BS EN 12393-3:2013

BSI Standards Publication

Foods of plant origin — Multiresidue methods for the determination of pesticide residues by GC or LC-MS/MS

Part 3: Determination and confirmatory tests

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National foreword

This British Standard is the UK implementation of EN 12393-3:2013. It supersedes BS EN 12393-3:2008 which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee AW/275, Food analysis - Horizontal methods.

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

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Compliance with a British Standard cannot confer immunity from legal obligations.

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Aliments d'origine végétale - Méthodes multirésidus de détermination de résidus de pesticides par CPG ou CL-SM/SM - Partie 3: Détermination et essais de confirmation

Pflanzliche Lebensmittel - Multiverfahren zur Bestimmung von Pestizidrückständen mit GC oder LC-MS/MS - Teil 3: Verfahren zur Bestimmung und Absicherung

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Foreword

This document (EN 12393-3:2013) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

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of an identical text or by endorsement, at the latest by May 2014, and conflicting national standards shall be withdrawn at the latest by May 2014.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN 12393-3:2008.

This document will supersede EN 12393-3:2008 with the following significant technical changes:

a) introduction of the LC-MS/MS as a recommended technique for the determination of pesticide residues;

b) deletion of method L as no longer in use;

c) deletion of old Annex B with considerations concerning MS confirmation;

d) addition of a new Annex B with suitable GC-MS/MS operating conditions;

e) addition of new Annex C with typical LC-MS/MS operating conditions.

EN 12393, *Foods of plant origin — Multiresidue methods for the determination of pesticide residues by GC or LC-MS/MS*" is divided into three parts:

— Part 1 "*General considerations*" provides general considerations with regard to reagents, apparatus, gas chromatography, etc., applying to each of the analytical selected methods;

— Part 2 "*Methods for extraction and clean-up*" presents methods M, N and P for the extraction and clean-up using techniques such as liquid-liquid partition, adsorption column chromatography or gel permeation column chromatography, etc.;

— Part 3 "*Determination and confirmatory tests*" gives some recommended techniques for the qualitative and the quantitative measurements of residues and the confirmation of the results.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Introduction

This European Standard comprises a range of multi-residue methods of equal status: no single method can be identified as the prime method because, in this field, methods are continuously developing. The selected methods included in this European Standard have been validated and/or are widely used throughout Europe.

Because these methods can be applied to the very wide range of food commodities/pesticide combinations, using different systems for determination, there are occasions when variations in equipment used, extraction, clean-up and chromatographic conditions are appropriate to improve method performance, see Clause 3.

1 Scope

This European Standard gives guidance on some recommended techniques for the determination of pesticide residues in foods of plant origin and on confirmatory tests.

The identity of any observed pesticide residue is confirmed, particularly in those cases in which it would appear that the maximum residue limit has been exceeded.

2 Normative references

Not applicable.

3 General

The methods specified in this European Standard permit identification and quantification of pesticide residues by gas chromatography using selective detectors or liquid chromatography with tandem-mass spectrometric detector (LC-MS/MS).

All relevant results require confirmation of identity and quantity.

The procedures listed for confirmation such as alternative GC columns, alternative GC detectors, high-performance liquid chromatography (HPLC), column fractionation, derivatisation, spectral measurements, etc. are all of value.

Results obtained using mass spectrometry (MS) present the most definitive evidence for confirmation/identification purpose.

As already described in the introduction, in certain occasions it is possible to improve the method performance by variations in equipment used, extraction, clean-up and chromatographic conditions. Such variations shall be always clearly documented and demonstrated to give valid results.

4 Determination

4.1 General

4.1.1 Identification

A number of parameters can be employed to determine the identity of an analyte present in the sample extract. This includes:

a) retention time of the analyte in question (RT) or, even better, the retention time ratio against the ISTD (Rt(A)/Rt(ISTD)) obtained from the same run (the simultaneous use of columns of different polarity improves this type of identification);

b) in case of MS or MS/MS detection, the relative abundance of simultaneously recorded signals (in general 3 ions are required in MS applications and 2 SRM transitions in MS/MS);

c) the application of high resolution mass spectrometry;

d) in case of MS with electron impact ionisation the comparison of the full scan mass spectrum of a suspected peak (when indicated after subtraction of background) with spectral libraries;

e) the quantification of equivalent amounts with different specific GC detectors, such as electron capture (ECD), nitrogen-phosphorous (NPD) or flame photometric (FPD) detector.

The parameters obtained for the analyte to be identified in the sample extract are compared with those obtained for the pesticides in the calibration solution(s). Should a higher degree of certainty be required for the confirmation of the analyte identity, additional measures may be necessary, such as the use of different chromatographic separation conditions or the evaluation of additional m/z or SRM-transitions. The occurrence of several stable isotopes of certain elements (e.g. Cl, Br, S) may be very helpful to identify substances by MS techniques.

For more information about the required identification criteria, see [1].

4.1.2 Quantification

For quantification, a chromatographic system calibrated with an sufficient number of appropriately distributed calibration points has to be used. The precision of calibration has to fulfil minimum requirements. Make sure that all the measurements are performed within the calibrated range of the system. In exceptional cases only, single-level calibration may be used. It has to be checked that the response of analytes present in complex mixtures does not differ from the response of separate analytes. Mixtures of isomers, degradation products and derivatives of analytes may require special conditions during calibration.

For calibration, either standards in solvents or standards prepared in blank matrix (matrix-matched standards) may be used. If matrix effects during GC injection or atmospheric pressure ionisation cannot be excluded, the use of matrix-matched standards or, even better, a quantification by standard addition has to be preferred. To detect instable detector response or such errors, which influence the amount of the analyte in the final extract, one or more internal standards should be added either to extracts or before extraction. To consider specific losses of individual analytes or their matrix effects, stable isotope labelled standards (if available) may be added to the sample before extraction.

All signals automatically identified by software tools may be considered as potential pesticide residues. However, any final quantification of relevant pesticide residues should be based on visual inspection of chromatograms. Before this European Standard can be used to quantify pesticides which are not tested before, a complete initial method validation is required. In all other cases, an on-going performance verification is sufficient to demonstrate the accuracy of the analytical method in a given laboratory.

For more information about the required quantification criteria, see [1] in its current version.

4.2 Gas chromatography (GC)

4.2.1 General

The detectors (see EN 12393-1:2013, 3.4) should be properly adjusted, according to the manufacturers' instructions. Variations in detector sensitivity should be checked periodically by verifying the linearity of the calibration curves using standard solutions of pesticides.

The measurement may be performed using various instruments, instrument parameters and columns. Some suitable instrument parameters and columns are listed in Annexes A and B.

For suitable experimental conditions of GC-MS measurement, see [2].

For suitable experimental conditions of GC-MS/MS measurement, see [3].

It has been found in practice that equivalent results can be achieved despite the adoption of different GC conditions, and different vendors of instruments. On the other hand, specifying standard GC parameters does not guarantee that the quality of the results generated will be identical.

4.2.2 GC columns

Columns should be conditioned for at least 24 h at a temperature near the maximum recommended operating temperature with the type of stationary phase used and should then be tested for their efficiency and selectivity at the required operating temperature using standard mixtures of pesticides. The end of the column should always be disconnected from the detector during conditioning.

Pure (oxygen-free) and dry (water-free) nitrogen, hydrogen or helium should be used as carrier gas. The flow rate depends on the size and type of column used. Generally, ensure that gas flow rates are controlled as accurately as possible. Gas purification filters should be installed for all gas supplies and replaced regularly.

Finally, make sure that the GC conditions (column length, stationary phase type, injector, detector and column temperatures, gas flow rates, etc.) are such that the separation of the pesticides likely to be present is as complete as possible.

Fused silica columns having an internal diameter of 0,20 mm to 0,35 mm and a length of between 10 m and 60 m have proved particularly satisfactory because of their separation efficiency, service life and mechanical properties. Wide bore columns having an internal diameter of 0,5 mm to 0,8 mm may also be useful in some cases.

The following stationary phases are frequently used as coatings:

— Methyl polysiloxane	equivalent to SE-30, OV-1, OV-101, DB-1, SPB-1, BP-1, HP-1, ULTRA-1, RTx-1, AT-1, CPSil-5, etc.
— Methyl 5 % phenyl polysiloxane	equivalent to SE-54, OV-23, DB-5, SPB-5, BP-5, HP-5MS, ULTRA 2, RTx-5, CPSil-8, VF-5ms, etc.
— Methyl 50 % phenyl polysiloxane	equivalent to OV-17, DB-17, SPB-7, BP-10, HP-17, RTx-17, AT-50, etc.
— 6 % Cyanopropylphenyl 94 % methyl polysiloxane	equivalent to DB-1301, RTx-1301, HP-1301, etc.
— Methyl 7 % cyanopropyl 7 % phenyl polysiloxane	equivalent to DB-1701, CPSil-19, RTx-1701, AT-1701 OV-1701, CP-SIL-19-CB, BP-10, SPB-7, etc.
— 50 % Cyanopropyl-phenyl 50 % dimethyl polysiloxane	equivalent to SP-2330, CP-Sil 43 CB, OV-225, Rtx-225, BP-225, 007-225, etc.
— Polyethylene glycol	equivalent to DB-Wax, Supelcowax 10, Super-ox, CPWax-52, Stabilwax, BP-20, HP-20M, AT-Wax, etc.

4.2.3 Injection techniques

Various injection techniques are useful such as split/splitless injection or programmed temperature vaporisation (PTV) injection.

The applicability of these techniques depends on the apparatus used and on special requirements.

4.2.4 GC determination

The measurement may be performed using various columns, instruments, acquisition parameters and GC detectors. Widely used specific detectors are electron capture (ECD), nitrogen-phosphorous (NPD) and flame photometric (FPD) detectors. Nowadays, GC is more often combined with single stage or tandem mass spectrometers (MS or MS/MS). Some instrument parameters and columns are listed in Annexes A and B.

Using a mass spectrometer, the determination is often more selective, because either the intensity of a number of previously selected ion is monitored (“SIM mode”) or, after registration of complete mass spectra, an equivalent number of ion chromatograms is reconstructed from the acquired spectra. Mass spectrometers are typically used with electronic impact ionisation (usually 70 eV). For some analytes chemical ionisation (positive or negative) offers better selectivity and sensitivity.

Better selectivity than those obtained with specific detectors or GC-MS is offered by tandem mass spectrometric detection, which allows the selection of intense ions by the first mass filter and the observations of their fragments with the second mass filter.

4.3 Liquid chromatography with tandem-mass spectrometric detection (LC-MS/MS)

4.3.1 General

The measurement may be performed using various instruments, instrument parameters and columns.

Some instrument parameters and columns are listed in Annex C.

Beside extensive tuning, the use of tandem mass spectrometry requires for each analyte a substantial set of instrument parameters. For suitable experimental conditions of LC-MS/MS measurement, see CEN/TR 15641:2007 [4]. Nevertheless, individual tuning of the compounds on the instrument that is used for measurement usually provides better sensitivities.

It has been found in practice that equivalent results can be achieved despite the adoption of different LC-MS/MS conditions, and different makes of instruments. On the other hand, specifying standard LC-MS/MS parameters does not guarantee that the quality of the results generated will be identical.

4.3.2 LC columns

For a sufficient separation of the pesticides reversed-phase (RP) columns have been proved to provide good results. Different column dimension can be used. RP columns having an internal diameter of 2,1 mm and a length of 150 mm have proved particularly satisfactory because of their separation efficiency, service life and mechanical properties. Shorter columns (50 mm x 2,1 mm) also have been used with good separation efficiency. If better separation efficiency, or faster analyses are needed, Ultra Performance Liquid Chromatography (UPLC) columns can be used. For polar pesticides, it is advisable to use modified RP phase columns in order to ensure a better retention. An exemplary choice of LC columns is given in Annex C.

4.3.3 LC-MS/MS determination

The measurement may be performed using various columns, instruments and acquisition parameters. For ionisation of separated analytes electrospray ion sources are most often used, but also ion sources offering atmospheric pressure chemical ionisation or atmospheric pressure photo ionisation may have advantages for individual analytes. Also for LC-MS/MS some instrument parameters and columns are listed in Annex C.

In LC-MS/MS measurements, generally for all analytes intense precursor ions have to be selected with the first mass filter and these ions are fragmented in a collision cell. Finally, the response of specific fragments of the precursor ions are recorded after their selective transmission through a second mass filter.

5 Confirmatory tests

5.1 General

Negative results (residues below the reporting limit) can be considered confirmed if the recovery and the response at the lowest calibrated level were acceptable. Positive results (residues at or above the RL) usually require additional confirmation. Generally, confirmation is needed when MRLs appear to be exceeded. Please note that many aspects of confirmation are very similar to identification, which are explained in 4.1.1.

5.2 Confirmation of GC results obtained with specific detectors

Even if a suspected pesticide is recorded at the correct retention time on two columns by ECD, NPD or FPD, it is recommended to confirm relevant results generally by mass spectrometry. The only exception are frequently found and previously confirmed residues. Whenever possible, GC results should be confirmed by LC-MS/MS.

5.3 Confirmation of results by mass spectrometry (MS or MS/MS)

Results using mass spectrometry (MS) present the most definitive evidence for confirmation/identification purposes and therefore, it is usually the confirmatory technique of choice.

Mass spectrometric confirmation by measurements in the selected ion measurement mode relies on proper selection of diagnostic ions. The (quasi) molecular ion is a diagnostic ion that should be included whenever possible. Alternatively, full scan spectra (when indicated after subtraction of background) can be recorded and compared to library spectra, given the analyte signals occur with sufficient intensity. If some differences are observed between a library spectrum and that obtained from the suspected pesticide residue, spectra of reference materials should be recorded with the same instrument.

The different ionisation modes (electron impact, chemical ionisation), tandem-mass spectrometric detection (MS/MS) or the use of high resolution mass spectrometry in combination with GC or LC may further improve the confirmation.

For more information about the required confirmation criteria, see [1].

Annex A
(informative)

Typical GC operating conditions

A.1 Operating conditions 1

Column:	Fused silica capillary; DB-5[1)] (30 m long, 0,25 mm i.d.; film thickness 0,25 µm)
Column temperature:	110 °C isothermal for 2 min, programmed to rise at 6 °C/min from 110 °C to 245 °C, isothermal at 245 °C for 2 min
Detector:	Electron-capture detector, temperature 350 °C
Injector:	Programmable temperature vaporizer (PTV)
PTV program:	Time (min) minus 0,15 Split open minus 0,10 PTV temperature 40 °C 0,20 Split close 0,25 PTV temperature 250 °C 2,00 Split open 4,00 PTV temperature 40 °C
Split flow rate:	50 ml/min

A.2 Operating conditions 2

Column:	Fused silica capillary: DB-1701[2)] (30 m long, 0,53 mm i.d.; film thickness 1,0 µm)
Column temperature:	80 °C isothermal for 1 min, programmed to rise at 30 °C/min from 80 °C to 150 °C and at 5 °C/min from 150 °C to 280 °C
Detector:	Electron-capture detector, temperature 280 °C
Injector:	Programmable temperature vaporizer (PTV)
PTV program:	Time (min) minus 0,15 Split open minus 0,10 PTV temperature 40 °C 0,20 Split close 0,25 PTV temperature 250 °C 2,00 Split open 4,00 PTV temperature 40 °C

1) DB-5 is a capillary column for GC containing a stationary phase based on methyl 5 % phenyl polysiloxane. For equivalent columns, see 4.2.2.

2) DB-1701 is a capillary column for GC containing a stationary phase based on methyl 7 % cyanopropyl 7 % phenyl polysiloxane. For equivalent columns, see 4.2.2.

A.3 Operating conditions 3

Column:	Fused silica capillary; DB-1[3)] (30 m long, 0,25 mm i.d.; film thickness 0,25 µm)
Column temperature:	Programmed to rise at 50 °C/min from 50 °C to 150 °C and at 10 °C/min from 150 °C to 250 °C
Detector:	Thermionic detector in P or N/P mode, temperature 275 °C
Injector:	Temperature 250 °C

A.4 Operating conditions 4

Column:	Fused silica capillary; HP-5MS[4)] (30 m long, 0,25 mm i.d.; film thickness 0,25 µm)
Column temperature:	2 min 70 °C, programmed to rise at 25 °C/min from 70 °C to 170 °C, at 3 °C/min from 170 °C to 210 °C, and at 3 °C/min from 210 °C to 290 °C
Detector:	MSD 5973N inert, transfer line temperatur 280 °C solvent delay 4,0 min; low mass 50, high mass 460 amu; threshold 150; MS quadrupole 150 °C; MS ion source 230 °C; electron impact 70 eV
Injector:	Temperature 240 °C, Pulsed splitless, 200 kPa; Pulse time 1,0 min
Carrier gas:	Helium, constant flow 1,0 ml/min (initial pressure 60,8 kPa)

3) DB-1 is a capillary column for GC containing a stationary phase based on methyl polysiloxane. For equivalent columns, see 4.2.2.

4) HP-5MS is a capillary column for GC containing a stationary phase based on methyl 5 % phenyl polysiloxane. For equivalent columns, see 4.2.2.

Annex B
(informative)

Typical GC-MS/MS-operating conditions

B.1 Operating conditions 1

Instrument	Thermo; TSQ Quantum XLS, Trace GC Ultra with PTV + Backflush
Column	Fused-silica-capillary VF-5ms[5)] (30 m long, 0,25 mm ID, film thickness 0,25 µm; precolumn not covered, 2 m long, 0,32 mm ID)
Column temperature	50 °C isothermal for 0,6 min, programmed to rise at 15 °C/min from 50 °C to 180 °C, isothermal for 1 min, rise at 7 °C/min to 230 °C, rise at 3 °C/min to 280 °C, isothermal at 280 °C for 10 min
Backflush	Backflush between precolumn and analytical column, see PTV-Program Cleaning
Carrier gas:	Helium, 1,2 ml/min, constant flow
Injector	Programmable temperature vaporizer (PTV)
Injection	2 µl (MeCN), PTV solvent split

Table B.1 — Operating conditions 1 — PTV program

PTV-Program	Temperature	Time	Split-Flow
Injection	50 °C	0,2 min	100 ml/min
Evaporation	50 °C	0,6 min	100 ml/min
Transfer	14,5 °C/s to 250 °C	12,1 min, thereof	
Real transfer		2 min	splitless
First cleaning		10,1 min	20 ml/min
Cleaning (Backflush)	14,5 °C/s to 280 °C	31 min	50 ml/min

Transfer line temperature:	250 °C
Detector	EI-MSMS, 70 eV, source temperature 250 °C
Resolution Q1	0,7 u
Resolution Q3	0,7 u
Collision gas pressure	1,0 mTorr (Q2 CID gas Argon)

5) VF-5ms is a capillary column for GC containing a stationary phase based on methyl 5 % phenyl polysiloxane. For equivalent columns, see 4.2.2.

B.2 Operating conditions 2

Instrument	Agilent GC 7890; 7000A MSMS-System
Column	Fused-silica-capillary DB-5ms ultra inert[6)] (30 m long, 0,25 mm ID, film thickness 0,25 µm)
Column temperature	70 °C isothermal for 2,0 min, programmed to rise with 25 °C/min to 150 °C, 0 min isothermal, rise with 3 °C/min to 200 °C, and with 8 °C/min to 280 °C, isothermal at 280 °C for 15 min
Carrier gas:	Helium, constant pressure; fore pressure locked at RT 16,54 min for Chlorpyriphos-methyl
Injector	split/splitless
Injection	1 µl splitless
Transferline temperature:	280 °C
Detector	EI-MSMS, 70 eV, source temperature 280 °C
Resolution Q1	0,7 u
Resolution Q3	0,7 u
Gases for collision cell	1,5 ml/min N_2, 2,25 ml/min He

B.3 Operating conditions 3

Instrument	Chromtech Evolution with Agilent GC 6890N and JAS PTV Unis 2100
Column	Fused-Silica-capillary HP-5 MS (30 m long, 0,25 mm ID, film thickness 0,25 µm; precolumn uncoated, deactivated, 3 m long, 0,25 mm ID)
Column temperature	60 °C isothermal for 1,52 min, programmed to rise with 20 °C/min to 130 °C, and with 4 °C/min to 280 °C, isothermal at 280 °C for 6 min
Carrier gas	Helium, 1,2 ml/min, constant flow
Injector	Programmable temperature vaporizer (PTV)
Injection	5 µl to 10 µl, PTV solvent vent

Table B.2 — Operating conditions 3 — PTV program

PTV-Program			
Initial Temp	20 °C	Initial Time	0,45 min.
Rate	720 °C/min		
Final Temp	280 °C	Final Time	5 min
Vent Time	0,6 min	Vent Flow	20 ml/min
Purge Time	2 min	Purge Flow	20 ml/min

Transferline temperature:	280 °C
Detector	EI-MSMS, (QqQ), 70 eV, source temperature 230 °C
Resolution Q1	1,0 u

6) DB-5ms ultra inert is a capillary column for GC containing a stationary phase based on methyl 5 % phenyl polysiloxane. For equivalent columns, see 4.2.2.

Resolution Q3	1,0 u
Collision gas pressure	1,8 bar Argon
Scantime	0,2 s/scan to 0,5 s/scan

B.4 Operating conditions 4

Instrument	Waters Quatro micro GC; GC Agilent Typ:6890N, Injector Gerstel CIS4
Column	Fused-silica-capillary Restek Rxi®-5Sil MS[7)] (30 m long, 0,25 mm ID, film thickness 0,25 µm; precolumn uncoated, 10 m long, 0,25 mm ID)
Column temperature	40 °C isothermal for 2 min, programmed to rise with 30 °C/min to 220 °C, and with 5 °C/min to 260 °C, and with 20 °C/min to 280 °C, isothermal at 280 °C for 15 min
Carrier gas	Helium, 2 ml/min, constant flow
Injector	Programmable temperature vaporizer (PTV)
Injection	3 µl (MeCN), solvent vent mode

Table B.3 — Operating conditions 4 — Parameter and conditions

Parameter	Conditions
Split	at 0 min (injection) split valve open, split flow 50 ml/min 0 min to 0,5 min split valve open, split flow 20 ml/min 0,5 min to 2 min split valve closed 2 min to 6 min split valve open, split flow 50 ml/min at 6 min split valve open, split flow 20 ml/min (gas saver mode)
Temperature	0 min to 0,8 min at 50 °C programmed to rise at 0,8 min with 12 °C/s to 280 °C, hold 15 min

Transferline temperature:	260 °C
Detector	EI-MSMS, 70 eV, source temperature 200 °C
Resolution Q1	0,7 amu
Resolution Q3	0,7 amu
Collision gas pressure	2,3 mTorr (CID gas)

7) Rxi®-5Sil MS is a capillary column for GC containing a stationary phase based on methyl 5 % phenyl polysiloxane. For equivalent columns, see 4.2.2.

B.5 Operating conditions 5

Instrument	Varian 1200 Quadrupole MS/MS, Varian CP-3800 Gas Chromatograph
Column	Fused-silica-capillary FactorFour™ VF-5ms with EZ-Guard™ [8] (30 m coated column with 10 m integrated precolumn uncoated, 0,25 mm ID, film thickness 0,25 μm)
Column temperature	90 °C isothermal for 1,0 min, programmed to rise with 30 °C/min to 180 °C, 0,50 min isothermal, and with 5 °C/min to 280 °C, isothermal at 280 °C for 5,5 min. Post run: 320 °C isothermal for 10 min
Carrier gas	Helium, 1,0 ml/min, constant flow
Injector	Varian 1079 Programmable temperature vaporizer (PTV)
Injection	2 µl

Table B.4 — Operating conditions 5 — PTV program

PTV-Program			
Initial Temp	170 °C	Initial Time	0,1 min
Rate	180 °C/min		
Final Temp	280 °C	Final Time	40 min
Purge Time	1 min	Purge Flow	50 ml/min

Transferline temperature:	310 °C
Detector	EI-MSMS, 70 eV, source temperature 250 °C
Resolution Q1	0,7 u
Resolution Q3	0,7 u
Collision gas pressure	1,4 bar Argon
Scantime	0,5 s/scan (cycle time)

8) FactorFour™ VF-5ms with EZ-Guard™ is a capillary column for GC containing a stationary phase based on methyl 5 % phenyl polysiloxane. For equivalent columns, refer to 4.2.2.

Annex C
(informative)

Typical LC operating conditions

C.1 HPLC-System 1

For most LC-amenable compounds:

Column	Zorbax XDB C18, length 150 mm, inner diameter 2,1 mm, particle size 3,5 µm
Mobile phase A_1	Ammonium formate solution in water, c = 5 mmol/l
Mobile phase B_1	Ammonium formate solution in methanol, c = 5 mmol/l
Column temperature	40 °C
Injection volume	5 µl

Table C.1 — Flow rate and elution gradient

Time min	**Flow rate** µl/min	**Mobile phase A_1** %	**Mobile phase B_1** %
0	300	50	50
20	300	0	100
25	300	0	100
26	300	50	50
30	300	50	50

C.2 HPLC-System 2

For acidic compounds:

Column	Zorbax XDB C18[9)] length 150 mm, inner diameter 2,1 mm, particle size 3,5 µm
Mobile phase A_2	Acetic acid solution in water, ρ = 0,1 ml glacial acetic acid /l
Mobile phase B_2	Acetic acid solution in acetonitrile, ρ = 0,1 ml glacial acetic acid /l
Column temperature	40 °C
Injection volume	5 µl

Table C.2 — Flow rate and elution gradient

Time min	**Flow rate** µl/min	**Mobile phase A_2** %	**Mobile phase B_2** %
0	300	80	20
20	300	0	100
22	300	0	100
22,1	300	80	20
30	300	80	20

9) Zorbax XDB C18 is a reversed phase HPLC column with bonded C18 phase. Equivalent C 18 columns can be used.

C.3 HPLC system 3

For most LC-amenable compounds:

HPLC pump	HP1100 Binary Pump (G1312A)
Autosampler	HP1100 (G1313A)
Injector programme	draw 5 µl Mobile phase A_3 draw 1 µl sample wash needle with acetonitrile draw 2 µl Mobile phase A_3 draw 1 µl sample wash needle with acetonitrile draw 2 µl Mobile phase A_3 draw 1 µl sample wash needle with acetonitrile draw 2 µl Mobile phase A_3 draw 1 µl sample wash needle with acetonitrile draw 5 µl Mobile phase A_3
Column	Phenomenex Aqua 5 µ C18[10)] 125Å, 50 mm × 2 mm
Mobile phase A_3	Methanol/water 2+8 (*V/V*) with 5 mmol/l ammonium formate
Mobile phase B_3	Methanol/water 9+1 (*V/V*) with 5 mmol/l ammonium formate
Column temperature	20 °C

Table C.3 — Flow rate and elution gradient

Time min	**Flow rate** µl/min	**Mobile phase A_3** %	**Mobile phase B_3** %
0	200	100	0
11	200	0	100
23	200	0	100
25	200	100	0
33	200	100	0

10) Phenomenex Aqua 5 µ C18 is a polar endcapped reversed phase HPLC column with bonded C18 phase. Equivalent polar endcapped C18 columns can be used.

C.4 UPLC system 1

For most LC-amenable compounds:

HPLC system	Waters Aquity UPLC system
Column	Waters HSS T3[11)] 1,8 µm, 2,1 mm x 150 mm
Injection volume	2 µl
Mobile phase A	Methanol/water 1+20 (*V/V*) with 5 mmol/l ammonium acetate
Mobile phase B	Methanol
Column temperature	60 °C

Table C.4 — Flow rate and elution gradient

Time min	**Flow rate** µl/min	**Mobile phase A** %	**Mobile phase B** **%**
0	450	100	**0**
0,2	450	100	**0**
10,90	450	1	**99**
11,90	450	1	**99**
12,00	450	0,1	**99,9**
14,00	450	0,1	**99,9**
14,10	450	100	**0**
16,00	**450**	**100**	**0**

C.5 MS/MS system 1

MS/MS instrument	Applied Biosystems API 2000
Ion source	Turbo Ion Spray (ESI)

Table C.5 — Ion source and general parameters

Curtain gas	nitrogen, 35 psi	Gas 2 temperature	400 °C
Collision gas	nitrogen, 2 units	Resolution MS 1	unit
Ion spray voltage	5 500 V	Resolution MS 2	unit
Gas 1	nitrogen, 60 psi	Dwell time	25 ms
Gas 2	nitrogen, 60 psi	Focusing potential	360 V

11) Waters HSS T3 is universal, silica-based HPLC column with bonded C18 phase and compatible with 100 % aqueous mobile phase. Equivalent C 18 columns of similar particle size can be used.

C.6 MS/MS system 2

MS/MS instrument Micromass Quattro LC

Ion source Electrospray

Table C.6 — Ion source and general parameters

Nebulizer gas flow	nitrogen, 93 l/h	MS1 LM Resolution	14,7
Desolvation gas flow	nitrogen, 552 l/h	MS1 HM Resolution	14,7
Desolvation temp.	350 °C	MS2 LM Resolution	14,7
Capillary voltage	3 500 V	MS2 HM Resolution	14,7
Gas cell	(9,2 x 10^{-4}) mbar		

C.7 MS/MS system 3

MS/MS instrument Applied Biosystems API 5500

Ion source Turbo Ion Spray (ESI)

Table C.7 — Ion source and general parameters

Curtain gas	nitrogen, 40 psi	Gas 2 temperature	400 °C
Collision gas	nitrogen, 8 units	Resolution MS 1	unit
Ion spray voltage	5 500 V	Resolution MS 2	unit
Gas 1	air, 40 psi	Dwell time	variable
Gas 2	air, 50 psi		

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