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iDRIP

What goes down the drainpipes?

Illicit drugs, pollutants and other chemicals in recycled water and wastewater

In-house single laboratory validation report of analytical methods for CECs and OPCs determination.

S. Tavazzi, G. Mariani S., Skejo H., Lobo Vicente J. Chassaigne H, Vandecasteele I., Comero S. and B. M. Gawlik

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Contact information
Name: Simona Tavazzi
Address: Joint Research Centre, via Enrico Fermi 2749, TP 120, 21027 Ispra (VA)
Email: simona.tavazzi@jrc.ec.europa.eu
Tel.: +39 0332 783683

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Authors

Tavazzi, Simona, Directorate-General Joint Research Centre, Directorate D – Sustainable Resources, Unit D2 Water and Marine Resources

Mariani, Giulio, Directorate-General Joint Research Centre, Directorate D – Sustainable Resources, Unit D2 Water and Marine Resources

Skejo, Helle, Directorate-General Joint Research Centre, Directorate D – Sustainable Resources, Unit D2 Water and Marine Resources

Lobo Vicente Joana, Directorate General Joint Research Centre Directorate F – Health, Consumers & Reference Materials, Unit F5 Food and Feed Compliance.

Hubert Chassaigne Directorate General Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Unit F2 – Consumer Products Safety

Ine Vandecasteele, Directorate-General Joint Research Centre, Directorate B – Growth And Innovation, Unit B3 Territorial Developement

Comero, Sara, Directorate-General Joint Research Centre, Directorate D – Sustainable Resources, Unit D2 Water and Marine Resources

Gawlik, B.M., Directorate-General Joint Research Centre, Directorate D – Sustainable Resources, Unit D2 Water and Marine Resources

Abstract

The validation of an analytical method is a necessary step in controlling the quality of quantitative analysis. Method validation is an established process which provides documentary evidence that a system fulfils its pre-defined specification, or shows that an analytical method is acceptable for its intended purpose. The purpose of the present study was to develop and validate analytical procedures for the quantitative determination in wastewater and recycled water samples of substances selected in the framework of the exploratory project "iDRIP: What goes down the drainpipes?".

Analyte extraction was performed using a JRC in-house developed sampling device (i.e.: Mariani box) for on-site Solid Phase Extraction (SPE) of environmental water samples.

Two instrumental methods were developed and characterised:

- a multi-residual method based on LC-MS/MS analysis for quantitative determination of selected Contaminants of Emerging Concern (CECs), including: acesulfame K, sucralose, estrone (E1), 17β-estradiol (E2), 17α-ethynyl estradiol (EE2), carbamazepine, 10,11-dihydro 10,11-dihydroxy carbamazepine, diclofenac, ibuprofen, sulfamethazine and sulfamethoxazole in wastewater and recycled water samples;
- a multi-residual method based on GC-MS analysis for quantitative determination of selected organo-phosphorous compounds (OPCs).

Selectivity, detection and quantification limits, linearity study, matrix comparison, repeatability and intermediate precision, variability of trueness and recoveries were determined for both CECs and OPC compounds.

1 Introduction

The iDRIP Exploratory Research (Work-package id. 2747; Project id. 302 EUDEPRO) aims at the detection, identification, and monitoring of chemical residues and illicit substances that may accumulate in the environment (via wastewater), in people (via drinkable water consumption) or in irrigation water.

The project addresses the need of defining environmental contamination levels of chemicals of concern, their possible propagation patterns with the final aim of mapping population's pharmaceutical consumption and drug use.

The present document summarises the methodological characteristics of developed analytical procedures for detection and quantification of some CECs, including organo-phosphorous (OPCs) flame retardants.

2 Experimental set-up of methods validation

Different experiments were carried out for the characterisation of the developed procedures in terms of limit of detection and quantitation, linearity study, repeatability, intermediate precision, variability of trueness and recovery.

In our approach, calibration curves created from freshly prepared standards and quality control samples (QCs) on three different days were considered. Some of the experiments were used in the evaluation of different parameters.

The analyte/internal standard peak area ratios were used as target parameters for quantitation. A weighted (1/c) least-square regression analysis of data was performed in order to determine the calibration curve parameters and the coefficient of determination (R^2).

2.1 Selectivity

Selectivity of quantitative determination was accomplished by relative retention times and by operating in multiple reaction monitoring (MRM) mode using LC-MS/MS and in selected ion monitoring (SIM) mode using GC-MS. At least two MRM transitions or two selected fragment ions were recorded for each compound.

2.2 Limits of detection and quantification

The mean value of blank samples (b) and the relative standard deviation (RSD) served for LOD and LOQ estimations, in accordance with the following equations:

$$LOD = b + 3SD;$$

$$LOQ = b + 10SD.$$

2.3 Linearity study

The calibration standards in solvent including a blank sample (i.e.: zero sample) were freshly prepared in triplicate and processed on each day of validation.

The relationship (goodness of fit) between peak area ratios of analyte/IS and concentrations in the concentration range investigated was assessed by the coefficient of determination (R^2).

The acceptance criteria set for calibration curves was:

- $R^2 \geq 0.9900$ calculated over nine calibration curves.

2.4 Repeatability and intermediate precision

Three QCs were freshly prepared in reconstituting solution and analysed on three days at two spiking levels for a total of 9 independent sample preparations were considered for repeatability and intermediate precision evaluation.

The acceptance criterion for the RSD of the repeatability and intermediate precision was set to 30% at both spiking levels.

2.5 Variability of trueness

Due to the absence of Certified Reference Material (CRM) on the market, the trueness was evaluated as variability of target analytes concentration in QC samples prepared using spiking solutions coming from different weighs of standards. The average concentrations found in QC samples were compared to the added (theoretical) concentrations in order to estimate the variability of trueness as slope of the resulting regression line, expressed as a percentage. Values in the range 70-130 % were considered satisfactory.

2.6 Recovery

For CECs, the recovery was evaluated by extracting and analysing in triplicate 0.5-litre MilliQ water samples spiked with only native analytes prior to extraction. The internal standards were then added to the disks before the elution step with the aim of allowing an estimation of analytes loss during extraction.

The recovery was evaluated by comparing the ratios analyte/IS in spiked samples to the same ratios obtained by analysing a standard solution containing native compounds and the labelled solution at the same concentration level.

For OPCs, the recovery of labelled analogues was evaluated *vs* benzo(a)antracene d_{12}, used as syringe standard. The use of syringe standard allowed to determine the portion of labelled internal standards (added to real samples before processing) actually recovered during sample extraction, under the assumption that labelled internal standards mimic entirely the analytical behaviours of respective non-labelled analogues.

The recovery was evaluated by comparing the ratios labelled analogue/syringe standard in real sample to the same ratios obtained by analysing a standard solution containing labelled compounds and the syringe standard at the same concentration level.

3 Validation procedure and results

3.1 Extraction procedure

HLB SPE Disk (Hydrophilic/Lipophilic Balanced - Atlantic™ HLB-H) filtration/adsorption disks, previously cleaned and conditioned, were used for sample extraction and concentration in the framework of the recovery and matrix comparison evaluation.

Samples were filtered at an average flow of 0.140 l/min, using a transportable field sampling device developed by JRC (i.e.: Mariani box).

Briefly the device consists of a Teflon holder for the 47mm SPE Disk, a membrane pump, a digital flowmeter counter and a battery (12V-9A/h). All spare part were assembled in an aluminum box, as depicted in Figure 1.

Figure 1 JRC in house developed field sampling device

HLB disks' activation, drying and elution were performed using an automatic extractor (J2 Scientific).

SPE experimental conditions are summarized in Table 1.

Table 1 SPE experimental conditions

OASIS HLB disk	Volume (ml)	Solvent
Conditioning and pre-cleaning	3 x 20	Ethyl acetate
Conditioning and pre-cleaning	3 x 20	Methanol
Conditioning	1 x 20	Water
QC Sample Filtration		

OASIS HLB disk	Volume (ml)	Solvent
Drying	Under N_2 for 30 min at 20 ml/min	
Elution	3 x 20 ml	Ethyl acetate
Elution	3 x 20 ml	Methanol

A two fractions sequential elution was performed with 3 x 20 ml ethyl acetate (1st fraction) followed by 3 x 20 ml methanol (2nd fraction).All used solvents were Pesticide Analysis grade.

The ethyl acetate fraction was divided into two portions for the OPCs and polar compounds analysis, respectively.

The portion dedicated to OPCs analysis was concentrated under gentle nitrogen flow to 100 µl and submitted to HRGC-HRMS analysis. The portion dedicated to polar compounds analysis was added to the methanolic eluate, mixed and evaporated to dryness. The sample was reconstituted in 0.5 ml reconstituting solution and analysed by UHPLC-MS/MS.

3.2 Analytical Methods

3.2.1 UHPLC-MS/MS method

UHPLC experimental conditions for polar compounds chromatographic separation were set as follows:

Pumps:	Binary Solvent Manager, Model UPB, Waters (Milford, MA, USA).
Autosampler:	Sample Manager, Model UPA, Waters (Milford, MA, USA).
Detector:	QTRAP 5500, Applied Biosystems MDS SCIEX, (Foster City, CA, U.S.A) equipped with Turbo V™ ion source.
Flow rate:	600 µL/min
Injection volume:	10 µL
Analytical column:	Hypersil GOLD, 2.1x100 mm, 1.9 µm, Thermo Scientific
Mobile phase:	A: $CH_3CO_2NH_4$ 5 mM B: Acetonitrile: Methanol, 9:1 (%, v/v)
Reconstituting solution	Water:Methanol, 95:5 (% v/v), 0.1% formic acid

The chromatography was performed in gradient mode according to the scheme reported in Table 2.

Table 2 UHPLC gradient scheme

Time (min)	Mobile phase (A%)	Mobile Phase B (%)
0	95	5
1	95	5

Time (min)	Mobile phase (A%)	Mobile Phase B (%)
3	40	60
5	25	75
7	0	100
8	0	100
8.1	95	5
9.5	95	5

An ABSciex QTRAP5500 mass spectrometer equipped with Turbo V™ ion source was used for polar compounds analysis. The instrument was previously tuned and calibrated in electrospray mode using PPG's. Prior to analysis all the specific parameters were optimized infusing a 1 µg/mL standard solution of analytes and I.S.s.

The eluate from the column was introduced directly into the ion source. The rapid desolvatation and vaporization of the droplets minimizes thermal decomposition and preserves their molecular identity.

The data were collected using the software program Analyst 1.6.2.

All calculations were based on chromatographic peak area ratios for the MRM precursor-product ion transitions for analytes versus I.S.s.

The general operating conditions were as follows:

Scan Type: Scheduled MRM

Polarity: Polarity Switching Positive/Negative

Ion Source: Turbo Spray

Resolution Q1: Unit

Resolution Q3: Unit

MR Pause: 5.0000 msec

Curtain gas (CUR): 25.00

Collision Gas (CAD): Medium

Temperature (TEM): 550.00

IonSpray Voltage (IS): ± 4500.00

Ion Source Gas 1 (GS1) 55

Ion Source Gas 2 (GS2) 45

Target Scan Time 0.1 sec

MRM detection window 10 sec.

In Table 3 Q1 and Q3 selected masses for analytes and labelled analogues are reported.

Table 3: Q1 and Q3 selected masses for CECs and their labelled analogues

Analyte	Internal labelled standard	Q1 Mass	Q3 Mass
Acesulfame K 1		162	78
Acesulfame K 2		162	82
	Acesulfame K-D_4	166	86
Diclofenac		294	250
Diclofenac 2		294	214
	Diclofenac $^{13}C_6$	300	256
E1		269	145
E1 1		269	143
	E1 $^{13}C_3$ 1	272	148
E2		271	145
E2 1		271	143
	E2 d_4 1	275	187
EE2		295	145
EE2 1		295	143
	EE2 d_4 1	299	187
Ibuprofen		205	161
Ibuprofen 2		205	159
	Ibuprofen $^{13}C_3$	208	163
Sucralose		395	359
Sucralose 1		395	35
Sucralose 2		397	361
	Sucralose d_6	401	365
10,11-dihydro-10,11-dihydroxy-carbamazepine	Carbamazepine d_{10}	271	180
10,11-dihydro-10,11-dihydroxy-carbamazepine 1		271	210
10,11-dihydro-10,11-dihydroxy-carbamazepine 2		271	253

Analyte	Internal labelled standard	Q1 Mass	Q3 Mass
Carbamazepine	Carbamazepine d_{10}	237	194
Carbamazepine 2		237	165
	Carbamazepine d_{10}	247	204
Sulfamethazine		279	92
Sulfamethazine 1		279	124
	Sulfamethazine $^{13}C_6$	285	70
Sulfamethoxazole		254	156
Sulfamethoxazole 2		254	92
	Sulfamethoxazole $^{13}C_6$	260	98

3.2.2 GC-MS method

OPCs were separated on a HP-5ms UI 60 m long, 0.25 mm i.d. (inner diameter) and 0.25 µm film (Agilent J&W, USA).

Gas chromatographic conditions for OPCs were:

PTV injector with temperature program from 100 to 300 °C at 14.5 °C/s, splitless time 1 min., split flow 50 ml/min., constant flow at 1.5 ml min^{-1} of He, GC-MS interface at 300 °C and a GC program rate: 80 °C for 1 min., 10 °C min^{-1} to 250 °C for 5 min., then 5 °C min^{-1} to 300 °C for a final isotherm of 1 min.

In Table 4 are reported exact masses recorded and retention time in HRGC-HRMS for native compounds, internal and syringe labelled standards.

Table 4 HRGC-HRMS experimental conditions for OPCs analysis

Group number	m/z 1	m/z 2	RT	Analyte	Internal Standard	Recovery Standard
1	135.0657	167.1221	6.98	TEP-d_{15}	TNPP-d_{21}	Tert-phenyl-d_{14}
	127.0155	155.0468	7.11	TEP		
2	131.0375	151.0939	10.38	TNPP-d_{21}		
	122.9842	141.0311	10.56	TNPP		
3	131.0375	167.1221	13.63	TNBP-d_{27}		
	139.0155	155.0468	12.25	TNBP	TNBP-d_{27}	
	124.9998	155.0468	13.84	TIBP		
4	261.0598	263.0568	15.09	TCEP-d_{12}		
	248.9845	250.9786		TCEP	TCEP-d_{12}	
	277.0158	279.0128	15.65	TCPP-1		

Group number	m/z 1	m/z 2	RT	Analyte	Internal Standard	Recovery Standard
			15.78	TCPP-2		
			15.89	TCPP-3		
5	393.9775	395.9746	21.38	TDCPP-d_{15}		
	380.8939	382.9746	21.62	TDCPP	TDCPP-d_{15}	
6	299.1618	300.1652	22.42	TBOEP-$^{13}C_6$		
	303.1752	304.1785	22.43	TBOEP	TBOEP-$^{13}C_6$	
	343.1228	344.1306	22.69	TPhP-$^{13}C_{18}$		
	339.1503	341.1644	22.56	TPhP-d_{15}		
	325.0624	326.0702	22.70	TPhP	TPhP-$^{13}C_{18}$	
7	250.0389	251.0468	23.03	EHDP		
8	98.9842	113.1325	23.44	TEHP		
9	403.1893	419.2206	30.27	T35DMPP-d_9		
	367.1094	368.1172	26.85	TMPP-1	T35DMPP-d_9	
			27.35	TMPP-2		
			27.86	TMPP-3		
			28.37	TMPP-4		
	452.2111	453.2145	29.02	TIPPP		
	395.1407	410.1641	30.33	T35DMPP		

3.3 Selectivity

Quantification of selected analytes was performed using isotopic dilution method, implying the use of isotopically labelled analogues for both OPCs and polar compounds.

The concept based on the use of identification points (IPs) proposed by the EU Commission Decision 2002/657/EC, both for GC-MS and LC-MS/Ms analysis was used to identify and confirm the selected analytes in real samples.

The concept, originally defined for the determination of organic contaminants in food, has been widely used in a huge range of matrices, including environmental samples. It proposes a minimum number of IPs for the confirmation of a positive finding in real samples. Furthermore, the Decision requests that the deviation of the relative intensity (ion ratio) of recorded ions/MRM transitions must be within a certain percentage value compared to the reference standard and the retention time may not deviate more than 2.5%.

In the present report, the compounds were identified and confirmed based on:

- retention time comparison of the corresponding standard;
- ratios between two ions/MRM transitions.

3.3.1 LC-MS/MS

For the identification of selected analytes, the two most abundant MRM transition ions from the precursor ion were chosen and monitored. The first was used for quantitation purposes, whereas the second ('qualifier') was used to confirm the presence of the target compound in the sample. The quantitated analyte was identified by comparing the retention time of the corresponding standard and the ratio between two ions recorded (± 30 %), in the standard and in the water samples.

The selected mass transitions used for quantification and confirmation are reported in Table 3.

3.3.2 GC-MS

For the identification of organophosphorous compounds, SIM was used and two selected ions among the most abundant were recorded, one for quantitation purposes and the other for confirmation.

The quantitated analytes were identified by comparing the retention time of the corresponding standard and the presence of peak on both selected ions.

The selected ions used for quantification and confirmation are reported in Table 4.

3.4 Limit of detection (LOD) and limit of quantification (LOQ)

Method Detection and Quantification limits were estimated by analysing blank samples in the respective matrix.

The mean values of the blank samples (b) and standard deviation (SD) were calculated using the data output from these experiments. LOD and LOQ were estimated according to the formula reported in 2.2.

The results of the LOD and LOQ estimation for CECs and OPCs are shown in Table 5 and 6, respectively.

Table 5: LOD and LOQ for CECs

Analyte	LOD (ng/l)	LOQ (ng/l)
Acesulfame K	0.3	0.8
Sucralose	2.3	7.8
Carbamazepine	0.1	0.4
10,11-dihydro-10,11-dihydroxy-carbamazepine	0.1	0.4
Ibuprofen	1.8	6.1
Diclofenac	0.05	0.2
E1	0.1	0.2
E2	0.4	1.4
EE2	0.3	1.1
Sulfamethazine	0.7	2.4
Sulfamethoxazole	0.1	0.4

Table 6: LOD and LOQ for OPCs

Analyte	LOD (ng/l)	LOQ (ng/l)
TEP	3.0	6.2
TNPP	1.4	3.4
TIBP	1.1	2.6
TNBP	7.7	20.3
TCEP	2.1	6.0
TCPP	4	10.9
TDCPP	0.7	1.9
TBOEP	2.1	5.1
TPhP	0.6	1.7
EHDP	1.7	4.5
TEHP	0.1	0.2
TMPP	0.1	0.3
TIPPP	0.1	0.2
T35DMPP	0.1	0.2

Special care is recommended when evaluating these methodological parameters in the presence of matrix components which could interfere with analytes determination.

Indeed, the overall sensitivity of developed procedure could be affected by the different real matrix components.

As a rule of thumb, a proper verification of sensitivity parameters using real matrix samples should always be performed to guarantee the reliability of produced datasets.

3.5 Linearity study

The linearity study for CECs ands and OPCs considered the concentration ranges reported in tables 7 and 8, respectively.

Table 7: Calibration ranges for CECs (ng/l)

Analyte / Conc (ng/l)	A	B	C	D	E	F	QC L	QC H
Acesulfame K	9900	4950	495	49.5	4.95	0.99	1.485	2970
Sucralose	12100	6050	605	60.5	6.05	1.21	1.815	3630
CBZ	1270	635	63.5	6.35	0.635	0.127	0.1905	381
CBZ-DiOH	3450	1725	172.5	17.25	1.725	0.345	0.5175	1035
Ibuprofen	10400	5200	520	52	5.2	1.04	1.56	3120
Diclofenac	19800	9900	990	99	9.9	1.98	2.97	5940
E1	103	51.5	5.15	0.515	0.0515	0.0103	0.01545	30.9
E2	113	56.5	5.65	0.565	0.0565	0.0113	0.01695	33.9

EE2	106	53	5.3	0.53	0.053	0.0106	0.0159	31.8
Sulfamethazine	550	275	27.5	2.75	0.275	0.055	0.0825	165
Sulfamethoxazole	555	277.5	27.75	2.775	0.2775	0.0555	0.08325	166.5

Table 8: Calibration ranges for OPCs (ng/l)

Analyte / Conc (ng/l)	A	B	C	D	E	F	QC L	QC H
TNPP	3	15	30	150	300	3000	9	2250
TIBP	3	15	30	150	300	3000	9	2250
TNBP	3	15	30	150	300	3000	9	2250
TCEP	3	15	30	150	300	3000	9	2250
TCPP	3	15	30	150	300	3000	9	2250
TDCPP	3	15	30	150	300	3000	9	2250
TBOEP	3	15	30	150	300	3000	9	2250
TPhP	3	15	30	150	300	3000	9	2250
EHDP	3	15	30	150	300	3000	9	2250
TEHP	3	15	30	150	300	3000	9	2250
TMPP	3	15	30	150	300	3000	9	2250
TIPPP	3	15	30	150	300	3000	9	2250
T35DMPP	3	15	30	150	300	3000	9	2250

Because of the huge concentration range for polar compounds, for diclofenac, sulfamethazine, sulfamethoxazole, carbamazepine and 10,11-dihydro 10,11-dihydroxy carbamazepine the linearity studies were conducted separately in the sub-ranges indicated in tables 9-11.

For OPCs, the reported calibration curves are referred to separate cases: the ones indicated as "low range" include the zero sample (a blank sample spiked only with labelled IS) and S1-S5 calibration standards; the ones indicated as "high range" include the zero sample and S2-S6 calibration standards.

The coefficient of determinations (R^2) of calibration curves for each day of validation together with the resulting mean R^2 and the relative standard deviation (RSD%) are reported in table 9-12 for CECs and in Table 13-16 for OPCs, respectively.

Table 9: Coefficient of determination (R^2) values for calibration curves of CECs on Day 1 of validation.

Analyte	Day 1		
	R^2	R^2	R^2
Acesulfame K	0.9971	0.9983	0.9963
Diclofenac (1.9-99 ng/l)	1.0000	0.9999	1.0000
Diclofenac (990-19800 ng/l)	0.9674	0.9664	0.9822
E1	0.9999	0.9998	0.9998
E2	1.0000	1.0000	0.9994
EE2 (0.53-106)	1.0000	0.9926	0.9997
Ibuprofen	0.9995	0.9895	0.9927
Sucralose	0.9983	0.9803	0.9855
Sulfamethazine (27.5-550 ng/l)	0.9886	0.9723	0.9775
Sulfamethazine (0.0825-2.75 ng/l)	1.0000	1.0000	1.0000
Sulfamethoxazole (0.055-2.775 ng/l)	1.0000	0.9998	0.9999
Sulfamethoxazole (27.55-555 ng/l)	0.9777	0.9695	0.9901
Carbamazepine (0.127-6.35 ng/l)	1.0000	0.9998	0.9999
Carbamazepine (63.5-1270 ng/l)	0.9876	0.9679	0.9806
10,11-dihydro 10,11-dihydroxy carbamazepine (0.345-17.25 ng/l)	0.9999	0.9982	0.9998
10,11-dihydro 10,11-dihydroxy carbamazepine (172.5-3450 ng/l)	0.9819	1.0000	0.9992

Table 10: Coefficient of determination (R^2) values for calibration curves of CECs on Day 2 of validation

Analyte	Day 2		
	R^2	R^2	R^2
Acesulfame K	0.9992	0.9996	0.9985
Diclofenac (1.9-99 ng/l)	0.9999	1.0000	1.0000
Diclofenac (990-19800 ng/l)	na	0.9967	0.9973
E1	0.9966	0.9997	0.9999
E2	0.9972	0.9967	1.0000
EE2 (0.53-106)	0.9940	0.9866	0.9866

Analyte	Day 2		
Ibuprofen	0.9971	0.9921	0.9982
Sucralose	0.9995	1.0000	1.0000
Sulfamethazine (27.5-550 ng/l)	1.0000	0.9998	1.0000
Sulfamethazine (0.0825-2.75 ng/l)	0.9746	0.9868	0.9863
Sulfamethoxazole (0.055-2.775 ng/l)	1.0000	1.0000	1.0000
Sulfamethoxazole (27.55-555 ng/l)	0.9847	0.9847	0.9904
Carbamazepine (0.127-6.35 ng/l)	1.0000	1.0000	0.9999
Carbamazepine (63.5-1270 ng/l)	0.9902	0.9871	0.9867
10,11-dihydro 10,11-dihydroxy carbamazepine (0.345-17.25 ng/l)	1.0000	0.9999	1.0000
10,11-dihydro 10,11-dihydroxy carbamazepine (172.5-3450 ng/l)	0.9872	0.9932	0.9951

Table 11: Coefficient of determination (R^2) values for calibration curves of CEcs on Day 3 of validation

Analyte	Day 3		
	R^2	R^2	R^2
Acesulfame K	1.0000	0.9971	0.9994
Diclofenac (1.9-99 ng/l)	1.0000	1.0000	0.9999
Diclofenac (990-19800 ng/l)	0.9824	0.9985	na
E1	0.9998	0.9985	0.9999
E2	1.0000	0.9813	0.9770
EE2 (0.53-106)	1.0000	0.9847	0.9862
Ibuprofen	0.9550	0.9983	0.9906
Sucralose	1.0000	0.9983	0.9991
Sulfamethazine (27.5-550 ng/l)	1.0000	1.0000	0.9999
Sulfamethazine (0.0825-2.75 ng/l)	0.9845	0.9970	0.9994
Sulfamethoxazole (0.055-2.775 ng/l)	1.0000	1.0000	0.9999
Sulfamethoxazole (27.55-555 ng/l)	0.9867	0.9997	0.9991
Carbamazepine (0.127-6.35 ng/l)	0.9998	1.0000	0.9999

Analyte	Day 3		
Carbamazepine (63.5-1270 ng/l)	0.9993	0.9906	0.9988
10,11-dihydro 10,11-dihydroxy carbamazepine (0.345-17.25 ng/l)	1.0000	1.0000	0.9999
10,11-dihydro 10,11-dihydroxy carbamazepine (172.5-3450 ng/l)	0.9996	0.9992	0.9957

Table 12: Mean coefficient of determination (R^2) values for calibration curves (n=9) and relative standard deviation (RSD%) for CECs

Analyte	Mean R^2 (n=9)	RSD%
Acesulfame K	0.9984	0.13
Diclofenac (1.9-99 ng/l)	1.0000	0.00
Diclofenac (990-19800 ng/l)	0.9844	1.40
E1	0.9993	0.11
E2	0.9946	0.90
EE2 (0.53-106)	0.9923	0.65
Ibuprofen	0.9903	1.39
Sucralose	0.9957	0.74
Sulfamethazine (27.5-550 ng/l)	0.9931	1.11
Sulfamethazine (0.0825-2.75 ng/l)	0.9920	0.94
Sulfamethoxazole (0.055-2.775 ng/l)	0.9999	0.01
Sulfamethoxazole (27.55-555 ng/l)	0.9869	0.97
Carbamazepine (0.127-6.35 ng/l)	0.9999	0.01
Carbamazepine (63.5-1270 ng/l)	0.9876	0.96
10,11-dihydro 10,11-dihydroxy carbamazepine (0.345-17.25 ng/l)	0.9998	0.06
10,11-dihydro 10,11-dihydroxy carbamazepine (172.5-3450 ng/l)	0.9946	0.63

Table 13: Coefficient of determination (R^2) values for calibration curves of OPCs on Day 1 of validation

Analyte	Low Range			High range		
	Day 1					
	R^2	R^2	R^2	R^2	R^2	R^2
TEP	0.999	0.999	1.000	1.000	0.999	1.000
TNPP	0.997	0.998	0.997	1.000	1.000	1.000
TIBP	0.998	0.998	0.999	1.000	0.999	0.999
TNBP	0.997	0.996	0.997	0.999	0.999	0.999
TCEP	0.996	0.997	0.995	1.000	1.000	1.000
TCPP	0.998	0.997	0.997	1.000	1.000	1.000
TDCPP	0.998	0.998	0.996	1.000	1.000	1.000
TBOEP	0.998	0.999	0.999	1.000	1.000	1.000
TPhP	0.998	1.000	0.999	1.000	1.000	1.000
EHDP	0.990	0.993	0.988	1.000	0.999	0.999
TEHP	0.979	0.989	0.987	0.999	0.999	0.999
TMPP	0.999	0.999	1.000	1.000	1.000	1.000
TIPPP	0.997	0.997	0.999	0.999	0.999	0.999
T35DMPP	0.998	0.997	0.994	1.000	0.999	0.999

Table 14: Coefficient of determination (R^2) values for calibration curves of OPCs on Day 2 of validation

Analyte	Low Range			High range		
	Day 2					
	R^2	R^2	R^2	R^2	R^2	R^2
TEP	0.999	1.000	1.000	1.000	1.000	1.000
TNPP	0.999	0.998	0.997	1.000	1.000	1.000
TIBP	0.996	0.998	0.998	1.000	0.999	0.999
TNBP	0.997	0.995	0.996	1.000	0.999	0.999
TCEP	0.997	0.997	0.996	1.000	1.000	1.000
TCPP	0.997	0.996	0.998	1.000	1.000	0.999
TDCPP	0.996	0.996	0.997	1.000	1.000	0.999
TBOEP	0.998	0.997	0.999	1.000	1.000	1.000
TPhP	0.999	1.000	1.000	1.000	1.000	1.000
EHDP	0.995	0.995	0.995	1.000	1.000	1.000
TEHP	0.995	0.996	0.994	1.000	1.000	1.000
TMPP	0.998	1.000	1.000	0.999	0.999	0.999
TIPPP	0.996	0.996	0.997	0.999	0.999	0.999
T35DMPP	0.993	0.994	0.994	0.999	0.999	0.999

Table 15: Coefficient of determination (R^2) values for calibration curves of OPCs on Day 2 of validation

Analyte	Low Range			High range		
	Day 3					
	R^2	R^2	R^2	R^2	R^2	R^2
TEP	1.000	1.000	1.000	1.000	1.000	0.999
TNPP	0.996	0.995	0.998	1.000	1.000	1.000
TIBP	0.999	0.996	0.998	1.000	0.999	0.999
TNBP	0.997	0.995	0.998	0.999	0.999	0.999
TCEP	0.996	0.995	0.997	1.000	1.000	1.000
TCPP	0.999	0.997	0.994	1.000	0.999	1.000
TDCPP	0.996	0.997	0.997	1.000	1.000	0.999
TBOEP	0.991	1.000	0.997	1.000	1.000	1.000
TPhP	0.999	0.999	0.999	1.000	1.000	1.000
EHDP	0.989	1.000	0.993	1.000	0.999	1.000
TEHP	0.971	1.000	0.996	1.000	0.998	1.000
TMPP	1.000	1.000	1.000	0.999	0.999	0.999
TIPPP	0.991	0.999	0.998	0.999	0.999	0.999
T35DMPP	0.993	0.995	0.994	0.999	0.999	0.999

Table 16: Mean coefficient of determination (R^2) values for calibration curves (n=9) and relative standard deviation (RSD%) for OPCs.

Analyte	Low Range		High Range	
	Mean R^2 (n=9)	RSD%	Mean R^2 (n=9)	RSD%
TEP	1.000	0.1	1.000	0.01
TNPP	0.997	0.1	1.000	0.01
TIBP	0.998	0.1	0.999	0.02
TNBP	0.996	0.1	0.999	0.01
TCEP	0.996	0.1	1.000	0.01
TCPP	0.997	0.1	1.000	0.02
TDCPP	0.997	0.1	1.000	0.01
TBOEP	0.998	0.3	1.000	0.01
TPhP	0.999	0.1	1.000	0
EHDP	0.993	0.4	0.999	0.03
TEHP	0.990	0.9	0.999	0.05
TMPP	0.999	0.1	0.999	0.03
TIPPP	0.997	0.3	0.999	0.03
T35DMPP	0.995	0.2	0.999	0.02

For all analytes, the R^2 respect the set performance criteria of > 0.9900.

3.6 Repeatability and intermediate precision

For repeatability and intermediate precision, nine QCs at two concentration levels were tested on three different days. The results obtained using the one-way analysis of variance (ANOVA) are shown in table 17 and 18 for CECs and in tables 19 and 20 for OPCs, respectively.

Table 17 Repeatability and Intermediate precision for CECs QC L

Analyte	QC L	
	Repeatability	Reproducibility / Intermediate precision
Acesulfame K	5.8	10.2
Diclofenac	4.2	8.3
E1	8.5	10.8
E2	na	na
EE2	na	na
Ibuprofen	9.3	10.2
Sucralose	5	5.4
Sulfamethazine	7.5	8.8
Sulfamethoxazole	14.6	15.5
Carbamazepine	11.8	16.1
10,11-dihydro 10,11-dihydroxy carbamazepine	5	10.1

Table 18 Repeatability and Intermediate precision for CECs QC H

Analyte	QC H	
	Repeatability	Reproducibility / Intermediate precision
Acesulfame K	2.4	2.6
Diclofenac	5.3	8.3
E1	3.2	6.6
E2	3.9	8.5
EE2	4	6.9
Ibuprofen	12.9	13.1

Analyte	QC H	
	Repeatability	Reproducibility / Intermediate precision
Sucralose	13.3	14.1
Sulfamethazine	5.4	7.9
Sulfamethoxazole	8.9	12.9
Carbamazepine	10.2	11.5
10,11-dihydro 10,11-dihydroxy carbamazepine	3.9	7.9

Table 19 Repeatability and Intermediate precision for OPCs QC L

Analyte	QC L	
	Repeatability	Reproducibility / Intermediate precision
TEP	7.8	8.7
TNPP	4	4.6
TIBP	2.8	4.9
TNBP	3.6	3.7
TCEP	3	4.8
TCPP	2.9	5.1
TDCPP	2	2.9
TBOEP	9.6	17.3
TPhP	2.9	3.2
EHDP	14	18.4
TEHP	16.1	24.3
TMPP	5.3	7.0
TIPPP	7.0	8.9
T35DMPP	4.0	5.0

Table 20 Repeatability and Intermediate precision for OPCs QC H

Analyte	QC H	
	Repeatability	Reproducibility / Intermediate precision
TEP	5.4	7.6
TNPP	1.9	9
TIBP	4.1	5.2
TNBP	2.5	9

Analyte	QC H	
	Repeatability	Reproducibility / Intermediate precision
TCEP	3.1	9.1
TCPP	2	3.3
TDCPP	1.9	2.4
TBOEP	5	5.4
TPhP	1.2	12.2
EHDP	10.2	10.2
TEHP	8.8	23.8
TMPP	2.8	9.9
TIPPP	3.4	4
T35DMPP	2.5	2.5

3.7 Variability of trueness

The variability of trueness has been evaluated using the data from three QCs at low and high concentration levels analysed on three different days, for a total of nine independent replicates.

Using the LINEST function provided by Excel, regression lines, obtained by using the 'least-square method', were calculated, interpolating QCs back-calculated concentrations and the corresponding theoretical values. This approach was not appropriate for 17β-estradiol and 17α-ethynyl estradiol since only data form QCs spiked at high concentration levels were available.

Trueness variability was determined as slope % and is listed in Table 21 and 22 for CECs and OPCs, respectively.

Table 21: Results of trueness variability for CECs

Analyte	Slope	Variability of Trueness
Acesulfame K	1.03	103.5
Diclofenac	0.90	89.7
E1	1.06	106.4
Ibuprofen	0.89	89.1
Sucralose	0.90	89.6
Sulfamethazine	0.89	88.5
Sulfamethoxazole	0.89	88.7
Carbamazepine	0.92	91.7
10,11-dihydro 10,11-dihydroxy carbamazepine	0.88	88.5

Table 22: Results of trueness variability for OPCs

Analyte	Slope	Variability of Trueness
TEP	0.92	92
TNPP	0.90	90.5
TIBP	0.84	84
TNBP	0.84	84.4
TCEP	0.88	87.7
TCPP	0.90	89.8
TDCPP	0.88	88.1
TBOEP	1.06	105.8
TPhP	0.97	96.6
EHDP	0.94	94.5
TEHP	0.84	84.4
TMPP	0.80	80.2
TIPPP	0.74	74.2
T35DMPP	0.79	78.9

3.8 Recovery

The results of the recovery experiments for CECs, carried out using analyte-spiked MilliQ water samples extracted according to section 3.1 are listed in Table 23.

Table 23: Recovery for CECs

Analyte	REC %	RSD %
Carbamazepine	100.2	8.8
10,11-dihydro 10,11-dihydroxy carbamazepine	98.3	12.7
Ibuprofen	56.0	24.4
Diclofenac	127.4	14.6
Sucralose	112.2	8.3
Acesulfame K	1.8	30.4
E1	98.3	12.0
E2	95.7	21.0
EE2	120.4	23.1
Sulfamethazine	31.5	17.5
Sulfamethoxazole	9.4	21.7

The results of recovery experiment for OPCs evaluated *vs* benzo(a)anthracene-d_{12}, used as syringe standard according to Section 2.6 are listed in Table 24.

Table 24: Recovery for OPCs

Analyte	REC %	RSD %
TNPP-d_{21}	77.9	44.4
TNBP-d_{27}	103.4	36.6
TCEP-d_{12}	104.0	30.2
TDCPP-d_{15}	105.9	31.9
TBOEP-$^{13}C_6$	197.7	40.4
TPhP-$^{13}C_{18}$	102.2	36.4

Analyte	REC %	RSD %
T35DMPP-d_9	103.2	35.1

4 Conclusions

SPE-LC-MS/MS and SPE-GC-MS multi-compound methods developed and described in this report are fit for purpose for the quantitative determination of environmental contaminants selected in the framework of the exploratory iDRIP project.

References

G. Mariani, S, Tavazzi, S. Comero, G. Buttiglieri, B. Paracchini, H. Skejo, L. Alcande Sanz and B.M. Gawlik; Short-term isochronous stability study of contaminants of emerging concern in environmental water samples; EUR 28425 EN; doi:10.2760/488206

List of abbreviations and definitions

Chemical elements are identified by their respective symbols as defined by the International Union of Pure and Applied Chemistry (IUPAC). Throughout this report, the following abbreviations and symbols are used:

CAD	Collision Gas
CECs	Contaminants of Emerging Concern
CUR	Curtain Gas
CRM	Certified Reference Material
CXP	Collision Cell Exit Potential
E1	Estrone
E2	17α-estradiol
EE2	17β-ethinyl estradiol
EC	European Commission
EI	Electron Impact
EP	Entrance Potential
EU	European Union
GC	Gas chromatography
GS1	Ion Source gas 1
GS2	Ion Source gas 2
HLB	Hydrophilic-lipophilic balanced
IS	Internal standard/Ion Transfer voltage
ISO	International Organisation for Standardisation
JRC	Joint Research Centre
LC	Liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
MRM	Multiple reaction monitoring
MS	Mass spectrometry
OPCs	Organo phosphorous compounds
PPG	Polypropylene glycol
QC	Quality control sample
R^2	Coefficient of determination
RSD	Relative standard deviation
RT	Retention time
SD	Standard deviation
S/N	Signal to Noise
SPE	Solid-Phase Extraction
TEM	Temperature
UHPLC	Ultra-high-pressure liquid chromatography
WFD	Water Framework Directive

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