Short-term isochronous stability study of contaminants of emerging concern in environmental water samples

Stabilisation of chemical analytes using a novel sampling device

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Foreword
This report informs about the analytical activities performed by JRC, Directorate D, Unit 02, in the framework of the development and application of a novel device for on-site environmental water sample processing.
Acknowledgements

The kind collaboration of GianLuigi Buttiglieri in the execution of sampling campaign is gratefully acknowledged.
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Abstract

The report addresses the issue of short-term stability of contaminants of emerging concern (CECs) in the framework of environmental survey using the so-called isochronous stability test design. A novel on-site sampling device, developed in-house by JRC, was used for environmental water samples collection, allowing compounds stabilization on solid phase extraction disks. The statistical evaluation of analytical measurements, performed according to the defined isochronous scheme and under repeatability conditions (i.e.: in one single analytical run), proofed that the device can be used for a multi-residual sampling strategy for CECs. The feasibility of the isochronous stability test design to investigate stability of water samples, together with the advantages of on-site sample processing represent valuable improvements in the execution of environmental monitoring campaigns both in support to European Water Framework Directive (WFD) and to the characterization of alternative practices for water reuse opportunities.
1. Introduction

The analytical determination and quantification of trace-levels of so-called compounds of emerging concern in environmental water samples remains a challenging and demanding process in environmental research on occurrence and fate of chemical pollutants.

We understand under the term “compounds of emerging concern” (CECs) those chemical and microbial constituents that have not historically been considered as contaminants and which are therefore also not included in national or international regulation. In this report, CECs are in particular those substances not covered by the EQS Directive or other pieces of EU legislation.

The concern is mainly driven by research activities, proving the relevance of these constituents beyond a local scale and frequently related to municipal, agricultural, and industrial wastewater sources and pathways. As stated by the USGS (http://toxics.usgs.gov/investigations/cec/index.php, n.d.) “these newly recognized contaminants represent a shift in traditional thinking as many are produced industrially yet are dispersed to the environment from domestic, commercial, and industrial uses”.

CECs include groups of compounds categorized by end-use (e.g. pharmaceuticals, non-prescription drugs, personal care products, household chemicals, food additives, flame retardants, plasticizers, disinfection-by-products, and biocides), by environmental and human health effects (e.g. hormonally active agents, endocrine disrupting compounds [EDCs]), or by type of compound (e.g. chemical vs. microbiological, antibiotic resistance gens, phenolic vs. polycyclic aromatic hydrocarbons), as well as transformation products resulting from various biotic and abiotic processes, and mixtures of chemicals.

Although the available information indicate often a potential threat to environmental and human health, the available database is incomplete and does not allow a reliable and legally agreed definition of health and ecosystem relevant threshold values. Frequent monitoring for every potential chemical substance is neither feasible nor plausible. Currently, research is focusing on the development of a science-based framework to guide the identification of CECs that should be monitored or otherwise regulated, including the context of reclaimed water use, especially for potable use (Drewes, et al., 2013). A sound selection framework is needed that can provide a short list of meaningful indicator measurements that can address both human health relevance and assurance of proper performance of water treatment processes in addition to routine monitoring for compliance with guidelines and/or regulations.

To improve the knowledge base for the afore-mentioned science based framework, we developed a novel sampling device, which combines the steps of environmental sampling and pre-concentration of the analytes of interest, while enhancing the conversation properties of the sample taking. This issue is of particular relevance when investigating CECs, which may tend to quick chemical alteration or degradation, thus jeopardizing their proper identification and quantification.

More precisely, the purpose of the performed stability tests described hereafter is to investigate the influence of storage conditions on the stability of an analyte and its matrix. Stability evaluation is of utmost importance for analytical analysis of poorly investigated contaminants, for which generally no stability data have been collected and scarce knowledge of their presence and persistence in the environment is available.

Traditionally, one distinguishes two different kinds of stability test: long-term and short-term stability studies (Lamberty, Schimmel, & Pauwels, 1998). While long-term stability studies are generally performed to define longer shelf-life times, for instance in the case of certified reference materials (CRMs), retained samples or environmental specimen banking (Rudel, Bohmer, & Schrotter-Kermani, 2006), (Gies, Schroeter-Kermani, Ruedel, Paulus , & Wiesmueller, 2007) short-term stability studies are essential in the framework of environmental monitoring campaigns, as (in) stability of chemical compounds can dramatically affect the quality of the results.
The investigation of short-term stability of environmental samples during transport, shipment and the moment of analysis has always been hindered by logistic difficulties: thus, the deliveries of water samples imply the risk of specimen loss in case of accidental damage of glass containers, frequently used for samples to be used for organic trace analysis. In addition, these containers are usually quite costly, heavy to transport and require adding of chemical stabilisation agents to avoid for instance microbial degradation processes.

The scientific basis of short-term stability studies have been largely developed by scientific work conducted in the JRC. They are based on the statistical evaluation (e.g.: t-test) of a set of measurements, executed under repeatability conditions in samples stored at different conditions (i.e.: different time intervals and different temperatures).

The present technical document reports the evaluation of short-term stability of some selected contaminants of emerging concern (i.e.: OPCs, NSAID, beta blockers, mood stabilising drugs, antibiotics, insecticides, etc.), in the framework of an application study of a JRC in-house developed sampling device for on-site extraction of environmental waters, the so-called MARIANI-Box.

Details on this sampling device, its design and functioning as well as necessary steps of sample pre-treatment and analysis are reported together with the statistical dissertation of results obtained.
2. Sampling campaign

2.1 Sampling location and sample type

As a test case for the application of MARIANI-Box and this isochronous stability study for OPCs and selected contaminants of emerging concern (CECs) were performed on grey water samples collected in the context of a FP VII Project dealing with various aspects of water recycling in the context of a touristic facility. The demonstration site at the Samba Hotel is located in Lloret de Mar, Spain. As a touristic recreational resort it offers the advantage to have a well-characterised sewer system, whose chemical footprint reflects the typical use of certain categories of CECs.

The hotel at the demonstration site is structured in 441 air-conditioned rooms, surrounding green areas, outdoor pools, conference rooms, bars and restaurants. The SAMBA Hotel is certified according to the EMAS-scheme and is certified according to ISO 14001. The claimed water use at the site ranges from 25,000 to 34,000 m³/year (100 to 135 l/client/day).

Grey water samples used in this study are composed by water outlets from the hotel room showers, from taps, from swimming pools and filters and their backwashes.

Figure 1 shows a view of the Hotel Samba; Figure 2 gives details on grey water sampling location and collection.

Figure 1: Hotel Samba
2.2 Sampling device and sampling method

The MARIANI-Box, a JRC-in-house developed sampling device (Figure 3), is a flexible, versatile, and easy-to-use system. With a complete weight of 6 kg it is portable and designed for the on-site extraction of environmental water samples ranging from 1 to 20 litre volumes. It allows for the reduction of storage facility volume, while increasing the stability of samples. The stabilisation is due to the selective adsorption of the analytes on a specially coated filter.

As shown in Figure 3, the device consists basically of the following parts:

1. Holder SPE disk
2. Flow meter
3. Counter
4. Pump (Flow rate 0.1-0.2 l/min)
5. Battery
6. Water sampling line
7. Waste line
In the framework of this study the MARIANI-Box was equipped with a solid-phase extraction disk of 47mm. A SPE HLB disk (Hydrophilic/Lipophilic Balanced - Atlantic™ HLB-H, Horizon Technology Inc.) was used in all experiments.

Prior to sampling, the disks were pre-cleaned with 60 ml of ethyl acetate and activated with 60 ml of methanol followed by 20 ml of analytical grade water (Milli-Q water). For greywaters extraction, the pump of the box was used at an average flow rate of 0.140 l/min.

Collaborating partner was asked to compile a standard sampling bill, used by the JRC for the collection of all the relevant information about sampling. In particular, information about geographical coordinates, specific weather and filed conditions as well as photographic material was collected together with any other details considered important at the moment of sampling.

At selected time schedule, the loaded filters were further processed by drying under a gentle flow of nitrogen for 30 minutes, followed by spiking with a labelled internal standard mixture. A two fraction sequential elution was performed with 3 x 20 ml ethyl acetate (1’ fraction followed by 3 x 20 ml 0.1% NH₄OH in methanol (2’fraction)).

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 spiked with labelled recovery standards prior to HRGC-HRMS analysis.

The portion dedicated to polar compounds analysis was added to the methanolic eluate, spiked with the corresponding recovery standard, mixed and evaporated to dryness. The resulting sample was reconstituted in 0.5 ml of reconstituting solution and analysed. Analytical conditions are described below.

Analytical and field blank samples were included in the sampling campaign to assess possible contamination sources or losses.
3. Isochronous stability test design

The isochronous stability test design has been described in its principles elsewhere (Lisinger, et al., 2001) (Gawlik, et al., 2012) (Lisinger, van der Veen, Gawlik, Pauwels, & Lamberty, 2004) and was adopted to the needs of this study.

The schematic design of the stability study is represented by a bar chart in Figure 4. In total, eleven grey water samples were collected and stored accordingly, thus covering a total duration of 10 weeks.

The reference temperature (i.e.: 20°C) is the temperature at which the samples were always transported or kept at before the analysis, while the testing temperature (i.e.: 4°C) was the condition at which test samples were stored at for a selected period of time, before returning to reference temperature.

In details:

- 3 samples were stored at the reference temperature for all the duration of the study;
- 2 samples were initially stored at 20°C for 2 weeks and then placed at 4°C for 8 weeks;
- 2 samples were initially stored at 20°C for 4 weeks and then placed at 4°C for 6 weeks;
- 2 samples were initially stored at 20°C for 8 weeks and then placed at 4°C for 2 weeks;
- 2 samples were stored at 20°C for all 10 weeks.

At the end of the study, all samples were stored at the reference temperature until analysis.

Figure 4: Isochronous short-term stability test design
4. Materials and methods

4.1 Standards and chemicals

Analytical standards and labelled analogues were purchased on the market as detailed in Table 1.

Table 1 Native standards and labelled analogues

<table>
<thead>
<tr>
<th>Chemical classes</th>
<th>Chemical Compound</th>
<th>Brand</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organophosphate compound (OPCs) and labelled analogues</strong></td>
<td>Diethyl-phosphate, Diethyl-phosphate-d_{15}, Tri-n-propyl-phosphate, Tri-n-propyl-phosphate-d_{21}, Tris(butyl)-phosphate, Tris(isobutyl)-phosphate, Tris(butyl)-phosphate-d_{27}, Triphenyl-phosphate, Triphenyl-phosphate-d_{15}, mix o,m,p-Trityl-phosphate Tris(2,3-dichloropropyl)-phosphate</td>
<td>CHIRON AS (Trondheim, Norway).</td>
</tr>
<tr>
<td></td>
<td>Tris(1-chloro-2propyl)-phosphate, Tris(2-Chloroethyl)-phosphate and Tris(2-butoxyethyl)-phosphate</td>
<td>Accustandard (USA).</td>
</tr>
<tr>
<td></td>
<td>Tris(2-isopropylphenyl)-phosphate, Tris(3,5-dimethylphenyl)-phosphate, Tris(2-butoxy(^{13}C_{12})ethyl)-phosphate, Tris(1,3-dichloro-2-propyl)-phosphate-d_{15}, 2-Ethylhexyl-diphenyl phosphate, Tris(2-ethylhexyl)-phosphate, and Triphenyl-phosphate-^{13}C_{18}</td>
<td>Wellington Laboratories Guelph (Ontario, Canada).</td>
</tr>
<tr>
<td></td>
<td>Tris(3,5-dimethylphenyl)-phosphate-d_{9} and Tris(2-Chloroethyl)-phosphate-d_{12}</td>
<td>Hayashi Pure Chemical Ind., LTD (Japan).</td>
</tr>
<tr>
<td><strong>Contaminant of Emerging concern (CECs) and labelled analogues</strong></td>
<td>Atrazine, Atrazine deisopropyl, Diclofenac, Ibuprofen, Sulfamethoxazole, Metolachlor d_{6}</td>
<td>Dr. Ehrenstorfer</td>
</tr>
<tr>
<td></td>
<td>MCPA, Metolachlor, Terbutylazine and Terbutylazine-desethyl</td>
<td>Fluka</td>
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<tr>
<td></td>
<td>1-H-Benzotriazole, 2,4-D, 5-methyl-1-H-benzotriazole, Bezafibrate, Carbamazepine, DEET, Ketoprofen, Ketoprofen-d_{3}</td>
<td>Sigma Aldrich</td>
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<tr>
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<td>10,11-dihydro-10,11-dihydroxy-carbamazepine</td>
<td>Spectra 2000</td>
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<td>Chemical Compound</td>
<td>Brand</td>
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<td>------------------------------------------</td>
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<td></td>
<td>Benzotriazole d₄ and Metopropol-d₇</td>
<td>CDN Isotopes</td>
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<td>CBZ d₁₀, DEET d₆, Gemfibrozil d₆,</td>
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<td>Diclofenac ¹³C₆</td>
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<td></td>
<td>Simazine</td>
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</table>

All organic solvents used were *Residue Analysis Grade* (Sigma-Aldrich, Buchs SG, Switzerland).

Adsorbent filter Atlantic HLB-H 47mm was purchased from Horizon Technology Inc. (Salem, New Hampshire, USA).

### 4.2 Instrumental analysis

Two different analytical methods were developed and applied for the analysis of semivolatiles and polar compounds.

Semivolatile compounds were analysed by HRGC-HRMS.

Polar compounds were analysed by UHPLC-MS/MS.

#### 4.2.1 HRGC-HRMS method for OPCs analysis

OPCs were analysed on double HRGC (Thermo Trace GC Ultra, Thermo Electron, Bremen, Germany), coupled with a DFS high resolution mass spectrometer HRMS (Thermo Electron, Bremen, Germany) operating in the EI-mode at 45 eV with a resolution of >10000.

Isotope dilution technique was used for their quantitative determination.

Two most abundant ions were selected coming from the fragmentation and chosen on the basis of close elution of different OPCs and the dynamic mass range of the HRMS.

Table 2 and Table 3 report the gas chromatographic and mass spectrometric conditions, respectively.
Table 2 HRGC experimental conditions for OPCs

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<tr>
<th>PTV injector</th>
<th>Initial T (°C)</th>
<th>Final T (°C)</th>
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<td>Splitless</td>
<td>Split flow</td>
<td>He constant Flow</td>
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Table 3 HRMS experimental conditions for OPCs

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<td>410.1641</td>
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</table>
4.2.2 UHPLC-MS/MS for polar compound analysis

Multi-residual UHPLC-MS/MS method developed included the analytes reported in Table 4:

Table 4 Analytes selected in multi-residual UHPLC-MS/MS method

<table>
<thead>
<tr>
<th>Compound</th>
<th>Use/Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>1H-benzotriazole</td>
<td>Corrosion inhibitor</td>
</tr>
<tr>
<td>5-methyl-1H-benzotriazole</td>
<td>Transformation product of 1H-benzotriazole</td>
</tr>
<tr>
<td>Bezfibrate</td>
<td>Fibrate (hypolipidemic agent)</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td></td>
</tr>
<tr>
<td>Diclofenac</td>
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<td>NSAID</td>
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</tr>
<tr>
<td>Naproxen</td>
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<td>Estrogen</td>
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<tr>
<td>17β-Estradiol</td>
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</tr>
<tr>
<td>17α-Ethyl-estradiol</td>
<td>Synthetic estrogen</td>
</tr>
<tr>
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<td>Beta blockers</td>
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<tr>
<td>Metopropol</td>
<td></td>
</tr>
<tr>
<td>2.4-D</td>
<td></td>
</tr>
<tr>
<td>Atrazine</td>
<td>Herbicide</td>
</tr>
<tr>
<td>Simazine</td>
<td></td>
</tr>
<tr>
<td>Atrazine-desethyl</td>
<td>Transformation product of atrazine</td>
</tr>
<tr>
<td>Carbamazepina</td>
<td>Anti-epileptic drug</td>
</tr>
<tr>
<td>10,11-dihydro, 10,11-dihydroxy-carbamazepine</td>
<td>Metabolite of carbamazepine</td>
</tr>
<tr>
<td>DEET</td>
<td>Insecticide</td>
</tr>
<tr>
<td>Simazine</td>
<td>herbicide</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
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</tr>
<tr>
<td>Trimethoprim</td>
<td>Antibiotic</td>
</tr>
</tbody>
</table>

4.3.2.1 UHPLC conditions

The experimental conditions for polar compounds UHPLC-MSMS analysis are reported in Table 5.
Table 5 UHPLC experimental conditions for polar compounds chromatographic separation

<table>
<thead>
<tr>
<th>Pumps:</th>
<th>Binary Solvent Manager, Model UPB, Waters (Milford, MA, USA).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autosampler:</td>
<td>Sample Manager, Model UPA, Waters (Milford, MA, USA).</td>
</tr>
<tr>
<td>Detector:</td>
<td>QTRAP 5500, Applied Biosystems MDS SCIEX, (Foster City, CA, U.S.A) equipped with Turbo V™ ion source.</td>
</tr>
<tr>
<td>Flow rate:</td>
<td>600 µL/min</td>
</tr>
<tr>
<td>Injection volume:</td>
<td>5 µL</td>
</tr>
<tr>
<td>Analytical column:</td>
<td>Acquity UPLC BEH C18, 1.7 µm, 150 x 2.1 mm(Waters) equipped with Acquity UPLC Column In-line filter</td>
</tr>
<tr>
<td>Mobile phase:</td>
<td>A: water: methanol 90:10 (%, v/v), 0.1% acetic acid</td>
</tr>
<tr>
<td></td>
<td>B: acetonitrile -methanol (50:50, % v/v), 0.1 % acetic acid</td>
</tr>
<tr>
<td>Reconstituting solution</td>
<td>A:B, 90:10, % v/v</td>
</tr>
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</table>

The chromatography was performed in gradient mode according to the scheme reported in the following Table 6.

Table 6 UHPLC gradient scheme.

<table>
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<tr>
<th>Time (min)</th>
<th>Mobile phase (A%)</th>
<th>Mobile Phase B (%)</th>
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<td>0</td>
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<tr>
<td>1</td>
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<tr>
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</tr>
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4.3.3.2 QTRAP 5500 MS/MS operative conditions

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 1.6.1

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
Ion Spray 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

Table 7 reports the specific operational parameters, tuned for each analysed compounds.

<table>
<thead>
<tr>
<th>Analyte ID</th>
<th>Internal standard</th>
<th>Q1</th>
<th>Q3</th>
<th>RT</th>
<th>DP</th>
<th>EP</th>
<th>CE</th>
<th>CXP</th>
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<td>104</td>
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<td>6.67</td>
<td>218</td>
<td>10</td>
<td>45</td>
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<tr>
<td>Simazine 13C3</td>
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<td>6.67</td>
<td>218</td>
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<td>Simazine 13C3</td>
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<td>205</td>
<td>134</td>
<td>6.67</td>
<td>218</td>
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<td>27</td>
<td>13</td>
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<tr>
<td>Analyte ID</td>
<td>Internal standard</td>
<td>Q1</td>
<td>Q3</td>
<td>RT</td>
<td>DP</td>
<td>EP</td>
<td>CE</td>
<td>CXP</td>
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<td>150</td>
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<td>22</td>
<td>13</td>
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<td>254</td>
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<td>150</td>
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<tr>
<td>Sulfamethoxazole $^{13}$C$_6$</td>
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<td>98</td>
<td>70</td>
<td>10</td>
<td>36</td>
<td>13</td>
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</tr>
<tr>
<td>Trimethoprim</td>
<td>Trimethoprim $^{13}$C$_3$</td>
<td>291</td>
<td>123</td>
<td>293</td>
<td>10</td>
<td>34</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Trimethoprim 1</td>
<td>Trimethoprim $^{13}$C$_3$</td>
<td>291</td>
<td>230</td>
<td>293</td>
<td>10</td>
<td>33</td>
<td>13</td>
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<tr>
<td>Trimethoprim $^{13}$C$_3$</td>
<td></td>
<td>294</td>
<td>126</td>
<td>221</td>
<td>10</td>
<td>33</td>
<td>13</td>
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</tr>
<tr>
<td>Trimethoprim $^{13}$C$_3$ 1</td>
<td></td>
<td>294</td>
<td>133</td>
<td>221</td>
<td>10</td>
<td>32</td>
<td>13</td>
<td></td>
</tr>
</tbody>
</table>


4.3. Quality Assurance and Quality Control

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.

Levels of analytical blanks and field blank obtained during the sample preparation were at least 10 times lower than the reported concentrations for all compounds studied. The blank level was not subtracted. The reported detection limits were calculated on the bases of a signal to noise ratio of 3/1.

4.4 Isochronous stability test: data treatment

The study of the degradation of the selected substances was first carried out for a total time of 10 weeks and then, a shorter interval of 4 weeks was also considered.

In the case of the 4 week stability only the first seven samples of Figure 1 were used for the computation. This shorter stability interval was mainly adopted on substances which showed a degradation over 10 weeks.

Equations used to evaluate the stability are based on a linear model and are described by (Lamberty, Schimmel, & Pauwels, 1998) and (Lisinger, van der Veen, Gawlik, Pauwels, & Lamberty, 2004). The main equations and parameter are:

slope of the linear model: $b$

standard error of the slope: $u_b = \frac{S_{x,y}}{\sqrt{\sum (x - \bar{x})^2}}$

where

$x = x$ (time) values of the measurement points

$\bar{x}$ = average over all $x$
\[
s_{x,y} = \sqrt{\frac{\sum (y - \hat{y})^2}{n-2}}
\]

with

\( y \) = measured concentration
\( \hat{y} \) = concentration from the regression line
\( n \) = total number of measurements

Using a t-test, the slope of the linear model is tested by comparing the ratio \( b/u_b \) to the t-value with a 95% level of confidence and \( n-2 \) degrees of freedom.

If the slope is statistically equal to zero, indicating absence of degradation, then the uncertainty of the stability after \( x \) weeks of storage (shelf life) can be computed.

Linsinger et al. [2, 4] express that under this condition, the uncertainty could be approximated by the product of the standard error of the slope (\( u_b \)) and the time value (\( x \)).

Alternatively, given a fixed uncertainty, the shelf life for the investigated analyte could be computed using the same formula.
5. Results

In this section, the isochronous short-term stability results are summarised. The stability for detected substances in grey water samples was computed for both a 10 week and a 4 week duration time.

Table 8 shows the t-test results for detected substances: when TRUE, the slope was significantly equal to zero and no degradation occurred; when FALSE the slope was significantly different from zero indicating instability of the analyte.

<table>
<thead>
<tr>
<th>Compound</th>
<th>t-test: 10 week</th>
<th>t-test: 4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-H-Benzotriazole</td>
<td>TRUE</td>
<td></td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>TRUE</td>
<td></td>
</tr>
<tr>
<td>CBZ_met</td>
<td>FALSE</td>
<td>TRUE</td>
</tr>
<tr>
<td>DEET</td>
<td>TRUE</td>
<td></td>
</tr>
<tr>
<td>Diclofenac</td>
<td>FALSE</td>
<td>TRUE</td>
</tr>
<tr>
<td>E1</td>
<td>TRUE</td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>FALSE</td>
<td>TRUE</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>TRUE</td>
<td></td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>TRUE</td>
<td></td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>TRUE</td>
<td></td>
</tr>
<tr>
<td>Naproxen</td>
<td>TRUE</td>
<td></td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>TRUE</td>
<td></td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>TRUE</td>
<td></td>
</tr>
<tr>
<td>TEP</td>
<td>FALSE</td>
<td>TRUE</td>
</tr>
<tr>
<td>TIBP</td>
<td>TRUE</td>
<td></td>
</tr>
<tr>
<td>TNBP</td>
<td>TRUE</td>
<td></td>
</tr>
<tr>
<td>TCEP</td>
<td>TRUE</td>
<td></td>
</tr>
<tr>
<td>TCPP</td>
<td>FALSE</td>
<td>TRUE</td>
</tr>
<tr>
<td>TDCPP</td>
<td>FALSE</td>
<td>TRUE</td>
</tr>
<tr>
<td>TBOEP</td>
<td>FALSE</td>
<td>TRUE</td>
</tr>
<tr>
<td>TPhP</td>
<td>FALSE</td>
<td>TRUE</td>
</tr>
<tr>
<td>EHDP</td>
<td>FALSE</td>
<td>TRUE</td>
</tr>
<tr>
<td>TMPP</td>
<td>FALSE</td>
<td>TRUE</td>
</tr>
</tbody>
</table>

In Table 9 the results of the stability study in terms of uncertainty and shelf life are summarised:

- The graphs shows the uncertainties of the stability at 10 weeks (black dotted lines), when no degradation is observed, and at 4 weeks (blue dotted lines). The solid dots represent the samples used for the 4 weeks stability study, while both solid and empty dots are used for the computation of the 10 weeks stability.
- The first column express the results for the 10 week study. The first line indicates the computed uncertainty at 10 weeks shelf life and the second line is the computed life time at a chosen 10% of uncertainty.

- The second column express the results for the 4 week study. The first line indicates the computed uncertainty at 4 weeks shelf life and the second line is the computed life time at a chosen 10% of uncertainty.

For 1H-benzotriazole, the uncertainty resulted to be 18% and 12% at 10 and 4 weeks shelf-life, respectively, while its transformation product, 5-methyl-1H-benzotriazole, was not detected in the samples.

For fibrates, the calculated uncertainty was 21% and 11% for the gemfibrozil while bezafibrate was not detected in grey water samples.

For NSAIDs, the calculated uncertainty was 18, 21 and 22% for ibuprofen, ketoprofen and naproxen, respectively, at 10 weeks shelf-life and 7%, 11% and 12% at 4 weeks shelf-life.Diclofenac showed instability at 10 weeks and an uncertainty at 4 weeks shelf-life of 5%.

The uncertainty of the stability of the anti-epileptic drug carbamazepine resulted to be 21% at 10 weeks shelf-life and 11% at 4 weeks shelf-life. Its more active metabolite, 10,11-dihydro-10,11-dihydroxy carbamazepine, resulted to be unstable at 10 weeks shelf-life and to have an uncertainty of 6% at 4 weeks shelf-life.

Among the monitored estrogens, estrone showed an uncertainty of the stability of 56% and 35%, respectively at 10 and 4 weeks shelf-life, 17β-estradiol resulted to be unstable at 10 weeks and an uncertainty of 7% was calculated at 4 weeks shelf-life. 17α-ethinyl estradiol was not detected in the samples.

For pesticides, the uncertainty calculated for the insecticide DEET was 18% and 7%, respectively at 10 and 4 weeks shelf-life; atrazine, desethyl-atrazine and simazine were not detected in the samples.

For antibiotic drugs, the uncertainties of stability were 19% and 25% for sulfamethoxazole and trimethoprim, respectively, at 10 weeks shelf-life and were 5.5% and 14% at 4 weeks shelf-life.

For OPcs, uncertainty at 10 weeks shelf-life was evaluated only in the case of TIBP, TNBP and TCEP where it was evaluated to be 27%, 28% and 29%, respectively.

At 4 weeks shelf-life, the uncertainty of stability was 5.5% for TEP, 29% for TIBP, 34% for TNBP, 31% for CEP, 11% for TCP, 5.9% for TDCPP, 6.6% for TPhP, 9.8% for EHDP and 4.3% for TMPP.

The same data were used to evaluate the shelf life at 10% uncertainty, both over 10 and 4 weeks storage at 20°C.

For 1H-benzotriazole, the shelf-life resulted to be 6 weeks when evaluated over 10 weeks and 3 weeks when evaluated over 4 weeks.

For fibrate gemfibrozil, the shelf-lives were 5 and 4 weeks respectively over 10 and 4 weeks.

For NSAIDs, the shelf lives were 6 weeks for ibuprofen in both case study, 5 and 4 weeks for ketoprofen and 5 and 3 weeks for naproxen.

For carbamazepine the shelf life was 5 and 4 weeks in the investigated time and storage conditions, while for 10,11-dihydro-10,11-dihydroxy carbamazepine, it was 7 weeks in the 4 weeks study.

For estrone a shelf life of 2 weeks resulted in the 10 weeks study and of 1 week in the 4 weeks study; for 17β-estradiol, which resulted unstable over 10 weeks, a shelf-life of 6 weeks resulted in the 4 weeks study.

For DEET the shelf-life of 6 weeks resulted in both 10 and 4 weeks evaluation.
For sulfamethoxazole, the shelf-lives resulted to be 5 and 7 weeks, respectively in 10 and 4 weeks studies and for trimethoprim it resulted in 4 and 3 weeks, respectively.

Shelf life at 10% uncertainty for OPCs was the following; 7 weeks for TEP, 1 week for TIBP, 1 week for TNBP, TCEP and TCPP, 7 weeks for TDCPP, 3 weeks for TBOEP, 6 weeks for TPhP, 4 weeks for EHDP and 9 weeks for TMPP. The results are graphically represented in Table 9.

Table 9 Uncertainty of the stability and shelf life at 10 weeks and 4 weeks. Solid dots represent the samples used for the 4 weeks stability study

<table>
<thead>
<tr>
<th>Compound</th>
<th>10 weeks</th>
<th>4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-H-Benzotriazole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncertainty at 10 weeks shelf life:</td>
<td>18%</td>
<td>12%</td>
</tr>
<tr>
<td>Shelf life at 10% uncertainty:</td>
<td>6 weeks</td>
<td>3 weeks</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncertainty at 10 weeks shelf life:</td>
<td>21%</td>
<td>11%</td>
</tr>
<tr>
<td>Shelf life at 10% uncertainty:</td>
<td>5 weeks</td>
<td>4 weeks</td>
</tr>
<tr>
<td>CBZ_met</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncertainty at 4 weeks shelf life:</td>
<td>6,0%</td>
<td></td>
</tr>
<tr>
<td>Shelf life at 10% uncertainty:</td>
<td>7 weeks</td>
<td></td>
</tr>
<tr>
<td>DEET</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncertainty at 10 weeks shelf life:</td>
<td>18%</td>
<td></td>
</tr>
<tr>
<td>Shelf life at 10% uncertainty:</td>
<td>6 weeks</td>
<td></td>
</tr>
<tr>
<td>CBZ_met</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncertainty at 4 weeks shelf life:</td>
<td>6,7%</td>
<td></td>
</tr>
<tr>
<td>Shelf life at 10% uncertainty:</td>
<td>6 weeks</td>
<td></td>
</tr>
<tr>
<td>Compound</td>
<td>10 weeks</td>
<td>4 weeks</td>
</tr>
<tr>
<td>------------</td>
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<td>---------</td>
</tr>
<tr>
<td>Diclofenac</td>
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<td></td>
<td>Uncertainty at 4 weeks shelf life: 4,7%</td>
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<td>Shelf life at 10% uncertainty: 8 weeks</td>
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<tr>
<td>E1</td>
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<td></td>
<td>Uncertainty at 10 weeks shelf life: 56%</td>
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<tr>
<td></td>
<td>Uncertainty at 4 weeks shelf life: 35%</td>
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<tr>
<td></td>
<td>Shelf life at 10% uncertainty: 2 weeks</td>
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<td></td>
<td>Shelf life at 10% uncertainty: 1 week</td>
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</tr>
<tr>
<td>E2</td>
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</tr>
<tr>
<td></td>
<td>Uncertainty at 10 weeks shelf life: 6,9%</td>
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</tr>
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<td></td>
<td>Shelf life at 10% uncertainty: 6 weeks</td>
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<tr>
<td>Gemfibrozil</td>
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<td></td>
<td>Uncertainty at 10 weeks shelf life: 21%</td>
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</tr>
<tr>
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<td>Uncertainty at 4 weeks shelf life: 11%</td>
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<td></td>
<td>Shelf life at 10% uncertainty: 4 weeks</td>
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</tr>
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<td>Ibuprofen</td>
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<td>Uncertainty at 10 weeks shelf life: 18%</td>
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</tr>
<tr>
<td></td>
<td>Uncertainty at 4 weeks shelf life: 6,9%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shelf life at 10% uncertainty: 6 weeks</td>
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<tr>
<td></td>
<td>Shelf life at 10% uncertainty: 6 weeks</td>
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</tr>
<tr>
<td>Compound</td>
<td>10 weeks shelf life:</td>
<td>4 weeks shelf life:</td>
</tr>
<tr>
<td>------------------</td>
<td>----------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>Uncertainty: 21%</td>
<td>Uncertainty: 11%</td>
</tr>
<tr>
<td>Naproxen</td>
<td>Uncertainty: 22%</td>
<td>Uncertainty: 12%</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>Uncertainty: 19%</td>
<td>Uncertainty: 5,5%</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>Uncertainty: 25%</td>
<td>Uncertainty: 14%</td>
</tr>
<tr>
<td>TEP</td>
<td>Uncertainty: 5,5%</td>
<td>Shelf life: 7 weeks</td>
</tr>
</tbody>
</table>

Shelf life at 10% uncertainty:
- Ketoprofen: 5 weeks
- Naproxen: 5 weeks
- Sulfamethoxazole: 5 weeks
- Trimethoprim: 4 weeks
- TEP: 7 weeks
<table>
<thead>
<tr>
<th>Compound</th>
<th>10 weeks</th>
<th>4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uncertainty at 10 weeks shelf life: <strong>27%</strong></td>
<td>Uncertainty at 4 weeks shelf life: <strong>29%</strong></td>
</tr>
<tr>
<td></td>
<td>Shelf life at 10% uncertainty: <strong>4 weeks</strong></td>
<td>Shelf life at 10% uncertainty: <strong>1 weeks</strong></td>
</tr>
<tr>
<td>TIBP</td>
<td>Uncertainty at 10 weeks shelf life: <strong>28%</strong></td>
<td>Uncertainty at 4 weeks shelf life: <strong>34%</strong></td>
</tr>
<tr>
<td></td>
<td>Shelf life at 10% uncertainty: <strong>4 weeks</strong></td>
<td>Shelf life at 10% uncertainty: <strong>1 weeks</strong></td>
</tr>
<tr>
<td>TNBP</td>
<td>Uncertainty at 10 weeks shelf life: <strong>29%</strong></td>
<td>Uncertainty at 4 weeks shelf life: <strong>31%</strong></td>
</tr>
<tr>
<td></td>
<td>Shelf life at 10% uncertainty: <strong>3 weeks</strong></td>
<td>Shelf life at 10% uncertainty: <strong>1 weeks</strong></td>
</tr>
<tr>
<td>TCEP</td>
<td>Uncertainty at 4 weeks shelf life: <strong>11%</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shelf life at 10% uncertainty: <strong>1 weeks</strong></td>
<td></td>
</tr>
<tr>
<td>TCPP</td>
<td>Uncertainty at 4 weeks shelf life: <strong>5.9%</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shelf life at 10% uncertainty: <strong>7 weeks</strong></td>
<td></td>
</tr>
<tr>
<td>Compound</td>
<td>10 weeks</td>
<td>4 weeks</td>
</tr>
<tr>
<td>----------</td>
<td>----------</td>
<td>---------</td>
</tr>
<tr>
<td><strong>TBOEP</strong></td>
<td>Uncertainty at 4 weeks shelf life: 14%</td>
<td>Shelf life at 10% uncertainty: 3 weeks</td>
</tr>
<tr>
<td><strong>TPhP</strong></td>
<td>Uncertainty at 4 weeks shelf life: 6,6%</td>
<td>Shelf life at 10% uncertainty: 6 weeks</td>
</tr>
<tr>
<td><strong>EHDP</strong></td>
<td>Uncertainty at 4 weeks shelf life: 9,8%</td>
<td>Shelf life at 10% uncertainty: 4 weeks</td>
</tr>
<tr>
<td><strong>TMPP</strong></td>
<td>Uncertainty at 4 weeks shelf life: 4,3%</td>
<td>Shelf life at 10% uncertainty: 9 weeks</td>
</tr>
</tbody>
</table>
6. Conclusion

The isochronous stability study approach was, for the first time, applied to an environmental dataset obtained on greywater samples stabilised on a polymeric phase by the mean of the JRC in-house developed sampling device. The results obtained proofed that the device can be used for a multi-residual sampling strategy for CECs resulting in enhanced sample stability with increased analytical performances.

More precisely, the statistical evaluation of collected dataset allowed the identification of the following trends:

- For the stability at 10 weeks the computed uncertainty of stability ranges between 18% and 56% while for the 4 weeks stability the uncertainty varies in the range 5.5 – 35%.
- Generally, the substances which are found to be stable at 20°C for 10 weeks show a higher uncertainty of the stability at 10 weeks than the uncertainty at 4 weeks, except for the compounds TIBP, TNBP and TCEP. This could be due to a lower concentration of the analyte measured at 2 weeks compared to the following weeks. These substances also show a low shelf life at 10% uncertainty compared to the other investigated substances.
- Other analytes with a low computed shelf life at 10% uncertainty are E1 and TCPP.
- 1H-Benzotriazole, carbamazepine, DEET, estrone, gemfibrozil, ibuprofen, ketoprofen, naproxen, sulfamethoxole, trimethoprim, TIBP, TNBP and TCEP resulted to be stable over 10 weeks, when stored at 20°C.
- For 10, 11-dihydro, 10, 11-dihydroxy-carbamazepine, diclofenac, 17β-estradiol, TEP, TCPP, TDCPP, TBOEP, TPhP, EHDP and TMPP a statistically significant decrease of the concentration over time was measured indicating instability in the storage conditions.
- When stability was computed for a period of 4 weeks, all the detected analytes are significantly stable when stored at 20°C.
7. References


### 7. 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:

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAD</td>
<td>Collision Gas</td>
</tr>
<tr>
<td>CECs</td>
<td>Contaminants of Emerging concern</td>
</tr>
<tr>
<td>CUR</td>
<td>Curtain Gas</td>
</tr>
<tr>
<td>CRM</td>
<td>Certified reference material</td>
</tr>
<tr>
<td>CXP</td>
<td>Collision Cell Exit Potential</td>
</tr>
<tr>
<td>DG</td>
<td>Directorate-General</td>
</tr>
<tr>
<td>E1</td>
<td>Estrone</td>
</tr>
<tr>
<td>E2</td>
<td>$17\beta$-estradiol</td>
</tr>
<tr>
<td>EE2</td>
<td>$17\alpha$-ethinyl estradiol</td>
</tr>
<tr>
<td>EC</td>
<td>European Commission</td>
</tr>
<tr>
<td>EDC</td>
<td>Endocrine disrupting chemical</td>
</tr>
<tr>
<td>EI</td>
<td>Electron Impact</td>
</tr>
<tr>
<td>EP</td>
<td>Entrance Potential</td>
</tr>
<tr>
<td>EU</td>
<td>European Union</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>GS1</td>
<td>Ion Source gas 1</td>
</tr>
<tr>
<td>GS2</td>
<td>Ion Source gas 2</td>
</tr>
<tr>
<td>HLB</td>
<td>Hydrophilic-lipophilic balanced copolymer</td>
</tr>
<tr>
<td>HRGC-HRMS</td>
<td>High resolution gas chromatography-high resolution mass spectrometry</td>
</tr>
<tr>
<td>IES</td>
<td>Institute for Environment and Sustainability</td>
</tr>
<tr>
<td>IS</td>
<td>Internal standard/Ion Transfer</td>
</tr>
<tr>
<td>JRC</td>
<td>Joint Research Centre</td>
</tr>
<tr>
<td>LC</td>
<td>Liquid chromatography</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>LOQ</td>
<td>Limit of quantification</td>
</tr>
<tr>
<td>MRM</td>
<td>Multiple reaction monitoring</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>NSAID</td>
<td>Nonsteroidal anti-inflammatory drug</td>
</tr>
<tr>
<td>OPC</td>
<td>Organophosphorus compounds</td>
</tr>
<tr>
<td>PPG</td>
<td>Polypropylene glycol</td>
</tr>
<tr>
<td>PS</td>
<td>Priority substances</td>
</tr>
<tr>
<td>QC</td>
<td>Quality control sample</td>
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<tr>
<td>R2</td>
<td>Coefficient of determination</td>
</tr>
<tr>
<td>RSD</td>
<td>Relative standard deviation</td>
</tr>
<tr>
<td>RT</td>
<td>Room temperature / retention time</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>S/N</td>
<td>Signal to Noise</td>
</tr>
<tr>
<td>SPE</td>
<td>Solid-phase extraction</td>
</tr>
<tr>
<td>TEM</td>
<td>Temperature</td>
</tr>
<tr>
<td>UHPLC</td>
<td>Ultra-high-pressure liquid chromatography</td>
</tr>
<tr>
<td>WFD</td>
<td>Water Framework Directive</td>
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</tbody>
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