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Water quality — Multi-compound class methods —

Part 1: Criteria for the identification of target compounds by gas and liquid chromatography and mass spectrometry

Qualité de l'eau — Méthodes d'analyse de composés multi-classes —

Partie 1: Critères pour l'identification de composées cibles par chromatographie en phase gazeuse ou liquide et spectrométrie de masse

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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 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

A list of all parts in the ISO 21253 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

The use of gas chromatography (GC) and liquid chromatography (LC) in combination with mass spectrometric (MS) detection is common in many analytical standards. This detector is a powerful tool provided it is properly used. This document gives the criteria for the identification of target compounds in various types of water. This document shall be used in combination with specific analytical standards or in combination with any GC-MS and LC-MS procedure. The result of the procedure described is identified, indicated or absent.

NOTE See Annex A for recommendations for the most commonly used techniques.

This document is generally based on ISO 22892[5].

Water quality — Multi-compound class methods —

Part 1: Criteria for the identification of target compounds by gas and liquid chromatography and mass spectrometry

1 Scope

This document specifies the criteria for mass spectrometric identification of target compounds in water samples and is applicable to environmental samples in general. This document is intended to be used in conjunction with standards developed for the determination of specific compounds. If a standard method for analysing specific compounds includes criteria for identification, those criteria are followed.

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 6107 (all parts), *Water quality — Vocabulary*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 6107 (all parts) and the following apply.

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/

3.1
diagnostic ion
selected fragment ion, molecular ion or other characteristic ion from the mass spectrum of the *target compound* (3.7) with the highest possible specificity

[SOURCE: ISO 22892:2006, 3.6]

3.2
identification point
result of mass spectrometric investigation or other investigations/information to identify a component in environmental matrices

[SOURCE: ISO 22892:2006, 3.7]

3.3
relative retention time
ratio between the retention time of the *target compound* (3.7) and the retention time of the *retention time standard* (3.4)

[SOURCE: ISO 22892:2006, 3.4]

3.4
retention time standard
compound that is added to the sample (or to the sample extract) and to the calibration standard solution, and used to calculate the *relative retention times* (3.3) of the *target compounds* (3.7)

[SOURCE: ISO 22892:2006, 3.3]

3.5
selected ion monitoring
SIM
measurement of the intensity of selected *diagnostic ions* (3.1) only

3.6
standard compound
target compound (3.7) with the highest possible purity, which can be used as a reference during the analysis

Note 1 to entry: Any impurities should not have influence on the mass spectrum of the standard compound.

[SOURCE: ISO 22892:2006, 3.2]

3.7
target compound
selected component, the presence or absence of which is being established

Note 1 to entry: This definition can also apply to a derivative of the original compound which is formed during an intentional derivatization procedure.

[SOURCE: ISO 22892:2006, 3.1]

3.8
calibration standard
solution prepared from a secondary standard and/or stock solutions and used to calibrate the response of the instrument with respect to analyte concentration

[SOURCE: ISO 18073:2004, 3.1.2]

3.9
calibration solution
solution used to calibrate the instrument, prepared from (a) stock solution(s) or from a certified standard

[SOURCE: ISO 17294-1:2004, 3.4]

4 Abbreviated terms

APCI	atmospheric pressure chemical ionization
CI	chemical ionization
Da	Dalton
ECD	electron capture detector
EI	electron ionization
EI-GC-MS	electron ionization-gas chromatography-mass spectrometry
ESI	electrospray ionization

FWHM	full width at half maximum
GC	gas chromatography
GC-MS	gas chromatography-mass spectrometry
HRMS	high resolution mass spectrometry
LC	liquid chromatography
LC-MS	liquid chromatography-mass spectrometry
mDa	milliDalton
MRM	multiple reaction monitoring
MS	mass spectrometry
MS^n	mass spectrometry
MTBE	methyl tertiary-butyl ether
m/z	mass to charge ratio
NPD	nitrogen-phosphorus detector
OCP	organo chlorine pesticides
PFPD	pulsed flame photometric detector
PID	photoionization detector
SIM	selected ion monitoring
S/N	signal to noise ratio
SRM	selected reaction monitoring
TAME	tertiary amyl methyl ether
UV/Vis	ultraviolet – visible spectroscopy

5 Principle

A target compound is identified if the measured values meet the criteria specified in this document or in the standard in which the procedures are described to analyse the target compound. Criteria are based on the relative retention times and the intensity ratio of diagnostic ions, and other relevant factors. Additional information regarding diagnostic ions from specific international standards on the analysis of the target compound can be used. The principle of identification points is used (see Annex D).

6 Apparatus

As this document is complementary to other standards using GC-MS and LC-MS, it is assumed that the instrumentation used meets the requirements of those standards and a detailed description is not within the scope of this document. The minimum acquisition requirements for low resolution and high resolution mass spectrometry are summarized in Tables 1 and 2.

Table 1 — Minimum acquisition requirements for low resolution mass spectrometry

Mass range:	Peaks (masses) with a $S/N < 3$ are not taken into consideration and the lower end of the scan range is limited to 35 (to avoid the measurement of oxygen and nitrogen) to the highest mass of the target compound +10 unified atomic mass units (u) in full scan measurements.
Scan rate:	Minimum of 7 scans per peak.
Scan mode:	Full scan or SIM.
Mass resolution:	To be tuned on nominal resolution, the peak width at half-height of every tune mass should not exceed 0,7 DA.

Table 2 — Minimum acquisition requirements for high resolution mass spectrometry

Scan rate:	Minimum of 7 scans per peak.
Mass resolution:	The resolution shall be greater the 10 000 FWHM for the used mass range.
Mass accuracy:	The mass accuracy should be ≤5 ppm.

7 Identification of target compounds

IMPORTANT — The equipment shall be operated, and the determination shall be carried out by suitably trained staff.

7.1 Procedure for identification of organic compounds with chromatography-mass spectrometry

The procedure to qualify a component consists of three steps (see the flow scheme in Figure 1).

7.1.1 Step 1

Chromatographic evaluation (see 7.2): the relative retention time shall fulfil the specified criteria.

Proceed to step 2 only if step 1 is positive.

7.1.2 Step 2

Mass spectrometric evaluation (see 7.3): gathering identification points using mass spectrometric data. For qualification, the principle of identification points is used (see EN 16693[1]).

Identification points can be obtained from mass spectrometric data, but also using other analytical information.

7.1.3 Step 3

Additional analytical confirmation evaluation (see 7.4).

The following classification can be obtained.

a) Identified (see 7.5.1): The target compound is present in the analysed extract if at least 3 identification points are obtained.

b) Indicated (see 7.5.2): The target compound may be present if only 1 or 2 identification points are obtained.

c) Absent (below the detection limit) (see 7.5.3): No identification points are obtained using mass spectrometry.

In case the classification result "indication" is unwanted, then any result with less than 3 identification points shall be regarded as not identified.

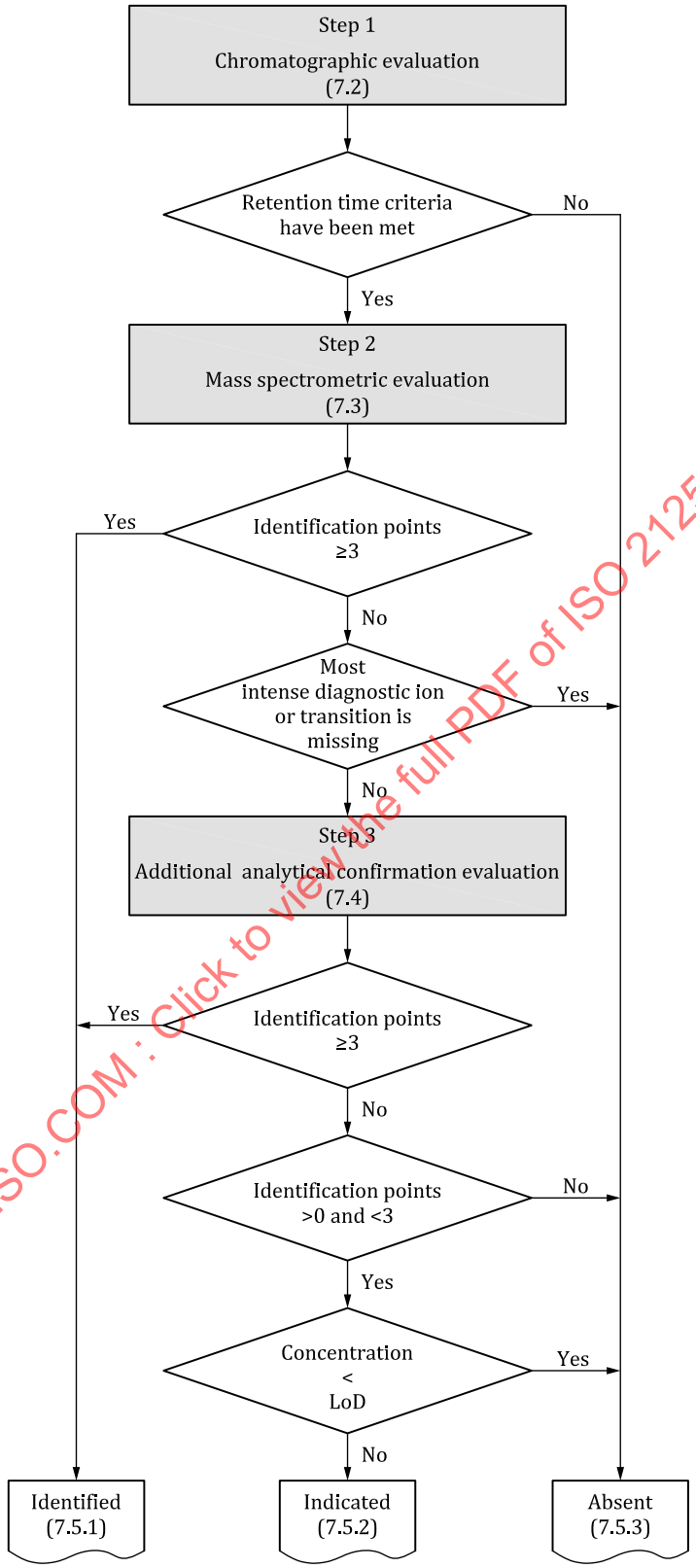

Figure 1 — Flow scheme for the identification of a target compound using three steps

7.2 Step 1: Chromatographic separation

For GC-MS procedures, the gas chromatographic separation shall be carried out using capillary or a packed column. For LC-MS procedures, the chromatographic separation shall be carried out using a

suitable LC column. The minimum acceptable retention time for the analyte under examination shall be twice the retention time corresponding to the void volume of the column. The (relative) retention time of the analyte in the sample shall match the (relative) retention time of the calibration standard within a specified retention time window. The retention time window shall be commensurate with the resolving power of the chromatographic system. The ratio of the chromatographic retention time of the analyte to that of the retention time standard, i.e. the relative retention time of the analyte, shall correspond to that of the calibration solution at a tolerance of ±2,5 % for LC and ±0,5 % for GC.

NOTE If specific retention time criteria are given in another standard, these can be followed.

7.3 Step 2: Mass spectrometric evaluation

7.3.1 Mass spectrometric detection

Mass spectrometric detection shall be carried out by employing MS-techniques such as recording of full mass spectra (full scan) or selected ion monitoring (SIM), as well as MS^n techniques such as selected reaction monitoring (SRM), multiple reaction monitoring (MRM) or other suitable MS or MS^n techniques in combination with appropriate ionization modes. In high-resolution mass spectrometry (HRMS), the resolution shall typically be greater than 10 000 FWHM for the entire mass range.

a) Full scans: When mass spectrometric determination is performed by the recording of full scan spectra, the presence of all measured diagnostic ions (the molecular ion, characteristics adducts of the molecular ion, characteristic fragment ions and isotope ions) with a relative intensity of more than 10 % in the reference spectrum of the calibration standard is obligatory. In HRMS, a partial scan (of limited mass range) may be adequate to yield sufficient diagnostic ions for identification.

b) SIM: When mass spectrometric determination is performed by fragmentation, the molecular ion can be one of the selected diagnostic ions (the molecular ion, characteristics adducts of the molecular ion, characteristic fragment ions and the isotope ions). Whenever possible, the selected diagnostic ions should be independent from each other, for example not exclusively originate from the same part of the molecule, nor exclusively be isotope ions. The S/N ratio for each diagnostic ion shall be ≥ 3:1.

c) Full scan and SIM: The relative intensities of the detected ions, expressed as a percentage of the intensity of the most intense ion or transition, shall correspond to those of the calibration standard, either from calibration standard solutions or from spiked samples, at comparable concentrations, measured under the same conditions, within the tolerances given in Table 3.

Table 3 — Maximum permitted tolerances for relative ion intensities using a range of mass spectrometric techniques

Relative intensity	Maximum allowed tolerance in EI-GC-MS	Maximum allowed tolerance CI-GC-MS, CI-GC-MS^n, EI-GC-MS^n, LC-MS, LC-MS^n
>50 % to 100 %	±10 %	±30 %
>20 % to 50 %	±15 %	
>10 % to 20 %	±20 %	
≤10 %	±50 %	±50 %

7.3.2 Selection of diagnostic ions

If available, three diagnostic ions shall be selected for each target compound. Their intensities shall be determined in the calibration standard solution either from a calibration standard solution or from spiked samples at comparable concentrations measured under the same conditions, as the peak area or peak height of the corresponding extracted ion current chromatograms. Their intensities shall be determined using at least three injections. The relative intensities are calculated as the ratio of the determined peak heights (or areas) and the peak height (or area) of the most intensive diagnostic ion.

NOTE Due to overloading, the ratios of the diagnostic ions can change.

Diagnostic ions may also be specified in the standard method being used. It is not always possible to obtain three diagnostic ions (for instance for polycyclic aromatic hydrocarbons). In that case, select the available ions. Diagnostic ions should have a high "uniqueness value" (see ISO 22892[5]). Annex C gives selection criteria for diagnostic ions.

Co-eluting substances may influence the peak shape. As long as the peak of interest can be separately integrated, it may be used.

Criteria for the retention time of the selected diagnostic ions are related to the peak maxima of the extracted ion current chromatograms.

Diagnostic ions should originate from the analyte under investigation only. This implies that, theoretically, all diagnostic ions belonging to one and the same analyte have the same retention time. If the retention time of one selected diagnostic ion differs from the retention times of the other diagnostic ions from the same analyte, a co-eluting substance or a partly-separated substance giving the same mass may be present. In this case, the particular diagnostic ion cannot be used.

The accuracy of the retention time depends on the number of scans within the chromatographic peak and hence, on the scan rate. Because the scan rate is limited, small differences in the retention times of the diagnostic ions should be allowed. A suitable criterion for the allowed difference in retention times of the diagnostic ions of an analyte shall not be greater than 40 % of the peak width at half the peak height in a single run. Therefore, the differences in retention times of the peak maxima of all the selected diagnostic ions in the extracted ion current chromatograms belonging to the same analyte shall not be greater than 40 % of the peak width at half the peak height. For most analyses, whether GC or LC, this means an acceptable difference of 1 s. These criteria apply for both the calibration standard solution and the sample.

7.3.3 Assigning identification points

The qualification of the results is different depending on the mass spectrometric technique used.

When full scan spectra are recorded, a minimum of three ions shall be present with a relative intensity of ≥10 % of the base peak. The molecular ion shall be included if it is present in the reference spectrum with a relative intensity of ≥10 %. If computer aided library searching is used, critical match factors described in Annex B shall be applied. Variability in the spectra caused by the sample matrix and the detector performance shall be checked.

When mass fragments are measured using anything other than full-scan techniques, or when library matching is not possible, a system of identification points shall be used to interpret the data. For the confirmation of any substance, three identification points are required. Tables 4 and 5 shows the number of identification points that each of the basic mass spectrometric techniques can earn. However, in order to qualify for the identification points required for confirmation and the sum of identification points to be calculated:

— at least one ion ratio shall be measured;

— all relevant measured ion ratios shall meet the criteria described in Table 3;

— a maximum of three separate techniques can be combined to achieve the minimum number of identification points;

— each ion may only be counted once;

— GC-MS using electron impact ionization is regarded as being a different technique to GC-MS using chemical ionization;

— transition products include both product ions and n^{th} generation product ions.

Table 4 — Identification points for frequently used MS techniques, provided criteria are met

MS resolution	Typical MS systems	MS acquisition mode	Source[d]	Identification points per source
Unit mass resolution	Single MS: for example quadrupole, iontrap, TOF	Full scan, limited *m/z* range, SIM	Ion	1
	MS^n: for example triple quadrupole (MS/MS), iontrap, Q-trap, Q-TOF, Q-orbitrap	Selected or multiple reaction monitoring (SRM, MRM)	Precursor ion	1
			Transition products	1,5
Accurate mass measurement	High resolution MS: for example (Q)-TOF, (Q)-Orbitrap, FT-ICR-MS, Sector MS	Full scan, limited *m/z* range, SIM[a,b,c]	Ion	2
		Fragmentation with or without precursor-ion selection or combinations thereof	Precursor ion	2
			Transition products	2,5

Each ion may only be counted once.

NOTE 1 GC-MS using electron impact ionization is regarded as being a different technique to GC-MS using chemical ionization.

NOTE 2 Transition products include both product ions and n^{th} generation product ions.

NOTE 2 See Table D.14 for additional examples on how to calculate id points; table and criteria are adapted from References [6] and [9].

[a] Preferably including the molecular ion, (de)protonated molecule or adduct ion.

[b] Including at least one fragment ion.

[c] Mass accuracy ≤ 5 ppm (or < 1 mDa for m/z < 200).

[d] $S/N \geq 3$ (in case noise is absent, a signal should be present in at least five subsequent scans).

Table 5 — Examples of additional identification points per alternative technique, provided criteria are met

Source	Identification points per source	Remark
Absence of any other ions in full scan	1	Diagnostic ions in full scan $S/N > 3$ (in case noise is absent, a signal should be present in at least five subsequent scans)
Column with other polarity	1	
Analyte spike/standard	1	
Other analytical techniques	1	Every other selective detector For GC; ECD, PFPD, NPD, PID For LC UV/Vis, fluorescence
Combination of ionization techniques, for example GC-MS (both EI and CI; positive/negative)	3	1 (EI) + 1 (CI positive) + 1 (CI negative)

7.4 Step 3: Additional analytical confirmation evaluation

Additional confirmation information can be used to obtain extra identification points when in step 2 not enough identification points are obtained for identification. In Table 5, some examples are given for additional identification points that can be obtained using other analytical information.

7.5 Reporting the presence of target compounds

7.5.1 Identification

The analysed target compound is identified if at least 3 identification points are obtained [see 7.1.3 a)].

The relative retention time shall comply with 7.2. The relative intensities of all the selected ions measured in the sample meet the relative ion intensities criteria (see Table 3) in comparison with the calibration standard preceding or following the sample.

7.5.2 Indication

There is an indication for the presence of the analysed target compound in the sample if:

— the requirements for the relative retention time are met (7.2);

— only 1 or 2 identification points are obtained;

— the most intensive diagnostic ion is present ($S/N > 3$);

— the quantifying diagnostic ion is present ($S/N > 3$);

— the intensity of the missing diagnostic ion(s) is $S/N < 3$;

— the intensity of the quantifying diagnostic ion is higher than the intensity of the same quantifying diagnostic ion at the limit of reporting.

In case the classification result "indicated" is unwanted, then any result with less than 3 identification points shall be regarded as not identified.

7.5.3 Absence of the target compounds (<detection limit)

The target compound is absent in the sample (not identified and no indication for its presence), if:

— the requirements for the relative retention time are not met;

— or no identification points are obtained.

8 Test report

The test report in addition to the specifications given in the analytical International Standard applied, shall contain at least the following information:

a) a reference to this document, i.e. ISO 21253-1:2019;

b) complete identification of the sample;

c) the results of the identification procedure, identified, indicated or absent as performed according to this document;

d) any operations not prescribed in this document, which might have affected the results.

Annex A
(informative)

Recommendations for the most commonly used techniques

A.1 Overview

This annex gives recommendations for the most commonly used MS^n techniques.

A.2 Recommendation for selected ion monitoring mode

The relative intensity of the detected ions should be expressed as a percentage of the intensity of the most intensive diagnostic ion, by dividing the peak area or height of the diagnostic ions by the area or height of the most intensive diagnostic ion.

A.3 Recommendations for tandem mass spectrometric detection and identification

For tandem mass spectrometry, the following recommendations apply.

— Two precursor-product ion transitions should be monitored.

— In some cases, the combination of a single precursor-product ion pair may be sufficiently unique to be definitive. If a laboratory chooses to use only one precursor-product ion pair for identification instead of two precursor-product ion pairs, validation data are required documenting the uniqueness of the transition.

— The mass resolution of the first mass analyser should be set to at least to unit resolution. When more than one precursor-product ion pair is monitored, the relative abundance of a diagnostic ion should preferably be determined from the peak area or height of integrated selected reaction monitoring chromatograms.

— The ion ratios should be calculated by dividing the peak area or height of the diagnostic ion by the peak area or height of the most intensive diagnostic ion.

A.4 Recommendations for performing accurate mass measurement

Accurate mass measurement provides the opportunity to determine the elemental composition of an ion. While accurate mass measurement cannot distinguish isomeric structures, it is often sufficient to determine the number of carbon, oxygen, hydrogen, nitrogen and other atoms in the molecule.

Report the mass accuracy as parts per million (ppm), by using the following Formula (A.1):

$$a_{\mathrm{m}} = \frac{m_{\mathrm{m}} - m_{\mathrm{t}}}{m_{\mathrm{t}}} \times 10^{6} \qquad \text{(A.1)}$$

where

a_{m} is the mass accuracy, in parts per million (ppm);

m_{m} is the measured mass, in mass to charge ratio (m/z);

m_{t} is the theoretical mass, in mass to charge ratio (m/z).

Preferably, four decimals should be used when expressing m_{m} and m_{t}.

Annex B
(normative)

Criteria for full scan measurement

When the recording of full scan spectra is mandatory, all measured diagnostic ions shall have a relative intensity greater than 10 % in the reference spectrum of the calibration standard. Diagnostic ions can be:

— the molecular ion;

— characteristics adducts of the molecular ion;

— characteristic fragment ions;

— isotope ions.

NOTE 1 The recording of full scan spectra is sometimes prescribed in legislation or agreed upon with a client.

The following requirements shall be met.

— For GC analysis a full scan should begin at a *m/z* value of 35 Da, avoiding the inclusion of ions arising from permanent gases. A partial scan may begin at an *m/z* value greater than any abundant ion due to the derivatizing agent (such as the *m/z* 73 ion arising from trimethylsilyl derivatives) or chemical ionization reagent.

For GC-MS^n and LC-MS^n techniques in Table 3, all diagnostic ions in the product ion scan with a relative abundance greater than 10 % shall be present.

NOTE 2 The relative abundance of the diagnostic ions can be obtained from a single spectrum at the peak apex or averaged spectra or integration of peak areas of extracted ion profiles.

The relative abundances of each of the diagnostic ions greater than 10 % are within the limits specified in Table 3.

For full scan measurement, computer-aided library searching can also be used. When mass spectra library searching is used, both the test samples and the calibration solution shall be so similar to the library that they exceed a critical match factor. The critical match factor for a user library is 90 % (self-made library) and for a reference library 70 % (e.g. NIST[1)] mass spectral library).

1) NIST mass spectral library are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

Annex C
(informative)

Diagnostic ions to be used for identification using GC-MS^n and LC-MS^n

C.1 Selection criteria for diagnostic ions

Diagnostic ions should have a high "uniqueness value". When selecting diagnostic ions, the following recommendations should be followed:

— high *m/z* values are preferred due to their higher significance;

— even mass fragments are preferred over odd ones;

— if possible, the molecular ion should be selected as one of the diagnostic ions;

— the intensity of diagnostic ions is preferably greater than 15 % in relation to the base peak in the spectrum;

— if characteristic isotope clusters are present in the mass spectrum (e.g. chlorine), two diagnostic ions should be selected from one isotope cluster. Isotopes can be very characteristic for complex compounds, i.e. organotin;

— if during the sample preparation, the target compounds have been derivatized with a reagent with low specificity, only one of the ions M+ and M_{der}^+ should be selected as a diagnostic ion (M+ is the molecular ion of the derivatized target compound);

— an adduct ion may be used as a diagnostic ion, but not if the molecular ion is also used. Preferably sodium and potassium adduct ions should not be used as diagnostic ions;

— in the selection of the diagnostic ions, potential column artefacts should be taken into consideration, avoiding corresponding masses (e.g. *m/z* 73, *m/z* 207, *m/z* 281).

EXAMPLE Methyl tertiary-butyl ether and tertiary amyl methyl ether have *m/z* 73 as diagnostic ion.

C.2 Reference documents with recommended diagnostic ions

The following reference documents give recommended diagnostic ions which can be used for identification:

— ISO 22892:[5];

— CEN/TR 15641[12].

Annex D
(informative)

Examples of calculating identification points

D.1 Overview

This annex gives examples of the calculation of identification points for the following techniques:

— low resolution EI-GC-MS (D.2);

— low resolution LC-MS^n (D.3);

— high resolution MS^n (D.4);

— low resolution GC-MS^n (D.5);

— additional examples (D.6).

D.2 Example of calculating identification points for low resolution EI-GC-MS

D.2.1 General

Compound: alachlor, diagnostic ions 160, 188, 146.

The three selected diagnostic ions have the following relative intensities: 100 %, 82 % and 26 %.

D.2.2 Identification based on relative retention time

Table D.1 — Retention times and relative retention times, in minutes

	Calibration standard		Sample 1	
	Retention time	Relative retention time	Retention time	Relative retention time
Atrazine-D5	33,86	—	33,85	—
Ion 1 (m/z 160)	36,33	1,072 9	36,33	1,073 3
Ion 2 (m/z 188)	36,34	1,073 2	36,31	1,072 7
Ion 3 (m/z 146)	36,31	1,072 4	36,36	1,074 2

The relative retention time is calculated on retention time ion/retention time atrazine-D5 (internal standard). See Table D.1.

The relative retention time of ion 1 (160) in sample 1 differs from the relative retention time of the calibration standard by 0,04 %.

[(1,073 3 − 1,072 9)/1,072 9] × 100 % = 0,04 %

The relative retention time of ion 2 (188) in sample 1 differs from the relative retention time of the calibration standard by 0,05 %.

[(1,072 7 − 1,073 2)/1,073 2] × 100 % = −0,05 %

The relative retention time of ion 3 (146) in sample 1 differs from the relative retention time of the calibration standard by 0,16 %.

[(1,074 2 − 1,072 4)/1,072 4] × 100 % = 0,16 %

Each of these differences is less than ±0,5 % from relative retention time in the last measured calibration standard solution.

The criteria for retention time have been met. Mass spectrometric evaluation is necessary for further identification.

D.2.3 Example based on maximum allowed deviation of the relative intensities

Table D.2 — Peak area

	Peak area ion 1	Peak area ion 2	Peak area ion 3
Calibration standard	1 286 162	1 054 653	334 402
Sample 1	260 850	195 876	76 992

Table D.3 — Relative intensity of ion 2/ion 1 and ion 3/ion 1 based on peak area

	Relative intensity peak area ion 2/ion 1	Relative intensity peak area of ion 3/ion 1
Calibration standard	0,820	0,260
Sample 1	0,751	0,295

The relative intensity of a diagnostic ion is determined from the peak area or the height of integrated selected ion chromatograms. See Tables D.2 and D,3.

The relative intensity of ion 2/ion 1 (based on peak area) of sample 1 is: 195 876/260 850 = 0,751.

The relative intensity of ion 3/ion 1 (based on peak area) of sample 1 is: 76 992/260 850 = 0,295.

The deviation of ion 2 in sample 1 is: [(0,751 − 0,820)/0,820] × 100 % = −8,4 %.

The deviation of ion 3 in sample 1 is: [(0,295 − 0,260)/0,260] × 100 % = 13,5 %.

The relative intensity of ion 2 is 82 %. The maximum allowed deviation of ion 2 is ± 10 %. The relative intensity of ion 3 is 26 %. The maximum allowed deviation of ion 3 is ± 15 %. On the basis of three diagnostic ions, 3 identification points have been obtained.

D.2.4 Conclusion

The criteria for both retention time (see D.2.2) and ion relative intensities (see D.2.3) have been met and 3 identification points are obtained. In this case, the target compound is considered as being identified (Figure 1 and Table 4).

D.3 Example of calculating identification points for low resolution LC-MS^n

D.3.1 General

Compound: Imidacloprid; precursor ion, *m/z* 256; product ions *m/z* 209, 175.

The two selected product ions have the following relative intensities: 100 % and 75 %.

D.3.2 Identification based on relative retention time

Table D.4 — Relative retention times, in minutes

	Calibration standard Relative retention time	Sample 2 Relative retention time
Product ion 1 (*m/z* 209)	1,050 0	1,052 6
Product ion 2 (*m/z* 175)	1,050 0	1,052 6

The relative retention time of the analyte shall correspond to that of the calibration solution at a tolerance of ±2,5 % for LC.

The relative retention time of ion 1 (*m/z* 209) in sample 2 differs from the relative retention time of the calibration standard by 0,25 %:

[(1,052 6 – 1,050 0)/1,050 0] × 100 % = 0,25 %

The relative retention time of ion 2 (*m/z* 175) in sample 2 differs from the relative retention time of the calibration standard by 0,25 %:

[(1,052 6 – 1,050 0)/1,050 0] × 100 % = 0,25 %

Each of these differences in relative retention time between the sample and the last measured calibration standard is less than ±2,5 %.

The criteria for retention time have been met. Mass spectrometric evaluation is necessary for further identification.

D.3.3 Example based on maximum allowed deviation of the relative intensities

Table D.5 — Peak area

	Peak area ion 1	Peak area ion 2
Calibration standard	13 716 160	10 313 807
Sample 2	936 220	683 449

Table D.6 — Relative intensity of product ion 2/ion 1

	Relative intensity peak area ion 2/ion 1
Calibration standard	0,752
Sample 2	0,730

The relative intensity of product ion 2/ion 1 (based on peak area) of the calibration standard is: 10 313 807/13 716 160 = 0,752. See Tables D.5 and D.6.

The relative intensity of product ion 2/ion 1 (based on peak area) of sample 2 is: 683 449/936 220 = 0,730.