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## **Preventing Recalcitrant Organic Mobile Industrial chemicals for Circular Economy in the soil-sediment-water System**

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### **D1.2 – Targeted methods for relevant iPM(T) substances in waters**

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## Executive Summary

This document is the result of WP1, Task 1.1, Subtask 1.1.3 “Targeted methods for iPM(T)s occurring in the water cycle (CSIC, BAFG, BWB)”.

Within this subtask, four different analytical methods have been developed/adapted for the determination of iPM(T)s in different water matrices (groundwater, surface water and wastewater).

Their design and experimental conditions are marked by the physical-chemical properties of the targeted compounds, the instrumentation available, the water matrix under study and the required analytical performance, primarily in terms of limits of quantification (LOQs) that should be ideally lower than the corresponding predicted no-effect concentrations (PNEC). The reported methods respond to the needs observed in PROMISCES case studies (CS#1, CS#2, CS#3, and CS#7). The target compounds analysed in each case have been selected based on previous suspect screening methods, knowledge of local activities and stakeholders, and/or previously reported data.

Three of the four reported methods are based on liquid chromatography-tandem mass spectrometry (LC-MS/MS), and from these, one method includes direct injection of the sample in the system and two methods include on-line solid phase extraction step. The fourth method is a gas chromatography-mass spectrometry (GC-MS)-based method used for determination of chlorinated solvents and other volatile organic compounds (VOCs) in groundwater samples.

The number of compounds measured with each method is 26 (BAFG), 21 (BWB), 42 (CSIC) and 59 (CSIC), respectively. In terms of methods performance, all of them can detect the target compounds in the ng/L range or lower, while accuracy and repeatability are satisfactory. The methods developed are cost-effective, simple and fast, and easily reproducible in other labs provided that the instrumentation is available. Table 1 compiles the main features of the developed methods developed.

Table 1: Comparative summary of the four methods (for abbreviations, see Table 2)

	<b>BAFG</b>	<b>BWB</b>	<b>CSIC 1</b>	<b>CSIC 2</b>
<b>Matrix</b>	Surface water	Drinking, ground, surface water, wastewater	WWTP effluent	Groundwater
<b>Separation</b>	UPLC	UPLC	UPLC	GC
<b>Analyser</b>	Triple quadrupole	Orbitrap (HRMS)	Q-TOF (HRMS)	Single quadrupole MS
<b>Sample preparation</b>	Direct injection	Online-SPE	On-line SPE	PT
<b># PMTs analytes</b>	26	21	42	59
<b># Internal standards</b>	16	8	22	3
<b>LOQ (ng/L)</b>	1-200	12 – 369	0.02 - 321	8.4-6400

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**Table 2: List of abbreviations, acronyms and dimensions.**

ACN	Acetonitrile
bbCID	Broadband Collision Induced Dissociation
CE	Collision Energy
CS	Case Study
DI	Direct injection
EIS	Extracted Internal Standard
ESI	Electrospray Ionisation
GC	Gas Chromatography
H <sub>2</sub> O	Water
HRMS	High Resolution Mass Spectrometry
IS	Internal Standard
LC	Liquid Chromatography
LOD	Limit of Detection
LOQ	Limit of Quantification
LVI	Large Volume Injection
MDL	Method Detection Limit
MeOH	Methanol
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometer
MS/MS	Tripple Quatrupole Mass Spectrometer
NIS	Non-extracted Internal Standard
NTS	Non-Target Screening
PCE	Perchloroethylene
PT	Purge-and-Trap
PTFE	Polytetrafluoroethylene
RO	Reversed Osmosis
RPM	Rounds Per Minute
RT	Retention Time
S/N	Signal to Noize Ratio
SPE	Solid Phase Extraction
TCE	Trichloroethylene
TOF	Time-of-flight
UPLC	Ultra-high performance liquid chromatography
VOCs	Volatile organic compounds
WP	Work Package
WWTP	Wastewater Treatment Plant

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## 1 Introduction

The acronym iPMTs stands out for industrial substances that are persistent in the environment, mobile in the aquatic environment, and toxic, thereby presenting an inherent hazard to the environment. The study of these substances is relatively recent, hence the knowledge available on their occurrence and potential impact on the water cycle and the environment in general, as well as methods for their analysis are still scarce. In this context, one of the objectives of PROMISCES was to develop LC- and GC-MS based targeted methods for the quantification of relevant iPMTs in ground-, surface and wastewater, for their application in different case studies as well as experiments conducted within the project. The aim of the present deliverable is to compile and describe such methods. Targeted compounds were selected on the basis of former monitoring data (e.g. benzotriazoles and organophosphates), already known suspects (melamine, sulfonated aliphates/benzenes/benzoic acids, guanidines, etc.) and outcomes of non-target screening (NTS) approaches conducted in the case studies and associated work packages (WP2, WP4) presented in MS2. This deliverable reports mainly upon activities conducted in WP1, Task 1.1, Subtask 1.1.3 “Targeted methods for iPM(T)s occurring in the water cycle (CSIC, BAFG, BWB)”.

## 2 iPM(T) analysis in waters

This chapter describes the analytical methods developed by BAFG, BWB and CSIC for their use in the cases studies and conducted experiment CS#1, CS#2, CS#3 and CS#7.

<b>CS # &amp; location</b>	<b>Partners</b>	<b>Objectives</b>
CS#1 – Berlin, Germany	BWB, KWB, BAFG, UBA, DELTARES, BRGM	PFAS and iPM(T) substances fate and remediation in the semi-closed urban water cycle
CS#2 – Upper Danube basin (to Budapest)	TU WIEN, DELTARES, BUWW, BRGM, BAFG, KWB	Sources, pathways, fate and transport of PFAS in the Danube basin semi-closed water cycle
CS#3 – Montornès del Vallès WWTP, Catalonia, Spain	EUT, CSIC, CBT, BDS	Water reuse from a wastewater treatment plant with a high share of industrial wastewater for agricultural irrigation
CS#7 – Besos-Tordera aquifers, Catalonia, Spain	EUT, ESOLVE, BRGM, DELTARES, CSIC, IPGP, SINAPTEC, UBA	Groundwater remediation: - site 1 = Besos (COVs), - site 2 = Tordera (AFFF)

## 2.1 Analysis of iPM(T)s in surface water via LC-MS/MS (BAFG)

### 2.1.1 General

The goal of this method is to confirm, quantify and monitor iPM(T) substances tentatively identified by suspect screening of samples from CS#1 and CS#2 in surface water. For this purpose, a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was adapted from an existing liquid chromatography-high resolution mass spectrometry (LC-HRMS) method already in use for suspect screening in Subtasks 2.2.3 and 2.2.4. The chromatographic separation is almost identical to the one of the LC-HRMS method, enabling a straightforward integration of new compounds.

The substances included up to now in the method are listed in table 3.

Table 3: List of target analytes included in the BAFG LC-MS/MS method.

#	Substance	CAS No.	Industry sector
1	Benzotriazole	95-14-7	Corrosion inhibitor
2	1-Hydroxy-Benzotriazole	123333-53-9	Organic synthesis
3	Diglyme	111-96-6	Organic synthesis, solvent
4	Triglyme	203-977-3	Organic synthesis, solvent
5	Tetraglyme	143-24-8	Organic synthesis, solvent
6	Ethyltriphenyl phosphonium	1530-32-1	Organic synthesis
7	Methyltriphenyl phosphonium	1779-49-3	Organic synthesis
8	Tetrabutyl phosphonium	2304-30-5	Organic synthesis
9	(Methoxymethyl)triphenyl phosphonium	4009-98-7	Organic synthesis
10	Tetrabutyl ammonium	1643-19-2	Organic synthesis, solvent, plasticizer
11	Tetrapropyl ammonium	1941-30-6	Organic synthesis, solvent, plasticizer
12	Caffeine	58-08-2	Food industry
13	Acesulfame	55589-62-3	Food industry
14	Diphenyl urea	102-07-8	Intermediate
15	Saccharine	81-07-2	Food industry
16	Sucralose	56038-13-2	Food industry
17	Denatonium	3734-33-6	Additive for toxic liquids and solvents (bitter taste)
18	Tolyl biguanide	93-69-6	Antioxidant also used in paints and coatings
19	Propiconazole	60207-90-1	Fungicide also used for wooden construction material
20	Tebuconazole	107534-96-3	Fungicide also used for wooden construction material
21	Terbutryne	886-50-0	Algicide for paints

22	Cybutryne	28159-98-0	Antifouling agent
23	Triphenyl phosphineoxide	791-28-6	Byproduct from organic synthesis
24	Cyclamate	139-05-9	Food industry
25	Acridone	578-95-0	Fluorescence dye
26	Acridine9-carboxylic acid	332927-03-4	Fluorescence dye

### 2.1.2 Chemicals and reagents

Reference standards and isotopically labelled standards are purchased from Sigma Aldrich, TRC and Dr. Ehrenstorfer. LC-MS grade acetonitrile is purchased from Merck. Formic acid and acetic acid are purchased from Sigma Aldrich.

Stock solutions of analytes are prepared in MeOH and stored at -26°C pending analysis. A 10-point external calibration is performed with concentration levels between 0.1 ng/L and 5000 ng/L. Calibration standards are prepared by diluting stock solutions with ultrapure water. To each, internal standard mixture is added at a concentration of 200 ng/L

### 2.1.3 Sample collection, preservation and pre-treatment

Samples sent to BAFG by project partners are stored in the refrigerator at 5°C until analysis.

Approx. 10 mL of sample are filtered through 0.45 µL PTFE syringe filters. 980 µL of filtered sample are spiked with 20 µL of internal standard mix, resulting in an internal standard concentration of 200 ng/L.

### 2.1.4 Analysis

Chromatographic separation is achieved with a Zorbax Eclipse Plus C18 column (Agilent Technologies) using an Agilent 1260 infinity (Agilent Technologies). Aliquots of the sample under investigation (80 µL) are directly injected into the system without further pre-treatment. Ultrapure water with 0.1% formic acid (eluent A) and acetonitrile (eluent B) are used as eluent with the gradient shown in Table 4:

Table 4: Chromatographic gradient used for analysis of iPM(T)s in the BAFG LC-MS/MS method.

Time	Eluent A	Eluent B
0 min	100%	0%
1 min	100%	0%
2 min	80%	20%
16.5 min	0%	100%
22 min	0%	100%
22.1 min	100%	0%
25 min	100%	0%

Mass spectrometric analysis is performed with a Sciex Triple Quad 6500+ tandem mass spectrometer equipped with an electrospray ionisation (ESI) source. The method described here includes two separate runs – one with positive ionization and the other one with negative ionization mode. Target detection and quantification was achieved by means of multiple reactions monitoring using the following parameters shown in Table 5.

**Table 5: Experimental conditions used for analysis of iPM(T)s in the BAFG LC-MS/MS method.**

Substance	ESI +/-	RT / min	Precursor m/z	Fragment 1 m/z	CE 1	Fragment 2 m/z	CE 2	Internal standard
Benzotriazole	+	6.60	120.0	65.0	29	39.0	45	Benzotriazol-d4
1-Hydroxy-Benzotriazole	+	5.80	136.0	64.0	45	91.1	28	Benzotriazol-d4
Diglyme	+	5.23	135.2	59	17	103	11	Tetraglyme-d6
Triglyme	+	5.59	179.0	59	21	103	11	Tetraglyme-d6
Tetraglyme	+	6.40	223.1	103.0	14	59.0	28	Tetraglyme-d6
Ethyltriphenyl phosphonium	+	8.50	291.2	183.1	61	108.1	49	Methyl-d3-triphenylphosphonium bromide
Methyltriphenyl phosphonium	+	8.10	277.1	183.1	59	108.1	51	Methyl-d3-triphenylphosphonium bromide
Tetrabutyl phosphonium	+	10.50	259.3	76.0	53	90.0	47	Methyl-d3-triphenylphosphonium bromide
(Methoxymethyl)triphenyl phosphonium	+	8.60	30.1	183.1	54	185.1	31	Methyl-d3-triphenylphosphonium bromide
Tetrabutyl ammonium	+	9.80	242.3	142.0	34	100.0	45	Tetra-d28-propylammonium bromide
Tetrapropyl ammonium	+	6.50	186.2	114.1	34	142.1	28	Tetra-d28-propylammonium bromide
Caffeine	+	6.10	195.1	138.0	28	110.0	30	Caffeine 13C3
Acesulfame	-	4.80	162.0	82.0	-22	78.0	-38	Acesulfam-d4
Diphenyl urea	-	11.20	211.0	92.0	-15	-	-	-
Saccharine	-	5.70	182.0	105.8	-26	42.0	-52	Saccharine-d4
Sucralose	-	6.30	395.0	35.0	-38	-	-	Sucralose-d6
Denatonium	+	8.60	325.2	86.3	28	91.2	50	Benzotriazol-d4
Tolyl biguanide	+	5.50	192.1	60.1	28	116.0	40	Tramadol-d6
Propiconazole	+	13.80	342.1	159.0	45	-	-	Propiconazole-d5
Tebuconazole	+	13.20	308.1	70.0	49	125.0	45	Tebuconazole-d6
Terbutryne	+	12.50	242.0	186.0	25	91.0	38	Terbutryn-d5
Cybutryne	+	12.70	254.0	198.0	26	83.0	41	Cybutryne-d9
Triphenyl phosphineoxide	-	5.4	177.8	79.9	-40	95.8	-32	-
Cyclamate	+	8.80	196.0	167.1	43	139.1	71	Cyclamate-d11
Acridone	+	8.80	196.0	167.1	43	139.1	71	Carbamazepine-15N13C
Acridine9-carboxylic acid	+	5.00	224.1	196.0	37	167.0	57	Carbamazepine-15N13C

RT: retention time, CE: collision energy

### 2.1.5 Performance

Limits of quantification (LOQ) have been determined for surface water, considering a minimum signal-to-noise ratio (S/N) of 10 for the first transition and S/N > 3 for the second transition (see Table 6). As described above, the list of compounds for this method might be expanded by further compounds identified by suspect screening in Subtasks 2.2.3 and 2.2.4. Therefore, further validation parameters in terms of recoveries, etc. have not been finally determined.

Table 6: LOQs achieved for analysis of iPM(T)s in the BAFG LC-MS/MS method.

#	Substance	LOQ (ng/L)
1	Benzotriazole	100
2	1-Hydroxy-Benzotriazole	70
3	Diglyme	20
4	Triglyme	40
5	Tetraglyme	60
6	Ethyltriphenyl phosphonium	2
7	Methyltriphenyl phosphonium	2
8	Tetrabutyl phosphonium	1
9	(Methoxymethyl)triphenyl phosphonium	10
10	Tetrabutyl ammonium	1
11	Tetrapropyl ammonium	5
12	Caffeine	40
13	Acesulfame	10
14	Diphenyl urea	10
15	Saccharine	50
16	Sucralose	200
17	Denatonium	5
18	Tolyl biguanide	5
19	Propiconazole	5
20	Tebuconazole	2
21	Terbutryne	1
22	Cybutryne	5
23	Triphenyl phosphineoxide	40
24	Cyclamate	10
25	Acridone	1
26	Acridine9-carboxylic acid	10

### 2.1.6 Application to real samples

Once the validation has been completed, the LC-MS/MS method developed for the detection of iPMT substances in surface water can be applied to samples from CS#1.

## 2.2 Analysis of iPM(T)s in waters via LC-MS/MS (BWB)

### 2.2.1 General

The aim of this method is to quantify relevant iPM(T) substances for the Berlin area in treated and nontreated wastewater as well as in surface waters (CS#1). The evaluation of the relevance of the compounds includes a persistent occurrence in the semi-closed water cycle and an industrial source present in the area. For analysis an LC-HRMS method was developed with large volume injection and online SPE. The method is designed to have a high throughput of samples by a minimum on sample handling. The method also includes several pharmaceutical analytes (see Table 7).

Table 7: List of target analytes included in the BWB LC-MS/MS method.

#	Compound	Acronym	CAS No.	Substance category
1	1H-Benzotriazol	-	95-14-7	Industrial Chemicals
2	Tolyltriazol	-	29385-43-1	Industrial Chemicals
3	2-Methylthiobenzthiazol	MTBT	615-22-5	Industrial Chemicals
4	Tributylphosphate	TBP	126-73-8	Industrial Chemicals
5	Tris(2-chloro-1-(chloromethyl)ethyl) phosphate	TCEP	13674-87-8	Industrial Chemicals
6	Tris(2-chloroisopropyl)phosphate	TCPP	13674-84-5	Industrial Chemicals
7	Triphenylphosphate	TPP	115-86-6	Industrial Chemicals
8	N,N-Diethyl-3-methylbenzamide	DEET	134-62-3	Pesticide
9	Nicotin	Nicotin	13674-84-5	Others
10	o,p-Dichlorodiphenyltrichloroethane	o,p-DDA (-CO <sub>2</sub> )	50-29-3	Industrial Chemicals
11	p,p-Dichlorodiphenyltrichloroethane	p,p-DDA (-CO <sub>2</sub> )	50-29-3	Industrial Chemicals
12	4-Acetylaminoantipyrine	AAA	83-15-8	Pharmaceutical
13	Dioxypyramidon	AMDOPH	519-65-3	Pharmaceutical
14	(1-Methyl-2-phenyl)-hydrazide	AMPH	38604-70-5	Pharmaceutical
15	4-Dimethylaminoantipyrine	DMAA	58-15-1	Pharmaceutical
16	Dimethylpyrazolon	DP	3201-28-3	Pharmaceutical
17	4-Formylaminoantipyrine	FAA	1672-58-8	Pharmaceutical
18	Phenazon		60-80-0	Pharmaceutical
19	Propyphenazon		479-97-5	Pharmaceutical
20	Diclofenac		15307-86-5	Pharmaceutical
21	Metoprolol		56392-17-7	Pharmaceutical

### 2.2.2 Chemicals and reagents

The chemicals and reagents used, together with their manufacturer and description, are listed in Table 8.

Table 8: List of chemicals and reagents used in the BWB LC-MS/MS method.

Chemical	Manufacturer	Description
Methanol	Honeywell	Chromasolv LC-MS Ultra, tested for blind values
Ultrapure Water	Berrytec GmbH	Easy UP(UVUF) TOC with Merck KGaA EDS-Pak
Formic Acid	Biosolve	ULC-MS grade
Ammonium acetate	Sigma Aldrich	For mass spectrometry
Reference Standard	Manufacturer	Description
Calibration Standards (Single Substances)	Campro	Concentration 10-100 mg/L in MeOH
Quality control standards (Single Substances)	Ehrenstorfer	Concentration 10-100 mg/L in MeOH
Internal Standards (Single Substances)	LGC	Concentration 100 µg/L in MeOH

For an exact quantification a calibration with at least five different concentrations is used. For dilution of the standards, ultrapure water is used. The concentration range of the calibration curve is from 0.010 µg/L to 1 µg/L. In addition, 25 µL (equal to 100 ng/L) of the internal standard is also added to each calibration solution. Each vial should contain a volume of 1500 µL. For quality control a standard substance of the same compounds but from a different manufacturer is also diluted to a concentration of 100 ng/L in the same way as the calibration standard. The concentrations of this control standard are documented.

At the beginning of the sequence, the standard series and the control standard is measured. If there are more than 30 samples, the 0.04 µg/L calibration point is prepared and measured again approximately in the middle of the sequence as a quality control. The signal of the blank sample before the first calibration point should be at maximum one third of the lower limit of quantification, otherwise the limit of quantification cannot be retained.

### 2.2.3 Sample collection, preservation and pre-treatment

The sample should be collected with caution, to avoid any contamination from any material (especially Tubing Sealing, O-rings). The sample is filled bubble-free into a 50 mL ground glass bottle and transported refrigerated. The sample is stored in the refrigerator at 5 °C until measurement (within 2 weeks) or deep frozen at -25 °C.

An aliquot of the sample is filled in a 1.5-mL-vial and diluted, if necessary, with ultrapure-water on a ratio of 1:5 to 1:50 depending on the matrix. To each sample 25  $\mu\text{L}$  of Internal standard solution (equals 100 ng/L) is added. The final volume should be 1500  $\mu\text{L}$ .

#### 2.2.4 Analysis

Thermo Fisher Scientific Dionex Labmate 3000 UHPLC system coupled to an Thermo Fisher Scientific QExactive Orbitrap high resolution mass spectrometer is used. The ionisation is realised through an ESI source. The Labmate 3000 system is equipped with a second pair of eluent pumps which is used as column cleaning eluents. A second Labmate 3000 pump system is used for the online SPE column. An analytical column an ACUITY UPLC HSS T3 (2.1x50 mm, 1.8  $\mu\text{m}$ ) is used at a temperature of 40 °C. An online SPE column Thermo Fisher Hypersil GOLD aQ (20x2.1 mm) is used with an injection volume of 1000  $\mu\text{L}$ .

The separation is achieved by using a binary gradient mobile phase consisting of ultra-pure water with 1 % (v/v) methanol and 0.1 % (v/v) formic acid (A) and HPLC-MS grade methanol with 0.1 % (v/v) formic acid (B) at a constant flow of 0.6 mL/min. The mobile phase gradient starts at 1 % B and increases over 7 minutes to 95 % B at a static flow of 0.6 mL/min. A clean-up of the analytical column is performed with 99 % B for 2.5 minutes and is followed by a reconstitution step on start conditions at 99 % A for 3 minutes.

The ESI source is operating in negative or positive ion mode (see Table 9). A mass calibration with Thermo Fisher Calibration Solution is performed each day prior to measuring. The capillary spray voltage is set to 3.6 kV. The sheath gas flow is set to 40 and the aux gas flow to 20 at 300 °C. The quantification of the measured samples is performed with Thermo Fisher Scientific Tracefinder 4.1 software. The sample peaks are quantified via the relative response ratio obtained through the internal standard. The linear calibration curve should have a correlation coefficient ( $R^2$ ) of more than 0.99 for each compound and is weighted by  $1/x$ .



Table 9: List of target analytes included in the BWB LC-MS/MS method and main experimental conditions.

#	Compound	Retention time in min	ESI +/-	Exact Mass m/z	Internal Standard (IS) acronym	Molecular Formula	Internal Standard (IS) exact mass m/z
1	1H-Benzotriazol	4.10	+	120.05562	Benzotriazol d4	<sup>12</sup> C <sub>6</sub> H <sub>4</sub> N <sub>3</sub>	124.08073
2	Tolyltriazol	5.05	+	134.07127	Benzotriazol d4	<sup>12</sup> C <sub>6</sub> H <sub>4</sub> N <sub>3</sub>	124.08073
3	MTBT	6.95	+	182.00927	Benzotriazol d4	<sup>12</sup> C <sub>6</sub> H <sub>4</sub> N <sub>3</sub>	124.08073
4	TBP	7.90	+	267.17197	TBP d27	<sup>12</sup> C <sub>12</sub> D <sub>27</sub> O <sub>4</sub> P	294.34144
5	TCEP	5.95	+	284.96115	TBP d27	<sup>12</sup> C <sub>12</sub> D <sub>27</sub> O <sub>4</sub> P	294.34144
6	TCPP	7.05	+	327.00811	TBP d27	<sup>12</sup> C <sub>12</sub> D <sub>27</sub> O <sub>4</sub> P	294.34144
7	TPP	7.70	+	327.07807	TBP d27	<sup>12</sup> C <sub>12</sub> D <sub>27</sub> O <sub>4</sub> P	294.34144
8	DEET	6.45	+	192.13829	DEET d7	<sup>12</sup> C <sub>12</sub> H <sub>10</sub> D <sub>7</sub> NO	199.18223
9	Nicotin	7.55	+	163.12298	-	-	-
10	o,p-DDA (-CO <sub>2</sub> )	7.60	-	235.00868	-	-	-
11	p,p-DDA (-CO <sub>2</sub> )	7.70	-	235.00868	Diclofenac d4	<sup>12</sup> C <sub>14</sub> H <sub>7</sub> D <sub>4</sub> Cl <sub>2</sub> NO <sub>2</sub>	300.04907
12	AAA	3.90	+	246.1237	Phenazon d3	<sup>12</sup> C <sub>11</sub> H <sub>9</sub> D <sub>3</sub> N <sub>2</sub> O	192.12107
13	AMDOPH	4.70	+	264.13427	Phenazon d3	<sup>12</sup> C <sub>11</sub> H <sub>9</sub> D <sub>3</sub> N <sub>2</sub> O	192.12107
14	AMPH	5.25	+	165.10224	Phenazon d3	<sup>12</sup> C <sub>11</sub> H <sub>9</sub> D <sub>3</sub> N <sub>2</sub> O	192.12107
15	DMAA	3.45	+	232.1444	Dihydrocarbamazepin	<sup>12</sup> C <sub>15</sub> H <sub>14</sub> N <sub>2</sub> O	239.11789
16	DP	2.70	+	113.07094	-	-	-
17	FAA	3.80	+	232.10805	Phenazon d3	<sup>12</sup> C <sub>11</sub> H <sub>9</sub> D <sub>3</sub> N <sub>2</sub> O	192.12107
18	Phenazon	4.40	+	189.10224	Phenazon d3	<sup>12</sup> C <sub>11</sub> H <sub>9</sub> D <sub>3</sub> N <sub>2</sub> O	192.12107
19	Propyphenazon	6.10	+	231.14919	Phenazon d3	<sup>12</sup> C <sub>11</sub> H <sub>9</sub> D <sub>3</sub> N <sub>2</sub> O	192.12107
20	Diclofenac	7.55	+	296.02396	Diclofenac d4	<sup>12</sup> C <sub>14</sub> H <sub>7</sub> D <sub>4</sub> Cl <sub>2</sub> NO <sub>2</sub>	300.04907
21	Metoprolol	4.70	+	268.19072	Propranolol d6	<sup>12</sup> C <sub>16</sub> H <sub>15</sub> D <sub>6</sub> NO <sub>2</sub>	266.20217

### 2.2.5 Performance

LOQs listed in Table 10 are calculated as described in detail in DIN 32645:2008-11. Briefly, at least 10 calibration solutions are diluted in a blank water. The highest calibration concentration should be 10 times the expected limit of quantification. Through extrapolation within a confident area the LOQ is calculated. For wastewater a 1 to 5 dilution of the samples is conducted, hence the LOQ is calculated times 5.

For the recovery 10 individual samples have been spiked with a concentration of 100 ng/L of the calibration standard. The recovery below is calculated without blank subtraction.

Table 10: LOQs and recovery of the BWB method.

#	Compound	LOQ (drinking, ground and surface water) in ng/L	LOQ (wastewater) in ng/L	Recovery (100 ng/L in drinking water) In %	Relative Standard Deviation (n=10) in %
1	1H-Benzotriazol	11	54	100	3
2	Tolyltriazol	21	107	117	10
3	MTBT	44	218	116	11
4	Tributylphosphate	47	237	106	6
5	Tris(2-chloroethyl) phosphate	42	209	103	7
6	Tris(2-chloroisopropyl)phosphate	90	448	102	5
7	Triphenylphosphate	94	472	101	6
8	DEET	25	123	98	7
9	Nicotin	147	736	130	12
10	o,p-DDA	16	82	104	8
11	p,p-DDA	16	80	105	8
12	4-Acetylaminoantipyrine	14	70	91	10
13	Dioxypyramidon	25	124	103	7
14	AMPH	22	109	100	4
15	DMAA	19	94	109	5
16	Dimethylpyrazolon	17	85	95	8
17	4-Formylaminoantipyrine	15	73	89	11
18	Phenazon	16	81	100	4
19	Propyphenazon	18	92	100	7
20	Diclofenac	22	109	88	9
21	Metoprolol	12	58	101	4

### 2.2.6 Application to real samples

The developed LC-MS/MS method for the detection of iPMT substances in different water matrices can be applied to samples from CS#1.

## 2.3 Analysis of iPM(T)s in treated wastewater via LC-MS/MS (CSIC 1)

### 2.3.1 General

The goal of this method is to confirm, quantify and monitor iPM(T) substances tentatively identified in Subtask 4.2.1 by suspect screening of samples from Montornès del Vallès WWTP (CS#3), plus other substances identified as relevant in a similar study conducted in another WWTP receiving mainly urban and agricultural wastewaters. The method developed is based on on-line SPE-LC-MS/MS. The substances listed in Table 11 are included in the method.

Table 11: List of target analytes included in the CSIC LC-MS/MS method.

Nº	Compound	CAS No.	Substance category
1	1,2,3-Benzotriazole	95-14-7	Industrial Chemicals
2	1,8-Diazabicyclo [5.4.0]undec-7-ene	6674-22-2	Industrial Chemicals
3	10,11- Dihydroxycarbamazepine	35079-97-1	Pharmaceuticals (met.)
4	2,4-Diaminotoluene	95-80-7	Industrial Chemicals
5	2-Amino-4-cresol	95-84-1	Industrial Chemicals
6	2-Aminophenol	95-55-6	Personal Care Products
7	2-Ethyl-1,5-dimethyl-3,3-diphenylpyrrolinium	21409-27-8	Pharmaceuticals (met.)
8	2-Ethylhexyl diphenyl phosphate	1241-94-7	Industrial Chemicals
9	2-Methoxy-5-methylaniline	120-71-8	Industrial Chemicals
10	3,5-di-tert-Butyl-4-hydroxybenzoic acid	1421-49-4	Industrial Chemicals
11	(4+5)-Methylbenzotriazole	29878-31-7	Industrial Chemicals
12	6-Methoxyquinoline	5263-87-6	Industrial Chemicals
13	6-Methyl-2-pyridinemethanol	1122-71-0	Industrial Chemicals
14	Bis(2-ethylhexyl)amine	106-20-7	Industrial Chemicals
15	Caffeine	58-08-2	Others
16	Caprolactam	105-60-2	Industrial Chemicals
17	Carbamazepine	298-46-4	Pharmaceuticals
18	Carbendazim	10605-21-7	Pesticides
19	Dibutyl adipate	105-99-7	Personal Care Products
20	Dibutyl hydrogen phosphate	107-66-4	Industrial Chemicals
21	Dibutyl phthalate	84-74-2	Industrial Chemicals
22	Diethyl phthalate	84-66-2	Personal Care Products

Nº	Compound	CAS No.	Substance category
23	Diuron	330-54-1	Pesticides
24	Flecainide	54143-55-4	Pharmaceuticals
25	Galaxolidone	507442-49-1	Personal Care Products
26	MDMA	42542-10-9	Drugs of abuse
27	Melamine	108-78-1	Industrial Chemicals
28	N,N'-Diphenylguanidine	20277-92-3	Industrial Chemicals
29	N-Phenyl-1-naphthylamine	90-30-2	Industrial chemicals
30	O-Desmethyl Venlafaxine	93413-62-8	Pharmaceuticals (met.)
31	Ofloxacin	82419-36-1	Pharmaceuticals
32	Secbumeton	26259-45-0	Pesticides
33	Sitagliptin	486460-32-6	Pharmaceuticals
34	Sulpiride	15676-16-1	Pharmaceuticals
35	Temazepam	846-50-4	Pharmaceuticals
36	Terbutryn	886-50-0	Pesticides
37	Theophylline	58-55-9	Others (met.)
38	Tributyl phosphate	126-73-8	Industrial Chemicals
39	Tributylamine	102-82-9	Industrial Chemicals
40	Triethyl phosphate	78-40-0	Industrial Chemicals
41	Tris(2-butoxyethyl) phosphate	78-51-3	Industrial Chemicals
42	Venlafaxine	93413-69-5	Pharmaceuticals

### 2.3.2 Chemicals and reagents

Standards of the 42 target compounds and 22 isotopically labelled standards (see Table 12) were purchased from either Merck (Darmstadt, Germany) or Toronto Research Chemicals (Toronto, ON, Canada). Stock solutions of known concentrations of the individual compounds and mixtures of them were prepared in methanol and kept in the refrigerator at  $-20\text{ }^{\circ}\text{C}$  until their use. Solvents were all LC-HRMS grade. Acetonitrile (ACN) and water ( $\text{H}_2\text{O}$ ) for LC-HRMS were obtained from Thermo Fisher Scientific Inc. (Waltham, MA, USA). The same water was used for sample preparation. Formic acid (>98%) and methanol (MeOH) were purchased from Merck (Darmstadt, Germany).

For quantification of the compounds, calibration standards are prepared with ultrapure water. The concentration range of the calibration curve goes from  $1\text{ ng/L}$  to  $6\text{ }\mu\text{g/L}$  and the internal standard mix is added at a final concentration of  $0.5\text{ }\mu\text{g/L}$ . The standard at  $0.5\text{ }\mu\text{g/L}$  is used as quality control. At the beginning of the sequence, the blanks and the calibration curve series are measured and a quality control is analysed every 10 samples.

### 2.3.3 Sample collection, preservation and pre-treatment.

To avoid any contamination from the processing material, the sample is collected and handled using as far as possible glass material. During the field sampling procedure, 10 mL of sample is poured into a 15 mL falcon tube containing the isotopically labelled compounds at a final concentration of 0.5 µg/L, in order to take into account any possible degradation of the compounds. The sample is transported refrigerated and stored at -22°C until analysis (between 3 days to 2 weeks).

Prior to analysis, 2 mL of the spiked defrosted sample is transferred into Eppendorf tube and centrifuged at 10,000 rpm and 20°C for 10 min. Then, the resulting supernatant is poured into a LC vial and ready for analysis.

### 2.3.4 Analysis

Analysis is performed in an online SPE–LC–QTOF-MS/HRMS system consisting of an ISOLUTE® ENV+ On-line SPE cartridge (30 x 2.1 mm, 40 µm) from Biotage (Uppsala, Sweden) for the preconcentration and purification of samples and a Luna Omega 3 µm Polar C18 (50 x 2.1 mm) HPLC column from Phenomenex (Torrance, CA, USA) for chromatographic separation coupled to an Impact II ESI ultra high-resolution QTOF mass spectrometer from Bruker Daltonics GmbH & Co. (Billerica, MA, USA).

The column oven is set at 30 °C and the sample compartment at 10 °C. Conditioning and equilibration of the online extraction column is performed with 1 mL of ACN and 1 mL of water, respectively, and washing after sample loading (1 mL) with 1.7 mL of 5% of ACN in water. An injection volume of 5 µL and a flow-rate of 0.3 mL min<sup>-1</sup> are used for the analysis. The mobile phase composition in positive ionization mode consisted of (A) H<sub>2</sub>O and (B) ACN, both with 0.1 % formic acid, and in negative ionization mode of (A) H<sub>2</sub>O with 5 mM ammonium acetate and (B) ACN. Initially, the gradient elution program run at 95 % (A) for 1 min and then increased to 97 % (B) in 17 min, it was held at 97% for 2.5 min, and finally returned to the initial conditions in 1 min with a linear gradient, which was maintained for 2.5 min.

Mass spectrometry analyses is performed with the VIP-HESI source in positive and negative ionization. Ion source conditions are: capillary voltage, +2500 V in positive mode, -2500 V in negative mode; dry gas, 8 L min<sup>-1</sup>; dry temperature, 220 °C. Fullscan spectra are recorded between 70 *m/z* to 1,000 *m/z* at a collision energy of 6.0 eV. The fragmentation data is obtained in two acquisition modes, AutoMSMS, a data dependant mode (DDA), and bbCID, a data independent mode (DIA), both with a nominal collision energy (n.c.e) of 25 eV with a ramped scans range between 25 – 60 eV (100% to 240 % n.c.e.).

Identification and quantification of the target compounds is performed using both TASQ® and Compass Data Analysis softwares from Bruker Daltonics GmbH & Co. (Billerica, MA, USA) according to SANTE/11312/2021 guideline on analytical quality control and method validation procedures [EC, 2021]. Target compounds are quantified using the signal of their protonated molecular ion ([M+H]<sup>+</sup>) and confirmed when minimum 2 ions with mass accuracy ≤ 5 ppm matched reference spectra of the compounds (including the [M+H]<sup>+</sup> and at least one adduct or fragment ion with < 1 mDa for *m/z* < 200). Detailed information with the retention time and the selective ions for the identification of analytes in samples is shown in Table 12.

Table 12: Targeted compounds, retention time, [M+H]<sup>+</sup> ion used for quantification, ions selected for identification confirmation, and internal standard used for quantification.

N°	Compound	RT (min)	[M+H] <sup>+</sup> m/z	Ion 1 m/z	Ion 2 m/z	Ion 3 m/z	IS	IS ion m/z
1	1,2,3-Benzotriazole	5.90	120.0561	120.0556	92.0494	-	Benzotriazole-d4	124.0686
2	1,8-Diazabicyclo[5.4.0]undec-7-ene	3.60	153.1224	153.1384	125.1066	96.0707	4-Methyl-benzotriazole-d3	137.0763
3	10,11-Dihydroxycarbamazepine	6.57	271.1083	236.0468	210.0703	180.0627	Carbamazepine-d10	247.1655
4	2,4-Diaminotoluene	1.79	123.0922	123.0674	106.0648	97.0073	2,4-Diaminotoluene-d3	126.0977
5	2-Amino-4-cresol*	1.53	124.0762	124.0749	106.5100	107.0493	4-Methyl-benzotriazole-d3	137.0763
6	2-Aminophenol	1.80	110.0605	110.0593	92.0487	-	4-Methyl-benzotriazole-d3	137.0763
7	2-Ethyl-1,5-dimethyl-3,3-diphenylpyrrolinium	9.05	278.1907	249.1252	234.1034	219.0714	EDDP-d3	281.2097
8	2-Ethylhexyl diphenyl phosphate	16.28	363.1725	363.1347	251.0209	-	Tris(2-butoxyethyl)phosphate-d27	426.3755
9	2-Methoxy-5-methylaniline	4.56	138.0774	123.0557	106.0550	94.0562	Carbendazim-d3	195.0758
10	3,5-di-tert-Butyl-4-hydroxybenzoic acid	12.45	251.1646	195.1034	177.0363	-	Tris(2-butoxyethyl)phosphate-d27	426.3755
11	(4+5)-Methylbenzotriazole	6.95	134.0577	106.0548	105.0343	95.0400	4-Methyl-benzotriazole-d3	137.0763
12	6-Methoxyquinoline	4.66	160.0597	145.0378	117.0457	89.0300	Carbendazim-d3	195.0758
13	6-Methyl-2-pyridinemethanol*	1.53	124.0762	124.0750	106.0645	79.0535	4-Methyl-benzotriazole-d3	137.0763
14	Bis(2-ethylhexyl)amine	11.72	242.2847	130.1460	71.0788	-	Diuron-d6	239.0373
15	Caffeine	5.25	195.0882	138.0661	110.0602	138.0661	Caffeine-d3	198.0865
16	Caprolactam	4.90	114.0918	96.0714	79.0468	-	ε-Caprolactam-d10	124.1546
17	Carbamazepine	9.05	237.1012	194.0761	192.0607	194.0761	Carbamazepine-d10	247.1655
18	Carbendazim	4.72	192.0772	160.0345	132.0424	105.0344	Carbendazim-d3	195.0758
19	Dibutyl adipate	14.48	259.1909	185.0980	147.0496	129.0412	Dibutyl phthalate-d4	283.1847
20	Dibutyl hydrogen phosphate	13.60	211.1099	116.9832	98.9746	-	Tris(2-butoxyethyl)phosphate-d27	426.3755
21	Dibutyl phthalate	14.75	279.1596	149.0079	205.0646	-	Dibutyl phthalate-d4	283.1847
22	Diethyl phthalate	11.30	223.0970	149.0084	177.0367	-	Diethyl phthalate-d4	227.1221
23	Diuron	10.29	233.0238	187.9469	159.9549	187.9469	Diuron-d6	239.0373

24	Flecainide	8.70	415.1450	398.0773	232.0731	209.0008	Caffeine-d3	198.0865
25	Galaxolidone	14.83	273.1855	273.1855	255.1743	240.1505	Dibutyl phthalate-d4	283.1847
26	MDMA	5.30	194.0983	163.0594	135.0310	133.0520	EDDP-d3	281.2097
27	Melamine	1.34	127.0731	127.0597	110.0345	85.0423	Melamine-15N3	130.0506
28	N,N'-Diphenylguanidine	6.02	212.1187	195.0723	119.0488	105.0346	N-N'-Diphenylguanidine-d10	222.1595
29	N-Phenyl-1-naphthylamine	14.13	220.1118	205.0672	143.0581	128.0490	N-phenyl-13C6-1-naphthylamine	226.1327
30	O-Desmethyl venlafaxine	5.78	264.1967	246.1604	201.1072	107.0388	Venlafaxine-d6	284.2496
31	Ofloxacin	5.69	362.1516	318.1292	261.0774	221.0500	Ofloxacin-d3	365.1704
32	Secbumeton	7.58	226.1668	170.0866	142.0582	114.0550	Secbumeton-d5	231.1981
33	Sitagliptin	6.89	408.1262	391.0594	235.0563	193.0501	Caffeine-d3	198.0865
34	Sulpiride	4.45	342.1488	213.9948	112.1010	213.9948	S-(-)-Sulpiride-d3	345.1675
35	Temazepam	10.19	301.0735	283.0344	255.0422	228.0337	EDDP-d3	281.2097
36	Terbutryn	9.36	242.1200	186.0803	158.0486	138.0635	Terbutryn-d3	245.1368
37	Theophylline	4.65	181.0717	124.0501	181.0716	-	Venlafaxine-d6	284.2496
38	Tributyl phosphate	13.60	267.1720	98.9745	116.9832	-	Tributyl phosphate-d27	294.3419
39	Tributylamine	7.91	186.2221	186.2024	130.1459	-	4-Methyl-benzotriazole-d3	137.0763
40	Triethyl phosphate	7.49	183.0776	98.9744	116.9830	80.9655	Tris(2-butoxyethyl)phosphate-d27	426.3755
41	Tris(2-butoxyethyl) phosphate	14.21	399.2506	299.1304	243.0744	199.0524	Tris(2-butoxyethyl)phosphate-d27	426.3755
42	Venlafaxine	7.20	278.2119	260.1742	215.1210	121.0528	Venlafaxine-d6	284.2496

RT: retention time, IS: internal standard, \*overlapping compounds quantified together

### 2.3.5 Performance

Method performance was assessed in terms of linearity, reproducibility, matrix effects (ME), recovery (RE) and both LODs and LOQs.

The method exhibited a satisfactory linear response (weighted by  $1/X$ ), with regression coefficients ( $r^2$ ) larger than 0.99 for all compounds. The linear range was localised mainly between 1 ng/L and 4 µg/L.

Recoveries of the online system were mainly between 70 and 130%. Method recoveries were calculated by comparing the results of the triplicate analysis of a water sample spiked with the target compounds at 1 µg/L with those of the off-line analysis of a standard solution at an equivalent concentration.

LOD and LOQ values were found in the ng/L scale for all compounds, with minimum values of 0.006 and 0.018 ng/L for Tributylamine and maximum values of 97 and 321 ng/L for 2,4-Diaminotoluene (see Table 13). Method LODs and LOQs were calculated as three and ten times, respectively, the ratio between the estimated concentration and the signal-to-noise ratio for each compound in the treated wastewater samples.

Table 13: LODs and LOQs, recoveries and matrix effects of the CSIC method based on on-line SPE-LC-HRMS.

Nº	Compound	LOD (ng/L)	LOQ (ng/L)	Recovery at 1 µg/L (%)	Relative Standard Deviation (n=3) %
1	1,2,3-Benzotriazole	0.826	2.73	125	4
2	1,8-Diazabicyclo[5.4.0]undec-7-ene	0.016	0.051	113	4
3	10,11-Dihydroxycarbamazepine	13.2	43.6	131	7
4	2,4-Diaminotoluene	97.4	321	154	29
5 + 13	2-Amino-4-cresol + 6-Methyl-2-pyridinemethanol	1.01	3.32	15	33
6	2-Aminophenol	3.92	12.9	208	12
7	2-Ethyl-1,5-dimethyl-3,3-diphenylpyrrolinium	0.045	0.149	101	0.3
8	2-Ethylhexyl diphenyl phosphate	5.87	19.4	7	1
9	2-Methoxy-5-methylaniline	0.118	0.390	144	2
10	3,5-di-tert-Butyl-4-hydroxybenzoic acid	7.71	25.4	140	26
11	(4+5)-Methylbenzotriazole	0.523	1.73	98	28
12	6-Methoxyquinoline	0.332	1.10	128	3
14	Bis(2-ethylhexyl)amine	0.364	1.20	83	12
15	Caffeine	12.5	41.3	99	3



Nº	Compound	LOD (ng/L)	LOQ (ng/L)	Recovery at 1 µg/L (%)	Relative Standard Deviation (n=3) %
16	Caprolactam	1.11	3.67	131	6
17	Carbamazepine	0.946	3.12	82	16
18	Carbendazim	0.100	0.332	135	2
19	Dibutyl adipate	0.660	2.18	133	7
20	Dibutyl hydrogen phosphate	0.211	0.697	115	17
21	Dibutyl phthalate	30.2	99.8	72	8
22	Diethyl phthalate	8.18	27.0	186	32
23	Diuron	3.64	12.0	89	10
24	Flecainide	0.241	0.795	99	3
25	Galaxolidone	16.7	55.2	49	6
26	MDMA	0.281	0.93	106	1
27	Melamine	1.98	6.53	4	1
28	N,N'-Diphenylguanidine	0.113	0.371	105	2
29	N-Phenyl-1-naphthylamine	0.326	1.08	70	5
30	O-Desmethyl venlafaxine	0.462	1.52	87	2
31	Ofloxacin	1.35	4.46	64	3
32	Secbumeton	0.063	0.208	103	16
33	Sitagliptin	1.46	4.83	79	12
34	Sulpiride	0.408	1.35	117	5
35	Temazepam	3.61	11.9	99	9
36	Terbutryn	0.266	0.879	150	14
37	Theophylline	11.4	37.8	134	7
38	Tributyl phosphate	0.414	1.37	111	16
39	Tributylamine	0.006	0.018	93	10
40	Triethyl phosphate	0.192	0.634	59	42
41	Tris(2-butoxyethyl) phosphate	0.299	0.985	57	6
42	Venlafaxine	0.204	0.672	72	13

### 2.3.6 Application to real samples

The method developed by CSIC for analysis of PMTs in treated wastewaters has been used in laboratory and pilot experimental studies conducted with spiked synthetic water and real effluent water of the Montornès del Vallès WWTP with the objective of evaluating the removal efficiency of different electrochemical advanced oxidation conditions in Task 4.2 and CS#3.

## 2.4 Analysis of VOCs in groundwater via GC-MS

### 2.4.1 General

The goal of this method was to determine chlorinated solvents and other volatile organic compounds (VOCs) in groundwater (GW) samples from Besos-Tordera aquifers (CS#7) by using a Purge-and-Trap (PT) technique coupled with GC-MS. The 59 substances included in the method are listed in Table 14.

Table 14: Target compounds, CAS number, registered ion(s), and LODs and LOQs in ng/L of the CSIC method for chlorinated solvents and other VOCs in groundwater based on PT-GC-MS.

#	Compound	CAS	Quantif. ion	Identif. ion 1	Identif. ion 2	LOD ng/L	LOQ ng/L
1	1,1,1,2-tetrachloroethane	630-20-6	131	133	117	5.6	19
2	1,1,1-trichloroethane	71-55-6	97	99	101	15	49
3	1,1,2,2-tetrachloroethane	79-34-5	84	86	95	44	148
4	1,1,2-trichloroethane	79-00-5	98	84	100	21	69
5	1,1-dichloroethane	75-34-3	64	65	83	14	47
6	1,1-dichloroethylene	75-35-4	62	96	63	2.7	9.2
7	1,1-dichloropropylene	563-58-6	75	39	110	13	42
8	1,2,3-trichlorobenzene	87-61-6	181	183	147	4.0	13
9	1,2,3-trichloropropane	96-18-4	76	110	77	49	164
10	1,2,4-trichlorobenzene	120-82-1	181	183	184	11	35
11	1,2,4-trimethylbenzene	75-01-4	105	120	119	3.1	10
12	1,2-dibromo-3-chloropropane	96-12-8	157	75	155	30	99
13	1,2-dibromoethane	106-93-4	107	109	93	15	49
14	1,2-dichlorobenzene	95-63-6	147	149	111	4.5	15
15	1,2-dichloroethane	107-06-2	62	49	43	6.2	21
16	1,2-dichloropropane	78-87-5	64	63	42	2.6	8.8
17	1,3,5-trimethylbenzene	95-50-1	120	105	119	7.3	24
18	1,3-dichlorobenzene	541-73-1	146	148	75	6.3	21
19	1,3-dichloropropane	142-28-9	76	41	115	3.7	12
20	1,4-dichlorobenzene	106-46-7	146	148	75	5.3	18
21	2,2-dichloropropane	594-20-7	78	41	79	6.1	20
22	2-chlorotoluene	95-49-8	92	126	89	11	36
23	4-chlorotoluene	106-43-4	92	126	125	3.7	12
24	4-isopropyltoluene	99-87-6	119	134	91	11	38
25	benzene	71-43-2	78	77	52	3.6	12

#	Compound	CAS	Quantif. ion	Identif. ion 1	Identif. ion 2	LOD ng/L	LOQ ng/L
26	bromobenzene	108-86-1	78	51	156	24	81
27	bromochloromethane	74-97-5	50	131	129	174	580
28	bromodichloromethane	75-27-4	83	85	47	17	57
29	bromoform	75-25-2	173	171	175	6.3	21
30	bromomethane	74-83-9	94	96	93	261	870
31	carbon tetrachloride	56-23-5	117	119	121	90	300
32	chlorobenzene	108-90-7	112	77	114	53	177
33	chloroethane	75-00-3	64	66	65	12	41
34	chloroform	67-66-3	83	85	47	7.0	23
35	chloromethane	74-87-3	47	36	52	23	76
36	cis-1,2-dichloroethylene	156-59-2	61	96	98	6.7	22
37	cis-1,3-dichloropropylene	10061-01-5	75	39	77	26	88
38	dibromochloromethane	124-48-1	129	49	48	4.3	14
39	dibromomethane	74-95-3	175	94	96	35	115
40	dichlorodifluoromethane	75-71-8	85	87	50	2.8	9.2
41	ethylbenzene	100-41-4	91	65	51	1911	6371
42	hexachlorobutadiene	87-68-3	226	188	260	3.7	12
43	isopropylbenzene	98-82-8	105	120	77	11	37
44	methylene chloride	75-09-2	49	84	51	11	38
45	m-xylene/p-xylene	108-38-3	91	106	105	5.2	18
46	naphthalene	106-42-3	127	51	64	3.0	10
47	n-butylbenzene	104-51-8	91	92	105	6.2	21
48	n-propylbenzene	103-65-1	91	92	65	2.6	8.4
49	o-xylene	91-20-3	91	105	77	3.4	11
50	sec-butylbenzene	135-98-8	106	91	77	46	153
51	styrene	100-42-5	77	105	133	59	196
52	tert-butylbenzene	98-06-6	120	92	134	55	182
53	tetrachloroethylene	95-47-6	166	164	129	8.2	27
54	toluene	127-18-4	91	56	43	20	65
55	trans-1,2-dichloroethylene	156-60-5	61	96	98	46	155
56	trans-1,3-dichloropropylene	10061-02-6	75	39	77	38	125
57	trichloroethylene	108-88-3	130	63	95	6.5	19
58	trichlorofluoromethane	75-69-4	102	104	66	15	49
59	vinyl chloride	79-01-6	62	63	64	44	148

#### 2.4.2 Chemicals and reagents

Standards of the 59 target compounds and 3 internal standards (fluorobenzene, 4-bromofluorobenzene and 1,2-dichloroethane-d4) are purchased from Merck (Darmstadt, Germany). A 2000 µg/mL VOC Mix solution in MeOH is obtained from Dr. Ehrenstorfer (GmbH, Augsburg, Germany) and kept in the refrigerator at –20 °C until its use. Solvents are all GC-MS grade. MeOH and water are obtained from Merck (KGaA, Darmstadt, Germany). The same water is used for sample preparation.

### 2.4.3 Sample collection, preservation and pre-treatment

Groundwater samples were collected in triplicate in 40 mL glass vials and stored in the dark at -18 °C until analysis.

### 2.4.4 Analysis

Analysis is performed using a Purge-and-Trap (PT) technique coupled with GC-MS without any pre-processing of the groundwater samples. As it is shown in Table 15, the PT manually dispenses 10 mL of the water sample or a standard solution. The samples placed in the vial are purged for 11 min by a stream of helium at 40 mL/min and trapped in an adsorbent. After desorption at 250 °C for 4 min, the VOCs are transferred directly into a Trace GC coupled to a MS equipped with a CP-Select 624 CB capillary column (60 m x 250 µm x 1.4 µm). The column is set at 40 °C during 10 min, ramped 50–150 °C at 5 °C/min, and to 210 °C at 15 °C/min, and this temperature is held for 10 min. The injector is operated in split mode and helium is used as carrier gas.

The MS is operated in full-scan acquisition mode in the  $m/z$  35-380 Da range. Quantification is performed by the internal calibration method using fluorobenzene, 4-bromofluorobenzene and 1,2-dichloroethane-d4 as internal standards. Nine-point calibration curves are prepared using deionized water just before instrumental analysis. Limits of detection (LOD) and quantification (LOQ) were also determined for each compound (Table 14).

Table 15: Simplified features of PT-GC-MS.

<b>P&amp;T</b>	
Valve oven temperature	180 °C
Sample volume	10 mL
Purge time	11 min
Purge flow	40 mL/min
Purge temperature	20 °C
<b>GC</b>	
Run time	46 min
Temperature program	Initial: 40 °C (hold time: 10 min); Post run: 50 °C; Rate: 5 °C/min; Value: 150 °C (hold time: 0 min); Rate: 15 °C/min; Value: 210 °C (hold time: 10 min)
Injector volume	1 µL
Inlet temperature	220 °C
Mode	Split
Split ratio	50:1
Split flow	50 mL/min
Column	CP-Select 624 CB (60 m x 250 µm x 1.4 µm)
<b>MS</b>	
Acquisition mode	Full-scan
$m/z$ range	35-380

#### 2.4.5 Performance

Method performance was assessed in terms of linearity, reproducibility, ME, recovery, and both LODs and LOQs.

The method exhibited a satisfactory linear response, with regression coefficients ( $r^2$ ) larger than 0.99 for all compounds. The linear range was localised mainly between 250 ng/L and 50 µg/L. Recoveries were mainly between 58 and 117%. LOD and LOQ values were found in the ng/L scale for all compounds, with minimum values of 2 and 5 ng/L and maximum of 1910 and 6370 ng/L (see Table 14). Method LODs and LOQs were calculated as three and ten times the signal-to-noise ratio, respectively.

#### 2.4.6 Application to real samples

This method has been employed to characterize contamination patterns caused by chlorinated solvents and other VOCs in one of the Besos-Tordera aquifers considered in CS#7. The results can be found in D2.2 (Characterization of PFAS and chlorinated solvent contamination in two aquifers in North-eastern Spain). Additionally, this method has been used to identify potential chlorinated by-products generated during laboratory remediation treatments conducted in task 3.4.

### 3 Conclusions

Three LC-MS-based methods for determination of iPMTs in different water matrices and one GC-MS-based method for determination of chlorinated solvents and other VOCs in groundwater have been developed within PROMISCES in WP1, Subtask 1.1.3. All of them are simple and fast methods easily reproducible in other laboratories with similar instrumentation. The use of internal standards for quantification in all four methods contributes to compensate potential matrix effects and obtain more accurate and reliable results. The application of extraction/purification steps prior to analysis in 3 of them also helps reducing matrix effects and improving LODs.

Notwithstanding this, it may be worth noting that, due to the considerably high concentration of many industrial chemicals in the water matrices, a dilution of the samples is often needed in order not to saturate the MS detector. The methods described for iPMTs have several target compounds in common, such as benzotriazoles and phosphates, since they were found to be relevant industrial contaminants in previous suspect screening studies conducted in the CSs. Table 16 shows the whole list of PMTs measured with the three described LC-MS based methods. This list includes in total 84 compounds. Most of them are industrial chemicals but there are also some pharmaceuticals and pesticides of concern in the CS.

The application of these methods in diverse experiments and case studies has permitted to put into evidence the widespread presence of PMTs in the water cycle and the persistence in the environment of some of them. These results, which will be presented in detail in other deliverables, will help police makers and water authorities to adopt specific actions to counteract this situation and maximise water reuse in future water scarcity scenarios.

**Table 16: List of PMTs analysed with the various LC-MS-based methods developed.**

N°	Method BAFG	Method BWB	Method CSIC	CAS N°	Substance category
1	-	(1-Methyl-2-phenyl)-hydrazide	-	38604-70-5	Pharmaceutical
2	-	-	(4+5)-Methylbenzotriazole	29878-31-7	Industrial Chemicals
3	(Methoxymethyl)triphenyl phosphonium	-	-	4009-98-7	Organic synthesis
4	Benzotriazole	1H-Benzotriazol	1,2,3-Benzotriazole	95-14-7	Industrial Chemicals, Corrosion inhibitor
5	-	-	1,8-Diazabicyclo [5.4.0]undec-7-ene	6674-22-2	Industrial Chemicals
6	-	-	10,11-Dihydroxycarbamazepine	35079-97-1	Pharmaceuticals (met.)
7	1-Hydroxy-Benzotriazole	-	-	123333-53-9	Organic synthesis
8	-	-	2,4-Diaminotoluene	95-80-7	Industrial Chemicals
9	-	-	2-Amino-4-cresol	95-84-1	Industrial Chemicals
10	-	-	2-Aminophenol	95-55-6	Personal Care Products
11	-	-	2-Ethyl-1,5-dimethyl-3,3-diphenylpyrrolinium	21409-27-8	Pharmaceuticals (met.)
12	-	-	2-Ethylhexyl diphenyl phosphate	1241-94-7	Industrial Chemicals
13	-	-	2-Methoxy-5-methylaniline	120-71-8	Industrial Chemicals
14	-	2-Methylthiobenzthiazol	-	615-22-5	Industrial Chemicals
15	-	-	3,5-di-tert-Butyl-4-hydroxybenzoic acid	1421-49-4	Industrial Chemicals
16	-	4-Acetylaminoantipyrine	-	83-15-8	Pharmaceutical
17	-	4-Dimethylaminoantipyrine	-	58-15-1	Pharmaceutical
18	-	4-Formylaminoantipyrine	-	1672-58-8	Pharmaceutical
19	-	-	6-Methoxyquinoline	5263-87-6	Industrial Chemicals
20	-	-	6-Methyl-2-pyridinemethanol	1122-71-0	Industrial Chemicals
21	Acesulfame	-	-	55589-62-3	Food industry
22	Acridine9-carboxylic acid	-	-	332927-03-4	Fluorescence dye
23	Acridone	-	-	578-95-0	Fluorescence dye
24	-	-	Bis(2-ethylhexyl)amine	106-20-7	Industrial Chemicals
25	Caffeine	-	Caffeine	58-08-2	Food industry
26	-	-	Caprolactam	105-60-2	Industrial Chemicals
27	-	-	Carbamazepine	298-46-4	Pharmaceuticals
28	-	-	Carbendazim	10605-21-7	Pesticides
29	Cybutryne	-	-	28159-98-0	Antifouling agent
30	Cyclamate	-	-	139-05-9	Food industry
31	Denatonium	-	-	3734-33-6	Additive for toxic liquids and solvents (bitter taste)
32	-	-	Dibutyl adipate	105-99-7	Personal Care Products
33	-	-	Dibutyl hydrogen phosphate	107-66-4	Industrial Chemicals

N°	Method BAFG	Method BWB	Method CSIC	CAS N°	Substance category
34	-	-	Dibutyl phthalate	84-74-2	Industrial Chemicals
35	-	Diclofenac	-	15307-86-5	Pharmaceutical
36	-	-	Diethyl phthalate	84-66-2	Personal Care Products
37	Diglyme	-	-	111-96-6	Organic synthesis, solvent
38	-	Dimethylpyrazolon	-	3201-28-3	Pharmaceutical
39	-	Dioxypyramidon	-	519-65-3	Pharmaceutical
40	Diphenyl urea	-	-	102-07-8	Intermediate
41	-	-	Diuron	330-54-1	Pesticides
42	Ethyltriphenyl phosphonium	-	-	1530-32-1	Organic synthesis
43	-	-	Flecainide	54143-55-4	Pharmaceuticals
44	-	-	Galaxolidone	507442-49-1	Personal Care Products
45	-	-	MDMA	42542-10-9	Drugs of abuse
46	-	-	Melamine	108-78-1	Industrial Chemicals
47	Methyltriphenyl phosphonium	-	-	1779-49-3	Organic synthesis
48	-	Metoprolol	-	56392-17-7	Pharmaceutical
49	-	N,N-Diethyl-3-methylbenzamide	-	134-62-3	Pesticide
50	-	-	N,N'-Diphenylguanidine	20277-92-3	Industrial Chemicals
51	-	Nicotin	-	13674-84-5	Others
52	-	-	N-Phenyl-1-naphthylamine	90-30-2	Industrial chemicals
53	-	o,p-Dichlorodiphenyltrichloroethane	-	50-29-3	Industrial Chemicals
54	-	-	O-Desmethyl Venlafaxine	93413-62-8	Pharmaceuticals (met.)
55	-	-	Ofloxacin	82419-36-1	Pharmaceuticals
56	-	p,p-Dichlorodiphenyltrichloroethane	-	50-29-3	Industrial Chemicals
57	-	Phenazon	-	60-80-0	Pharmaceutical
58	Propiconazole	-	-	60207-90-1	Fungicide also used for wooden construction material
59	-	Propyphenazon	-	479-97-5	Pharmaceutical
60	Saccharine	-	-	81-07-2	Food industry
61	-	-	Secbumeton	26259-45-0	Pesticides
62	-	-	Sitagliptin	486460-32-6	Pharmaceuticals
63	Sucralose	-	-	56038-13-2	Food industry
64	-	-	Sulpiride	15676-16-1	Pharmaceuticals
65	Tebuconazole	-	-	107534-96-3	Fungicide also used for wooden construction material
66	-	-	Temazepam	846-50-4	Pharmaceuticals
67	Terbutryne	-	Terbutryn	886-50-0	Pesticides, Algicide for paints



N°	Method BAFG	Method BWB	Method CSIC	CAS N°	Substance category
68	Tetrabutyl ammonium	-	-	1643-19-2	Organic synthesis, solvent, plasticizer
69	Tetrabutyl phosphonium	-	-	2304-30-5	Organic synthesis
70	Tetraglyme	-	-	143-24-8	Organic synthesis, solvent
71	Tetrapropyl ammonium	-	-	1941-30-6	Organic synthesis, solvent, plasticizer
72	-	-	Theophylline	58-55-9	Others (met.)
73	Tolyl biguanide	-	-	93-69-6	Antioxidant also used in paints and coatings
74	-	Tolyltriazol	-	29385-43-1	Industrial Chemicals
75	-	Tributylphosphate	Tributyl phosphate	126-73-8	Industrial Chemicals
76	-	-	Tributylamine	102-82-9	Industrial Chemicals
77	-	-	Triethyl phosphate	78-40-0	Industrial Chemicals
78	Triglyme	-	-	203-977-3	Organic synthesis, solvent
79	Triphenyl phosphineoxide	-	-	791-28-6	Byproduct from organic synthesis
80	-	Triphenylphosphate	-	115-86-6	Industrial Chemicals
81	-	-	Tris(2-butoxyethyl) phosphate	78-51-3	Industrial Chemicals
82	-	Tris(2-chloro-1-(chloromethyl)ethyl) phosphate	-	13674-87-8	Industrial Chemicals
83	-	Tris(2-chloroisopropyl)phosphate	-	13674-84-5	Industrial Chemicals
84	-	-	Venlafaxine	93413-69-5	Pharmaceuticals

## 4 References

EC, “Guidance document on analytical quality control and method validation for pesticide residues analysis in food and feed SANTE 11312/2021,” Sante/11312/2021, pp. 1–57, 2021, [Online]. Available: [https://ec.europa.eu/food/system/files/2022-02/pesticides\\_mrl\\_guidelines\\_wrkdoc\\_2021-11312.pdf](https://ec.europa.eu/food/system/files/2022-02/pesticides_mrl_guidelines_wrkdoc_2021-11312.pdf)