify individual compounds. As an identification criterion, the retention time of the compound shall not deviate by more than $\pm$ 2 %, and in any case not more than 10 s, from that of the reference compound measured under the same conditions. For the purpose of this International Standard, a compound shall be deemed to have been identified if the absorption spectrum of the compound coincides with that of the reference compound at the respective retention time in the chromatogram of the reference solution (the explanations and instructions of the spectrometer manufacturer shall be observed).

NOTE Compounds which absorb at the detection wavelengths and have a retention time similar to those of the compounds to be investigated will interfere with the determination. This is more likely to occur when examining samples other than ground water and drinking water. Depending on the quality of resolution, these interfering compounds may lead to unresolved peaks, and these will affect the accuracy of the results. Additionally, unsymmetrical peaks and peaks broader than those obtained with the reference compound are also an indication of interference. Information on the identity and the chromatographic purity of a compound, deduced from its retention time, can be obtained from the absorption spectrum of the compound. The identification can be validated by repeating the separation using a column packing material of different polarity (see 8.3.2) or, for some compounds, by using a mass spectrometer as a detector.

9 Calibration

9.1 General

Correct calibration will require a knowledge of the retention times of the reference compounds. These shall be determined with solutions of the reference compounds under the specified chromatographic conditions.

Design the method of determination such that the measured signal is a linear function of the concentration for every compound to be determined. Match the calibration range to the actual requirements. For example, when examining groundwater, choose a calibration range of 0,5 µg/l to 5 µg/l for the reference compounds.

There are two different procedures for plotting the calibration graphs:

a) calibration of part of the method using external standards (see 9.2);

b) calibration of the complete method using external standards (see 9.3).

For both procedures, prepare the calibration graphs using at least five different concentrations distributed as evenly as possible over the calibration range, if possible carrying out the analyses for all the compounds mentioned in Table 1 in one operation. The calibration graph prepared for a particular compound applies only to the concentration range covered and shall be checked regularly by analysing reference solutions or check samples, depending whether the results are calculated as in 10.1 or 10.2, respectively.

Table 2 gives a key to the subscripts used in the text below.

Table 2 — Key to subscripts used

Subscript	Meaning
i	Compound
e	Quantity measured in calibration
g	Complete method

9.2 Calibration of part of the method using external standards

This calibration applies solely to the HPLC step and shall always be performed since it is needed for determining the recovery rate (see 9.4) of individual compounds in the sample preparation steps and, possibly, for carrying out the method.

To calibrate part of the method using external standards, plot a calibration graph for every compound to be determined by injecting reference solutions of various concentrations (at least five), taking care to inject the same volume for calibration as for measurement. To draw the graph, plot the measured values, y_{ie} (peak area or peak height), for each compound i on the vertical axis and the associated mass concentration, ρ_{ie}, in micrograms per litre, on the horizontal axis. Use the series of measured values thus obtained to establish the linear regression function (1) as follows:

$$y_{ie} = m_i \cdot \rho_{ie} + b_i \tag{1}$$

where

y_{ie} is the measured response for compound i at concentration ρ_{ie} (the units will depend on the method of evaluation, e.g. area units);

m_i is the slope of the best-fit straight line for compound i (equivalent to the response factor for the particular compound), e.g. in area units × litres per microgram (l/µg);

ρ_{ie} is the mass concentration of substance i in the working reference solution, in micrograms per litre (µg/l);

b_i is the intercept of the best-fit straight line for substance i on the vertical axis, in units which will depend on the method of evaluation, e.g. area units.

When analysing samples, check the validity of this calibration regularly by analysing reference solutions during a series of measurements. Retention times can also be updated during this operation. If the measured values deviate from the associated nominal values by no more than 10 %, the original calibration is still valid and can still be used. However, if any of the measured values are greater, it may be necessary to prepare fresh reference solutions and, if the result is confirmed, recalibration will be necessary.

NOTE Under the specified chromatographic conditions, calibration using an external standard usually remains valid over a fairly long period of time. Variations are frequently due to changes in the concentrations of the reference solutions or malfunctioning of the HPLC equipment.

9.3 Calibration of the complete method using external standards

Before carrying out calibration of the complete method, determine and, if necessary, optimize the mean recovery rate of each compound in the sample (see 9.4).

To calibrate the complete method, spike at least five samples of water (5.1) with different concentrations of the compound to be determined, e.g. add 1 ml of the reference solutions (5.13) concerned to 1 l of water. Analyse the spiked water as specified in Clause 8.

Draw a calibration graph for each compound to be determined by plotting the measured values, y_{ieg} (peak area or peak height), for each compound i on the vertical axis and the associated mass concentration, ρ_{ieg}, in micrograms per millilitre, on the horizontal axis and determine the best-fit straight line from the pairs of values, y_{ieg} and ρ_{ieg}, by linear regression using Equation (2):

$$y_{ieg} = m_{ig} \cdot \rho_{ieg} + b_{ig} \tag{2}$$

where

y_{ieg} is the measured response for substance i at concentration ρ_{ieg} (the units will depend on the method of calculation, e.g. area units);

m_{ig} is the slope of the best-fit straight line for substance i, e.g. in area units × litres per microgram (l/µg);

ρ_{ieg} is the mass concentration of substance i in the spiked water, in micrograms per litre (µg/l);

b_{ig} is the intercept of the best-fit straight line for substance i on the vertical axis, in units which will depend on the method of calculation, e.g. area units.

Check the complete method regularly or as in the determination of the recovery rate (see 9.4) by analysing test samples during a series of analyses, particularly if changes occur in sample processing, e.g. if a new batch of adsorbent is used.

The recovery rate, A_i, is used in conjunction with the basic calibration and shall be checked regularly. If the measured recovery rates agree with the mean recovery rate, $\overline{A}_i$, to within the within-laboratory variance, the calibration can be assumed to be stable and A_i can be used. If this is not the case, redetermine A_i (see 9.4).

9.4 Determination of recovery rates

Reliable recovery rate values are obtained if the recovery rate is determined at various concentration levels evenly distributed over the working range, these levels preferably being the same as in the calibration of part of the method using external standards. The value determined for the mean recovery rate, $\overline{A}_i$, of a compound can be used for calibration of the complete method.

To determine the mean recovery rate of a compound, spike samples of water (5.1) with the compound in question by adding e.g. 1 ml of the appropriate reference solution to 1 l of water and analysing the solution as specified in Clause 8.

Drinking water which does not give a blank value may be used instead of grade 1 water (5.1) to determine the recovery rate.

Using the calibration graph prepared in 9.2, calculate the recovery rate at each concentration from Equation (3) and then the mean recovery rate, $\overline{A}_i$, from Equation (4):

$$A_{i,N} = \frac{\rho_{i,N\text{rec}}}{\rho_{i,N\text{def}}} \tag{3}$$

where

$A_{i,N}$ is the recovery rate of compound i at concentration N;

$\rho_{i,N\text{rec}}$ is the recovered mass concentration of compound i at concentration N, calculated using Equation (1), in micrograms per litre (µg/l);

$\rho_{i,N\text{def}}$ is the given mass concentration of compound i at concentration N, in micrograms per litre (µg/l).

$$\overline{A}_i = \frac{\sum A_{i,N}}{n} \tag{4}$$

where

$\overline{A}_i$ is the mean recovery rate of compound i;

$A_{i,N}$ is as in Equation (3);

n is the number of individual measured values of A_i.

NOTE With the ratio of the mass of adsorbent to sample volume specified in 8.1, a high recovery rate is usually obtained for the extraction step. Low or variable values for the recovery rate indicate effects due to differences in the sample matrix and/or difficulties in sample processing, particularly in concentrating the extracts by evaporation (see 4.2, 4.3 and 8.2).

10 Evaluation

10.1 Calculation of individual results using the basic calibration

Calculate the mass concentration, ρ_i, of compound i, in micrograms per litre, in the water sample using Equation (5) (having run both the sample and the reference solutions under the same conditions):

$$\rho_i = \frac{(y_i - b_i)\, V_0}{m_i \cdot V_\text{P} \cdot \overline{A}_i} \tag{5}$$

where

y_i is the measured value for compound i in the water sample, e.g. in area units;

m_i, b_i see Equation (1);

V_0 is the volume of the sample extract, in millilitres;

V_P is the volume of the extracted water sample, in millilitres;

$\overline{A}_i$ see Equation (4).

10.2 Calculation of individual results by calibration with external standards covering the complete procedure

Calculate the mass concentration, ρ_{ig}, of substance i, in micrograms per litre, in the water sample using Equation (6):

$$\rho_{ig} = \frac{(y_{ig} - b_{ig})}{m_{ig}} \tag{6}$$

where

y_{ig} is the measured value of substance i in the water sample, e.g. in area units;

m_{ig}, b_{ig} see Equation (2).

11 Expression of results

The analytical results obtained with this International Standard are subject to an uncertainty which needs to be taken into account when interpreting the results. Methods have been elaborated for the evaluation of the uncertainty, allowing the estimation of uncertainty from validation data obtained within a particular laboratory, routine quality assurance data (range control charts or mean control charts) as well as other validation and accreditation data. Preferably, the uncertainty is stated as the extended uncertainty. For this purpose, the combined standard uncertainty — expressed as the standard deviation or the variation coefficient — is multiplied by an extension factor of 2. This corresponds to a confidence level of about 95 %.

The uncertainty is estimated using the repeatability standard coefficient (CV_R) (see Annex D), multiplied by 2. The derived extended uncertainty, U, of the method serves as an guide, but it cannot replace the estimation of the uncertainty from intralaboratory data.

NOTE The uncertainty is concentration- and matrix-dependent, and its value increases when the working range of the method is decreased.

For a different concentration range, the uncertainty can be estimated from laboratory quality assurance data (e.g. range control cards for duplicate determinations). Another method is interlaboratory testing. The uncertainty of measurement may vary considerably with the type of sample.

The mass concentration of the analytes determined shall be reported in micrograms per litre to two significant figures, but to only one significant figure below a concentration of 1 µg/l.

EXAMPLES

2,4,6-Trinitrotoluene	11 µg/l
Hexogen	8,9 µg/l
1,3,5-Trinitrobenzene	0,6 µg/l

12 Test report

The test report shal include at least the following information:

a) a reference to this International Standard (ISO 22478);

b) all details necessary for complete identification of the water sample;

c) the results, expressed as specified in Clause 11;

d) if applicable, details of any circumstances that may have affected the results.

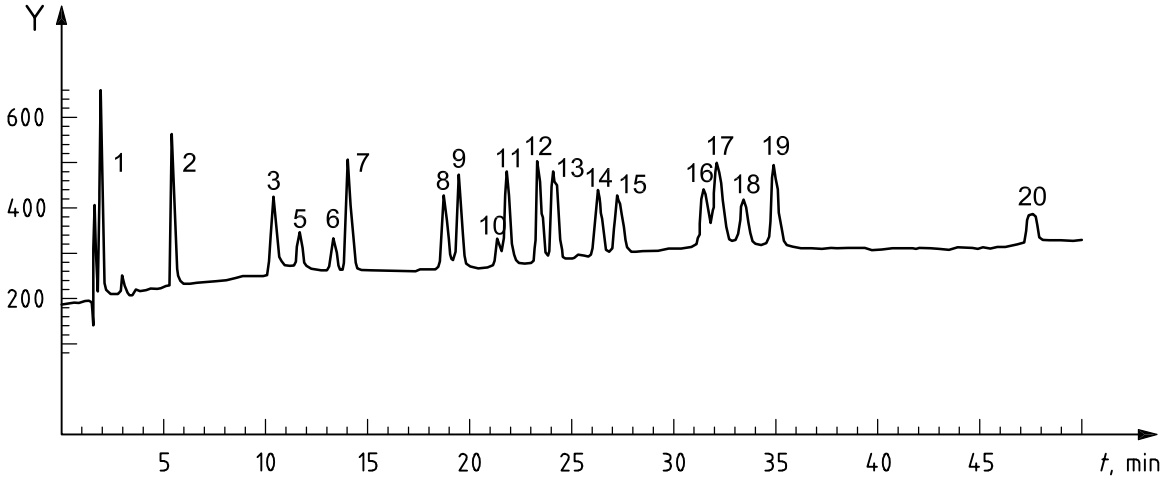
Annex A
(informative)

Examples of HPLC chromatograms

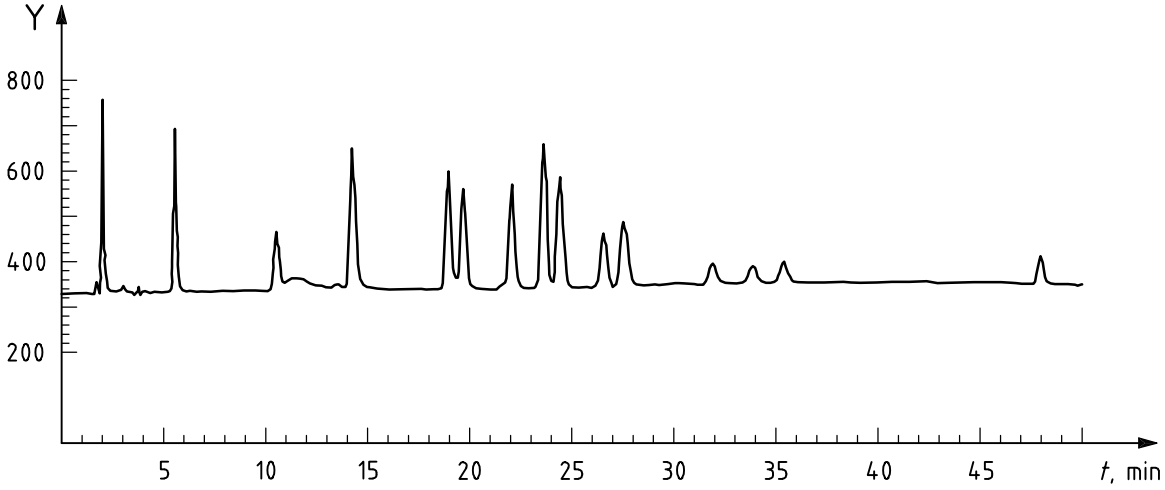
A.1 Chromatography conditions for chromatograms shown in Figure A.1

Analytical column:	LiChrospher 100 RP-18 [1)], 250 mm × 3 mm, 5 µm	
Mobile phase (flow rate 0,4 ml/min):	A: Methanol (CH_3OH), B: water	
Gradient (linear):	0 min to 10 min:	40 % to 50 % A
	10 min to 22 min:	50 % A
	22 min to 32 min:	50 % to 65 % A
	32 min to 42 min:	65 % A
	42 min to 43 min:	65 % to 100 % A
	43 min to 53 min:	100 % A
	53 min to 70 min:	40 % A
Column temperature:	30 °C	
Volume injected:	25 µl	
Detection:	PDA detector (wavelengths selected: 210 nm, 230 nm, 254 nm, 360 nm)	
Peak identification:	See Table 1	

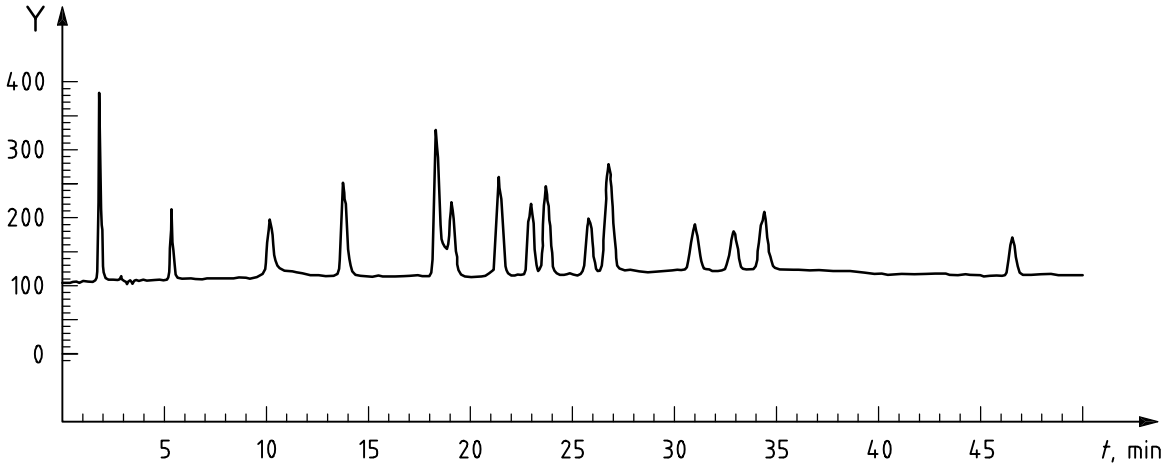
1) LiChrospher 100 RP-18 is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.



a) Detection at 210 nm



b) Detection at 230 nm

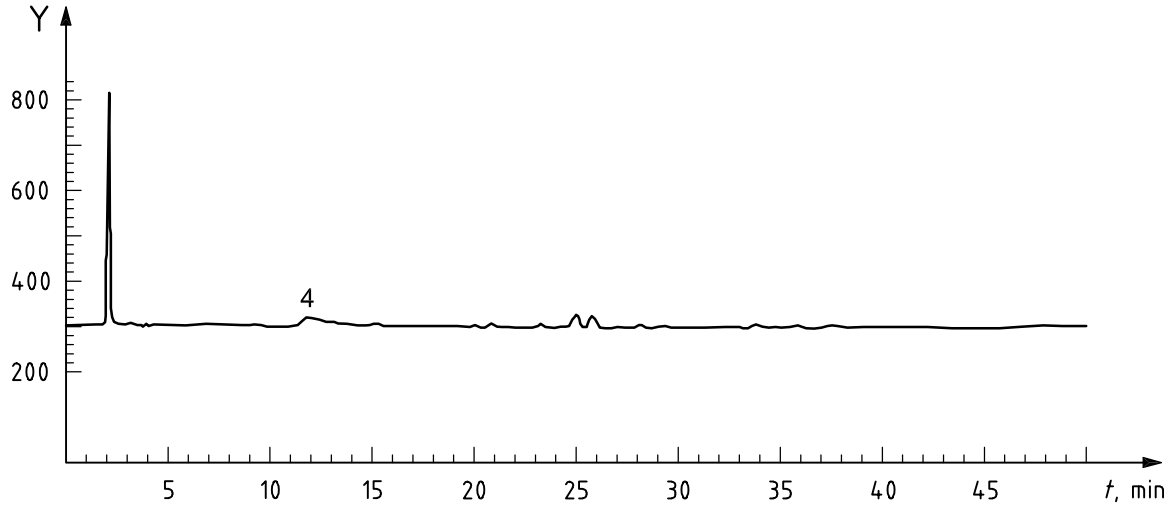


c) Detection at 254 nm

Key

Y detector response

Figure A.1 — Set of example chromatograms No. 1



d) Detection at 360 nm

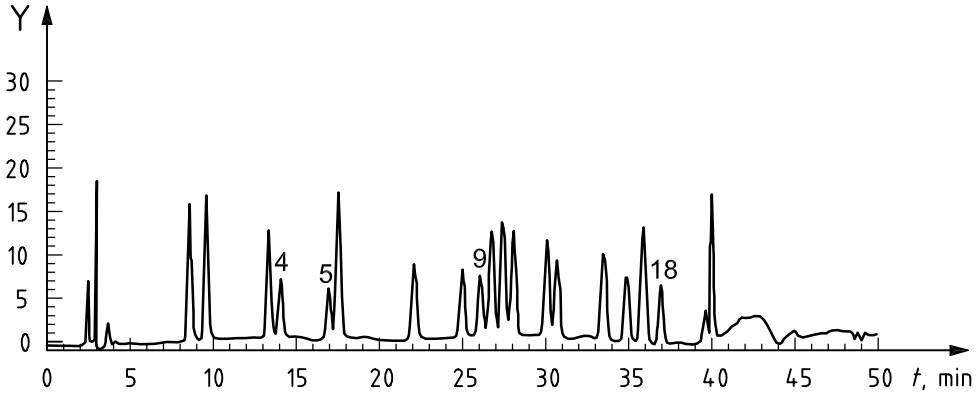
Key
Y detector response

Figure A.1 (*Continued*)

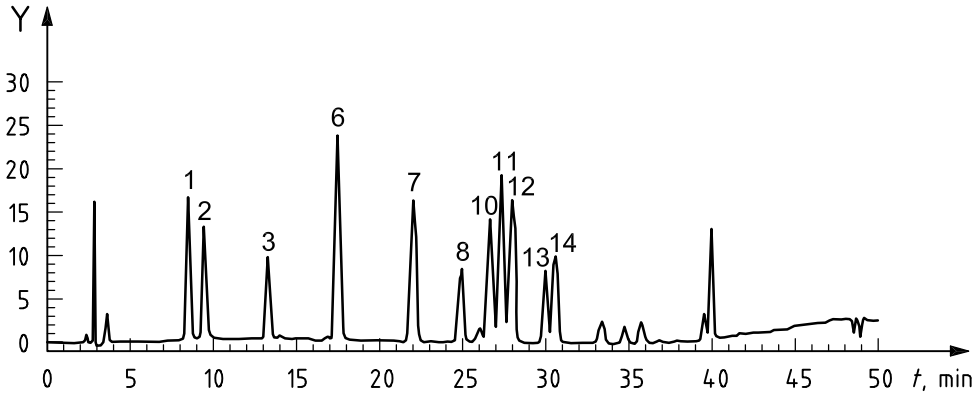
A.2 Chromatography conditions for chromatograms shown in Figure A.2

Analytical column:	Purospher [2], 250 mm × 3 mm, 5 µm	
Mobile phase (flow rate 0,4 ml/min):	A: acetonitrile (CH_3CN)/methanol (CH_3OH) = 20 + 80 parts by volume	
	B: 10 mmol/l sodium dihydrogen phosphate solution adjusted to a pH of 4,5 with orthophosphoric acid	
Gradient (linear):	0 min to 28 min:	35 % to 55 % A
	28 min to 40 min:	55 % to 85 % A
	40 min to 50 min:	85 % A
	50 min to 51 min:	85 % to 35 % A
	51 min to 71 min:	35 % A
Column temperature:	36 °C	
Volume injected:	10 µl	
Detection:	PDA detector (wavelengths selected: 210 nm, 235 nm, 278 nm, 378 nm)	
Peak identification:	See Table 1	

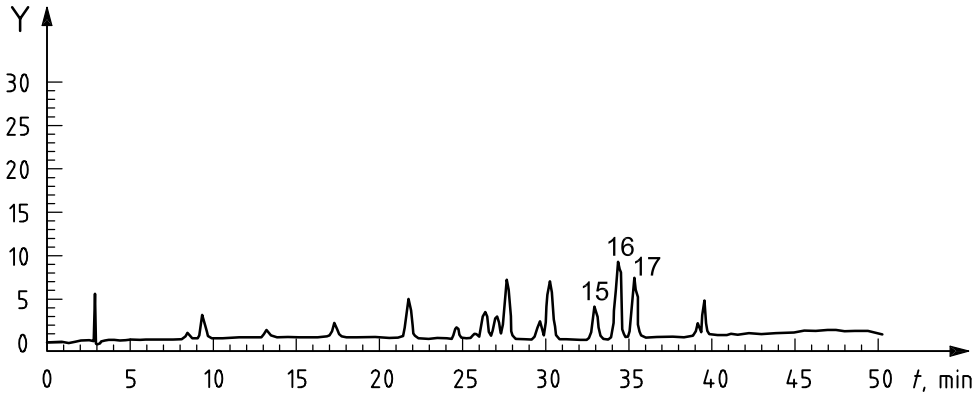
2) Purospher is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.



a) Detection at 210 nm



b) Detection at 235 nm

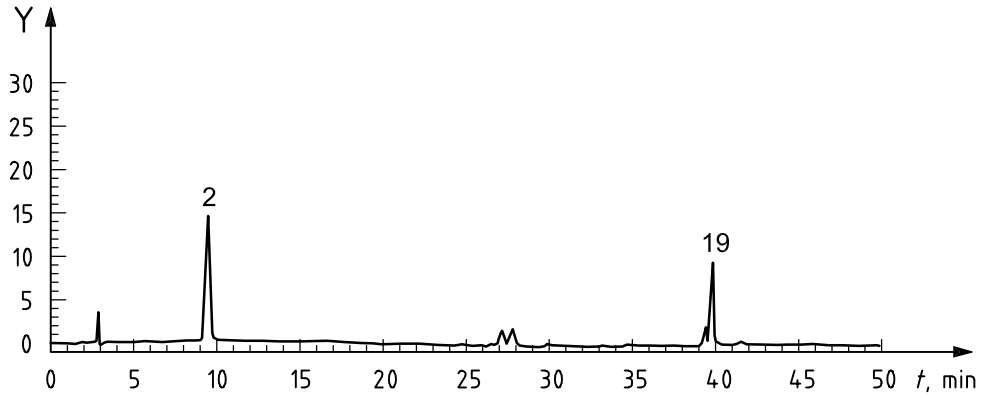


c) Detection at 278 nm

Key

Y detector response

Figure A.2 — Set of example chromatograms No. 2



d) Detection at 378 nm

Key

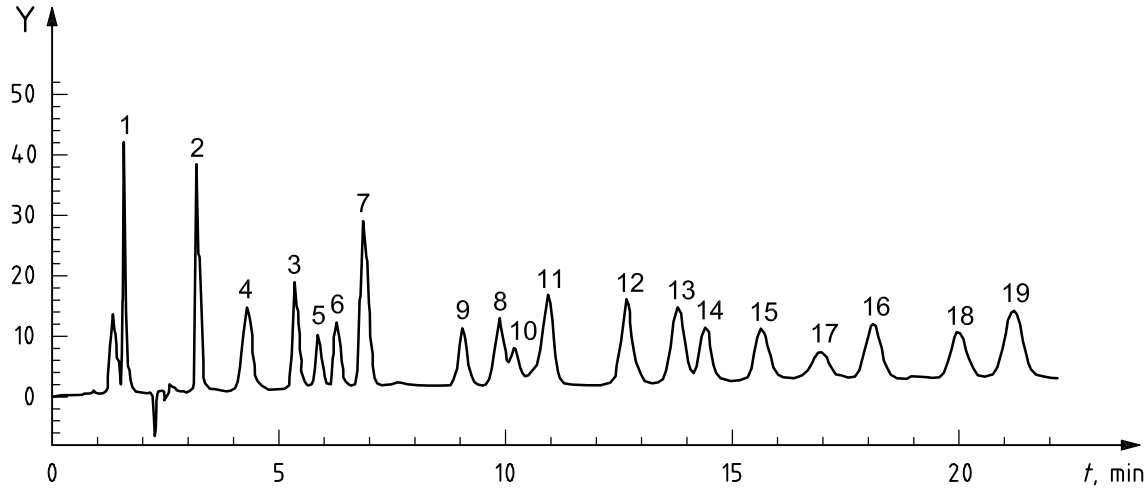
Y detector response

Figure A.2 (*Continued*)

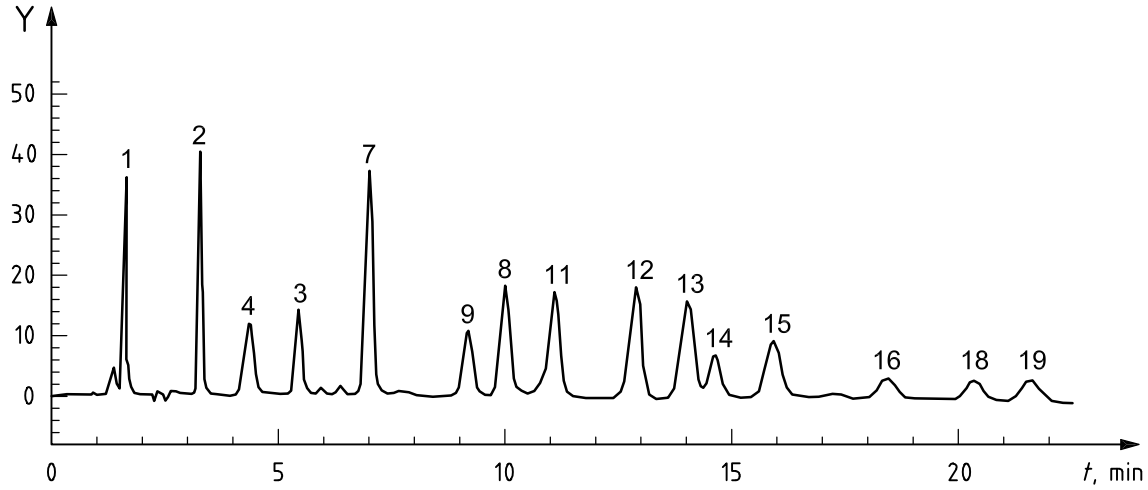
A.3 Chromatography conditions for chromatograms shown in Figure A.3

Analytical column:	LiChrospher 100 RP-18 [3)], 250 mm × 3 mm, 5 µm
Mobile phase (flow rate 0,55 ml/min):	A: methanol (CH_3OH), B: water
Isocratic elution:	A/B (53 + 47 parts by volume)
Column temperature:	21 °C
Volume injected:	20 µl
Detection:	PDA detector (wavelengths selected: 210 nm, 230 nm, 254 nm, 360 nm)
Peak identification:	See Table 1

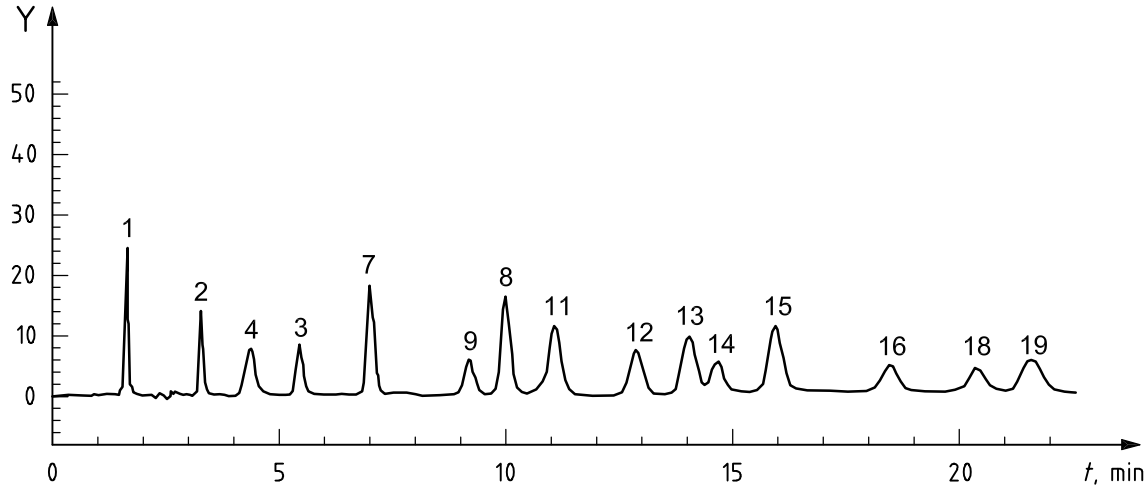
3) LiChrospher 100 RP-18 is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.



a) Detection at 210 nm



b) Detection at 230 nm

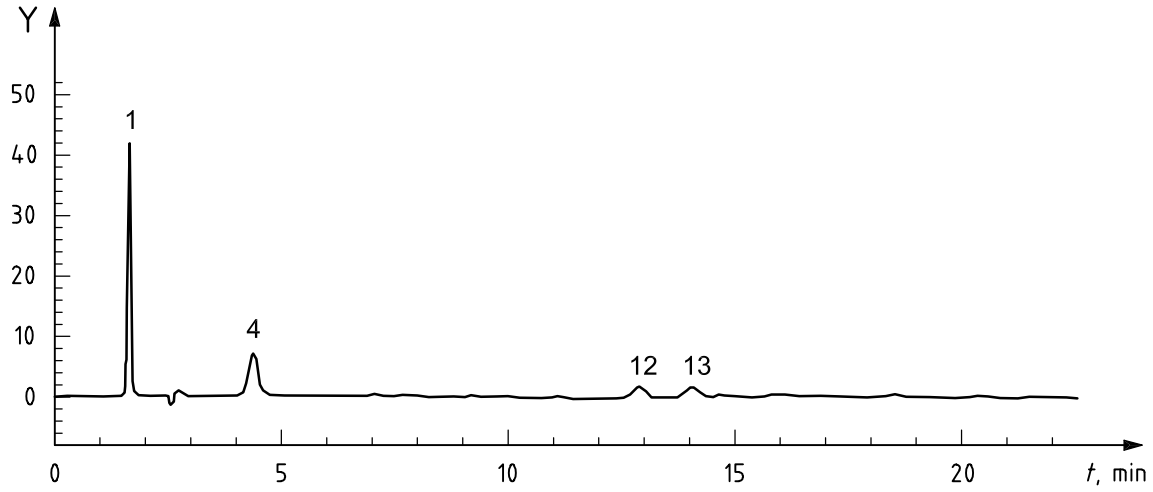


c) Detection at 254 nm

Key

Y detector response

Figure A.3 — Set of example chromatograms No. 3



d) Detection at 360 nm

Key

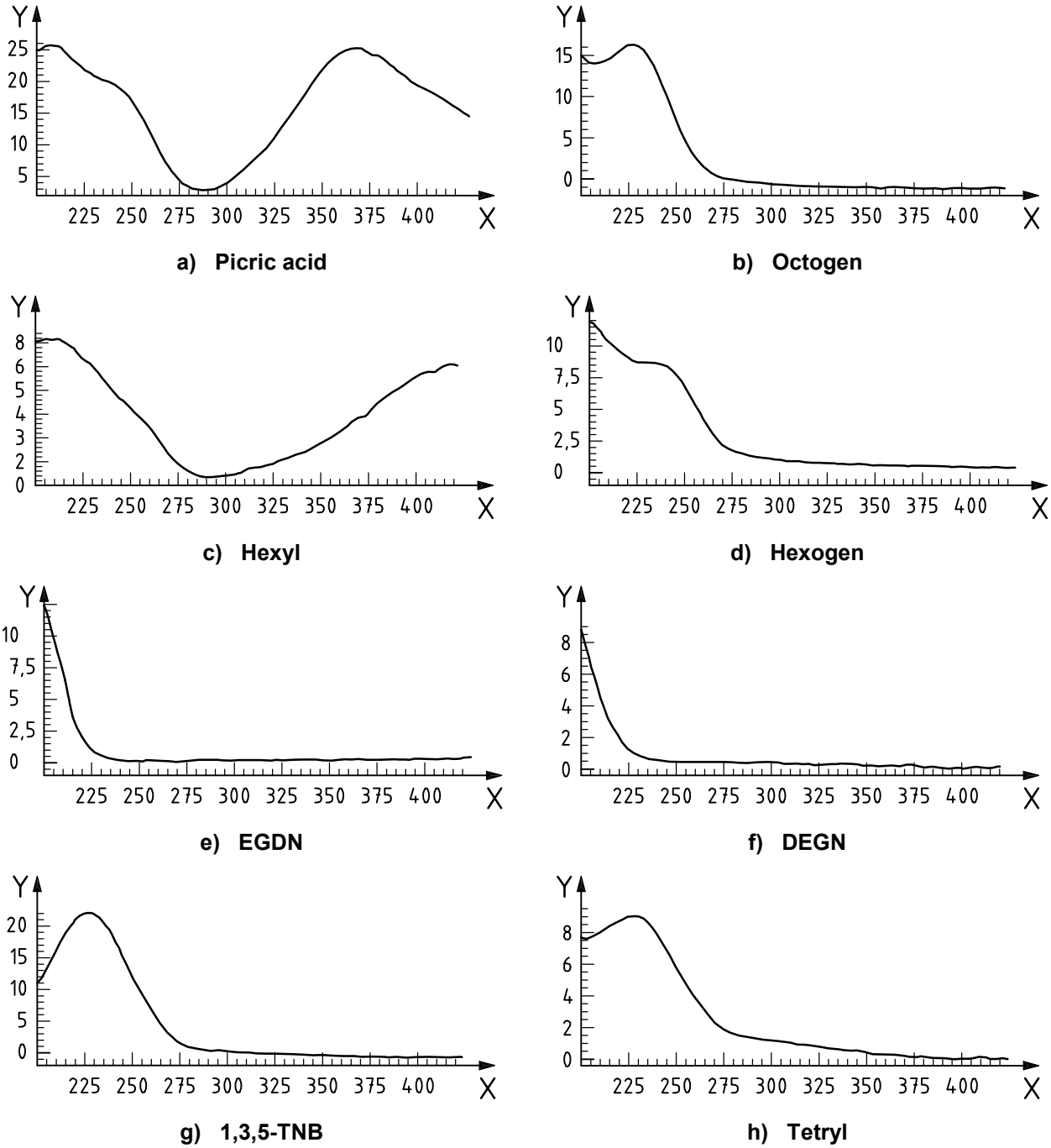
Y detector response

Figure A.3 (*Continued*)

Annex B
(informative)

UV spectra of compounds from Table 1

See set of example chromatograms No. 3 (Clause A.3) for conditions.

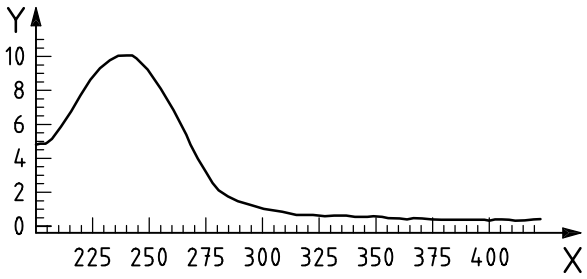


Key

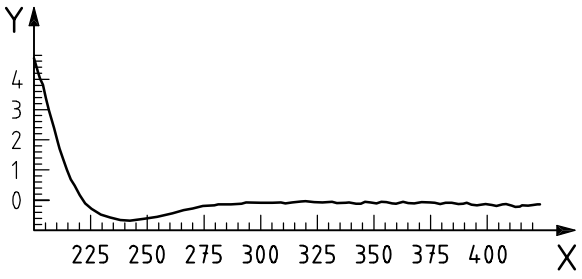
X wavelength (nm)

Y detector response

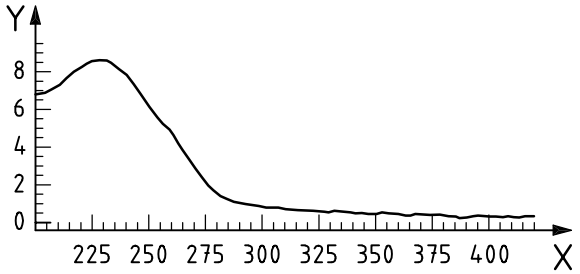
Figure B.1 — UV spectra of selected explosives from Table 1



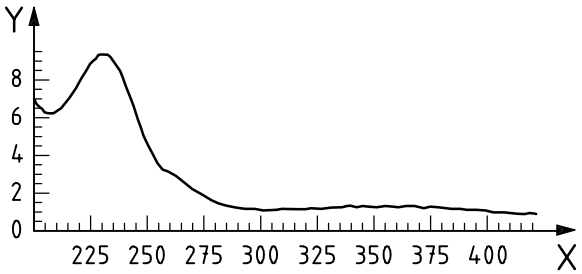
i) 1,3-DNB



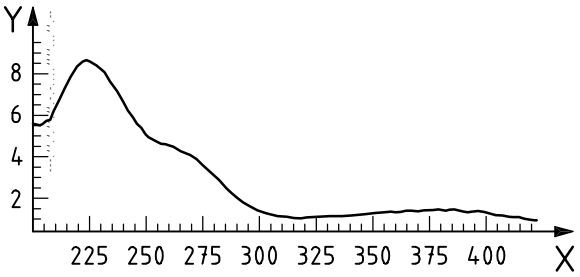
j) Nitroglycerine



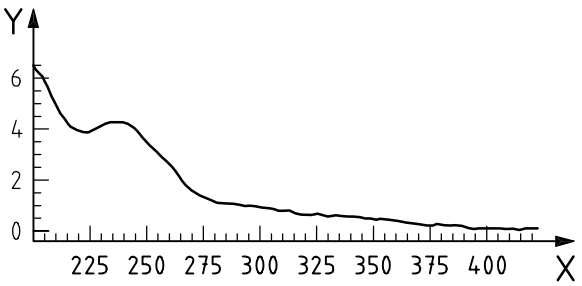
k) 2,4,6-TNT



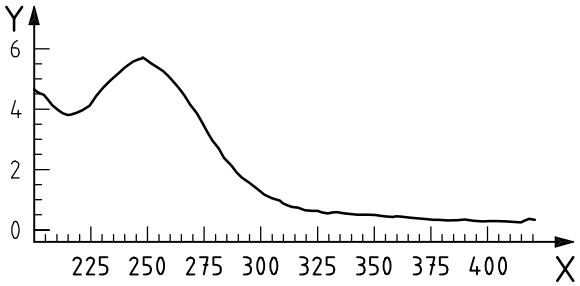
l) 4-A-2,6-DNT



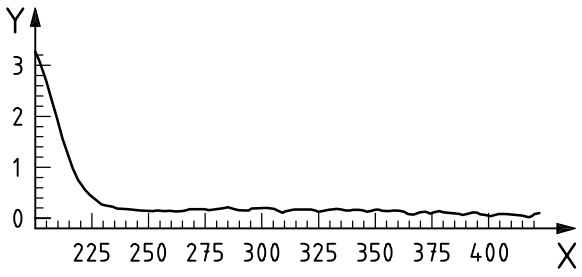
m) 2-A-4,6-DNT



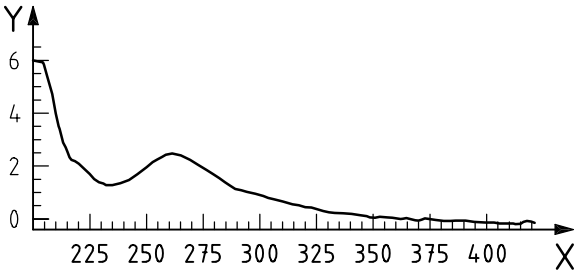
n) 2,6-DNT



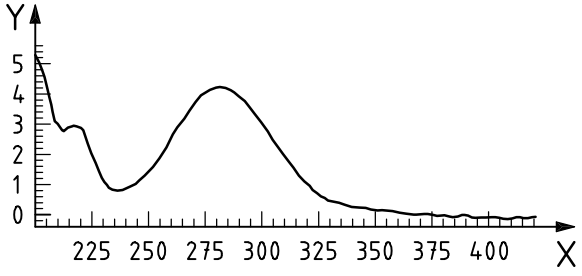
o) 2,4-DNT



p) Nitropenta

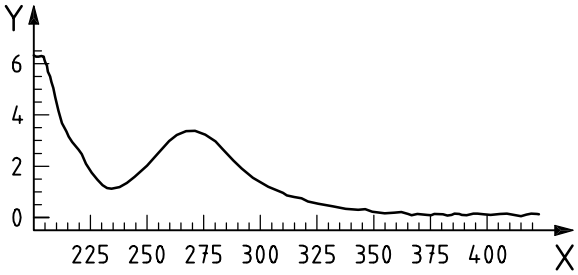


q) 2-NT



r) 4-NT

Figure B.1 (*Continued*)



s) 3-NT

Key

X wavelength (nm)

Y detector response

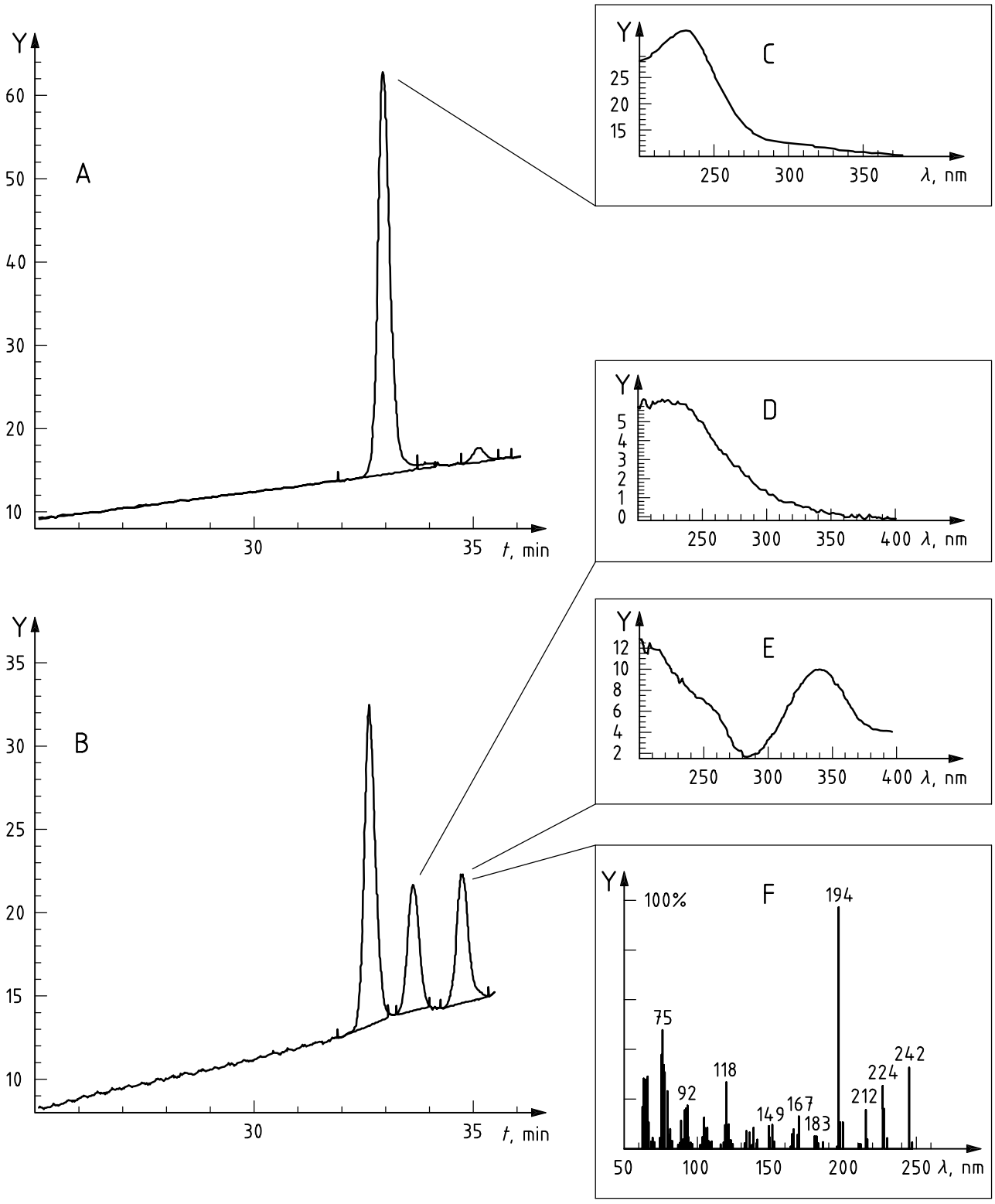
Figure B.1 (*Continued*)

Annex C
(informative)

Interference due to degradation of tetryl (CE) on exposure to daylight

Severe and rapid degradation of tetryl can be expected even in the case of a short exposure of samples, their organic solvent extracts or methanolic reference solutions to daylight. Two products in particular are observed whose signals are generally observed 1 min to 2 min later than that of tetryl, depending on the particular chromatographic conditions (see Figure C.1, products 1 and 2). With exposure of the samples to daylight for up to 5 min, both products are generally observed, but almost exclusively product 2 in the case of longer exposures. Complete degradation of tetryl may occur.

In order to avoid interference due to the degradation of tetryl, it is essential to avoid exposure to light as far as possible during sample preparation and/or to use brown-glass storage vessels.



Key

Y detector response

A HPLC chromatogram of tetryl before exposure to daylight

B HPLC chromatogram as in A after sample vial has been exposed to daylight for 5 min

C UV spectrum of tetryl before exposure to daylight

D, E UV spectra of products 1 and 2, respectively

F mass spectrum (obtained by GC-MS) of product 2

Figure C.1 — Examples of chromatograms of tetryl reference solutions showing formation of degradation products

Annex D
(informative)

Precision data

An interlaboratory trial carried out in January 1999 using spiked drinking water and ground water samples yielded the results shown in Table D.1. Additional experiments carried out using spiked surface water samples demonstrated the applicability of the method to this type of sample matrix.

Table D.1 — Results of interlaboratory trial

Sample	Parameter	l	n	n_{AP} %	$\bar{\bar{x}}$ µg/l	x_{exp} µg/l	η %	s_R µg/l	CV_R %	s_r µg/l	CV_r %
A	Hexogen, RDX	12	48	7,69	5,89	6,31	93,31	0,812	13,79	0,378	6,42
	Hexyl	11	44	0	9,31	9,28	100,33	1,385	14,88	0,401	4,30
	1,3-Dinitrobenzene	12	48	0	1,53	1,55	98,52	0,319	20,90	0,191	12,51
	2,4,6-Trinitrotoluene, TNT	10	39	17,02	3,83	4,53	84,61	0,354	9,24	0,232	6,05
	4-Amino-2,6-dinitrotoluene	12	48	7,69	6,66	7,00	95,18	0,395	5,92	0,221	3,32
	2,6-Dinitrotoluene	13	49	3,92	0,78	0,75	104,55	0,087	11,12	0,062	7,89
	2,4-Dinitrotoluene	12	47	0	0,62	0,63	98,39	0,101	16,35	0,049	7,96
	2-Nitrotoluene	12	46	0	1,93	2,30	84,07	0,443	22,91	0,288	14,90
	Ethylene glycol dinitrate	11	42	8,70	7,75	9,02	85,92	1,917	24,73	0,663	8,56
B	Hexogen	12	47	9,62	0,59	0,63	93,37	0,082	13,90	0,059	10,05
	Octogen	12	48	7,69	1,22	1,24	98,62	0,111	9,05	0,074	6,09
	Nitroglycerine	11	41	0	1,12	1,26	89,02	0,271	24,18	0,219	19,53
	Nitropenta	13	51	1,92	1,95	2,00	97,29	0,272	13,99	0,188	9,66
	1,3,5-Trinitrobenzene	8	32	27,27	0,25	0,25	101,75	0,026	10,33	0,024	9,56
	4-Amino-2,6-dinitrotoluene	9	36	10,00	0,27	0,25	106,52	0,043	16,21	0,023	8,63
	2-Amino-4,6-dinitrotoluene	13	52	0	0,52	0,50	104,22	0,065	12,53	0,035	6,74
	3-Nitrotoluene	12	48	0	1,42	1,62	87,41	0,261	18,46	0,197	13,90
	4-Nitrotoluene	12	46	4,17	1,63	1,90	85,95	0,258	15,81	0,207	12,65
	Diethylene glycol dinitrate	11	44	15,38	0,71	0,77	92,65	0,104	14,61	0,081	11,40
	Picric acid	11	44	0	1,16	1,03	112,42	0,199	17,18	0,067	5,82
	Tetryl (see Annex C)	7	25	0	0,73	1,54	47,45	0,464	63,48	0,121	16,52

l	is the number of laboratories;	η	is the recovery rate;
n	is the number of measurements;	s_R	is the reproducibility standard deviation;
n_{AP}	is the percentage of outliers;	CV_R	is the reproducibility coefficient of variation;
x_{exp}	is the accepted true value;	s_r	is the repeatability standard deviation;
$\bar{\bar{x}}$	is the overall mean;	CV_r	is the repeatability coefficient of variation.

Samples

A Drinking water B Ground water

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