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BSI Standards Publication

Influence of materials on water intended for human consumption — GC-MS identification of water leachable organic substances

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National foreword

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The UK participation in its preparation was entrusted to Technical Committee EH/6, Effects of materials on water quality.

A list of organizations represented on this committee can be obtained on request to its secretary.

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Influence of materials on water intended for human consumption - GC-MS identification of water leachable organic substances

Influence sur l'eau des matériaux en contact avec l'eau destinée à la consommation humaine - Identification par CG-SM de substances organiques lixiviables à l'eau

Identifizierung mittels GC-MS von durch Wasser auslaugbaren organischen Substanzen aus Materialien für den Kontakt mit Trinkwasser

This European Standard was approved by CEN on 29 November 2014.

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Contents

Page

Foreword

This document (EN 15768:2015) has been prepared by Technical Committee CEN/TC 164 “Water supply”, the secretariat of which is held by AFNOR.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by July 2015 and conflicting national standards shall be withdrawn at the latest by July 2015.

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This document describes methods of identification only, and should not be used or quoted as a specification.

References to this document should indicate that the methods of identification used are in accordance with EN 15678:2015.

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Introduction

Organic substances that migrate from products containing such substances have, when used in contact with water intended for human consumption, the potential to cause health concerns for consumers. The potential health effects of these chemicals are assessed in three stages as follows:

a) preparation of migration waters by exposing a portion of the material to water under controlled conditions;

b) analysis of the migration waters;

c) assessment of the identities and concentrations of the substance detected.

The analysis of organic substances present in migration waters can involve two different types of analytical methods as follows:

d) a screening method, which allows a variety of substances to be detected and a semi-quantitative assessment to be made of their concentrations;

e) accurate quantitative methods for the determination of specific target substances known to be present in the chemical formulations of the materials.

This standard describes the analytical procedures based upon gas chromatography and mass spectrometry (GC-MS) used to screen migration waters for organic substances derived from finished products such as pipes, protective coatings, membranes, etc. This method is suitable for migration waters from all products that can potentially release organic chemicals into water when they are used in contact with water intended for human consumption, and which are the subject of an application for approval by the national regulatory body. It may be one of several methods that form part of the overall approval process. The method may also be used as part of an approval audit process. The method does not provide accurate quantitative results and other analytical methods are recommended for accurate quantitative determination of specific target substances.

1 Scope

This European Standard describes a method for detecting and identifying organic chemicals that are amenable to GC-MS analysis using the procedures described and which can migrate from a product into water intended for human consumption. This European Standard does not provide all the necessary tools to completely identify all the substances that are detected. A method of semi-quantitatively estimating the concentrations of the organic substances detected is also provided, however, concentrations should only be seen as indicative.

NOTE The method to be used for the preparation of migration waters is specified by separate ENs, as noted below.

2 Normative references

EN 12873-1, *Influence of materials on water intended for human consumption - Influence due to migration - Part 1: Test method for factory-made products made from or incorporating organic or glassy (porcelain/vitreous enamel) materials*

EN 12873-2, *Influence of materials on water intended for human consumption - Influence due to migration - Part 2: Test method for non-metallic and non-cementitious site-applied materials*

EN 12873-3, *Influence of materials on water intended for human consumption - Influence due to migration - Part 3: Test method for ion exchange and adsorbent resins*

EN 12873-4, *Influence of materials on water intended for human consumption - Influence due to migration - Part 4: Test method for water treatment membranes*

EN ISO 3696, *Water for analytical laboratory use - Specification and test methods (ISO 3696)*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
u
atomic mass unit, defined as 1/12 of the mass of a single atom of carbon-12 in the gas phase (i.e. unbound), at rest and in its ground state

3.2
asymmetry factor
A_s
measure of the absorption of a compound during gas chromatographic analysis

Note 1 to entry: The asymmetry factor (A_s) can be derived from Formula (1).

$$A_s = \frac{(a+b)}{2b} \quad (1)$$

where

a is the distance from the leading edge of the peak at the point on the baseline where the perpendicular dropped from the peak maximum crosses it;

b is the corresponding distance from the trailing edge of the peak.

Locate the apex of the peaks that require their asymmetry values calculated. For each peak, drop a perpendicular line down at a right-angle to the baseline.

Note 2 to entry: Some manufacturer's GC-MS software packages allow the calculation of peak asymmetries to be produced automatically. Check the criteria used for the measurement, as some packages perform the calculation at 5 % of the peak height.

3.3
electron impact ionization
ionization by a beam of electrons

3.4
GC-MS
analytical instrument comprising a gas chromatograph (GC) linked to a mass spectrometer (MS)

3.5
GC-MS general survey analysis
acquisition of a series of mass spectra (up to several thousand) during the course of a gas chromatographic run, by operating the mass spectrometer in a continuous cyclic scanning mode over a wide *m/z* range

3.6
injection standard
organic substance added to the final solvent extract prior to analysis

Note 1 to entry: An injection standard is added to allow the recoveries of the internal standards to be calculated, by providing a means of normalizing the GC-MS responses for solvent extracts and GC-MS standards.

3.7
internal standard
organic substance added to the migration water at a known concentration prior to the commencement of the analysis

Note 1 to entry: Internal standards are added for the following reasons (a) to demonstrate that the analysis has been undertaken successfully, and (b) to provide a reference to allow other substances detected to be quantified. Ideally, the internal standards should not be present in the migration waters being analysed; for this reason, isotopically labelled standards are used.

3.8
test water
water used to prepare the migration water, as specified in EN 12873-1, EN 12873-2, EN 12873-3 or EN 12873-4 depending on the material being tested

3.9
laboratory blank
water, as specified in EN ISO 3696, known to contain negligible levels of contamination, to which internal standards (3.7) have been added and which is then analysed in the same way as the migration water

Note 1 to entry: Laboratory blanks are used to check for potential contamination of either migration waters or solvent extracts which may occur within the laboratory during the course of the analysis.

3.10
migration water
aqueous solution that results from leaving test water in contact with the test material under the specified test conditions

3.11
procedural blank
aqueous solution that results from leaving test water in contact with tanks or containers identical to those used to prepare the migration water in the absence of the test material

Note 1 to entry: Procedural blanks are used to check for potential contamination of migration waters that can arise during the migration procedure. For example, substances can migrate from the tanks or containers used into the test water, or aerial contamination can occur if volatile substances are present in the laboratory atmosphere. Further details are provided in the European Standards regarding the preparation of migration waters.

3.12
mass spectrometric resolution
measure of the capability of a mass spectrometer to correctly distinguish two mass spectral peaks, having sequential mass to charge (m/z) values, as separate peaks

Note 1 to entry: If z =1, this is generally denoted by m_2 / (m_2 - m_1), where m_2 has the higher m/z value and m_1 has the lower m/z value.

Note 2 to entry: Mass spectrometer set up so that the resolution is 650 will satisfactorily resolve and assign the correct masses to mass spectral peaks at m/z 649 and m/z 650. Although the mass spectrometric resolution of a mass spectrometer using a magnetic field for mass resolution remains constant throughout the mass range scanned, a quadrupole mass spectrometer is usually operated so that unit mass resolution is achieved over the mass range scanned (i.e. at m/z 200 the resolution is 200, whereas at m/z 500 the resolution is 500).

3.13
m/z
mass (m)-to-charge (z) ratio of an ion

Note 1 to entry: As most ions produced by electron impact ionization are singly charged, this ratio usually corresponds to the mass of an ion. However, exceptionally, ions can possess multiple charges.

3.14
solvent extract
solution containing substances partitioned from the migration waters into the extraction solvent (in this case dichloromethane)

3.15
total ion current
TIC
sum of all the separate ion currents carried by the individual ions contributing to a single mass spectrum

3.16
TIC chromatogram
graphical representation of the TIC versus time

3.17
Linear Retention Index

$$\text{Linear Retention Index} = (n \times 100) + 100 \times \left(\frac{(RT_x - RT_n)}{(RT_{n+1} - RT_n)} \right) \quad (2)$$

where

n is the number of carbon atoms in n-alkane prior to substance of interest;

RT_n is the retention time (min) of n-alkane;

RT_{n+1} is the retention time (min) of next n-alkane;

RT_x is the retention time (min) of substance of interest

3.18
threshold value
concentration below which detection and/or identification is uncertain

4 Principle

A mixture of isotopically-labelled internal standards is added to each of the migration waters to be analysed immediately before the extraction solvent dichloromethane is added. After solvent extraction, the extract is concentrated, an injection standard added, and the resultant concentrated extract is then analysed by GC-MS to determine the identity of any organic chemicals that may be present above a pre-set threshold value.

The mass spectrometer is used in a repetitive full-scan mode operating with positive electron impact ionization and the mass spectra produced are recorded by, and stored on, the GC-MS data system.

Wherever possible, each substance detected above the threshold value is identified, and semi-quantified by reference of its TIC to the TIC responses obtained for the internal standards.

NOTE The procedure to be used to identify organic substances from their mass spectra is given in Annex B.

5 Reagents

5.1 General

Only reagents of analytical grade shall be used, except where specified otherwise. All reagents shall be of sufficient purity to ensure that they do not give rise to interferences during the GC-MS analysis.

NOTE Contamination can arise from various sources, e.g. the laboratory atmosphere, glassware, plastics and rubber materials. The use of procedural blanks and laboratory blanks assists in detecting and identifying the source of any contamination and are used to correct results for the effects of contamination.

5.2 Required reagents

5.2.1 Reagent water, having a conductivity of < 2 mS/m, a total organic carbon content of < 0,2 mg/l carbon, and free from organic contaminants which can interfere with the GC-MS analysis of the extracts. Suitable water can be prepared by reverse osmosis, de-ionization or distillation.

5.2.2 Hydrochloric acid, concentrated (density 1,18 g/ml).

5.2.3 Hydrochloric acid solution (6 mol/l), prepared as follows:

— slowly add (0,5 ± 0,01) l of concentrated hydrochloric acid (5.2.2) to (0,5 ± 0,01) l of reagent water (5.2.1);

— this solution should be replaced on a six monthly basis.

NOTE Care is needed in preparing this solution which can generate heat.

5.2.4 Sulfuric acid, concentrated (density 1,84 g/ml).

5.2.5 Sulfuric acid solution (0,5 mol/l), prepared as follows:

— slowly add (14,0 ± 0,5) ml of sulfuric acid (5.2.4) to (300 ± 5) ml of reagent water (5.2.1) and make up to (500 ± 5) ml with reagent water;

— this solution should be replaced on a yearly basis.

NOTE Care is needed in preparing this solution which can generate heat.

5.2.6 Sodium hydroxide solution (0,5 mol/l), prepared as follows:

— dissolve (20,0 ± 0,1) g of sodium hydroxide pellets in reagent water (5.2.1) and make up to 1 l;

— replace this solution on a two weekly basis.

NOTE Care is needed in preparing this solution which can generate heat.

5.2.7 Dichloromethane, glass distilled grade, batches should not be used until they have been verified as free from significant contamination, see Note 2.

NOTE 1 Other grades can be suitable but it is necessary to demonstrate that any impurities present do not interfere with the detection of substances of interest or the internal standards, or introduce unacceptable contamination, see Note 2.

NOTE 2 Generally any peak in the solvent after contaminants have been concentrated by evaporation (200 ml of solvent reduced to 500 µl) with an estimated concentration greater than 1 µg/l is likely to interfere with the method.

5.2.8 Acetone, glass distilled grade, batches should not be used until they have been verified as free from significant contamination, see Note 2.

NOTE 1 Other grades can be suitable but it is necessary to demonstrate that any impurities present do not interfere with the detection of substances of interest or the internal standards, or introduce unacceptable contamination, see Note 2.

NOTE 2 Generally any peak in the solvent after the acetone has been diluted 5 times with dichloromethane with an estimated concentration greater than 1 µg/l is likely to interfere with the method.

5.2.9 Ascorbic acid solution, prepared as follows:

— dissolve (4,0 ± 0,1) g of ascorbic acid in reagent water (5.2.1) and make up to 1 l. Before use, extract the ascorbic acid solution with two 100 ml aliquots of dichloromethane, discarding the solvent afterwards. Transfer the ascorbic acid solution to a suitable screw-capped bottle. The solution is stable for use for one month after preparation.

5.2.10 Sodium sulphate (anhydrous), prepared as follows:

— remove any organic contaminants by heating at (500 ± 50) °C for ≥ 4 h, and store so that rehydration is minimized and re-contamination cannot occur.

5.2.11 Internal standards

Use the following isotopically-labelled (≥98 atom % D) substances:

— d_6-benzene;

— d_{21}-2,6-di-t-butyl-4-methylphenol (d_{21}-BHT);

— d_5-chlorobenzene;

— d_{34}-hexadecane;

— d_8-naphthalene;

— d_{10}-phenanthrene;

— d_5-phenol;

— d_{62}-squalane;

— d_{10}-p-xylene.

5.2.12 Internal standards stock solutions

Make up individual standards stock solutions in acetone (5.2.8) as follows:

— d_6-benzene (2,0 ± 0,05) mg/ml;

— d_{21}-BHT (8,0 ± 0,20) mg/ml;

— d_5-chlorobenzene (2,0 ± 0,05) mg/ml;

— d_{34}-hexadecane (1,0 ± 0,02) mg/ml;

— d_8-naphthalene (1,0 ± 0,02) mg/ml;

— d_{10}-phenanthrene (2,0 ± 0,05) mg/ml;

— d_5-phenol (8,0 ± 0,20) mg/ml;

— d_{10}-p-xylene (1,0 ± 0,02) mg/ml.

Due to its volatility, it is difficult to make standard solutions of d_6-benzene by weighing; it is recommended that suitable volumes of d_6-benzene are measured using calibrated micro-syringes or positive displacement pipettes, based on its density (0,950 g/ml), and injected below the surface of the acetone.

Make up the following stock solution in dichloromethane (5.2.7)

— d_{62}-squalane (8,0 ± 0,20) mg/ml.

The stock solutions are stable for at least 12 months, provided they are stored in the dark at (−18 ± 5) °C.

5.2.13 Internal standards intermediate solution

The internal standards intermediate solutions are ten-fold dilutions of each of the individual standard stock solutions, see 5.2.12. Prepare the intermediate solutions as follows:

— place (2,5 ± 0,025) ml of the d_{62}-squalane stock solution (5.2.12) in a 25 ml volumetric flask;

— gently evaporate the dichloromethane, using nitrogen blow down, until it is completely removed. This can be verified by evaporating to constant mass;

— place (2,5 ± 0,025) ml of each of the other eight individual standard stock solutions (5.2.12) into the volumetric flask, ensuring that the d_6-benzene stock solution is added last, and make up to the graduation mark with acetone (5.2.8). The d_6-benzene stock solution is added last in order to minimize any potential evaporative losses of this standard.

This solution is stable for at least six months provided it is stored in the dark at (−18 ± 5) °C.

5.2.14 Internal standards spiking solution

The internal standards spiking solutions are hundred-fold dilutions of each of the individual standard stock solutions, see 5.2.12. Prepare the spiking solutions as follows:

— add (1,00 ± 0,01) ml of the internal standards intermediate solution (5.2.13) to (8 ± 0,5) ml acetone in a 10 ml volumetric flask;

— make up to the graduation mark with acetone.

Store this solution in the dark at (−18 ± 5) °C. This solution should be renewed every three months, or sooner if during its use any indication is obtained that the concentrations of any of the internal standards have changed.

5.2.15 Injection standard

Use d_{10}-1-methylnaphthalene (≥ 98 atom % D).

5.2.16 Injection standard stock solution

Prepare the injection standard stock solution as follows:

— weigh (25 ± 0,02) mg d_{10}-1-methylnaphthalene (5.2.15) and add to approximately 20 ml dichloromethane contained in a 25 ml volumetric flask;

— shake to dissolve and then make up to the graduation mark with dichloromethane.

This solution contains 1 mg/ml and is stable for 12 months provided it is stored in the dark at (−18 ± 5) °C.

5.2.17 Injection standard spiking solution

Prepare the injection standard spiking solution as follows:

— take 1 ml of the injection standard stock solution (5.2.16) and add to approximately 5 ml of dichloromethane contained in a 10 ml volumetric flask.

— make up to the graduation mark with additional dichloromethane.

This solution contains 100 µg/ml and is stable for at least three months provided it is stored in the dark at (−18 ± 5) °C.

5.2.18 Internal standards GC column test solution

Internal standards GC column test solution are five hundred-fold dilution of each of the individual standard stock solutions, see 5.2.12 and a one hundred-fold dilution of the injection standard stock solution, see 5.2.16. Prepare the test solutions as follows:

— add (200 ± 5) µl of the internal standards intermediate solution (5.2.13) and (1 000 ± 10) µl of the injection standard spiking solution (5.2.17) to (7 ± 1) ml of dichloromethane in a 10 ml volumetric flask then make up to the graduation mark with dichloromethane.

— to avoid potential losses of the most volatile internal standard (d_6-benzene) a calibrated micro-syringe or positive displacement pipette fitted with a needle or tip of sufficient length to allow the tip of the syringe needle or pipette tip to be introduced below the meniscus of the dichloromethane prior to expelling the syringe or pipette contents should be used.

This solution is stable for three months provided it is stored in the dark at (−18 ± 5) °C. This solution should be renewed, if during its use, an indication is obtained that the concentrations of any of the internal standards have changed.

See also A.3 for suggested solutions to be used for independently assessing GC-MS performance.

6 Apparatus

6.1 Vessels, containers etc., constructed of a material, such as glass or polytetrafluoroethylene (PTFE) that is inert under the specified test conditions.

6.2 Cleaning, in accordance with either the following procedure or with an alternative procedure demonstrated to be equally effective in removing all detectable traces of organic substances from the apparatus. Laboratory glassware shall be cleaned by washing with a biodegradable laboratory detergent specially designed for the removal of organic materials, followed by rinsing with hydrochloric acid solution (5.2.3) and finally by thoroughly rinsing with reagent water (5.2.1).

6.3 Concentration apparatus, that is specifically designed to allow the solvent extract (3.14) to be reduced in volume from 200 ml to approximately 500 µl. The volume of the concentrate shall at no time be less than 50 µl. There are several commercial systems available consisting of suitable glass vessels which hold the dried dichloromethane extract. These can be gently heated under a stream of inert gas such as nitrogen in order to concentrate the extract whilst minimizing losses of the more volatile substances. The temperature of the dichloromethane under evaporation should not exceed the manufacturers recommended value. In addition, a source of dry nitrogen can both assist in the evaporation process and minimize contamination of the concentrated extract. Any concentration apparatus capable of meeting these requirements is suitable, see A.1 for the procedure for checking the suitability of concentration apparatus.

Other items of equipment (such as the classical Kuderna-Danish technique) can be suitable, but during the concentration process (which will usually proceed in several steps) it is essential that losses of volatile substances are minimized. The response of the most volatile internal standard (d_6-benzene) should be checked to ensure that losses of this substance in the concentration step do not exceed 50 %, see A.1.

6.4 Capillary gas chromatograph, with temperature gradient facility interfaced to a mass spectrometer (6.7) and capable of meeting the operating conditions specified in 8.3.

6.5 GC capillary column, fused silica coated with a non-polar bonded phase which performs equivalently to a 5 %-Phenyl, 95 %-Dimethylpolysiloxane phase (e.g. DB-5 or BP-5) and is capable of meeting the operating conditions specified in 8.3. Other column types capable of giving equivalent or better performance characteristics in this test may be used, providing suitable validation data is produced to justify the substitution.

6.6 Carrier gas (for GC-MS system) has a concentration of helium (99,999 % purity, or better).

6.7 Mass spectrometer, which is capable of operating in the electron impact ionization mode covering the *m/z* range 20 to 650 provided that the mass spectrometric resolution (3.12) is sufficient to allow unit mass resolution at the highest mass recorded, with a scan cycle time of ≤ 1 s.

6.8 Mass spectrometry data system, which is capable of acquiring data from the mass spectrometer under the conditions in 6.7, and which can produce total ion current (TIC) chromatograms, background subtracted averaged mass spectra and TIC peak areas. It shall also be capable of producing hard-copy outputs of TIC chromatograms and mass spectra.

6.9 Mass spectral library, available on the mass spectrometry data system (6.8).

7 Storage of migration waters, procedural blanks and laboratory blanks

Start the analysis of migration water samples as soon as possible following collection. If necessary, store the migration water samples, procedural blanks and laboratory blanks under the same conditions, i.e. in the absence of light at (4 ± 2) °C, for a maximum of 2 d (48 h) before solvent extraction is undertaken.

The migration waters for analysis should be prepared in accordance with appropriate ENs.

NOTE If storing chlorinated migration waters first neutralize by the addition of 1 ml ascorbic acid solution (5.2.9) per 500 ml.

8 Method of analysis

8.1 Extraction procedure

8.1.1 Transfer (500 ± 10) ml of the migration water sample to a separating funnel. If any migration waters have been prepared using chlorinated water, neutralize by the addition of 1 ml ascorbic acid solution (5.2.9).

8.1.2 Add (100 ± 2) µl of the internal standards spiking solution (5.2.14) into the sample using a calibrated syringe or positive displacement pipette, ensuring that the tip of the syringe needle or pipette tip is below the surface of the sample. Insert the stopper and swirl the contents of the separating funnel gently for a few seconds to mix.

8.1.3 Check the pH of the sample and adjust to pH (2 ± 0,2), if necessary, by dropwise addition of either sulfuric acid solution (5.2.5) or sodium hydroxide solution (5.2.6) as appropriate.

8.1.4 Add dichloromethane (50 ± 5) ml (5.2.7) to the spiked pH adjusted sample in the separating funnel and insert the stopper. Shake the separating funnel for a total of (3 min ± 20 s), releasing any excess pressure as necessary. Remove the dichloromethane (lower layer) into a flask (capacity at least 250 ml). Add a further (50 ± 5) ml of dichloromethane to the separating funnel and repeat the extraction step. Add the second aliquot of dichloromethane to the flask in which the initial solvent extract is stored, so that the two extracts are combined.

For safety reasons during the solvent extraction step, excess pressure (which can build up in the stoppered separating funnel) should be dissipated via the separating funnel tap when the separating funnel is inverted. As dichloromethane vapour is hazardous, dissipation of pressure should be undertaken with the separating funnel in a fume hood.

NOTE Extraction (shaking) time can be reduced following demonstrable evidence that this does not compromise the extraction process. An appropriate validation would need to be provided to the accreditation body and included in the scope of the laboratory method.

8.1.5 Change the pH of the sample in the separating funnel to pH of (10 ± 0,2) by dropwise addition of sodium hydroxide solution (5.2.6).

Approximately 6 ml of sodium hydroxide solution (5.2.6) is required.

8.1.6 Add a further (50 ± 5) ml of dichloromethane (5.2.7) to the separating funnel and repeat the extraction step. Add the aliquot of dichloromethane to the flask in which the initial two solvent extracts at pH of 2 is stored, so that the extracts are combined. Add a final (50 ± 5) ml of dichloromethane to the separating funnel, repeat the extraction step and add the aliquot of dichloromethane to the solvent extract storage flask.

8.1.7 Dry this combined solvent extract. Various methods may be used for drying solvent extracts e.g. freezing or addition of small amounts of sodium sulphate (5.2.10). Any of these may be used provided that they do not affect adversely the performance of this method. If a drying column is used, the walls of the column should be gently rinsed with one or two millilitres of dichloromethane and the rinsings added to the extract.

8.1.8 Evaporate the dried dichloromethane to approximately 500 µl using the appropriate apparatus (6.3). Add (50 ± 2) µl of the injection standard spiking solution (5.2.17) to the concentrated extract. If the final concentrated volume of the extract is 500 µl, then the concentration of the injection standard will be identical to that of the internal standards GC column test solution (5.2.18). Transfer the concentrated extract to a suitable amber vial and crimp-cap it.

The chosen apparatus and procedure used shall be demonstrated to be suitable before they are used, see A.1.

8.1.9 Analyse prepared extracts, or store at (−18 ± 5) °C for maximally 10 days. Prolonged storage requires validation by the laboratory to ensure no loss/gain of leachable substances.

8.2 GC-MS analysis

8.2.1 Safety precautions

Safety precautions should be observed when working with GC-MS systems. GC-MS systems typically operate from a nominal mains voltage (220-240 V AC; exceptionally, some operate from a '3-phase' 415 V AC supply), however, certain parts or components of some mass spectrometers (which utilize a magnetic field for mass resolution) can be at a very high electrical potential (up to 8 kV) relative to earth; other mass spectrometers utilize radio-frequency radiation and DC voltages for mass separation. Due care is necessary in the operation of GC-MS systems.

8.2.2 Mass spectrometer operating parameters

Follow the manufacturer's instructions for the mass spectrometer to set the following parameters:

— ionization mode: positive electron impact (EI);

— electron energy: 70 eV;

— mass range: minimum 20 u to 650 u;

— scan speed: ≥ 1 scan per second;

— scan mode: repetitive.

8.2.3 Setting up the mass spectrometer and data system

Follow the manufacturer's instructions relating to optimizing the performance and sensitivity of the mass spectrometer, mass calibration and data acquisition and processing.

8.2.4 Initial tuning and mass calibration of the mass spectrometer

Follow the manufacturer's instructions. All of the major reference peaks in the mass range covered in the calibration table held on the MS data system shall be found in the scan(s) used for calibration purposes.

8.3 Setting up the GC-MS system

Install the GC column according to the manufacturers' instructions and verify its performance (e.g. in terms of separation number and adsorption) against the column performance data supplied by the manufacturer. Proprietary standard solutions are available for this purpose (see A.3).

A simple way for testing a new column performance versus the previous column is to inject the Test Solution (5.2.18) and comparing the respective sets of data.

Provided the general performance of the column is satisfactory, use the internal standards GC column test solution (5.2.18) to establish the initial performance of the column for the internal standards. Use the same GC temperature program for this purpose as that used for the GC-MS analysis of the concentrated solvent extracts (see Note).

On the first occasion that this analysis is being carried out, the GC column test solution (5.2.18) shall be analysed once a day on five separate days to obtain (i) typical spectra of the internal standards and (ii) typical TIC peak areas for all of the internal standards.

NOTE The requirements for the GC-MS GC run-programme can be complied with by using a GC column of length 50 m to 60 m with an internal diameter of either 0,25 mm or 0,32 mm, coated with a bonded phase equivalent to DB-5 (phase film thickness 0,25 µm), an initial temperature of 30 °C for 4 min, linearly programmed at 8 °C/min to a final temperature of 300 °C and maintaining this for 20 min. Other columns and/or conditions can also be suitable.

Ensure that:

a) the temperature programming rate does not exceed 12 °C/min at any time;

b) all of the internal standards are detected on the TIC chromatogram;

c) d_6-benzene is separated from the solvent peak and that the retention time of d_{62}-squalane is between 35 min and 45 min;

d) the asymmetry factors, A_s, (3.2) for the peaks obtained for d_5-phenol and d_8-naphthalene are within the range 0,65 to 2,0. In the event that this requirement is not met, investigate the cause and correct before continuing with the analysis. If necessary, install a new GC column;

e) the sensitivity of the mass spectrometer is set so that acceptable spectra are obtained for those internal standards, which are present at the threshold concentration (2 µg/l) and the mass spectra obtained for the internal standards present at the highest level (16 µg/l) are not saturated by, if necessary, adjusting the sensitivity of the mass spectrometer. The TIC peak areas for the internal standards should be noted;

f) the mass spectra obtained from the GC-MS system performance test correspond closely to mass spectra previously acquired for these internal standards on the same GC-MS system under identical operating conditions;

g) the m/z value of the base peak is consistent, and that the intensities of 6 to 10 peaks having an intensity > 10 % of the base peak do not vary by more than 30 % of their intensity when compared to previously acquired spectra;

h) the high mass ions (>300 u) in the mass spectra for d_{62}-squalane are correctly mass measured after the mass spectrometer mass calibration. If this is not the case, recalibrate the mass spectrometer before continuing with the analysis.

8.4 GC-MS operating conditions for analysis of solvent extracts

Analyse concentrated solvent extracts using identical conditions to those used for checking the performance of the GC-MS system using the internal standards GC column test solution (5.2.18) (see Note 1).

The “fitness for purpose” of the GC-MS system (with reference to items a to h of 8.3) should be checked prior to using the system for this analysis. Check the performance of the GC-MS system before every batch of concentrated solvent extracts run, using the internal standards GC column test solution (5.2.18), and regularly during the batch if batch sizes are greater than six. For example, analyse the internal standards GC test column solution (5.2.18) after every sixth concentrated solvent extract and check the criteria given in 8.3 (items a to h) to ensure that the performance of the system has not deteriorated. If the performance of the system has deteriorated stop the analysis; investigate, and correct the cause of the failure before continuing with the analysis.

Depending on the types and concentrations of substances present in the extracts from the migration waters, compliance with the asymmetry requirement (item d of 8.3), particularly for d_5-phenol, may not be possible. If this is the case, this fact should be noted in the analytical report.

8.5 Production of required outputs from the GC-MS data system

Ensure the following outputs are obtained for each of the GC-MS runs carried out on concentrated solvent extracts:

— a hard copy or data file in a commonly used format of the TIC trace (covering the mass range scanned);

NOTE If a solvent delay is included as part of the data acquisition, the TIC trace will not include a peak for the solvent. This is acceptable.

— the retention times correct to ± 1 s of the peak maximum of every peak detected on the TIC chromatogram, including the internal standards;

— the peak areas of every detected peak, including the internal standards;

— hard copies of a mass spectrum obtained for each of the substances detected which are considered to originate from the sample; this shall be the best spectrum obtainable, normally obtained by background subtraction and averaging of several mass spectra.

Substances detected which are not considered to arise from the sample or which are not internal standards, are included in the above requirements. However, an indication should be given as to which of the substances detected fall into this category, along with their probable origin, e.g. contaminants in the solvent used for the solvent extraction, or substances present in the test water.

With the prior agreement of the client and the other key users of the information, the mass spectrum may be provided in an agreed electronic format, such as a PDF file.

9 Quality assurance (QA) and quality control (QC) procedures

9.1 The mass calibration of the mass spectrometer

Verify the mass calibration on each occasion that a batch of concentrated solvent extracts is analysed. Use the calibrant normally used for mass calibration for this purpose. Recalibrate the mass spectrometer if any of the calibrant masses are incorrectly assigned. See also A.3.

9.2 The performance of the GC-MS system

Check the performance of the GC-MS system on each occasion that a batch of concentrated solvent extracts is to be run by analysing the internal standards GC column test solution (5.2.18) prior to analysing the concentrated solvent extracts. Compare the response (TIC peak area) obtained for each internal standard to that obtained when setting up the GC-MS system (item e of 8.3). Provided that the peak areas are within 50 % of those initially determined, acceptable mass spectra are obtained for those internal standards present at the lowest concentration and the mass spectra of the internal standards present at the highest concentration are not saturated, and the asymmetry factors are in accordance with item d of 8.3, the performance is considered acceptable, see also A.3.

9.3 The performance of the method

When analysing the concentrated solvent extracts, consider the performance of the method acceptable provided the following criteria are satisfied:

— all of the internal standards are detected in the GC-MS TIC chromatogram;

— the recoveries of the internal standards d_8-naphthalene, d_{10}-phenanthrene and d_{62}-squalane are ≥ 50 % (A.2).

NOTE 1 The absence of any of the internal standards, or recoveries < 50 % of the three internal standards specified above, in the GC-MS TIC chromatogram indicates that either the extraction step has not been carried out correctly, or the concentration of the solvent extract has not been carried out correctly, or the GC-MS system is not functioning correctly.

NOTE 2 An injection standard (d_{10}–1-methylnaphthalene; 5.2.16) present in the internal standards GC column test solution and added to the concentrated solvent extracts from the blanks and migration water samples is used to normalize the GC-MS responses for the TIC traces from the extracts of the various solvent extracts and the internal standards GC column test solution.

NOTE 3 It is not possible to specify a numerical limit of detection, because this will vary with the nature of the substance detected (see 9.4). A threshold value of 2 µg/l is used instead of a limit of detection. The threshold value is the concentration below which detection and/or identification is uncertain and was set following analysis of the results of inter-laboratory trials.

9.4 Performance of the analyst

The performance of the analyst in identification of unknowns shall be checked using a suitable proficiency testing scheme.

9.5 Uncertainty of the method

It is not possible to quantify the uncertainty of the quantification as this depends on many variables including the relative responses of the identified substance and the internal standard used for estimating the concentration. This can vary by a factor of 10 and is the primary reason why estimations of concentration are only semi-quantitative. Other sources of variability, while high when compared to what is expected of quantitative methods, are unlikely to contribute significantly to the uncertainty budget and if needed an estimate of the expanded uncertainty (k = 2) of 1 log is reasonable.

10 Expression of results

10.1 Semi-quantitative estimation of concentrations of substances detected

Quantify each detected substance by comparing its response to the appropriate internal standard, as follows:

— those substances having retention times from solvent front, through d_6-benzene and up to d_8-naphthalene are quantified relative to d_5-chlorobenzene;

— those substances eluting between d_8-naphthalene and d_{34}-hexadecane are quantified relative to d_{21}-BHT;

— those substances eluting after d_{34}-hexadecane are quantified relative to d_{10}-phenanthrene.

Use the following Formula (3) to provide the concentration of a substance D of each substance detected in a migration water sample:

$$D_D = \frac{P_D \times I}{P_S} \quad (3)$$

where

D_D is the concentration of a substance D (in µg/l);

P_D is the peak area of a substance D;

P_S is the peak area of the internal standard;

I is the internal standard concentration (in µg/l).

In cases where an internal standard used for quantification co-elutes with another substance, the next nearest internal standard (in terms of GC retention time) present at 4 µg/l or 16 µg/l shall be used, with the exception of d_5-phenol, which is not used for quantification. If both the BHT-d_{21} and phenanthrene-d_{10} are co-eluting with other compounds, then the squalane-d_{62} may be used as an alternative internal standard instead.

NOTE 1 The concentration calculated on the basis of an internal standard assumes that both the substance and the internal standard have equal losses during the extraction of the sample and the extract preparation and have an identical response on the GC-MS TIC trace. Adjustment of concentration [D] for any of these factors is not necessary within the scope of this standard. Guidance on the interpretation of semi-quantitative results is given in Annex C.

NOTE 2 If a substance has been positively identified (10.2 a) further analysis may be possible to provide a quantitative result. This does not form part of this method.

10.2 Identification of substances detected

Use four categories to define the confidence level associated with the identities of the detected substances, as follows:

a) A positive identification (P) indicates that the mass spectrum and GC retention time are the same as those obtained from a pure standard of the substance run under the identical GC-MS conditions on the GC-MS system used to analyse the concentrated solvent extract;

b) A tentative identification (T) indicates that a possible identity has been obtained either from computerized library searching of a mass spectral database supported by interpretation of the mass spectral data and when possible by chromatographic information, or interpretation by an experienced mass spectroscopist. However, a pure standard has either not been run under identical GC-MS conditions on the GC-MS system used to analyse the concentrated solvent extract or is not available;

c) An unknown (U) is any substance not covered by either of the above categories. The four most intense peaks in the mass spectrum, in decreasing order of intensity (e.g. 147, 43, 71, 91), should be noted in the tabulation of results, together with a potential molecular ion (if observed, and is not one of the four most intense ions in the mass spectrum). Any partial interpretation by an experienced mass spectroscopist should also be included;

d) No identification (N) indicates that the peak of the GC spectrum is not identified by any of the above categories. The four most intense peaks in the mass spectrum, in decreasing order of intensity (e.g. 147, 43, 71, 91), should be noted in the tabulation of results. The mass spectrum is attached to the report.

In addition to using any automated library searching for substance identification, an experienced analyst should verify the results, i.e. the plausibility and or identification.

NOTE 1 Further information on identification can be found in Annex B and on the interpretation of results in Annex C.

NOTE 2 It is sometimes difficult to establish whether a molecular ion has been observed in the mass spectrum of an unidentified substance as the highest mass ion detected could not be the molecular ion. As with any interpretation of mass spectral data, expert judgement can be required.

10.3 Reporting of results

Tabulate the results from the GC-MS analysis and include the following information, for every peak detected on the TIC chromatograms obtained for extracts of the migration waters, procedural blanks and laboratory blank which are estimated to be present in the migration water at or above the threshold value (8.3 e)):

a) retention time;

b) relative retention time with respect to d_{10}-1-methylnaphthalene;

c) linear retention index;

d) substance name for tentative and positive identifications; in the case of substances reported as unknown or no identification the *m/z* values of the 4 most intense masses in decreasing order of intensity shall be listed;

e) confidence level, using the following abbreviations – P (positive), T (tentative), U (unknown) or N (no identification) (10.2);

f) TIC peak area

g) CAS number for positive identifications;

h) estimated concentration (expressed in µg/l to one place of decimals) for the migration waters and blanks. The concentrations reported for the migration waters should be corrected (i.e. blank subtracted) with respect to the concentrations detected in the procedural blanks (see Note 2);

i) for those substances detected in both the migration waters and the procedural blanks, calculate and report the ratio of non-corrected concentrations in migration waters and procedural blanks (see Note 1);

j) internal standard used for quantification;

k) probable origin (see Note 2).

If a substance has been detected at a higher concentration in the procedural blank than in the migration water, the blank-subtracted concentration will be a negative number that should be reported as 0,0.

NOTE 1 If a substance has been detected at a higher concentration in the migration water than in the procedural blank, the ratio of the two non-subtracted concentrations will be > 1. For some substances (mainly volatile substances such as chloroform which are sometimes present as impurities in the dichloromethane used for the solvent extraction), if this ratio is not > 5, it is difficult to be certain that the increased concentration in the migration waters compared to the procedural blank is due to migration from the product tested.

NOTE 2 The sources of the substances detected can fall into three categories, as follows:

a) contaminants in the dichloromethane used for the solvent extraction and other contaminants introduced in the laboratory where the sample processing is carried out – these will be detected in the laboratory blank;

b) contaminants introduced during the preparation of the migration water, e.g. substances present in the test water – these will be detected in the procedural blank;

c) substances migrating from the product being tested.

11 Test report

11.1 General

The test report shall include the following particulars:

a) a title (e.g. “Test Report – GC assessment of ……”) and the date of issue of the report;

b) a reference to this standard;

c) name and address of the analytical laboratory, and location where the migration tests were carried out if different;

d) unique identification of the test report (such as serial number), and on each page an identification in order to ensure that the page is recognized as a part of the test report, and a clear identification of the end of the test report;

e) name and address of the client placing the order.

11.2 Test results

Report of the GC-MS analysis undertaken on the migration waters, including the following:

a) copy of the TIC chromatograms (3.16) for the internal standards GC column test solution obtained on each analytical occasion;

b) data table listing the following for each sample and GC column test mix:

   1) the peak asymmetry values for d_5-phenol, d_8-naphthalene;

   2) the percentage recoveries for d_8-naphthalene, d_{10}-phenanthrene and d_{62}-squalane.

c) limits of detection for the deuterated internal standards and a description of the procedures used to obtain them;

d) description and results of the method validation (method performance) for the GC-MS method;

e) results from the GC-MS examination of each solvent extract reported in a tabular format, together with a copy of the TIC chromatogram for each solvent extract;

f) data tables listing the following:

   1) all peaks detected, including internal standards, which were “spiked” or calculated to be present at concentrations equivalent to the pre-set threshold value (8.3 e)) or greater in the leachates;

   2) those peaks considered not to originate from the product being tested with an indication of their possible origins;

   3) retention time of each peak listed and the identity of the substance;

   4) linear retention index of each peak listed;

   5) the calculated concentration of each peak in µg/l, together with the internal standard used to derive this estimate and an indication of the origin of the substance;

   6) those peaks which cannot be identified reported as “unknowns” or “no identification” with their four major ions (in decreasing order of intensity) and, where possible, the potential molecular ion;

   7) print-out (or copy) of the mass spectrum for each substance detected which is considered to originate from the product being tested;

   8) description of the basis on which peaks are identified (see 9.2);

   9) any comments on irregular peak shapes of reported substances.

g) any factors which could have affected the results, such as any incidents or any operating details not specified in this standard;

h) dates of test:

1) commencement and completion of preparation of migration water;

2) preparation of solvent extract;

3) GC-MS analysis of extract.

i) conditions of storage of:

1) migration water prior to extraction;

2) solvent extract prior to GC-MS analysis.

Annex A
(normative)

Additional procedural details

A.1 Checking suitability of apparatus used for concentrating solvent extracts

It is necessary to be able to reduce the volume of the dichloromethane solvent extracts from about 200 ml to between 50 µl and 500 µl without significant losses of volatile components which could be present in the leachate sample.

To verify that this can be satisfactorily achieved, it is recommended that a 500 µl portion of the internal standards GC column test solution (see 5.2.18) is diluted to 200 ml with dichloromethane, and the resulting solution concentrated to 500 µl, using appropriate apparatus or equipment. This concentrate shall be run on the GC-MS system under exactly the same conditions as used when using the GC column test standard solution for checking for satisfactory GC performance, and the TIC or RIC (reconstructed ion chromatogram) trace compared to a TIC or RIC trace obtained when the GC column test standard is run. Provided the loss of the most volatile internal standard, d_6-benzene, is not more than 50 % the technique used for the concentration of the solvent extracts is considered satisfactory.

A description of suitable equipment is in (6.3), but no commercial recommendation can be made for this process.

A.2 Procedure for calculation of recoveries of internal standards

A.2.1 General

Use either of the procedures given A.2.2 and A.2.3 to calculate recoveries of internal standards.

A.2.2 Procedure for calculation of recoveries of internal standards

The percentage recovery of each internal standard can be calculated using the Formula (4).

This is based upon the concentrations of each internal standard in the internal standards spiking solution (5.2.14), the injection standard spiking solution (5.2.17) and the volume of the leachate analysed. The final volume of the concentrated extract is not required for the calculation as the peak areas for the internal standards in each injection are normalized to allow a simple comparison to be made.

The respective ratios of the peak area of each internal standard to the peak area of the injection standard should be calculated for the injection of every extract. The same ratio should also be calculated for the two GC column test solutions (5.2.18) that bracket these samples.

$$R = \frac{P_e \times 100}{0{,}5 \times (P_{s1} + P_{s2})} \tag{4}$$

where

R is the recovery of internal standard, in percent;

P_e is the ratio of peak area of the internal standard chosen for the comparison against the peak area of the injection standard in the extract;

P_{s1}, P_{s2} are the peak areas of the internal standard chosen for the comparison against the peak area of injection standard, in the GC column test solutions (5.2.18) which bracket the injection of the extract.

A.2.3 Procedure for calculation of recoveries of internal standards (alternative version)

The percentage recovery of each internal standard can be calculated using the Formula (5).

This is based upon the concentrations of each internal standard in the internal standards spiking solution (5.2.14), the injection standard spiking solution (5.2.17) and the volume of the leachate analysed. The final volume of the concentrated extract is not required for the calculation as the peak areas for the internal standards in each injection are normalized to allow a simple comparison to be made.

The respective ratios of the peak area of each internal standard to the peak area of the injection standard shall be calculated for the injection of every extract. The same ratio shall also be calculated for the two GC column test solutions (5.2.18) that bracket these samples.

$$R = \frac{100 \times (P_e / IS_A)}{0{,}5 \times ((P_{s1} / IS_{s1}) + (P_{s2} / IS_{s2}))} \quad (5)$$

where

R is the recovery of internal standard, in percent;

P_e is the peak area of the internal standard chosen for the comparison in the sample extract;

P_{s1} is the peak area of the internal standard chosen for the comparison in the GC-MS test solution (5.2.13) before the injection of the extract;

P_{s2} is the peak area of the internal standard chosen for the comparison in the GC-MS test solution (5.2.13) after the injection of the extract;

IS_A is the peak area of the injection standard in the sample extract;

IS_{S1} is the peak area of the injection standard in the GC column test solution (5.2.18) before the injection of the extract;

IS_{S2} is the peak area of the injection standard in the GC column test solution (5.2.18) after the injection of the extract.

A.3 Standard solutions for checking GC column performance

Several chromatography supply companies produce mixtures specifically designed to evaluate the performance of GC columns, in terms of parameters such as column efficiency and adsorptive or ‘active’ sites. If the GC column used is from a manufacturer who does not provide a suitable test chromatogram, the column shall be evaluated before use with solvent extracts of migration waters, using this type of test mixture.

These solutions are sometimes referred to as ‘Grob mixtures’, but they were designed for assessing the condition of the analytical column using any type of detector, i.e. not necessarily a mass spectrometer.

Annex B
(informative)

Outline of general approach for identification of substances detected

B.1 General approach - Introduction

The data acquired during the GC-MS run for each solvent extract is normally stored on the mass spectrometry data system as a discrete data file that can be inspected either while the run is proceeding, or after the run has been completed.

The data is usually initially displayed on a data system display monitor as a total ion current (TIC) chromatogram or reconstructed ion chromatogram (RIC). Figure B.1 displays a typical diagram, as example. Each substance detected should appear as a peak on the TIC or RIC trace, and the mass spectra produced by each substance can be displayed on the monitor using the appropriate commands.

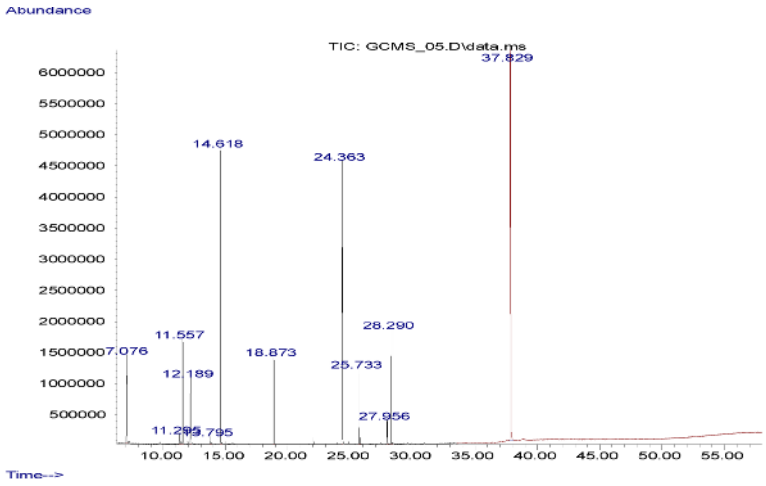

Figure B.1 — A typical Total Ion Chromatogram (TIC) of the internal standard solution

B.2 GC-MS Test Solution

The TIC for an extracted blank should show all nine of the deuterated internal standards, with no substance overloading. All the internal standards should elute as Gaussian peaks - phenol-d_6 and naphthalene-d_8 are used to measure column activity, and these may experience some small deformities. There should be very few extraneous peaks present, and these should be traceable to the solvents used for the spiking and/or extraction. The baseline may rise gently due to column bleed as the temperature programme reaches its maximum, and this may also fall away slightly as the temperature remains isothermal during the latter part of the injection. See Figure B.2.

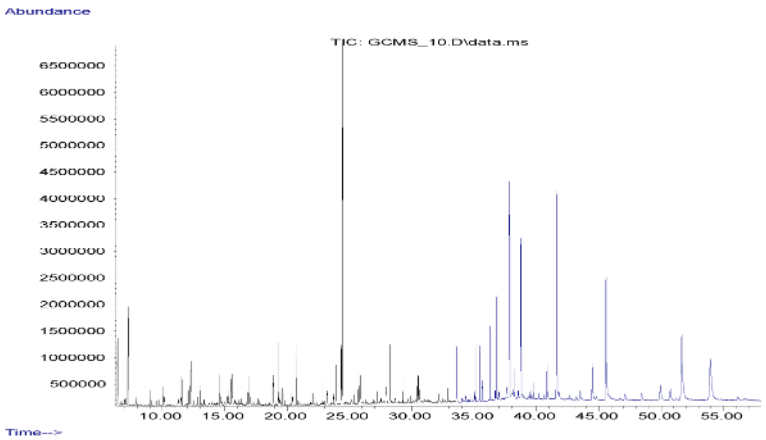

Figure B.2 — A typical Total Ion Chromatogram (TIC) of an extracted leachate

B.3 Typical Leachate

Having achieved a satisfactory injection, the substances in a leachate require identification.

Normally, the mass spectrum initially chosen for display will be that produced when the concentration of the substance of interest is at its maximum (i.e. at the top of the peak). However, if it is suspected that the eluting peak is a mixture (i.e. two or more substances are not satisfactorily separated by the GC column), or if the mass spectrum is saturated (due to the dynamic range of the mass spectrometer being exceeded), other spectra should be chosen for display.

The EI+ mass spectrum produced shall be examined to check that it is of sufficient quality. Information to look for includes the presence of isotopic ions in the appropriate quantities and ensuring that a background subtraction has been performed, either on a mass spectrum from a single scan or on an averaged spectrum, in order to remove extraneous ions that might be derived from either an overlapping substance and/or column bleed are removed.

Mass spectra can be averaged across a peak (provided it is considered that the peak is due to a single substance) to minimize any distortion of the spectra which can occur if the concentration of a substance entering the mass spectrometer changes significantly during the course of a single mass spectrometer scan.

This can occur when a GC peak is very sharp, e.g. only 2 s to 3 s wide. However, before averaging several spectra through a peak, each spectrum should be checked to ascertain whether any are saturated; if any are, due allowance should be made when assessing the resulting averaged spectrum.

B.4 Identifying substances

The mass spectrum obtained for each peak detected is generally initially inspected visually. Depending on the experience of the mass spectroscopist, it can be possible to identify the substance giving rise to the spectrum without recourse to reference mass spectra held in libraries (on the data system, or in reference books).

If the mass spectrum is not visually recognized, a library search is usually carried out on the data system. It is recommended that a reverse searching procedure is used. The closeness of the match between the unknown and the chosen library spectra is usually expressed in terms of three parameters - fit, purity and reverse fit.

However, the best match chosen by the data system does not necessarily lead to the identification of the unknown, and the mass spectroscopist has to apply his/her judgement, taking into account such factors as the GC retention time (see D.4), in order to decide whether the identification suggested by the computerized library search is accepted.

B.5 Overloading

An obvious indication that a mass spectrum is saturated, or overloaded, is when a peak has a “shark-fin” appearance, i.e. a leading edge. In this case, it may be possible to select a nearby spectrum from the back of the peak as it falls from the apex. Other clues to overloading may include when two or more *m/z* ions all have 100 % abundance.

Mass spectra from scans obtained before or after the intensity maximizes should be inspected to obtain a representative mass spectrum for the substance of interest, although if a single spectrum is chosen it should be ascertained that it is not distorted (‘skewed’).

Annex C
(informative)

Guidance on the interpretation of results

C.1 Introduction

C.1.1 The purpose of the method is to identify substances leaching from a product that may be of concern for the health of consumers of water. When considering whether the evidence available is sufficient to allow a decision, on suitability of the product for use in contact with drinking water, to be made, the Approver (see C.1.2) should take into account the level of confidence in the identification of the leaching substance and the semi-quantitative nature of the quantification.

C.1.2 This guidance puts into context the results of analysis using this method for the user of those results, the Notified Body, the national regulator or their appointed product certifying body, jointly referred to in this guidance as the Approver, making decisions on the suitability of products or materials for use in contact with drinking water. The results should not be considered in isolation but be used in conjunction with all other available information.

C.1.3 The guidance does not advise on how or when a toxicological assessment is carried out, nor does it advise on what decision should be made. It does advise on the way in which the information should be used.

C.2 Initial assessment of test report

C.2.1 The primary output of the test report is the identification of substances leaching into the test water. The identification of substances from their mass spectral data is a skilled process and highly dependent on the interpretative skills of the analyst. The test is also subject to contamination from reagents, apparatus and the laboratory environment. Correct identification and assignment of such contaminants is critical to the method as is the quality of the chromatographic and mass spectral data and the mass spectral libraries used to aid identification.

C.2.2 Because of the difficult nature of the interpretation of the mass spectra and the possibility of extra information being available to the Approver, it is recommended that before assessing the report, the Approver has to ensure (e.g. through a second opinion) that the test was carried out correctly and that the identification and quantitative estimates have been carried out correctly. In some cases, especially with unidentified substances (including those partially identified) the reviewer may be able to suggest possible alternative identifications to the analyst.

C.2.3 This peer review process is critical to satisfying the Approver's need for confidence in the quality of the information contained in the report and the expert appointed to carry out the review should be both competent and independent of the original analysis and interpretation.

C.2.4 Once the Approver is satisfied that the report is satisfactory and forms a basis for considering approval of the product or material, the health implications of the results can be assessed.

C.3 Assessment of results of analysis

C.3.1 If the report includes no substances of concern, further consideration is not needed and the report can be regarded as not containing anything that could be regarded as a barrier to approval of the product or material.

C.3.2 If substances of potential concern are identified (including substances that cannot be identified from their mass spectra), these need assessing toxicologically. Some identified substances will already have been

assessed and appear on a 'Positive List'[1].These can be assessed[2] directly from the information in the List and other information relating to the product. Other substances will require a full toxicological assessment[2] The semi-quantitative nature of the quantification, and the confidence in the identification should be taken into account when assessing the health implications of the toxicological assessment including reference to a 'Positive List'. For example, the implications may be different if the upper confidence level of the value is used when compared to the lower value. If there is no difference in the conclusion for the two values, then more accurate quantitative results are possibly not needed.

C.3.3 Once all the results have been assessed and if any indicate that there may be potential risks to health, the further information that would be needed to either confirm or dispel those concerns should be considered. Where this information is not immediately available steps should be taken, if possible, to obtain it. This may include further work for example:

— to identify substances that could not be identified from the mass spectra;

— and/or targeted analysis to confirm the identity of a tentatively or partially identified substance;

— and/or confirm the concentration of an identified substance;

— and or longer term die away studies to confirm the long term leaching range of a substance.

1) During the absence of any European "positive list" it shall be clearly indicated which reference list has been used.

2) Appropriate product type conversion factors should be used to convert the final migration rates to concentrations in drinking water, as well as applying other factors which may apply in specific cases such as worst case dilution rates.

Annex D
(informative)

Guidance on the identification of substances

D.1 A suggested strategy to assist in identifying substances in extracted leachates

Although the mass spectral library search can be very useful, there will be occasions when the library match either gives an erroneous result for identification or what may appear to be a sensible identification should be questioned as the suggested molecule is too small or too large for its position in the chromatogram.

There are six areas that merit attention in order to have the best opportunity to identify a substance:

a) how to resolve co-eluting peaks to give the mass spectrum of a single substance;

b) how to proceed if two unknown substances cannot be separated by normal means (e.g. by taking mass spectra from either side of the peak);

c) how to check the library identification (mass spectral and chromatographic information – retention time versus boiling point etc.);

d) how to report confidence in an identification;

e) how to report alternative identifications and when this is appropriate;

f) what to do if library search fails to assign a reasonable identification.

The above points are all covered with suggestions about how the analyst can at least report a tentative identification or interpretation of the available data in the absence of authentic standards.

D.2 Resolution of co-eluting peaks

Overlapping peaks can sometimes be observed by examination of the TIC, as a non-Gaussian peak implies that more than one substance is probably present. If the analyst suspects that more than one substance may be present then it is recommended that the analyst plots the major ions of the integrated peak at the apex as a series of overlaid extracted ion mass spectra. If these show that not all of the ions overlay and that there will now be more than one apex present, then there are at least two substances present.

In the event that more than one substance is present, the mass spectra for each of these extracted ion apexes should be examined to see if there are more appropriate contributory ions for each substance that can be plotted to assist in background subtraction.

Having established how many substances are probably present then it is usually possible to subtract one from another with the careful manual selection of a pair of mass spectra. The best method for achieving this is to firstly evaluate how much of a contribution a major interfering ion has to a substance of interest. Secondly, select the apex of the substance of interest and then to get a mass spectrum for background subtraction, select a spectrum from the interference where the intensity of the ion in question is equivalent to the interference in the substance of interest. When the peak of interest is close to the solvent front then dichloromethane (*m/z* 49, 51, 84, 86, 88) will have a significant contribution that needs to be taken account of.

If the latter is subtracted from the former then a purer mass spectrum is usually produced. The analyst will have to experiment with the technique but the results are usually library searchable or will make manual interpretation a lot easier.

Two possibilities remain regarding overlapping substances – are there two or more substances that may have come from the leachates or is the peak a single substance overlapping with an internal standard or a common interference such as a hydrocarbon?

From this comparison, it may be possible to decide if there is an interfering substance present. Characteristic ions to be aware of are those typical of alkanes (*m/z* 43, 57, 71), alkenes (*m/z* 41, 55, 69), and oxygenated substances (*m/z* 45, 59, 73).

The initial criterion for identifying a substance, or for declaring a suspected substance to be present, is that the spectrum generated should either agree with a published spectrum of the material, or be recognized by the operator from experience. It is important to realize however, that these in themselves are not a completely unambiguous identification.

D.3 Proceeding when background subtraction does not help

If, after plotting the extracted ions of a mass spectrum do not make it possible to separate two substances using background subtraction, the library search can often help. Examine the initial results, which at first appear to be totally wrong, but if the analyst looks carefully for points of similarity, then there may be one “hit” that can be accepted and then subtracted to give a second spectrum to search. The second search may reveal the identity of the second substance.

This method has to be used carefully, for the two substances to be closely eluting the analyst should check boiling points and retention index information (see D.4) to verify that the two substances have a realistic chance of co-eluting on an apolar column.

D.4 Tentatively identifying a substance using boiling points and retention indices

It is good practice, even with a good library match, to check that the selected substance has a realistic chance of eluting at the time stated on the chromatogram. There are two methods of doing this by checking the boiling point of a substance and any retention index information that may have been published. Although the majority of the substances that will be encountered in a leachate will not have data for these parameters, closely-related substances can be used as a possible source.

Good sources of boiling point information include some of the chemical catalogues, e.g. Aldrich, or online, the NIST WebBook: http://webbook.nist.gov is a good source of both boiling points and gas chromatographic retention indices. The verification of the boiling point of a substance is a good pointer, but calculating the retention index of a difficult to identify substance can help immeasurably.

The residence time of a component in a GC column, adjusted for the time taken for a non-retained substance, e.g. methane, is known as its retention time. The retention time of a component can be used to supplement its spectrum for identification purposes when necessary. Traditional methods used Kovats Indices for assigning a retention index. As the chromatographic programme is not a single linear ramp and a mass spectrometer is the detector, there will be no retention time for the solvent front (t_0) which means that Kovats Indices cannot be used. Instead, the method of Linear Retention Indices is employed which is based upon the retention time of an unknown peak expressed against the retention times for the two linear saturated alkanes that elute either side of it.

Use a suitable saturated alkane mixture (e.g. C_7 – C_{30} Saturated alkanes in hexane), prepare a 10 µg/ml solution in dichloromethane and inject it using the same GC-MS programme and standard parameters. Using the retention times of the two alkanes that elute immediately before and after the unknown substance, together with the retention time of the unknown substance, it is possible to calculate a Linear Retention Index (LRI) for the suspected peak using the formula below. These LRI will be close but not the same as Kovats indices, but they will give a strong indication as to whether a tentative identification is likely to correct.

$$\text{LRI} = (n \times 100) + 100 \times \left(\frac{(RT_x - RT_n)}{(RT_{n+1} - RT_n)} \right) \quad (6)$$

where

n is the number of carbon atoms in n-alkane prior to substance of interest;

RT_n is the retention time (min) of n-alkane;

RT_{n+1} is retention time (min) of next n-alkane;

RT_x is the retention time (min) of substance of interest.

Retention times can be useful in supporting the confirmation of the identity of substances which have spectra that are not very characteristic, e.g. those which display only one ion > 10 % relative intensity or display the characteristic of a series of substances of similar molecular formulae e.g. substituted aromatics or terpenes. However, in some cases the weak ions will be characteristic or structurally significant, for example weak molecular ions, and therefore the retention time information is possibly not needed.

On occasions where retention times are necessary to support confirmation, the retention time of the component in the sample shall agree with that of the standard to within 30 s or 2 %, whichever is the greater. On those occasions where it is suspected that matrix effects prevent agreement of the sample and standard retention times, spiked addition of the standard to the sample may be needed.

D.5 Library search results and identifying “unknown” substances

If a sample identity reported by a mass spectral library search is suspected and either no reference spectrum can be found or there are grounds to suspect that the reference spectrum is not of good quality, then a new reference spectrum may be produced by subjecting a standard material to GC-MS analysis. If this is done, and subject to satisfactory retention time agreement, then the spectrum of the standard shall be taken to be the definitive reference spectrum for the substance. This option is rarely available, and other options need to be considered. Individual laboratories can build this data up through identification of unknowns through the variously available proficiency testing schemes and their own individual production of a library of definitive reference spectra.

D.6 Interpretation of the mass spectrum

When there are one or more unknown peaks, some attempt should be made at manual interpretation. Depending upon how many significant ions are present, some information about a peak can usually be gleaned, especially when the major ions are at the low end of the mass spectrum, e.g. *m/z* 43, 57, 71, etc. This sort of information can be useful when there is a major ion above 200-300 Daltons, as it may indicate the length of an alkyl side-chain.

Obvious elements such as halogens (or less commonly sulphur) can usually be identified within a mass spectrum, and it may also be possible to estimate the number of carbon atoms in a substance by calculating the C^{13}/C^{12} ratio for various ions after taking into account the contribution of the (A+2) elements. The accepted value is that the amount of C^{13} present relative to C^{12} is at a ratio of 1,1 %.

Many mass spectral libraries will not contain all the possible substances without beginning to count any appropriate derivatives. Where chlorinated water has been used for leaching or chlorine has been added after the leaching then the potential for side-reactions is raised.

If a project also has any specific determinands then this information can be invaluable in assessing what substances may be discovered after leaching. There are many possible side reactions, but if the analyst has

any knowledge of the product formulation, then this in conjunction with some basic chemistry may indicate the type of substances that are present, e.g. esterification, polymerisation, degradation, etc.

Normally, an unambiguous identification of an unknown substance requires two separate techniques from the following list:

— a retention time match against an authentic standard;

— mass spectral library match;

— mass spectral interpretation using closely related substances as evidence;

— molecular mass confirmation by GC-MS (CI+ or CI-).

Although chemical ionization mass spectrometry lies outside the scope of this standard, the analyst should be aware that it may help to arrive at a more definite answer, especially when the suspected substance is highly toxic and/or illegal. The decision as to whether a mass spectrum includes the molecular ion (corresponding to the molecular weight) of the substance detected can also require expert judgement, as the highest detected mass is not necessarily the molecular ion. Additional mass spectrometric techniques (for example chemical ionization mass spectrometry) can be needed to be applied to confirm tentative molecular ions.

A particular structural isomer shall not be reported as the definitive answer unless either a retention time match against an authentic substance has been demonstrated, prior knowledge of GC column elution order eliminates other possibilities or another spectroscopic technique (which lies outside of this scope) such as ^{1}H NMR has been used.

D.7 Reporting confidence in a substance identification

This is more to do with how confident the analyst is with the quality of the mass spectra that have been generated. The absence of a molecular ion, however small, makes this harder, but manual interpretation of the mass spectrum is usually possible to get an idea of what the various fragment ions might be. Again, plotting the major ions as an extracted ion mass spectrum gives clues as whether an ion is really part of the substance of interest or not.

If there is any doubt concerning such an identification, it should be noted as a tentative identification and, if it is necessary to confirm the identification, a pure standard of the substance in question should be obtained and run on the GC-MS system in order to check the mass spectrum obtained and the GC retention time. The same principles apply to potential identifications resulting from manual inspection of mass spectral reference collections in books such as ‘The Eight Peak Index of Mass Spectra’.

It is inevitable that a significant proportion of the substances detected in many general survey GC-MS runs will only be tentatively identified, and that some will be unidentified, as the reference collections of mass spectra currently available represent a very small proportion (<10 %) of the known organic substances that are amenable to GC-MS analysis.

D.8 How to report alternative identifications and when this is appropriate

This situation will occur when there are probably more than two substances co-eluting at the peak apex selected for the automated library search. The analyst can also write macros that interrogate the data to give library matches and peak areas. These are best used to get a feel for how “dirty” a particular leachate might be and can make the processing a bit easier, but it is essential that the analyst not only manually integrates those peaks which are obviously not single substances but also individually checks each automated assignment. Invariably there will be at least one assignment that is totally wrong in every macro library search due to peak co-elution or interference from column bleed.

Although perhaps extreme, the analyst may also cross-check between injections, and even projects, if they get an answer that does not seem to fit. The information gained from this procedure can often indicate a possible substance identity from the retention index or else the mass spectrum can indicate suitable ions that can be extracted for peak de-convolution.

D.9 What to do if library search fails to assign a reasonable identification

If a library match is not available, or appears to have no relation to the mass spectrum being searched then the mass spectral library search parameters may require checking to make sure that the unknown peak is being searched against all the substances in the library database. If the library settings are not correct, large numbers of potential matches can be omitted from the final search because they have been dropped in the initial skim which only considers the eight most intense ions in the unknown peak.

The analyst should verify that the parameters used for the library search do not omit large portions of the database for a speedy result. Speed is not as important as getting the correct answer. Suitable recommended settings cannot be described to cover all manufacturers' instruments, but it is recommended that the operator ensures that the entire library is initially searched. Another option that may help in this process is to instruct the search strategy to ignore any duplicate CAS numbers.

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