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Abstract

The JRC organised in March 2018 a technical workshop on the analysis of the existing watch list (WL) substances for which the analysis is difficult and the three new WL substances of the Water Framework Directive (WFD) in order to share experience and knowledge on analytical methods and to identify the obstacles in developing methods that meet the required environmental quality standards (EQS) or predicted no effect concentrations (PNEC). The results from the first year of monitoring the substances of the 1st WL showed that some countries have found it very difficult to reach a satisfactory analytical limit of quantification (LOQ) for five out of the 17 substances (17-alpha-ethinylestradiol (EE2), 17-beta-estradiol (E2), azithromycin, imidacloprid, and methiocarb).

The most difficult WL substance to analyse is EE2 with its very low EQS value of 0.035 ng/L. Five countries have reported for EE2 LOQs ≤ EQS, and other four countries a close LOQ (0.05 - 0.1 ng/L). However, the workshop participants (experts from Member States and Island, Switzerland and Turkey) stressed that the analysis of EE2 at ultra-trace levels is complicated and work intensive because a good clean-up procedure and a very clean and sensitive LC-MS/MS instrument are obligatory.

The analytical methods for the three new WL substances amoxicillin, ciprofloxacin, and metaflumizone were discussed at the workshop and optimized methods for the water sample preservation and analysis for them, based on solid-phase extraction (SPE) followed by liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS) analysis, are presented in this report.

The workshop showed that many Member States (MS) are using today direct LC-MS/MS injection techniques. Direct LC-MS/MS injection should however only be performed for water soluble substances with low K_{OW} and $K_{d(sed)}$ values for which partitioning to suspended particulate matter (SPM) can be neglected. A literature study revealed that EE2 and E2 are strongly adsorbed to particulate matter and therefore have to be analysed in whole water samples. The adsorption properties of ciprofloxacin and amoxicillin are not totally clear, and therefore these antibiotics should preferably be analysed in whole water, or their negligible adsorption should be proven. An experimental analysis should be performed to confirm the comparability of the analysis of the dissolved water phase by direct LC-MS/MS injection and whole water analysis.

Finally, some MS asked to specify in detail the different methods for the determination or calculation of the Limit of Quantification (LOQ) including method validation, because the available general guidelines are not sufficient for a uniform approach. It remained unclear whether the LOQs of the presented methods are derived from appropriate real surface water samples with or without standard addition, or from calibration curves. For a better harmonisation of the methods, a consensus on the determination of the LOQ is necessary, which needs a thorough discussion on the minimum requirements for sample preparation and analysis, i.e. representative surface water matrix, standard addition, recovery, reproducibility, uncertainty, and multiplication factor.

1 Introduction

The Watch List (WL) under the Water Framework Directive (WFD) is a mechanism for obtaining high-quality Union-wide monitoring data on potential water pollutants for which insufficient monitoring data (or data of insufficient quality) are available. The main purpose of the WL is to determine the risk which the substances pose to the aquatic environment at EU level and to decide if Environmental Quality Standards (EQS) should be set for them (EU, 2008; 2013). Therefore, high quality measurements are expected from the Member States (MS) meaning that the monitoring methods shall be validated and reach the stated method detection (or quantification) limits for whole water measurements.

The first WL was established by Commission Decision 2015/495 (EU, 2015) and contained 10 (groups of) substances. The results from the first year of monitoring the substances of the 1st WL (Loos et al., 2018) show that some countries have found it very difficult to reach an analytical Limit of Quantification (LOQ) below the Predicted No-Effect Concentrations (PNECs) for five out of the 17 substances (17-alpha-ethinylestradiol (EE2), 17-beta-estradiol (E2), azithromycin, imidacloprid, and methiocarb (in case of azithromycin and methiocarb for the updated lower PNECs).

In accordance with the EQS Directive (EU, 2008; 2013), the WL is being revised, and three new substances have been included in the second WL: amoxicillin, ciprofloxacin and metaflumizone (EU, 2018; Loos et al., 2018).

The JRC organised on 1-2 March 2018 a technical workshop in order to support the MS and their activities under the WFD. MS experts came together to share their experience and knowledge on analytical methods for the existing problematic and new WL substances, to identify the obstacles in developing methods that meet (achieve) the required PNECs or EQSs, and to collaborate in method development by sharing technical details and information on method validation. In addition, the workshop facilitated MS experts to openly discuss and exchange opinions about good analytical practices through which actions for improvement by learning from each other were proposed. Laboratory experts discussed the possibility to facilitate the analysis of the WL substances for other MS.

Before the workshop, the JRC distributed a questionnaire to the participating laboratories in order to know the analytical methods applied and the LOQs achieved for these substances. The answers to the questionnaire are summarized in the Annex.

The workshop was attended by 35 experts from 21 countries (see participants list in the Annex).

2 Whole water analysis versus dissolved phase

According to the EQS Directive (EU, 2008; 2013), "whole water" analysis, including the particle bound fraction, should be performed for organic substances.

The workshop showed that many MS are using today direct LC-MS/MS injection techniques (Annex 3-10). Modern LC-MS/MS instruments can achieve with direct injection LOQs in the low ng/L range (Boix et al., 2015; Denadai and Bezerra Cass, 2015). Direct injection however can only give the dissolved water concentration of a chemical, because the samples are usually filtered before (or the particles are settled by precipitation).

Participants of the workshop raised the question of whether the three new substances were likely to bind to particles, and pointed out that if that was not the case, the analysis of the dissolved fraction (by direct injection techniques) would give the same result as the analysis of "whole water", while being easier to perform. Direct LC-MS/MS injection should only be performed for water soluble substances with low K_{OW} and $K_{d(sed)}$ values for which partitioning to suspended particulate matter (SPM) can be neglected.

In the absence of quantitative evidence comparing the concentration in dissolved fraction and in whole water, for different concentrations of SPM, the analysis of these three substances in dissolved fraction cannot be proven to be equivalent to the analysis of whole water. The recommended extraction and analytical methods therefore concern analysis in whole water.

3 New watch list substances

Amoxicillin (β-lactam antibiotic) and ciprofloxacin (fluoroquinolone antibiotic) are water soluble substances (3430 and 30 mg/L, respectively) with low log K_{OW} values (0.87 and 0.28, respectively) (Braschi, et al., 2013; Moarefian et al., 2014). Although several studies have reported the presence of both substances in wastewater effluent and surface water (Annex 11 and 12), their half-lives in surface water are relatively short due to rapid bio- and photodegradation (Amorim et al., 2014; Elizalde-Velázquez, 2016; Sturini et al., 2012). Reported half-lives in surface water are between 10 days (Van Doorslaer et al., 2014) and 2 h (Cardoza et al., 2005; Lam et al., 2003) for ciprofloxacin and the hydrolysis half-life of amoxicillin in water at pH 7 ca. 20 days (Braschi, et al., 2013). Amoxicillin is rapidly degraded in water by biotic and abiotic factors, yielding different intermediate products (Elizalde-Velázquez, 2016).

Ciprofloxacin tends to adsorb to particles with a log K_{oc} value of 4.8 l/kg for soil (Nowara et al., 1997) and log K_{oc} (or K_d) value of 4.3–4.9 l/kg (dependent on pH) for fine particulate matter (Belden et al., 2007; Cardoza et al., 2005). Thus, both adsorption and photo-degradation strongly influence ciprofloxacin fate in aquatic systems, although the dominant mechanism appears to depend upon the ambient SPM level (Cardoza et al., 2005; Speltini et al., 2011).

Metaflumizone is a hydrophobic substance with a water solubility of 0.00179 mg/L, log K_{OW} 4.2-4.9, and K_{oc} 30714 L/kg (Loos et al., 2018).

3.1 Analysis of amoxicillin and ciprofloxacin

The PNECs of amoxicillin and ciprofloxacin are 0.078 µg/L and 0.089 µg/L, respectively (Loos et al., 2018).

3.1.1. Methods presented by MS

Amoxicillin:

Analytical methods for amoxicillin were presented in the questionnaire by six countries (PT, AT, NL, DE, IT, CZ). All laboratories use LC-MS/MS analysis, two countries use SPE and four direct injection. In all the cases, with the exception of CZ, the LOQ is below the PNEC (Annex 8).

Austria (AT) uses both SPE and direct injection; they are able to reach an LOQ of 10 ng/L with however not very good (but acceptable) recovery using SPE.

Ciprofloxacin:

Analytical methods (LC-MS/MS) were presented by seven countries (HR, PT, AT, DE, SE, CZ, NL), two SPE and five direct injection methods. The LOQ is below the PNEC in all but one country (Annex 9).

In Portugal (PT), ciprofloxacin is analysed by direct injection or using SPE with disks (in whole water). The SPE disks allow to analyse both the substance present in SPM and in the dissolved fraction. PT reported that ciprofloxacin sources are related to human impact but also livestock because it is a degradation product of enrofloxacin, which is used for animal farming (Babic et al., 2013).

Sweden (SE) reported to have analytical problems with the deuterated ciprofloxacin internal standard.

For ciprofloxacin the conclusion from the workshop was that direct injection is, due to adsorption to sediment and SPM, probably not the appropriate analytical method and that

the samples should be analysed within 48h (or preserved properly). The use of SPE disks allow to perform the analysis of the whole water, as required by the legislation.

The partitioning behaviour to SPM, investigated after the workshop, is reported in section 5.

3.1.2. Recommended water sample preservation for amoxicillin and ciprofloxacin

Due to the fast degradation (or removal) of amoxicillin and ciprofloxacin in water, the samples should be analysed as fast as possible, preferably within one week. The water samples can be preserved in the laboratory in the freezer at -20°C (use of glass or HDPE bottles), but the stability and recovery should be tested for amoxicillin and ciprofloxacin (Llorca et al., 2014).

The water samples can also be stored (conserved) after SPE extraction by freezing the SPE cartridges or disks (Llorca et al., 2014). Another conservation method is the addition of ascorbic acid and sodium azide and storage in the fridge at 4°C (Vanderford et al., 2011).

US EPA (2010) recommends the water sample preservation (for macrolide antibiotics, quinoline antibiotics, beta-lactam antibiotics, sulfonamide antibiotics, tetracycline antibiotics, synthetic hormones and other pharmaceuticals and personal care products, PPCPs) with ascorbic acid (50 mg/L; dechlorinating agent) in amber high density polyethylene (HDPE) or glass containers, shipping and storage of samples in the dark at < 6°C. Then the extraction of samples should be done within seven days and analysis of the extracts as soon as possible (not to exceed 30 days). US EPA has shown the stability of ciprofloxacin under these conditions (preserved with ascorbic acid and stored at 4°C in HDPE bottles) for at least 14 days in WWTP effluent samples.

3.1.3. Proposed analytical method for amoxicillin and ciprofloxacin

Analytical methods, in all cases based on SPE-LC-MS/MS, for the analysis of these antibiotics in water samples have been described in the literature. Kazprzyk-Horden et al. (2007) developed a multi-residue analytical method for the determination of 28 basic/neutral pharmaceuticals (including amoxicillin and ciprofloxacin) in surface water and Gros et al. (2013) developed a fast and robust analytical method for the determination of 53 antibiotic residues (including amoxicillin and ciprofloxacin) in environmental water samples.

Mirzaei et al. (2017) optimized a multi-residue method to simultaneously analyse different classes of antibiotics, including β-lactam (amoxicillin and penicillin G), cephalosporin (ceftriaxone, cefixime, and cephalexin), macrolides (azithromycin and erythromycin), fluoroquinolone (ciprofloxacin) and nitro-imidazole (metronidazole) in water. The pH, the amount of Na_4EDTA and the volume of elution solvent were simultaneously optimised.

Preparation of analytical standards

Note that amoxicillin has to be dissolved in Milli-Q water because it is not stable in methanol (Gros et al., 2013; Kantiani et al., 2009; Mirzaei et al.; 2017). This stability problem of amoxicillin in methanol stock solution was noted as well before the workshop by experts from Belgium and was confirmed by some participants of the workshop (CZ; IT).

Ciprofloxacin can be dissolved in methanol. The addition of 100 µL NaOH (1 M) in 10 mL solution improves the solubility of ciprofloxacin (Gros et al., 2013; Ibanez et al., 2009). Mirzaei et al. (2017) dissolved fluoroquinolone antibiotics including ciprofloxacin in MeOH/HPLC water (50:50, v/v) with 0.2% hydrochloric acid.

The stock solutions have to be stored in the freezer and working standards (water for amoxicillin) should be prepared freshly (Gros et al., 2013; Kantiani et al., 2009; Mirzaei et al.; 2017). Tlili et al. (2016) report that drug residue standards could not be preserved for more than one month at –20°C in the freezer.

The water samples should not be filtered for whole water analysis. A sample volume of 100-200 mL should be sufficient but can be adjusted by finding a compromise between the need of sensitivity and method robustness.

SPE extraction procedure

- Addition of internal standards (deuterated or 13C), best directly after sampling and before storage of the samples.
- Addition of Na_2EDTA solution (0.1 M, final concentration of 0.1%); this improves the extraction efficiency for many antibiotics by lowering their complexation with metals or multivalent cations (Castiglioni et al., 2005; Gros et al., 2009; 2012; 2013; Kazprzyk-Horden et al., 2007; Mirzaei et al.; 2017).
- Adjustment of sample pH to ca. 3.0 with hydrochloric acid (HCl). For penicillins (amoxicillin) it is recommended to acidify the samples just before the extraction to avoid analyte losses due to degradation (Gros et al., 2013; Kazprzyk-Horden et al., 2007; Mirzaei et al.; 2017).
- Use of Oasis HLB cartridges (60 mg, 3 mL) or similar, or SPE disks.
- Conditioning of the cartridges / disks with methanol and water (pH 2-3); use of pH buffer such as formic acid.
- Extraction flow rate: ca. 5 ml/min.
- Elution with methanol; for amoxicillin maybe the recovery could be improved by using another solvent such as acetonitrile or ethylacetate, but this should be checked.
- Evaporation to dryness and reconstitution in the HPLC solvent.

LC-MS/MS analysis

Chromatographic solvents recommended: acetonitrile and water; it was noted by CZ that methanol is not suitable for the analysis of amoxicillin, and also DE confirmed that chromatography was better with acetonitrile.

Table 1: Possible MRM LC-MS/MS transitions.

m/z	**Amoxicillin**	**Ciprofloxacin**
MRM 1	366 > 349	332 > 288
MRM 2	366 > 114	332 > 231
MRM 3	366 > 160	332 > 245
MRM 4	366 > 208	

The ion ratios of two transitions should be used for compound identification (comparison with the analytical standard).

Table 2: SPE recoveries (%).

	Kazprzyk-Horden et al., 2007	Gros et al., 2013	Mirzaei et al., 2017
Amoxicillin	41-57	20-40	95
Ciprofloxacin	n.a.	55	85

The validation of the method including determination of LOD/Q has to be checked with a representative whole water sample to be sure that suspended matter and matrix effect are taken into account.

3.2 Analysis of metaflumizone

Metaflumizone has a PNEC value of 0.0654 µg/L (Loos et al., 2018).

The water sample preservation for metaflumizone is identical to that of amoxicillin and ciprofloxacin.

3.2.1. Analytical methods presented on the workshop

Analytical LC-MS/MS methods for metaflumizone were presented in the questionnaire by three countries (PT, AT, IT). However, only one country used SPE and two direct injection. The LOQ is below the PNEC in all cases. Metaflumizone is however a hydrophobic substance and therefore should not be analysed by direct injection.

3.2.2. Analytical method for metaflumizone

No scientific publication could be found for the analysis of metaflumizone in environmental samples. EFSA (2013) reports that metaflumizone E- and Z-isomer can be monitored in drinking water and surface water by LC-MS/MS (no extraction method given; LOQ 0.025 µg/L). BASF (2003) applied LLE of 50 mL tap- and surface water with dichloromethane followed by LC-MS/MS analysis (LOQ 0.05 µg/L). Slovenia (SI) applies a modified EN ISO 11369 method (1997) (SPE-LC-UV detection; probably the UV detection was modified to MS; LOQ 0.01 µg/L). England reported a SPE-Ultra-High-Definition (UHD) Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) MS method with an LOQ of 0.005 µg/L (Loos et al., 2018).

In summary, metaflumizone should be extracted from whole water samples by liquid-liquid extraction (LLE) or SPE disk or cartridge, followed by LC-MS/MS analysis (MRM transitions: 507 > 178 and 507 > 287 (m/z) (Loos et al., 2018).

The validation of the (extraction) method has to be checked with a representative whole water sample to be sure that suspended matter and matrix effect are taken into account.

4 Existing watch list substances

The results from the first year WL data show that some countries have had some difficulties to reach an analytical LOQ below the PNEC for five substances, i.e. EE2, E2, azithromycin, imidacloprid, and methiocarb (in case of azithromycin and methiocarb for the updated lower PNECs). The workshop was also dedicated to these substances and the aim was to share the experiences among the experts for the analytical approaches and in case of difficulties how to overcome them to improve the methodology.

The analytical methods had been summarized by the JRC in 2015 (Loos, 2015) and validated in 2016 (Tavazzi et al., 2016).

4.1 Analysis of 17-alpha-ethinylestradiol (EE2)

The EQS value of EE2 is 0.000035 µg/L = 0.035 ng/L.

The questionnaire results on EE2 show that not all MS that can measure the substance are able to reach the PNEC (CZ, AT, FR, NL, SK, PT, LT, DE, DK). Five countries (HR, SE, DE, IT, LV) have reported LOQs ≤ EQS (0.035 ng/L). CZ has achieved an LOQ of 0.05 ng/L, and AT, FR, and NL 0.1 ng/L.

Many different analytical strategies, including sample extraction, clean-up, and determination are applied in the countries. Analytical principles for EE2 are mainly based on SPE followed by LC-MS/MS, but also LLE and GC-MS/MS (after derivatisation) are used.

For example, Sweden (SE) uses a large volume (2L) SPE method while Italy (IT) uses a double SPE (off line SPE followed by on-line SPE-LC-MS/MS), and France (FR) a dansylation derivatisation SPE disk LC-MS/MS method.

Many experts (e.g. from AT, CZ, DE, SE) reported that a good clean-up procedure (silica, florisil, aminopropyl, or gel permeation chromatography, GPC) and a very clean and sensitive LC-MS/MS instrument are essential for the analysis of EE2 at very low concentration levels. In fact, Portugal (PT) mentioned that they were not able to reach the EQS value because of problems with the matrix effect of surface water samples, and that they are trying to solve this problem with a clean-up procedure. It was also reported (by AT and SE) that in order to reduce this matrix effect it is better to inject a very small volume of sample extract (AT: 2 µL; SE: 10 µL). In addition, it was reported by CZ that the less intensive, but more specific MS/MS transition (295.2 > 267.0) is better for real water samples because of the matrix effect. No agreement on the use of ammonia in the HPLC eluent (used by AT and CZ) could be made, because SE has found a better ionization without ammonia.

Croatia (HR) achieved the lowest LOQ (0.012 ng/L) of all countries by using a new direct injection method with an injection volume of 100 µL and a new LC-MS/MS instrument. Direct injection however should not generally be used for the analysis of EE2 in "whole water" samples because it is known that EE2 can adsorb to particles (log K_{OW} 4.2) (Andrasi et al., 2013; Nie et al., 2015; Yarahmadi et al., 2018). HR mentioned that ammonium fluoride is important for the mobile phase, but their method is not published yet.

The water samples for the analysis of estradiol hormones can be preserved with ascorbic acid and sodium azide and stored in the fridge at 4°C (reported by NL and in Vanderford et al. (2011); US EPA (2010)) or in the freezer at -20°C.

4.2 Analysis of 17-beta-estradiol (E2)

The EQS value of E2 is 0.0004 µg/L = 0.4 ng/L.

E2 is measured in 15 MS and most of them are able to reach the EQS. Since E2 and EE2 are analysed together with the same analytical method, the reader should refer to the

section above on EE2. Since the EQS of E2 (0.4 ng/L) is higher than for EE2, more MS are able to achieve an LOQ ≤ EQS (HR, DE, SE, AT, IT, NL, LV, LT, CZ, SK, FR, PT) (Annex 4).

The importance to analyse the samples as soon as possible after sampling was stressed by several experts, best within 24 h (DE, HR, FR). France (FR) has observed for E2 in surface water a loss of 20 % in 24 h.

4.3 Analysis of azithromycin

The updated PNEC value of azithromycin is 0.019 µg/L.

Azithromycin is measured in 15 MS, and most analytical methods are based on SPE followed by LC-MS/MS, but also direct injection techniques are used. SE, NL, PT, HR, LT, AT, and CZ have reported LOQs < PNEC (0.019 µg/L). Other MS have reported higher LOQs because the PNEC has been updated from 0.09 to 0.019 µg/L during the WL exercise.

Germany (DE) stressed during the workshop that it is important to analyse also azithromycin as soon as possible after sampling; otherwise the water samples should be kept frozen.

4.4 Analysis of imidacloprid

The PNEC value of imidacloprid is 0.0083 µg/L.

All analytical methods for imidacloprid are based on SPE followed by LC-MS/MS, or direct injection LC-MS/MS techniques, with the exception of one LC-TOF-MS protocol used in Lithuania (LT) and a high-resolution Orbitrap MS instrument used in Latvia (LV). HR, PT, LT, BE, DE, NL, and AT have reported LOQs < EQS (0.0083 µg/L). Other MS (IT, LV, SK, CZ, FR) have achieved an LOQ very close (0.009 or 0.01 µg/L) to the EQS (Annex 6).

Imidacloprid has a log K_{OW} value of 0.6, and therefore should be mainly present in the dissolved phase.

4.5 Analysis of methiocarb

The updated PNEC value of methiocarb is 0.002 µg/L.

Methiocarb has been analysed by SPE – LC-MS/MS or direct injection - LC-MS/MS. Also LLE was used in some laboratories, and GC-MS/MS in Belgium (BE) (Annex 7). The updated PNEC of 0.002 µg/L is already achieved in NL, PT, and AT (0.0021 µg/L); HR (0.002556 µg/L), and DE (0.005 µg/L) are very close.

For methiocarb, the partitioning behaviour between water and SPM was not discussed during the workshop. Methiocarb has a log K_{OW} of 2.9 and therefore is a medium polar organic substance. No information on the water – SPM partitioning behaviour could be found.

5 Partitioning of substances between water and SPM

5.1 Discussions at the workshop

The workshop showed that many MS are using today direct LC-MS/MS injection techniques (Annex 3-10). Since direct injection can only give the dissolved water concentration of a chemical in a water sample, a discussion on the partitioning of the WL substances between water and suspended particle matter (SPM) was started during the workshop. The question in particular was to which extent the substances (ciprofloxacin, EE2, and E2) adsorb to particles or SPM.

Slovakia (SK) stressed that for ciprofloxacin it seems to be important to analyse the "whole water", so that direct injection appears not to be the right analytical method. The offline SPE method with disks that also extracts the particulate fraction should be proposed. France (FR) said that SPE with cartridges does not consider (or extract) the particulates (depending on the amount of the total suspended solids, TSS). If the particulates are loaded with the water sample on the SPE cartridges, then a part of the chemical substances could however be eluted / extracted with the elution solvent.

FR presented a French initiative based on the need to understand the partitioning behaviour of regulatory chemical substances for a correct analysis in dissolved fraction or whole water. To answer the question they are collecting data on the partitioning of the substances in water. This information will help to choose the correct analytical method to use between direct injection, SPE, or LLE. An invitation to collaborate and share data was launched to the other experts attending the meeting.

Germany (DE) asked if an inter-laboratory study on partitioning could be organised and argued that if less than 25 % of a substance adsorbs to SPM, then direct injection could be acceptable.

The JRC (Geel) mentioned that CEN/TC230 (under Mandate M/424 of the European Commission) has already developed three standard methods for whole water analysis of hydrophobic substances (PAHs, BDEs, and organochlorine pesticides) in water with SPM content up to 500 mg/L and the use of SPE disks (EN 16691, EN 16694, EN 16693; 2015).

5.2 Scientific background on partitioning

After the workshop the JRC investigated the partitioning behaviour of pharmaceutical substances by studying relevant publications.

According to the EQS Directive (EU, 2008; 2013), "whole water" analysis, including the particle bound fraction, should be performed for organic substances.

It has been shown for lipophilic compounds (e.g. PAHs, brominated diphenyl ethers, BDEs) that the measurement of the dissolved fraction underestimates the total concentrations in water (Ademollo et al., 2012). For nonylphenol, a compound of medium lipophilicity (log K_{ow} 4.5), up to 50 % of the substance has been found adsorbed on the particles (Li et al., 2004; Patrolecco et al., 2006; Rusconi et al., 2015).

General principles of pollutant partitioning can be found in the literature (e.g., Vignati et al. (2009) and references therein). The Expert Group on Analysis and Monitoring of Priority Substances (AMPS) concluded in 2005 that no specific requirements could be made regarding which matrix (whole water, liquid or particulate phase) should be analysed, because variations in hydrological and environmental circumstances, which are reflected in the quantity and the quality of SPM, preclude rigid categorization and make it difficult to establish general rules for the choice of the appropriate matrix (Ademollo et al., 2012).

However, the first and simplest approach is to consider the hydrophobicity of the analyte, as recommended in the WFD CIS Guidance document No. 25 on sediment and biota monitoring (2010). The proposed rule of thumb is that compounds with log K_{ow} > 5 should

preferably be measured in sediments, or in SPM, while compounds with a log K_{ow} < 3 should preferably be measured in water. And "for compounds with a log K_{ow} between 3 and 5, the sediment matrix or suspended particulate matter is optional and will depend on the degree of contamination. If the degree of contamination for a hydrophobic compound is unknown or expected to be low, sediment should be an additional monitoring matrix (due to accumulation)" (Ademollo et al., 2012).

The WFD CIS Technical guidance document No. 27 for deriving Environmental Quality Standards (2011) however states in section 3.8 that "discrepancies between total and dissolved concentrations may only become evident for very hydrophobic substances … with a log K_{ow} above 6". Since the EQS are usually derived in standard laboratory toxicity and bioconcentration tests with low levels of total organic carbon, the EQS refer to the dissolved (bioavailable) concentrations.

5.3 Evidence from literature studies

Tlili et al. (2016) investigated in France the partitioning of 26 pharmaceuticals, including 18 antibiotics, between the dissolved water phase and SPM. Drug residues associated with SPM were extracted from filters by pressurized liquid extraction (PLE) combined with an on-line SPE-LC-MS/MS system. In the French rivers investigated (Canche and Cojeul Rivers; Nord-Pas-de-Calais, Northern France), the drug residues were present mainly in the dissolved phase. The concentrations of ciprofloxacin in the rivers were 6.6 and 6.4 ng/L in the dissolved phase, and 1.2 and 1.4 ng/L on SPM, respectively, which corresponds to approximately 20 % on SPM (**Table 3**).

Table 3: Average concentrations (ng/L) and percentage (%) on SPM for the two French rivers Canche and Cojeul for several antibiotics similar to ciprofloxacin.

	Dissolved phase (ng/L)	**SPM (ng/L)**	**% on SPM**
Ciprofloxacin	6.5	1.3	20
Danofloxacin	63.3	13.1	21
Difloxacin	24.9	4.9	20
Enrofloxacin	32.5	6.9	21
Norfloxacin	7.4	1.5	20
Ofloxacin	6.8	1.3	19
Orbifloxacin	26.4	5.8	22

Ferreira da Silva et al. (2011) investigated the occurrence of 43 pharmaceuticals and their distribution in surface water, suspended solids and sediments in the Ebro river basin in the Northeast of Spain. The study showed that some compounds are preferentially found bound to SPM and not detected in the dissolved phase of river water. For the analysis of suspended solids river water samples were filtered before extraction and analysis, and the filters were afterwards dried. These filters were extracted by pressurized liquid extraction (PLE), using a methanol–water mixture (1:2) as extraction solvent. Regarding the distribution of pharmaceuticals between water and SPM, it was found that around 70 % of the 43 pharmaceutical compounds measured were predominantly detected in the water phase, while remaining 30 % were predominantly found bound to SPM and sediments. The antibiotics sulfamethazine and chloramphenicol were totally found (100 %) in the SPM fraction. The macrolide antibiotic tylosin was found in water and SPM (ca. 60 %). **Table 4** shows the SPM percentages for several substances.

Table 4: Partitioning percentages (%) on SPM in the Ebro river basin (Ferreira da Silva et al. ; 2011).

Substance	Class	% on SPM
Sulfamethazine	Sulfonamide antibiotic	100
Chloramphenicol	Amphenicol antibiotic	100
Tylosin	Macrolide antibiotic	60
Josamycin	Macrolide antibiotic	20
Clarithromycin	Macrolide antibiotic	15
Clofibric acid	Chlorinated carboxylate	20
Clenbuterol	Bronchodilator asthma drug	20
Diclofenac	Nonsteroidal anti-inflammatory drug	18
Ranitidin	Antihistaminic drug	12
Bezafibrate	Lipid-lowering drug	5
Acetaminophen	Pain killer	4
Enalapril	Drug to treat high blood pressure	3
Carbamazepine	Anti-epileptic drug	3
Ibuprofen	Nonsteroidal anti-inflammatory drug	3

Generally, sorption of organic contaminants to solids (sediment, soil and solid in suspension) is governed by several processes, such as hydrophobic partitioning, ion exchange, complexation and hydrogen bonding. In case of polar and ionic pharmaceuticals the sorption properties cannot be evaluated from the log K_{ow} and normalization to organic carbon content does not give a straightforward relationship. In the study by Ferreira da Silva et al. (2011), it was found that the distribution may be affected by other properties, such as pKa values. Generally, it was observed that compounds with basic characteristics (pKa > 7) such as famotidine, timolol and nadolol show higher tendency to bound to suspended solids. High values of pKa indicate that these compounds are positively charged at pH conditions of river water and other interactions (cationic interactions, complexation, hydrogen bonding) can affect the fate of these compounds. In any case, the sorption of pharmaceuticals depends on both, the properties of the pharmaceuticals and suspended solids and the results obtained here showed that the analysis of suspended solids is very important, since a considerable number of pharmaceuticals were not detected in the dissolved phase of surface water samples, but were found bound to the solids in suspension. Therefore, analysis of water sample with filtration can underestimate the data regarding the occurrence of pharmaceuticals in the aquatic environment (Ferreira da Silva et al., 2011).

Cheng et al. (2017) investigated the multi-phase distribution of the antibiotics oxytetracycline, tetracycline, sulfadiazine, sulfamethazine, norfloxacin, and ofloxacin in the surface water from the largest lake (Baiyangdian Lake) in North China (norfloxacin and ofloxacin are fluoroquinolones similar to ciprofloxacin). The antibiotics were mainly present in the soluble (dissolved) phase, indicating high biological availability. The distribution of the antibiotics to SPM was relatively uniform, and ranged from 11 % for oxytetracycline to 24 % for norfloxacin to the total average dissolved concentration. Comparing the distribution of targeted antibiotics in both SPM and dissolved phase, tetracyclines and fluoroquinolones had stronger sorption potentials than sulfonamides.

Yang et al. (2011) investigated the occurrence and geochemical behaviour of nine pharmaceutical compounds along the Yangtze River Estuary (China) and its coastal area by sampling and analysing the pharmaceuticals in sediment, SPM, colloidal and soluble phases, and found only very small amounts in the SPM compartment (< 10 %).

Estrogenic steroid hormones, with sediment–water (log K_{oc}) and octanol–water partition coefficient (log K_{ow}) of 3.4 for E2 and 3.7 for EE2 and 4.0 for E2 and 4.2 for EE2, respectively, are non-polar hydrophobic organic compounds that adsorb easily onto organic carbon rich sediments (Lei et al., 2009; Sun et al., 2012; Yarahmadi et al.; 2018; Ying et al., 2002). River sediments likely act as environmental sink for these compounds (Yarahmadi et al.; 2018). Relatively little information on their water – SPM partitioning behaviour is however available in the literature.

Andrasi et al. (2013) investigated for the first time the partitioning of steroids (androsterone, coprostanol, cholesterol, stigmasterol, sitosterol, estriol, E2, EE2) between the dissolved and suspended phases of influent and effluent waste waters and river water samples of the Danube River. The particulates were collected on glass microfiber filters and extracted by ultrasound assisted solvent extraction. The distribution of the steroids revealed that their biggest part (relating to their total amounts), were present in the suspended phase, i.e. on average 71 % for waste water and 64 % for Danube River water samples (in the range of 28–54 % for E2 and 23–100 % for EE2).

Nie et al. (2015) investigated the occurrence and distribution of six selected estrogenic compounds (estrone (E1), E2, estriol (E3), EE2, 4-tert-octyphenol (OP), and bisphenol A (BPA)) in samples of surface water, SPM, and sediment in the Yangtze Estuary and its coastal areas (China) over four seasons. The estrogens were detected in both aqueous phase and SPM. However, EE2 was not detected in any of the surface water samples (LOD: 0.10–0.5 ng/L; LOQ: 0.30–2 ng/L). A mass balance for total estrogens between these two phases in the estuarine system showed that the SPM phase contributed between 16 % up to 88 %.

Yarahmadi et al. (2018) conducted in Canada an extensive environmental monitoring in an urban river impacted by multiple combined sewer overflows and WWTP discharge points. Temporal and spatial distributions of dissolved and particulate steroids (progesterone, testosterone, medroxyprogesterone, levonorgestrel, norethindrone, estrone (E1), E2, estriol (E3), and EE2) were investigated in sewage, WWTP effluents, receiving river water, sediments, and in drinking water plant intakes. The particulate phase (suspended matter) was collected on glass micro-fiber filters and was extracted by solvent-assisted ultrasonic extraction involving 2 cycles with 5 and 3 mL of methanol/acetone. Steroids were detected in both dissolved and particulate phases with mean concentrations from 21 ng/L to 389 ng/L in raw sewage and from 10 ng/L to 296 ng/L in treated wastewater. The particle-associated steroids represented 0–82% of their total concentration as some steroids like E1 and E3 were detected only in the dissolved phase while medroxyprogesterone (81%), norethindrone (71%), and EE2 (> 75%) were primarily detected in the particulate phase.

Table 5 gives the detected concentrations of E2 and EE2 in the dissolved and particulate phase of the WWTP effluents investigated, showing that 38 % of E2 and 71 % of EE2 were present on SPM.

Table 5: Concentrations of E2 and EE2 in WWTP effluents (ng/L); dissolved and particulate phase.

	WWTP 1		**WWTP 2**		**WWTP 3**		
	Dissolved	**Particulate**	**Dissolved**	**Particulate**	**Dissolved**	**Particulate**	**Average % on SPM**
E2	n.d.	40	157	59	147	17	38
EE2	n.d.	29	15	48	13	22	71

In the river water samples, the concentration of all the selected steroids were below the detection limits (LOD = 5–52 ng/L) in the dissolved water phase. In contrast, all the suspended particles contained EE2 and E2 with mean concentrations of 3259 ng/g and 1000 ng/g in spring and 461 ng/g and 647 ng/g in summer samples, respectively. Thus, E2 and EE2 were detected 100 % in all river water samples in the particulate phase Yarahmadi et al. (2018).

It can therefore be concluded that EE2 shows a high affinity to particles and should thus be analysed in whole water. The adsorption properties of ciprofloxacin and amoxicillin are not totally clear, and therefore these antibiotics should preferably be analysed in whole water, or their negligible adsorption should be proven.

6 Determination of LOQ

The Netherlands (NL) and other countries asked to specify in detail the different methods for the determination or calculation of the LOQ.

Commission Directive 2009/90/EC (laying down, pursuant to Directive 2000/60/EC of the European Parliament and of the Council, technical specifications for chemical analysis and monitoring of water status) specifies that MS shall ensure that laboratories or parties contracted by laboratories apply quality management system practices in accordance with EN ISO 17025 (2005) or other equivalent standards accepted at international level (e.g. DIN 32645; 2008; Eurachem Guide([1]); ICH Q2(R1); IUPAC, 2002).

Directive 2009/90/EC states: "The limit of quantification can be calculated using an appropriate standard or sample, and may be obtained from the lowest calibration point on the calibration curve, excluding the blank".

The LOD is the smallest measured content from which it is possible to deduce the presence of the analyte with reasonable statistical certainty. The LOD is numerically equal to three times the standard deviation of the mean of blank determinations ($n \geq 5$). The LOQ is the lowest content of the analyte which can be measured with reasonable statistical certainty. If both accuracy and precision are constant over a concentration range around the limit of detection, then the limit of quantification is numerically equal to six or 10 times the standard deviation of the mean of blank determinations ($n > 20$) (IUPAC, 2002).

The Eurachem Guide (2014) states: "The LOQ is the lowest level of analyte that can be determined with acceptable performance. "Acceptable performance" is variously considered by different guidelines to include precision, precision and trueness, or measurement uncertainty. In practice, however, LOQ is calculated by most conventions to be the analyte concentration corresponding to the obtained standard deviation (s) at low levels multiplied by a factor, *k*Q. The IUPAC default value for *k*Q is 10 and if the standard deviation is approximately constant at low concentrations this multiplier corresponds to a relative standard deviation (RSD) of 10 %. Multipliers of 5 and 6 have also sometimes been used which corresponds to RSD values of 20 % and 17 % respectively."

It must however be noted that these are general guidelines which are not sufficient for a uniform approach on the determination of the LOQ. Whether the LOQs in the presented methods of the questionnaire are derived from appropriate real surface water samples with or without addition, or from calibration curves, remained unclear. For a consensus on methods, a consensus on the determination of the LOQ is necessary. This needs a thorough discussion on the minimum requirements for sample preparation and analysis, i.e. representative surface water matrix, standard addition, recovery, reproducibility, uncertainty, and multiplication factor.

Italy (IT) stressed that for accreditation it is requested to check the recovery with your matrix (surface water in case of the WL). Recovery experiments under repeatability conditions at low (spiked) concentration levels should be performed with a real surface water matrix.

The laboratories should be asked in detail how they determine the LOQ in order to provide insight in the LOQ and comparability between them.

([1]) Eurachem is a network of organisations in Europe, having the objective of establishing a system for the international traceability of chemical measurements and the promotion of good quality practices. It provides a forum for the discussion of common problems and for developing an informed and considered approach to both technical and policy issues. Piotr Robouch of the European Commission is a co-author of the report. https://www.eurachem.org/index.php/mnu-about

7 Conclusions

The workshop was an excellent opportunity for the technical experts of the Member States (plus Island, Switzerland and Turkey) to meet each other in order to discuss the analytical methods and problems for the analysis of the watch list (WL) substances of the WFD. The usefulness of this information exchange was stressed by several participants. In addition, the purpose of the meeting was to give the opportunity, as follow up, to share the analytical protocols, experience and eventually to provide analytical service to those countries which could not measure properly some of the existing WL substances discussed during the workshop. It was remarked several times that the milestone of the workshop is to ensure the delivery of good data quality for the WL substances in order to drive decision-making, since it is the prerequisite to assess and evaluate their risk at EU level.

Furthermore during the meeting, several experts raised the question of the water fraction of analysis (whole water, dissolved or particulate phase). The EQS directive requires monitoring whole water for organics. Experts pointed out that the analysis of the dissolved fraction is easier because it is often performed by direct LC-MS/MS injection techniques and can deliver for polar water-soluble compounds a good approximation of the concentration in the whole water sample. However, DG ENV highlighted that in term of process, only a subgroup of the WG Chemicals mandated for this task could make proposals of guidelines on whether and in which cases the dissolved fraction could be a good approximation for the whole water fraction. The participants agreed that compounds with a log $K_{ow} < 3$ should preferably be measured in the dissolved water phase, and compounds with log $K_{ow} > 5$ in sediment, SPM, or biota. A more precise investigation of the partitioning behaviour is necessary for compounds with log K_{ow} between 3 and 5.

In view of the fitness check and evaluation of the WFD, the correction of discrepancies in different technical guidance documents and the examination of the partitioning and "whole water" analysis problematic (because the EQS refer to the dissolved (bioavailable) concentrations) are recommended.

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List of abbreviations and definitions

BDE	Brominated diphenyl ether
CEN	European Committee for Standardization
CIS	Common implementation strategy
DG ENV	Directorate General for Environment
EE2	17-alpha-Ethinylestradiol
E2	17-beta-Estradiol
EDTA	Ethylenediamine tetra acetic acid
EQS	Environmental quality standard
GC	Gas chromatography
GC-MS	Gas chromatography mass spectrometry
GC-MS/MS	Gas chromatography (tandem) triple quadrupole mass spectrometry
HR	High resolution
HPLC	High pressure liquid chromatography
JRC	Joint Research Centre
LC	Liquid chromatography
LC-MS/MS	Liquid chromatography (tandem) triple quadrupole mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
LLE	Liquid liquid extraction
MDL	Method detection limit
MRM	Multiple reaction monitoring
MS	Mass spectrometry or Member State
PAH	Polycyclic aromatic hydrocarbon
PLE	Pressurized liquid extraction
PNEC	Predicted no-effect concentration
PPCP	Pharmaceutical and personal care product
SLE	Solid liquid extraction
SPE	Solid-phase extraction
SPM	Suspended particulate matter
TOF	Time-of-flight
TSS	Total suspended solids
WG	Working Group (of the European Union or Commission)
WL	Watch list
WWTP	Waste water treatment plant

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Annexes

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The workshop was attended by 35 experts from 21 countries.

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Annex 3. Questionnaire. Analytical methods for 17-alpha-ethinylestradiol (EE2) (PNEC = 0.000035 µg/L)

Country	Analytical method	Extraction method / sample preparation	Extraction volume or *injection volume*	LOQ (µg/L)
HR	LC-MS/MS	Direct injection	*100 µl*	0.000012
SE	LC-MS/MS Separation by an UPLC dual chromatographic method (basic and neutral)	SPE Oasis HLB (200 mg)	2 L *(10 µl)*	0.00003
DE	LC-MS/MS	SPE	0.5 L *100 µl*	0.00003
IT	LC-MS/MS	Off-line SPE + on-line SPE 1 L of the sample are loaded onto SPE cartridge, the extract is concentrated to a little volume and reconstituted with 10 mL of water; 5 mL of this final extract are injected into an on-line SPE-UHPLC-MS/MS system.	1 L 5 mL	0.000035
LV	GC-MS/MS	SPE	0.4 L	0.000035
CZ	LC-MS/MS	LLE (with dichloromethane) GPC and SPE clean-up (Florisil)	0.8 L *(100 µl)*	0.00005
AT	LC-MS/MS	SPE	1 L	0.0001
FR	LC-MS/MS Dansylation derivatisation	SPE Speedisk Bakerbond H_2O-Philic DVB	1 L	0.0001
NL	GC-MS Derivatization	SPE	1 L	0.0001
SK	LC-MS/MS Derivatization with dansylchloride	LLL Hexane/DCM (3/2, v/v)	0.5 L	0.0003
PT	LC-MS/MS	SPE	1 L	0.0004
LT	LC-TOF-MS	SPE	5 L	0.00056
DE	LC-MS/MS	Direct injection	*100-200 µl*	0.001
DK	LC-MS/MS Derivatization	SPE	0.5-1 L	0.001

Annex 4. Questionnaire. Analytical methods for 17-beta-estradiol (E2)

(PNEC = 0.0004 µg/L)

Country	Analytical method	Extraction method / sample preparation	Extraction volume or *injection volume*	LOQ (µg/L)
HR	LC-MS/MS	Direct injection	*100 µl*	0.000053
DE	LC-MS/MS	SPE	0.5 L *100 µl*	0.00003
SE	LC-MS/MS Separation: UPLC Dual chromatographic method (basic and neutral)	SPE Oasis HLB (200 mg)	2 L *(10 µl)*	0.00003
AT	LC-MS/MS	SPE	1 L	0.0001
IT	LC-MS/MS	Off-line SPE + on-line SPE 1 L of the sample are loaded onto SPE cartridge, the extract is concentrated to a little volume and reconstituted with 10 mL of water; 5 mL of this final extract are injected into an on-line SPE-UHPLC-MS/MS system.	1 L 5 mL	0.0001
NL	GC-MS Derivatization	SPE	1 L	0.0001
LV	GC-MS/MS	SPE	0.4 L	0.00012
LT	LC-TOF-MS	SPE	5 L	0.00028
CZ	LC-MS/MS	LLE (with dichloromethane) GPC and SPE clean-up (Florisil)	0.8 L *(100 µl)*	0.0003
SK	LC-MS/MS Derivatization with Dansylchloride	LLE Hexane/DCM (3/2, v/v)	0.5 L	0.0003
FR	LC-MS/MS Dansylation derivatisation	SPE Speedisk Bakerbond H_2O-Philic DVB	1 L	0.0004
PT	LC-MS/MS	SPE	1 L	0.0004
DE	LC-MS/MS	Direct injection	*100-200 µl*	0.001
DK	LC-MS/MS Derivatization	SPE	0.5-1 L	0.001
BE	LC-MS/MS	online SPE	5.9 mL	0.002

Annex 5. Questionnaire. Analytical methods for azithromycin

(PNEC = 0.019 µg/L)

Country	Analytical method	Extraction method / sample preparation	Extraction volume or *injection volume*	LOQ (µg/L)
SE	LC-MS/MS Separation: UPLC Dual chromatographic method (basic and acid)	SPE Oasis HLB (200 mg)	0.05 L *(1 µl)*	0.0011
NL	LC-MS/MS	Direct injection	*100-1000 µl*	0.0036
PT	LC-MS/MS	SPE	1 L	0.005
HR	LC-MS/MS	Direct injection	*100 µl*	0.006236
LT	LC-TOF-MS	SPE	1 L	0.0063
AT	LC-MS/MS	SPE	0.1 L	0.01
CZ	LC-MS/MS	Direct injection	*250 µl*	0.01
DE	LC-MS/MS	Direct injection	*100 µl*	0.02
DE	LC-MS/MS	Direct injection	*100-200 µl*	0.02
FR	LC-MS/MS	LLE	1 L	0.02
CH	LC-MS/MS	Direct injection or SPE		0.05
DK	LC-MS/MS	Direct injection		0.05
IT	LC-MS/MS	Direct injection	*100 µl*	0.05
SK	LC-MS/MS	Direct injection	*500 µl*	0.09
LV	UHPLC-HRMS (Orbitrap)	SPE	0.5 L	0.09

Annex 6. Questionnaire. Analytical methods for imidacloprid

(PNEC = 0.0083 µg/L)

Country	Analytical method	Extraction method / sample preparation	Extraction volume or *injection volume*	LOQ (µg/L)
HR	LC-MS/MS	Direct injection	*100 µl*	0.001028
SE	LC-MS/MS Separation: UPLC Dual chromatographic method (basic and acid)	SPE	0.05 L *(1 µl)*	0.0013
PT	LC-MS/MS	SPE	0.5 L	0.002
LT	LC-TOF-MS	SPE	1 L	0.002
BE	LC-MS/MS	Direct injection	*50 µL*	0.005
DE	LC-MS/MS	Direct injection	*100 µl*	0.005
NL	LC-MS/MS	On-line SPE		0.006
AT	LC-MS/MS	Direct injection		0.0081
IT	LC-MS/MS	Direct injection	*100 µl*	0.009
LV	UHPLC-HRMS (Orbitrap)	SPE	0.5 L	0.009
SK	LC-MS/MS	Online SPE Oasis HLB	15 mL	0.009
CZ	LC-MS/MS	Direct injection	*250 µl*	0.008
FR	LC-MS/MS	LLE	1 L	0.01
DE	LC-MS/MS	Direct injection	*100-200 µl*	0.02
CH	LC-MS/MS	Direct injection or SPE		0.02
DK	LC-MS/MS	SPE	0.25 L	0.05

Annex 7. Questionnaire. Analytical methods for methiocarb

(PNEC = 0.002 µg/L)

Country	Analytical method	Extraction method / sample preparation	Extraction volume or *injection volume*	LOQ (µg/L)
NL	LC-MS/MS	Direct injection	*100-1000 µl*	0.00069
PT	LC-MS/MS	SPE	0.5 L	0.002
AT	LC-MS/MS	Direct injection		0.0021
HR	LC-MS/MS	Direct injection	*100 µl*	0.002556
DE	LC-MS/MS	Direct injection	*100 µl*	0.005
CZ	LC-MS/MS	Direct injection	*250 µl*	0.008
FR	LC-MS/MS	LLE	1 L	0.01
IT	LC-MS/MS	Direct injection	*100 µl*	0.01
SK	LC-MS/MS	Online SPE Oasis HLB	15 mL	0.01
LV	UHPLC-HRMS (Orbitrap)	SPE	0.5 L	0.01
SE	LC-MS/MS Separation: UPLC Dual chromatographic method (basic and acid)	SPE Oasis HLB (200 mg)	0.05 L *(1 µl)*	0.0195
DE	LC-MS/MS	Direct injection	*100-200 µl*	0.02
DK	LC-MS/MS	SPE	0.25 L	0.02
BE	GC-MS/MS	LLE Dichloromethane	1 L	0.04
LT	LC-MS/MS	SPE	1 L	0.04

Annex 8. Questionnaire. Analytical methods for amoxicillin

(PNEC = 0.078 µg/L)

Country	**Analytical method**	**Extraction method / sample preparation**	**Extraction volume or *injection volume***	**LOQ (µg/L)**
PT	LC-MS/MS	SPE	1 L	0.008
AT	LC-MS/MS	SPE	0.1 L	0.01
NL	LC-MS/MS	Direct injection	*100-1000 µl*	0.01
DE	LC-MS/MS	Direct injection	*100-200 µl*	0.02
IT	LC-MS/MS	Direct injection	*100 µl*	0.05
CZ	LC-MS/MS	Direct injection	*250 µl*	0.1

Annex 9. Questionnaire. Analytical methods for ciprofloxacin

(PNEC = 0.089 µg/L)

Country	**Analytical method**	**Extraction method / sample preparation**	**Extraction volume or *injection volume***	**LOQ (µg/L)**
HR	LC-MS/MS	Direct injection	*100 µl*	0.004598
PT	LC-MS/MS	Direct injection		0.015
AT	LC-MS/MS	SPE	0.5 L	0.02
DE	LC-MS/MS	Direct injection	*100-200 µl*	0.02
DE	LC-MS/MS	Direct injection	*100 µl*	0.025
SE	LC-MS/MS Separation: UPLC Dual chromatographic method (basic and acid)	SPE Oasis HLB (200 mg)	0.05 L *(1 µl)*	0.0318
CZ	LC-MS/MS	Direct injection	*250 µl*	0.05
NL	LC-MS/MS	Direct injection	*100-1000 µl*	0.2
IT	LC-MS/MS	Direct injection	*100 µl*	0.05

Annex 10. Questionnaire. Analytical methods for metaflumizone

(PNEC = 0.0654 µg/L)

Country	**Analytical method**	**Extraction method / sample preparation**	**Extraction volume or *injection volume***	**LOQ (µg/L)**
PT	LC-MS/MS	SPE		0.010-0.020
DE	LC-MS/MS	Direct injection	*100-200 µl*	0.02
AT	LC-MS/MS	Direct injection	*100 µl*	0.05
IT	LC-MS/MS	Direct injection	*100 µl*	0.05

Annex 11. Measured Environmental Concentrations (MECs) for amoxicillin.

Country	Source of monitoring data	MEC values (µg/L)	Reference
Iran	Kan River	0.018	Mirzaei et al.; 2017
Spain	Hospital wastewater, and urban WWTP effluent in Girona	0.218-0.258	Gros et al., 2013
Europe (90 samples from 18 countries)	WWTP effluents	< 0.025	Loos et al., 2013
France	Seine River	0.068	Dinh et al., 2011
Canada	Wascana Creek, Qu'Appelle River	0.080 (max)	Waiser et al., 2011
Italy	River Po	<0.002	Zuccato et al., 2010
Italy	River Arno	0.006 (mean); 0.010 (max)	Zuccato et al., 2010
UK (Wales)	River Taff and Ely	0.117 (median); 0.622 (max)	Kazprzyk-Horden et al., 2008
UK (Wales)	River Taff	<0.010 – 0.245	Kazprzyk-Horden et al., 2007
Italy	WWTP effluents	0.015 – 0.120	Castiglioni et al., 2005
Italy	Different WWTP effluents	0.0018 – 0.120	Andreozzi et al., 2004

Annex 12. Measured Environmental Concentrations (MECs) for ciprofloxacin.

Country	Source of monitoring data	MEC values (µg/L)	Reference
Iran	Kan River	0.012	Mirzaei et al.; 2017
France	Canche River (urban impact)	0.007	Tlili et al., 2016
Spain	Ter River downstream WWTP in Girona	0.072 (max)	Rodriguez-Mozaz et al., 2015
USA	River in Maryland	0.010 (upstream WWTP) 0.031 (max; downstream WWTP)	He et al., 2015
Worldwide monitoring data collected from 47 articles; in total 501 samples.	Sum of fluoroquinolones in surface water	0.026 (median)	Van Doorslaer et al., 2014
China	Wenyu River	0.066 (max)	Zhang et al., 2014
Poland	Gościcina and Reda Rivers	2.7 (max)	Wagil et al., 2014
90 samples from 18 European countries (EU-wide monitoring survey)	Ciprofloxacin in EU WWTP effluents	0.096 (mean) 0.264 (max)	Loos et al., 2013
Spain	Urban WWTP effluents in Girona	0.147 (max)	Gros et al., 2013
France	Seine River; Charmoise River, downstream WWTP	0.017; 0.135	Dinh et al., 2011
Italy	Surface water, River Po	0.009 (mean)	Zuccato et al., 2010
Italy	Surface water, River Arno	0.019 (mean)	Zuccato et al., 2010
China	Tonghui River	0.010 (median); 0.020 (max)	Xiao et al., 2008
China	Pearl River	0.459 (max)	Peng et al., 2008
USA	Upper Tennessee River	0.007 (median); 0.054 (max)	Conley et al., 2008
Finland	Vantaa River	0.025 (max)	Vieno et al., 2007
USA	Streams downstream WWTPs	0.170 (median); 0.360 (max)	Batt et al., 2006
France, Greece, Italy and Sweden	WWTP effluents	0.060 (median)	Andreozzi et al., 2003
Italy	Po and Lambro River	0.020 (median); 0.026 (max)	Calamari et al., 2003
Switzerland	WWTP effluent in Zuerich	0.071 (mean)	Golet et al. 2002
USA	Surface water	0.030 (max)	Kolpin et al., 2002

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