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Efficient Validation Strategies in Environmental Analytical Chemistry: A Focus on Organic Micropollutants in Water Samples

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Abstract

This article critically reviews analytical method validation and quality control applied to the environmental chemistry field. The review focuses on the determination of organic micropollutants (OMPs), specifically emerging contaminants and pesticides, in the aquatic environment. The analytical technique considered is (gas and liquid) chromatography coupled to mass spectrometry (MS), including high-resolution MS for wide-scope screening purposes. An analysis of current research practices outlined in the literature has been performed, and key issues and analytical challenges are identified and critically discussed. It is worth emphasizing the lack of specific guidelines applied to environmental analytical chemistry and the minimal regulation of OMPs in waters, which greatly affect method development and performance, requirements for method validation, and the subsequent application

to samples. Finally, a proposal is made for method validation and data reporting, which can be understood as starting points for further discussion with specialists in environmental analytical chemistry.

INTRODUCTION

The production and use of chemicals are indispensable aspects of the current worldwide economy and modern life. Many synthetic compounds are released into the aquatic environment via industrial and communal wastewater treatment plants, surface runoff, and release from solid materials and waste. Consequently, a large number of organic micropollutants (OMPs) are found in surface water and groundwater, particularly in densely populated areas. OMPs comprise many categories of substances, including contaminants of emerging concern (CECs), a wide group of compounds frequently used in our everyday lives yet barely regulated. Pharmaceutical active compounds (PhACs) are among the most frequently investigated and reported CECs in the water cycle (1), particularly antibiotics, owing to the current concern in the environmental field about antibiotic resistance (2–5).

The great diversity in chemical composition, from nonpolar to highly polar compounds and from low to high volatility, makes the detection and identification of potentially hazardous OMPs an analytical challenge (6). Because many OMPs are transformed via biotic and abiotic processes once released into the environment, not only the parent compounds but also their transformation products (TPs) should be investigated, which adds more analytical challenges.

A major challenge to evaluating water quality and protecting water resources from anthropogenic pollution is to improve monitoring and strengthen the comprehensive prioritization and risk assessment of complex mixtures (7). The role of advanced analytical chemistry is essential to this aim, and the potential of hyphenated chromatography-mass spectrometry (MS) for the investigation of OMPs in the environment is undisputed. At present, both liquid chromatography (LC) and gas chromatography (GC) coupled to MS or tandem MS (MS/MS) are widely used for quantitative analysis of OMPs (8). Owing to its excellent sensitivity and selectivity, wide linear dynamic range, and compatibility with aqueous samples, LC-MS(/MS) is frequently applied for the determination of CECs because of the medium-high polarity of most compounds, particularly TPs (9). Although quantitative analysis is essential for monitoring CECs in water, it can only be applied to a limited list of target compounds, whose reference standards are available. Therefore, wider-scope techniques are required for a realistic overview of the chemical pollution state of the aquatic environment. This is currently possible through the use of GC and/or LC coupled to high-resolution mass spectrometry (HRMS). A systematic collection of wide-scope target, suspect, and nontarget screening data at the European scale has been proposed to improve the spatial and temporal coverage and range of matrices available for risk assessment (10). This integration helps to extend the range of chemicals investigated in the aquatic environment and facilitates prioritization based on the environmental occurrence of compounds (11).

This context illustrates the need to apply appropriate analytical methods previously validated and that must be periodically subjected to quality control to support the reliability of data. Method validation is a requirement in target quantitative methods, but it is not yet clearly defined in HRMS-based screening methods, especially in suspect and nontarget screening. Recent initiatives (e.g., NORMAN or the Dutch norm NTA 8033) are focused on the development and harmonization of measurement methods for the detection of emerging chemicals in the environment (10, 12).

The worldwide implementation of legislation to protect the aquatic environment is key to the development of advanced (target) analytical methodology and may also establish the requirements

for method performance as a function of the concentration levels regulated. It is also essential for the development of specific advanced technologies for industrial and urban wastewater treatment as well as the implementation of wide-scope screening strategies (13). However, the list of OMPs currently regulated is relatively small, despite recent advances. Examples include one from the United States, which introduced the Contaminant Candidate List, or the European Union's Water Framework Directive, which also introduces the Watch List mechanism. The limited regulation of OMPs in water stems from the inherent complexity of this topic. Factors include social and economic implications, the extensive number of OMPs with distinct physico-chemical and toxic properties that might be regulated, the analytical difficulties of receiving a rapid response for a large number of compounds, and insufficient information available on the toxicity of many OMPs and on environmental risk assessment. Future advances in environmental legislation will have evident implications for the requirements of analytical methods applied.

Method validation is commonly based on reference guidelines in the specific area of research (14–20). Thus, some fields, such as pesticide residue analysis in food, have detailed guidelines, specifying the steps and criteria applied in this type of analysis. Unfortunately, environmental analytical chemistry has no detailed guidelines for the determination of OMPs, and most laboratories apply criteria based on other guidelines and fields of research. Moreover, specific issues dealing with environmental analysis and related to the type of samples and variability in chemical composition are missing. This is an impediment for analytical methods applied to environmental samples, particularly for the harmonization of methodologies.

This review critically discusses method validation in the field of environmental analytical chemistry. Due to the complexity of the subject and the large number of articles covering a great variety of contaminants, from heavy metals to OMPs, and in different sample matrices (e.g., water, soil, sediments, air, biota), it is necessary to manage the extensive information available. Considering the interest in the topic and the authors' expertise, this review focuses on two relevant groups of OMPs, pesticides and CECs; the latter case is limited to pharmaceutical residues. The environmental matrix considered is the aquatic environment, including surface water, groundwater, drinking water, and wastewater because of the relevance of water resources to our quality of life and health. The analytical methods reviewed and critically discussed are exclusively based on chromatography-MS [including GC, LC, low-resolution MS (LRMS), and HRMS]. The review mainly covers quantitative methods, which are predominant in environmental analytical chemistry. A brief section is devoted to screening methods based on HRMS because of the increasing interest of these methods. The selection of recent publications between 2017 and 2021 is a sample to benchmark current practices used for method validation.

VALIDATION OF ANALYTICAL METHODS IN ENVIRONMENTAL ANALYSIS: DEFINITIONS AND CURRENT FRAMEWORK

Method validation may include different parameters and is quite variable depending on whether it covers research articles focused on method development, design of new sorbents or techniques for improved analytical determinations, method application, or analysis that is regulatory framed and may have legal consequences.

In this section, we discuss the most frequent performance figures considered in method validation. To this end, selected guidelines from reputed international organizations are considered (**Table 1**). Some guidelines are of general application, such as those from Eurachem or AOAC (21, 22), whereas others are related to pesticides or pharmaceuticals/drugs commonly linked to water, food, or biological fluids (15–20).

We have also considered EU Directive 96/23/EC (14), which is related to pharmaceuticals and other residues in food and animals, as it has inspired validation protocols in many (research)

Table 1 Summary of analytical performance requirements from selected guidelines

Source	Application field	General performance	Chromatographic/mass spectrometric guidelines	Reference
Eurachem Guidelines	General guidelines	■ Trueness measured from CRMs; $n = 10$ ■ Precision; $n = 6$–15 ■ LOD = 3 times the SD of a blank or low-concentration spiked sample; $n = 10$ ■ LOQ = k times the SD of a blank or low-concentration spiked sample; $n = 10$ (k is a variable number, but recommended to be set to 10) ■ Qualitative method LOD: defined for a certain possibility of false negatives (normally 5%); $n = 10$ ■ Robustness: test alterations in method parameters that may affect performance ■ Selectivity: test 1 sample with suspected interferents ■ Blanks to be measured frequently ■ Calibration: to be checked in the expectable concentration range ±10% (recommended 20%); $n = 6$–10 levels, to be checked with each matrix	NA	21
AOAC International Guidelines	General guidelines	■ Trueness: should be typically in the 40–120% range for trace analysis. Use CRM; $n = 5$. If CRM unavailable, use spiked samples at 3 concentration levels, with 3 different samples; $n = 7$ ■ Precision: RSD should be typically <30% (repeatability) or 45% (reproducibility). To be measured at 3 concentration levels, with 3 different samples; $n = 7$ ■ LOD: should be 3/5 of the reporting level ■ LOQ: defined as blank signal + 10 times the SD of the blank signal ■ Qualitative method LOD: report false positive and false negative ratio at the critical concentration ■ Robustness: assessed in trueness/precision studies by using 3 different samples ■ Blanks are accounted for in LOQ calculation ■ Interlaboratory studies required	NA	22
EU Directive 96/23/EC	Residues in animals and animal products	■ Trueness: should typically be in the 50–120% range for trace analysis from spiked samples at different levels; $n = 6$. If CRM available, recovery = 90–110%; $n = 6$. To be checked with each batch of samples ■ Precision: as low as possible, assessed together with trueness ■ LOD: decision limit + 1.64 times the SD of sample spiked at low concentration; $n = 20$ ■ LOQ: defined as blank signal + 10 times the SD of the blank signal ■ Qualitative method LOD: lowest level where compound is detected in 95% of the samples; $n = 20$ ■ Robustness: test alterations in method parameters that may affect performance ■ Selectivity: analyze 20 samples/blanks spiked with potential interferents ■ Stability: check for stability of standards (4 weeks) and samples (20 weeks) ■ Perform blanks and (spiked) control samples at near the decision limit with each batch of samples ■ Calibration, at least $n = 5$ levels. Standard addition can be used	■ Chromatographic retention factor should be ≥1 ■ Retention time tolerance: 0.5% (GC) to 2.5% (LC) ■ MS identification point system ■ Record at least 3–4 ions (depending on analyte type) in MS or 2 in MS/MS ■ MS ion ratio tolerance: 10–50% (relative) depending on confirmatory ion intensity and MS technique	14

(*Continued*)

Table 1 (*Continued*)

Source	Application field	General performance	Chromatographic/mass spectrometric guidelines	Reference
EU SANTE/ 12682/2019	Pesticides in fruits, vegetables, and food	■ Trueness: should be typically in the 70–120% range. 30–140% sometimes acceptable if RSD < 20%. If outside 80–120%, sample concentrations can be corrected by the recovery factor. Trueness to be assessed at two concentration levels (LOQ or reporting level and higher concentration). To be checked with each batch of samples, if possible ■ Precision: typically, RSD should be lower than 20% ■ LOQ: defined the lowest concentration where trueness and precision meet the acceptance criteria ■ Qualitative method LOD: lowest level where compound is detected in 95% of the samples; $n = 20$ ■ Robustness: test different sample matrices (commodities) for performance ■ Selectivity: assessed from blanks by making sure that the signal of analytes is lower than 30% of the reporting level ■ Calibration: recommended to perform multilevel calibrations, including matrix-matched calibration, ILIS or standard addition. Other alternatives might be single-point calibration or the use of procedural spiked standards	■ Retention time tolerance: 0.1 min but can be higher if an ILIS is available and can be used for verification of the difference ■ If matrix effects exceed 20% enhancement/suppression, this should be addressed by appropriate calibration ■ Record at least 3 ions in MS or 2 in MS/MS (S/N > 3) ■ HRMS error tolerance: <5 ppm or 1 mDa for ions <200 *m/z* ■ MS ion tolerance: 30% (relative), not applicable in HRMS (ions should fully overlap)	15
US Pesticide Data Program	Pesticides	■ Trueness: should be typically in the 50–150% range. To be assessed quarterly with samples spiked at 2 × LOQ; $n = 7$ ■ Precision: use Horwitz approach to derive tolerance. Assessed together with trueness ■ LOD: defined for a S/N ≥ 3 for all MS ions, verified with spiked samples. In the case of water, to be reevaluated every 2 years ■ LOQ: defined for a S/N ≥ 10 for the quantifier ions, while remaining ions S/N ≥ 3, verified with spiked samples. In the case of water, to be reevaluated every 2 years ■ Qualitative method LOD: as LOD ■ QA samples spiked at 5 × LOQ to be analyzed with each batch. Besides, a sequence should contain blanks, spiked samples, and standards at LOD level. The response should not change >20–30% during the sequence ■ Calibration: multilevel ($n \geq 5$ if quadratic), suggested to start at LOQ, run with the sequence in order to verify its stability; single-point allowed (concentration in the sample should not differ >30% from the standard)	■ Retention time tolerance: 0.1 min if no internal standard available or 0.01% relative retention time if internal standard available ■ Record at least 3 ions in MS or 2 in MS/MS (S/N ≥ 3 in all cases) ■ MS ion tolerance: 30% (relative)	16, 17

(*Continued*)

Table 1 (*Continued*)

Source	Application field	General performance	Chromatographic/mass spectrometric guidelines	Reference
WADA (World Antidoping Agency)	Doping	■ LOD: same as qualitative LOD ■ Qualitative method LOD: level at which the false negative rate is ≤5%	■ Retention time tolerance: 0.5% if no internal standard available or 0.5–1% relative retention time, depending on the nature of the internal standard ■ Record at least 3 ions in MS or 2 in MS/MS (S/N ≥ 3 in all cases) ■ MS ion tolerance: 5–10% (absolute) or 20% (relative), depending on ion relative intensity. If more than the required ions are registered, all should meet these criteria	18, 19
SWGTOX (Scientific Working Group for Forensic Toxicology)	Toxicology	■ Trueness: should typically be in the 80–120% range. Assessed with 3 different matrices, spiked at 3 different levels; $n = 5$ ■ Precision: RSD < 20%. Assessed as trueness, but intrarun and interrun precision needs to be addressed ■ LOD: several possibilities (from noise, spiked samples/blank SD, lowest calibrator or decision limit, among others). Assessed with 3 different matrixes and on different independent runs ■ LOQ: several possibilities (lowest calibrator or decision limit or set to the lowest validation level where performance is met). Assessed with 3 different matrixes and on different independent runs ■ Qualitative method LOD: as LOD ■ Selectivity: investigate isotopically labeled potential interferences. Also check 10 different matrixes free of analytes and samples spiked with potential interferents ■ Stability: check for freeze-thaw and autosampler stability ■ QA: check for carryover by analyzing a blank sample after a high-concentration sample ■ Calibration: at least 6 different concentrations, matrix-matched calibration encouraged	■ Check matrix effects with 10 different matrices at two concentration levels; $n = 2$. If enhancement/suppression >25%, verify the impact on LOD or LOQ	20
US EPA Method 1694	Pharmaceuticals and personal care products in water, sediment, and biota	■ Trueness: quite variable depending on target compound (up to 8–180% range). Assessed with spiked reagent water; $n = 4$. Range in QCs highly variable and in verification standards 70–130% ■ Precision: quite variable depending on target compound (up to 71% RSD). Assessed with spiked reagent water; $n = 4$. Range in QCs highly variable ■ LOD: defined for S/N ≥ 2.5 ■ Blanks should be free of analytes or the analytes' signal should be <1/3 of reporting level ■ QA: spiked samples analyzed quarterly. In each batch/sequence inject a verification solution, blank, and spiked sample and check for internal standard recovery ■ Calibration: internal standard method; $n = 5$ levels	■ Retention time tolerance: 15 s ■ Record 1 MS/MS transition	23

Abbreviations: CRM, certified reference material; GC, gas chromatography; HRMS, high-resolution MS; ILIS, isotopically labeled internal standards; LC, liquid chromatography; LOD, limit of detection; LOQ, limit of quantification; MS, mass spectrometry; MS/MS, tandem MS; NA, not applicable; QA, quality assurance; QC, quality control sample; RSD, relative standard deviation; SD, standard deviation; S/N, signal-to-noise ratio.

applications. Importantly, the European SANTE (originally SANCO) guidelines for pesticide residues were initially derived from EU Directive 96/23/EC, but they have been evolving much faster over time (with >10 revisions) and are likely better adapted to current methods (15). The US Environmental Protection Agency (EPA) Method 1694 (23) is also included here as an enforcement method dedicated to CECs that also contains validation guidelines.

Some of these guidelines only cover generic issues (e.g., trueness), while others consider chromatographic-MS detection particularities. The main features on method validation are discussed below.

Trueness/Accuracy and Precision

Trueness/accuracy and precision are key parameters included in all method validation schemes. They are normally assessed together with the same experiments. Regarding trueness, most guidelines give the range of acceptable recoveries, which varies greatly from a minimum of ca. 40–80% (sometimes even lower) to a maximum recovery of 120–180%. These intervals are, however, indicative, as most guidelines assume that certified reference materials (CRMs) are available, but this is not the case in the analysis of OMPs in water in most cases. For instance, EU Directive 96/23/EC establishes much narrower criteria for CRMs (90–110%) than for spiked samples (50–120%). Similarly, the SANTE guidelines consider a general interval of 70–120% but allow 30–140% as far as the precision, expressed as relative standard deviation (RSD), is lower than 20%. Under certain circumstances, the concentrations in samples can be corrected by a recovery factor, when recoveries are outside 80–120% (15). This is an important point, i.e., whether concentrations need to be corrected by recovery, which is not always clearly discerned in the scientific literature, and is not covered in many guidelines. In the case of precision, guidelines also have different criteria, but 20–30% seems to be most frequent recommendation (15, 20, 22).

Besides the allowed interval, a further important point is the number of samples and replicates and the diversity of matrices to be assessed. These again vary greatly across the different guidelines. The US EPA Method 1694 (enforcement method for CECs in water) considers that trueness can be assessed with spiked reagent water ($n = 4$ replicates), provided that isotopically labeled internal standards (ILIS) are measured within each batch and that accuracy is reevaluated quarterly. Other guides are more exigent and require three concentration levels and the analysis of 10 replicates of each spiked sample (**Table 1**), while others establish the lowest spiked level as the limit of quantification (LOQ) or close to it (e.g., twice the LOQ) (16, 17) and/or require that up to three different matrices be investigated (20). Furthermore, in the case of precision, some standards (20, 22) consider it necessary to evaluate interrun/interday (intermediate) precision, not only intrarun (repeatability) precision, and have different acceptable ranges for both parameters.

The different criteria applied in the guidelines can be understood considering the specific applied fields. For example, some have legal implications and must meet reporting levels; in residue or toxicological analysis, it is relatively easy to obtain sample matrices free of analytes, which may not be so easy to do in environmental samples; and enforcement methods are normally linked to a large number of samples analyzed, therefore mitigating the cost of intensive validation approaches, but this may not always apply to the analysis of CECs in environmental samples.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and LOQ are relevant parameters included in all method validations. In general purpose guidelines, LOD/LOQ are normally set to the International Union of Pure and Applied Chemistry (IUPAC) definition relying on the standard deviation of a blank sample. However, this is not easy to assess with chromatography-MS detection. Some other standards,

such as SWGTOX, allow different protocols, which fit better with several analytical techniques. Guidelines linked to MS detection and most research articles normally utilize the signal-to-noise (S/N) ratio, where the LOD is typically set to S/N = 3 and LOQ to S/N = 10. Surprisingly, US EPA Method 1694 sets the LOD to S/N = 2.5 with only one MS/MS ion being recorded, which may be far from optimal. Conversely, the US Pesticide Data Program guidelines establish a S/N ≥ 3 for all ions at the LOD (not only for the quantifier ion), therefore guaranteeing a solid confirmation of identifications. Other guidelines consider the LOQ as the lowest calibrator, whereas a more exigent criterion is applied in SANTE, which sets the LOQ to the lowest concentration that meets all validation criteria (e.g., trueness and precision). Furthermore, SWGTOX indicates that LOD and LOQ need to be calculated with at least three different matrices.

Calibration and Quantification

Many analytical methods use calibration in solvent or matrix through a linear regression model. However, guidelines do not usually require linear calibration and allow other mathematical models as long as the number of points used is high enough (this varies from ca. 5 to 8). They recommend visual verification of the fitting or using statistical tools (e.g., residual analysis, lack of fit) rather than considering only the value of R or R^2. Several guides encourage the use of ILIS and matrix-match calibration, or standard addition, also permitting the use of single-point calibration if the concentration in the sample does not differ more than ca. 30% from the standard. Another requirement is that the calibration range should begin at (or near) the LOQ and span a range broad enough to cover the analyte concentrations in samples. Importantly, several guidelines require the calibration integrity to be checked, through the repeated injection of standards, along the analysis sequence (16, 17, 20, 21).

Robustness/Ruggedness

Robustness (ruggedness) refers to the capacity of an analytical method to remain unaffected by small variations in method parameters, thus providing an indication of the method's reliability during normal usage, for example, due to small changes in temperature, compositions of eluents, or, most importantly, to matrix variability. Although robustness is commonly underinvestigated in studies, several guidelines include the evaluation of this parameter by either testing different alterations in the method or validating the method trueness, precision, and sometimes LOD/LOQs with different matrixes. This last approach seems highly convenient in the case of chromatographic-MS analysis, particularly in LC-MS applied to environmental samples, owing to the relevant and variable matrix effects (see the subsection titled Matrix Effects below).

Selectivity and Specificity

This term refers to the capacity of an analytical method to not lead to false positives related to the presence of other chemicals that are relatively similar to the analyte in the samples. Some standards [e.g., Eurachem or SWGTOX (20, 21)] require that method performance is assessed with samples spiked with those potential interferents. Indeed, this is more relevant to nonselective detectors, but it should not be overlooked in MS, particularly because of potential cross talk from ILIS that produces some signal in the mass-to-charge ratio (m/z) ions of the native substances and signals from the matrix itself (20).

In any case, in LC-MS/MS methods, the presence of so-called visible interferent compounds is uncommon in samples due to the use of selective/specific MS/MS transitions. However, the presence of coeluting compounds may affect the ion intensity ratio if, for example, one of the transitions has interference, which increases the risk of reporting false negatives because of

the noncompliance of ion ratios. This problem is particularly relevant for unspecific transitions (e.g., neutral loss of H_2O), which should be avoided as far as possible (15).

In contrast to other fields (20), in chromatography-MS analysis of OMPs, it is complicated to evaluate selectivity/specificity due to the inherent characteristics of these techniques and the unknown variable composition of the environmental samples. Therefore, this parameter is not usually evaluated.

Stability

Some guidelines include the evaluation of reference standard and sample stability at different temperatures (14) or during freeze-thawing (20). Although stability of the analytes in samples and standard solutions is essential for producing accurate results, this parameter is not always sufficiently studied. The most common way to prevent potential degradation of environmental samples, particularly water, is freezing (e.g., $<-18°C$). Another strategy is the addition of preservatives (e.g., ascorbic acid to quench chlorine or sodium azide to hamper biological processes). Acidification of the sample is also a common practice, but this must be performed with care, as it may lead to the conversion of some acid analytes to neutral species, which are more hydrophobic and might be sorbed to, for example, sample containers. Filtration is another critical step that needs to be investigated, particularly for relatively hydrophobic analytes (24, 25).

Whenever acidification and other strategies to mitigate degradation are not fruitful, an alternative is to perform a rapid or in situ extraction of the compounds. This can be done through, e.g., solid phase extraction (SPE), and then preserving the SPE (or other extractant) material instead (26).

Blanks

Most guidelines indicate the need to check that blanks are free of analytes or to confirm that analyte concentrations are lower than the reporting level or LOQ. The possibility of blank-related problems depends strongly on the nature of the target analytes and can be relevant in plastic-related compounds (such as phthalates or organophosphate flame retardants), per-/polyfluoroalkylated substances (PFAS), and hydrophobic analytes due to cross-contamination (27, 28). Therefore, blanks should always be investigated in order to design strategies to minimize their impact on method performance, particularly on key parameters such as method LOD/LOQ, when true blanks are not available. In such cases, the LOD/LOQ can be calculated from the standard deviation of the blank and not from S/N in MS analysis.

Matrix Effects

Matrix effects are not always covered as such in guidance documents but intrinsically considered when validation is performed in several matrices. Although this problem can occur with other analytical techniques, it is especially relevant in LC-MS(/MS) methods due to the inherent mechanism of atmospheric-pressure ionization techniques, which results in signal suppression and less commonly in signal enhancement (29). Matrix effects greatly affect quantification if not efficiently eliminated or corrected. Produced by unknown matrix components that affect ionization of analytes, matrix effects can be evaluated by comparison of either the analyte signal when prepared in solvent and in matrix or calibration slopes in solvent and matrix. High concentrations of analytes in the original blank matrix can complicate this assessment, but this may be circumvented by using ILIS of the analytes as a proxy for matrix effect evaluation.

Several strategies can be used for matrix effect correction, reduction, and elimination. Examples include matrix-match calibration, the method of standard additions, application of cleanup steps, sample dilution, and the use of analyte-ILIS (30).

Although less common, matrix effects may also occur in GC-MS analysis (31, 32). These are more frequently encountered in the analysis of vegetable or biota samples, rather than aqueous samples because the matrix blocks injector active sites. This results in signal enhancement, which needs to be tackled in ways similar to those mentioned for LC-MS or by using analyte protectants (31). Luckily, in this case, LOD/LOQ are seldom negatively affected but are instead improved.

Identification: Chromatography

A critical point in any analytical method is granting confidence in the identification of analytes. In chromatography-based methods, identification is normally associated with retention time (Rt), supported by further evidence provided by the detector (typically MS information). Thus, it is necessary to establish which Rt deviation is acceptable when comparing standards and samples because the sample matrix may affect this parameter. Some guidelines set up a 0.01–2.5% variation on Rt or, preferably, relative Rt (to an internal standard). A 0.1-min threshold is also applied instead of relative deviation, but the use of internal standards is encouraged so that relative Rt can be calculated, as the internal standard can help identify Rt shifts due to the sample matrix. In addition, the EU Directive 96/23/EC (14) indicates that the analytes need to have a retention factor ≥ 1, i.e., an Rt at least twice the dead time. Although this is not essential in other guidelines, it is a good practice in LC-MS whenever possible. This is because the coelution of analytes with nonretained substances induces severe matrix effects, which may impair method performance.

Identification: Mass Spectrometry

Rt itself is not sufficient for a positive identification, and today confidence is gained by selective MS detection. The EU Directive 96/23/EC (14) established one of the first guidelines on the number of ions to be measured and the tolerances for ion ratios, depending on the particular technique used (HRMS, low-resolution MS, or MS/MS). Most current guidelines require at least three ions in single-stage MS methods or two transitions in MS/MS analysis. The ion ratio tolerance when comparing standards and samples differs in the different guidelines, as some provide a wider range for lower-intensity ions, while others establish a maximum deviation of 30% in all cases. Surprisingly, the US EPA Method 1694 only requires that one MS/MS transition be recorded (23). Indeed, this is certainly not enough, and updating this method from 2007 is required. However, ion ratios are not deemed important when a scanning HRMS technique is used to record full MS(/MS) spectra. In such cases, at least two ions with a maximum mass error of 5 ppm (1 mDa for small ions) are required. Alternatively, a library matching can be used instead of using ion ratios whenever the full spectrum is recorded (15).

Validation of Screening and Qualitative Methods

Screening and qualitative methods are especially useful in environmental analysis, particularly HRMS, which allows searching a large number of compounds in samples. Validation of these methods is not an easy task, particularly when a large list of compounds is included in the search. Method validation must ensure the robustness of the screening, independently of the samples' origin and matrix sample variability, as well as the reliable identification of the compounds down to certain concentrations. Thus, the screening LOD is the key parameter in validating screening methods. This is established as the lowest level tested for which the reliable detection and identification (i.e., it must also fit the criteria of identification) of an analyte are empirically demonstrated in at least 95% of the samples included in validation. This requires the analysis of typically 10–20 spiked samples (14, 15, 21).

Different approaches can be applied in HRMS-based screening, such as target (utilizing reference standards), suspect (using large databases of suspect compounds for the search and without standards), and nontarget (without apparent restrictions in the search of compounds, which do not necessarily have to be included in a suspect list) analysis. Validation of a wide-scope screening is, in principle, only possible in the target approach. It should be aimed at demonstrating the applicability of the screening to a large list of target compounds using reference standards, empirically establishing the screening LODs, and ensuring that the compounds detected are also correctly identified (33–39). A recent norm developed in The Netherlands dedicated to nontarget screening may also be useful if it reaches further agreement at the international level (12).

Suspect and nontarget approaches allow the tentative identification of the compounds, which should be confirmed in a subsequent step through a comparison with reference standards. Several tools allow higher confidence in tentative identifications, such as Rt or MS/MS spectra prediction (40–43). For example, ion mobility separation (IMS) in combination with HRMS makes the identification process easier and more secure in both target and suspect screening (44).

ENSURING METHOD PERFORMANCE DURING NORMAL OPERATION: THE IMPORTANCE OF QUALITY CONTROL

An essential step in addition to method validation is to regularly check for method performance during its application (e.g., for monitoring purposes). Although not all guidelines consider this topic, the most common practice is including the following in each sample batch: standards injected along the sequence to check signal stability, blanks to check for (cross-) contamination, and spiked individual or pooled samples, typically at or near the LOQ or decision limit, to check for method overall performance. However, the tolerance for deviations from the initial validation figures is not always specified or is highly variable.

A key question is whether the samples used in validation are representative of those that will be analyzed in subsequent studies. In other words, it is unclear whether the validation process can be considered reliable enough if, for example, only one sample (although analyzed in quintuplicate) was used for such validation. The answer seems rather clear: A single validation, even if the figures of merit are fully satisfactory, is not sufficient. The analytical method needs to be tested periodically in a variety of samples to ensure its correct application in environmental studies.

Ensuring the quality of data is commonly achieved by the analysis of quality control samples (QCs) included in the sample batch. QCs can be prepared from blank real-world samples spiked at different analyte concentrations. Ideally, a large number of QCs should be analyzed to obtain a good picture of the analytical problems affecting the samples included in a given study.

The use of real-world samples for the preparation of QCs and evaluation of matrix effects implies some difficulties, for example, finding a representative blank sample to be spiked. This is quite evident when monitoring PhACs or some illicit drug biomarkers in urban wastewater, as these compounds are commonly present in all the samples analyzed, sometimes at high concentrations. The fact that blanks used for QC preparation contain the compounds under study complicates the calculation of recoveries at low analyte concentrations, close to the LOQ. Therefore, it complicates not only the initial method validation but also the subsequent quality control at low concentrations (discussed further below).

CURRENT RESEARCH PRACTICES OUTLINED IN THE SCIENTIFIC LITERATURE

Even if we limit the search to publications related to OMPs in environmental samples, there are many articles covering method validation and/or reporting analyte concentrations, usually at

sub-μg/L levels. These ultratrace analyses in complex matrices may be affected by important errors, and, therefore, method validation and quality control are critical aspects to ensure reliable analysis. A critical review of the specialized literature may allow us to establish whether enough data on this issue exist and to discuss the essential parameters required to support the data reported. To address these issues, we reviewed articles published over five years (from 2017 to 2021), focusing on information on analytical validation figures, quality control/assurance, and data reporting.

The Scopus electronic database (Elsevier) was selected for the literature search. That was aimed at quantitative methods based on chromatography-MS analysis used to determine CECs, particularly PhACs and illicit drugs, as well as pesticides, as they are widely monitored and reported in the aqueous environment and may well represent other OMP analysis scenarios. The search query string used was [TITLE-ABS-KEY (mass AND spectr*) AND TITLE-ABS-KEY (wastewater) OR TITLE-ABS-KEY (surface AND water) OR TITLE-ABS-KEY (ground AND water) AND TITLE-ABS-KEY (pharmaceuticals) OR TITLE-ABS-KEY (pesticides) OR TITLE-ABS-KEY (antibiotics) OR TITLE-ABS-KEY (drugs AND of AND abuse) OR TITLE-ABS-KEY (illicit AND drugs) AND TITLE-ABS-KEY (determination) OR TITLE-ABS-KEY (quantification) OR TITLE-ABS-KEY (validation)] AND [LIMIT-TO (PUBYEAR, 2022) OR LIMIT-TO (PUBYEAR, 2021) OR LIMIT-TO (PUBYEAR, 2020) OR LIMIT-TO (PUBYEAR, 2019) OR LIMIT-TO (PUBYEAR, 2018) OR LIMIT-TO (PUBYEAR, 2017)].

The Scopus search (December 27, 2021) yielded 528 published articles. A first filter was applied, evaluating titles, abstracts, and conclusions and dividing the publications into four groups: (*a*) priority papers that provide information regarding method validation and/or references to previous validation supporting the analytical data provided (210 papers, 40% of articles searched); (*b*) papers reporting analyte concentration data without information on the method validation or previously developed method (128 papers, 24%); (*c*) off-topic publications (e.g., reviews, nonaqueous matrices, different analytical techniques) (153 papers, 29%); and (*d*) papers with limited access and/or in a different alphabet (37 papers, 7%).

As illustrated, approximately 25% of the publications (group 2) provided only concentration data of OMPs without any analytical evidence or support. The 210 papers included in group 1 were carefully reviewed and evaluated according to the information available on method validation and quality control. First, information was collected on compounds included in the study, the matrix analyzed, sample treatment, and the chromatography-MS technique used. Second, a score rank (**Supplemental Table 1**) was proposed for scoring (*a*) the validation strategy that was applied, (*b*) support to the data provided and the value of analytical information, (*c*) the depth of discussion on analytical issues, (*d*) how the LOQ was established, and (*e*) the level of quality assurance. Between 1 and 3 points were assigned to each topic evaluated, except for the validation strategy, where up to 6 points could be reached as a function of the information available. The scoring system, similar to that applied by others (45), must be understood as a global overview of how different issues affecting the subject treated are considered in the scientific literature. A higher score does not necessarily imply a higher quality of the paper, but it does mean that the article includes more analytical information and/or is more focused on analytical issues and provides more support to the data reported.

When information was available, the validation strategies seemed to have been well designed, according to the issues highlighted in this review. Approximately 90% of papers from group 1 obtained at least 13 points, i.e., ≥50% of the maximum number of points, with an average of 16–17, but only 13% got at least 21 points, i.e., ≥80% of the maximum score. Owing to space limitations, only the references with the highest scores are included in **Table 2** (**Supplemental Table 2** contains the full list of reviewed publications). In these papers, more emphasis is given to analytical issues, which are treated in more detail below.

Supplemental Material >

Table 2 Detailed information and scoring for each item of reviewed publications (2017–2021) with a score of 19 points or higher

Reference	Compounds	Sample type	Sample treatment	Technique	Validation strategy	Validation data	Analytical evidence	Analytical discussion	Quantification strategy	Identification criteria	LOQ establishment	QCs in batch	Score
47	Antib	SW	DI	LC-MS/MS	4	3	3	3	3	3	3	2	24
59	Chlorothalonil metabolites	GW	DI	LC-MS/MS	4	3	3	3	3	1	3	1	21
60	OMPs	WW	DI	LC-MS/MS	4	3	3	3	2	3	3	2	23
61	OMPs	WW	DI	LC-MS/MS	4	3	3	2	3	3	3	1	22
62	OMPs	WW	DLLME	GC-MS	4	3	3	2	2	2	2	1	19
63	Antipsychotics	WW	DLLME, SPE	LC-MS/MS	4	3	2	2	2	3	3	2	21
64	OMPs	SW, GW, TW	Evaporation	LC-MS/MS	4	2	3	2	3	3	1	1	19
65	OMPs	WW	HS-SPME	GC-HRMS	4	3	3	3	2	3	3	1	22
66	Pharm	SW	LTPE	LC-MS/MS	4	3	3	2	2	3	3	1	21
49	Pest	SW, WW	MSPE	LC-MS/MS	4	3	2	3	2	3	2	2	21
67	DOAs	WW	POCIS	LS	4	3	3	2	3	1	1	2	19
68	Antib, pest, pharm	WW	QuEChERS	LC-MS/MS	4	3	2	3	2	3	2	1	20
69	Captan	WW	QuEChERS	GC-MS	4	3	3	1	3	2	2	1	19
70	OMPs	SW, DW	SBSE	LC-MS/MS	4	3	2	3	2	2	2	1	19
71	Antib	GW, SW, WW	SPE	LC-MS/MS	4	3	2	3	3	2	3	1	21
72	Antib	WW, SW	SPE	LC-MS/MS	4	3	3	2	3	2	3	1	21
73	Antib	SW, GW, WW	SPE	LC-HRMS	4	3	2	3	3	2	2	1	20
74	Antib	WW	SPE	LC-MS/MS	4	3	3	2	2	3	2	1	20
75	Antib	SW	SPE	LC-MS/MS	4	3	2	2	2	3	3	1	20
76	Antib	WW, SW	SPE	LC-MS/MS	4	2	3	3	3	2	2	1	20
77	Antib	WW	SPE	LC-MS/MS	4	3	2	1	3	3	2	1	19
78	Antib	SW	SPE	LC-MS/MS	4	3	3	3	1	1	3	1	19
48	Benzodiazepines	SW	SPE	LC-MS/MS	4	2	2	1	3	3	3	2	20
79	DOAs	WW	SPE	LC-HRMS	4	3	2	3	3	3	3	2	23
80	DOAs	WW	SPE	LC-MS/MS	4	3	2	2	3	1	2	2	19
81	DOAs	WW, SW	SPE	LC-MS/MS	4	3	3	2	3	1	2	1	19
82	DOAs and Pharm	WW	SPE	LC-MS/MS	4	3	2	3	2	2	2	1	19
83	Drugs	WW	SPE	LC-MS/MS	4	3	3	3	3	2	2	1	21
84	Drugs	SW	SPE	LC-MS/MS	4	3	3	2	3	2	2	2	21
85	EC	WW, DW	SPE	LC-HRMS	4	2	2	3	2	3	2	1	19
86	EC	WW	SPE	LC-HRMS	4	2	2	1	2	3	2	3	19
87	EC	DW	SPE	LC-MS/MS	4	3	2	2	3	2	1	2	19
88	EC	SW	SPE	LC-MS/MS	4	1	2	3	3	1	2	3	19
50	*N*,*N*-dimethyl-amphetamine	WW	SPE	LC-HRMS	5	3	2	3	3	2	2	1	21

(*Continued*)

Table 2 (*Continued*)

Reference	Compounds	Sample type	Sample treatment	Technique	Validation strategy	Validation data	Analytical evidence	Analytical discussion	Quantification strategy	Identification criteria	LOQ establishment	QCs in batch	Score
89	NPS	WW	SPE	LC-MS/MS	4	3	2	2	3	3	3	2	22
90	NPS	WW	SPE	LC-MS/MS	4	3	3	3	3	2	2	1	21
91	NPS	WW	SPE	LC-MS/MS	4	3	3	2	3	2	2	1	20
92	NPS and DOAs	WW	SPE	LC-MS/MS	4	3	3	3	3	2	2	2	22
25	OMPs	WW	SPE	LC-MS/MS	4	3	3	2	3	2	3	1	21
93	OMPs	DW, SW, WW	SPE	LC-MS/MS	4	3	2	2	3	2	2	1	19
94	OMPs	WW	SPE	LC-MS/MS	2	3	3	3	1	2	3	2	19
95	OMPs	WW	SPE	LC-MS/MS	4	3	2	2	3	2	2	1	19
96	Opioids	WW, SW	SPE	LC-MS/MS	4	3	3	2	3	2	2	1	20
97	Pest	SW	SPE	LC-MS/MS	4	3	2	3	3	3	2	1	21
46	Pest	SW	SPE	LC-HRMS	4	3	2	3	1	3	2	2	20
98	Pest	SW	SPE	GC-MS/MS	4	3	2	1	3	3	2	2	20
99	Pest	WW	SPE	LC-MS/MS	4	2	3	2	2	3	3	1	20
100	Pest	SW, DW	SPE	LC-MS/MS	2	3	2	3	2	3	2	2	19
53	Pest	SW	SPE	LC-MS/MS	4	3	2	3	2	1	3	1	19
101	Pharm	WW	SPE	GC-MS	4	3	3	3	2	3	2	3	23
102	Pharm	WW	SPE	LC-MS/MS	4	3	2	3	3	3	3	2	23
103	Pharm	WW	SPE	LC-MS/MS	4	3	2	3	3	3	2	2	22
104	Pharm	SW	SPE	LC-MS/MS	2	3	3	3	3	3	2	2	21
105	Pharm	SW	SPE	LC-MS/MS	4	3	3	1	3	3	3	1	21
106	Pharm	WW, SW, DW	SPE	LC-MS/MS	4	3	3	3	2	2	2	1	20
107	Pharm	SW, GW	SPE	LC-MS/MS	4	3	3	2	3	2	2	1	20
108	Pharm	WW, SW, DW	SPE	LC-HRMS	2	3	3	3	3	2	2	1	19
109	Pharm	WW	SPE	LC-MS/MS	4	3	2	3	3	1	2	1	19
110	Pharm	WW	SPE	LC-MS/MS	4	3	3	2	2	2	2	1	19
111	Pharm	WW, DW	SPE	LC-MS/MS	4	3	2	3	1	2	3	1	19
112	PPCPs	SW	SPE	LC-MS/MS	4	3	2	3	1	3	2	2	20
113	OMPs	SW	SPME	GC-HRMS	4	3	2	3	3	2	3	1	21
114	OMPs	WW	SPME	LC-MS/MS	4	3	2	2	3	3	2	2	21
115	OMPs	SW	VALLME-SFO, DLLME-SFO	LC-MS/MS	4	3	3	3	1	3	3	2	22

Abbreviations: Antib, antibiotics; DI, direct injection; DLLME, dispersive liquid–liquid microextraction; DOAs, drugs of abuse; DW, drinking water; EC, emerging contaminant; GC, gas chromatography; GW, groundwater; HRMS, high-resolution MS; HS, headspace; LC, liquid chromatography; LOQ, limit of quantification; LTPE, low-temperature partitioning extraction; MS, mass spectrometry; MS/MS, tandem MS; MSPE, micro-solid phase extraction; NPS, new psychoactive substance; OMPs, organic micropollutants; Pest, pesticide; Pharm, pharmaceuticals; POCIS, polar organic chemical integrative sampler; PPCPs, pharmaceuticals and personal care products; QC, quality control sample; QuEChERS, quick, easy, cheap, effective, rugged, and safe; SBSE, stir bar sorptive extraction; SFO, solidification of floating organic droplets; SPE, solid phase extraction; SPME, solid phase microextraction; SW, surface water (including seawater); VALLME, vortex-assisted liquid–liquid microextraction; WW, wastewater.

As mentioned above, a critical issue when evaluating the validation protocols is the lack of guidelines for environmental analytical chemistry. Some papers used analog guidelines such as those from SANTE (46, 47) or the Commission Decision 2002/657/EC (48, 49), whereas most publications used arbitrary validation requirements and/or those previously published in the scientific literature. The use of spiked samples to perform method validation is a common standard due to the lack of CRMs for OMPs in water samples. Only one publication mentions the use of an interlaboratory study for the validation of an analytical method (50). Regarding the use of spiked samples, two different groups of studies were identified: those that used an authentic matrix for performing method validation, which obtained 4 points for validation strategy, and those that used ultrapure water, drinking water, or a different water for method validation, which obtained 2 points. The lack of CRMs inferred that the maximum score of 6 points for validation strategy could not be achieved.

Although most papers provide validation data in the main text (3 points for validation data), approximately 19% of publications move this information to the supplemental information (2 points) and focus on concentration data obtained and their evaluation. The remaining 8% referred to previous publications dealing with validation (1 point). Regarding the discussion of validation data, only 20% of publications provide a detailed interpretation of results (3 points for analytical discussion), and approximately 50% provide only general comments (1 point). This is a pivotal fact, particularly when some compounds are not satisfactorily validated, when critical problems or steps are observed, or where a detailed analytical discussion would be required to fully understand and interpret the reported data. To support the data obtained, only 22% of the papers provide visual evidence such as chromatograms and/or spectra at the established LOQ or near the LOQ or support the specificity or compound identification (3 points for analytical evidence). A total of 52% of publications provide only limited specific data (2 points), and the remaining 26% only provide summarized results (1 point). Note that several articles focus on the development of new materials or devices for sample treatment, such as SPE sorbents (51–53), solid phase microextraction [SPME (54, 55)], or novel methodologies such as bag-based liquid-phase microextraction (56). Some of these publications do not include validation data and/or do not provide a detailed discussion on the validation results, even though this information is critical to demonstrate the applicability of the new material.

The use of ILIS seems to be the gold standard in chromatography-MS analytical methods. Nearly 50% of the reviewed studies used ILIS for correction of both sample treatment errors/losses and matrix effects (3 points for quantification strategy). Another 18% used matrix-matched standards (2 points), although the limitations of this approach in environmental analysis are scarcely discussed. Finally, 35% of the studies used solvent calibration and/or the standard preparation for quantification was not specified (1 point).

It is remarkable that a key aspect such as compound identification is usually not described or scarcely discussed in regard to method validation and/or method application. For example, more than 40% of publications do not specify the criteria used to consider a compound as identified (1 point in identification criteria). Another 38% use arbitrary criteria indicated in the publication (2 points), and only 20% refer to a guideline (3 points) independent of whether it applies to the addressed topic.

Regarding LOQ establishment, only 14% of the publications used the most restrictive criteria, considering the LOQ as the lowest validated level in terms of accuracy and precision (3 points for LOQ establishment). Many papers (54%) used statistical estimation, considering the LOQ as a peak with S/N = 10 in a sample spiked close to the estimated LOQ (2 points). Approximately 30% used the same criterion but from samples spiked at much higher levels and/or in pure solvent (1 point).

One of the main weak aspects detected in our literature survey is the lack of quality control in the methods application. For example, 78% of the publications do not mention the use of QCs (1 point for QCs analyzed in batch) for assuring the quality of results and to support method reliability when applied to real-world samples. Another 20% mentioned the use of QCs during sample analysis (2 points), and only 2% (4 out of 210) showed and discussed the results on QCs included in the sample batch (3 points). Although some studies involved new materials for sample treatment (e.g., SPE sorbents) and method development, information on the analysis of QCs and acceptability criteria would also be of great interest if the method was applied to samples.

CRITICAL ISSUES AND ANALYTICAL CHALLENGES

Now that the relevant guidelines have been discussed, and in light of the literature reviewed, several key issues and analytical challenges can be highlighted in relation to method validation and subsequent application. The main topics can be summarized as follows.

1. There is a general lack of specific guidelines in environmental analytical chemistry, which leads to the application of criteria from other guidelines [e.g., EU Directive 96/23/EC or SANTE for (pesticide) residue analysis]. But these criteria do not necessarily consider the specifics of the analysis of OMPs in the (aquatic) environment.
2. Little or no regulation exists on most OMPs in waters. Although it has improved in the last five years, the existing regulation is usually limited to a few compounds, which are not necessarily representative of the whole group of OMPs. The absence of reference values implies difficulties in establishing key parameters such as LOQs (or LODs). This may lead to an unreasonable competition between reporting the most sensitive methods ever published and the lowest LODs or LOQs ever achieved. In some cases, there would be serious doubts about whether those values are realistic and achievable in day-to-day research.
3. There are numerous compounds of potential interest, possessing quite different physicochemical characteristics. An additional difficulty is that no universal methods exist; therefore, a combination of different methodologies is necessary to cover the task of determining such a wide group of compounds. Although many multiclass methods based on LC-MS/MS and GC-MS/MS already exist, some compounds cannot be determined using those methodologies owing to their special characteristics, such as high polarity (e.g., persistent mobile organic contaminants) or volatility, which require differential and sometimes individual methods (57).
4. Matrix composition of environmental samples is highly variable, which leads one to question whether a method validated in a given sample is applicable to other samples, even those of the same type (e.g., surface water or wastewater) but from different origins and locations.
5. It is difficult to acquire an elevated number of reference standards. There are also the associated problems derived from the high cost, expiration date, and lack of commercial reference standards (e.g., metabolites, ILIS and TPs).
6. Real-world representative blank samples are lacking. This makes method validation and preparation of QCs troublesome, particularly at low concentrations (e.g., near the LOQ). This situation usually occurs for many pharmaceuticals in wastewater.
7. There is a lack of CRMs for method validation, an approach that cannot be easily used for OMPs in the aquatic environment. Although a trend toward the organization of interlaboratory tests is observed, such tests are still limited to a few target analytes.

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In addition to the abovementioned general aspects of method validation and subsequent application, **Supplemental Table 3** summarizes critical questions specifically related to each parameter

evaluated. The issues highlighted in the table are commonly questioned by analytical chemists involved in this type of analysis and could be used for further discussion among specialists.

A PROPOSED PROTOCOL FOR METHOD VALIDATION AND TO SUPPORT THE QUALITY OF DATA IN ENVIRONMENTAL ANALYSIS

In this section, a thoughtful proposal is made for an efficient method validation and to support the quality of data reported in the analysis of OMPs in the (aquatic) environment. **Table 3** summarizes our proposal for method validation, distinguishing between the minimum requirements and an ideal situation, which would be balanced depending on the number of samples to be measured (e.g., a new method demonstration may not be as exigent as one used in regular monitoring) and the objectives of the study. Further key issues related to every parameter evaluated and relevant remarks on the function of the complexity and particularities of each parameter (e.g., problems of high concentrations of blank samples, deviations in the criteria established, calculation problems) are discussed in more detail in **Supplemental Table 3**. The summarized information in **Table 3** and complete information in **Supplemental Table 3** comprise a proposal that can be refined upon discussion with the scientific community.

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When reporting concentration data, researchers should find a robust approach to support the quality of data reported. For example, they should consider key aspects related to the quantification and identification of OMPs, the objective of the analyses, the legal requirements, if applicable, and requirements of the journals where their papers are published. Reporting large data sets of compounds quantified in samples with little or no analytical information may generate doubts about these data. In some cases, the compounds are problematic from an analytical perspective, and with the minimal information provided, the reliability of determinations may be questioned. For other researchers to trust the data, it is essential to provide key examples (e.g., chromatograms and mass spectra) of problematic compounds, support the proposed LOQs, and clearly specify the criteria used for the quantification, identification, and acceptance of data. Researchers must be aware that their data can be used for environmental risk assessment, to evaluate trends across time and space, and to establish legislation or control measures.

Next, some recommendations are given for the support of data reported from an analytical point of view.

- Method validation must be included in the article or the authors must make reference to such validation, considering the key issues discussed so that the reader can trust the values given for relevant parameters (e.g., LOQs).
- The criteria applied for the identification of compounds found in samples should be specified.
- Authors should include illustrative examples or figures of real-world positive samples found at low concentrations; if possible, these should be near the LOQ.
- Researchers should include illustrative examples or figures of real-world positive samples where the criteria for identification are applied (e.g., chromatograms corresponding to quantification and confirmatory ions, with information on ion ratios and deviations with respect to reference standards and QCs).
- QCs analyzed together with the samples should be included, indicating the criteria applied for acceptability of quantitative data. Authors should also report the recoveries in analysis of the QCs.
- Researchers should differentiate between identifications confirmed using reference standards and tentative identifications without the use of standards based on, e.g., matches with spectra libraries, the presence of several reported ions, or the interpretation of mass spectra.

Table 3 Proposal of guidelines for the validation of chromatographic-MS methods for OMPs determination in water samples. See Supplemental Table 3 for further details, examples, and remarks

Parameter	Minimal validation requirements	Optimal validation requirements	Acceptability criteria
Accuracy/trueness and precision	Perform validation by recovery experiments in real-world samples of the same type as those that will be subsequently analyzed, each spiked at two analyte concentrations (low between 1–10 times LOQ and high ca. 10–50 times the low level). To grant method robustness and performance with varying matrix composition, validation should be performed with at least 3 different samples of the same type, and the total number of analyses should be at least 6. Different combinations are possible (see **Supplemental Table 3** for examples).	Include another spiking level (medium concentration, in total 3 spiking levels). Increase the number of different spiked samples to at least 5 and the total number of analyses to at least 10.	Recoveries between 70 and 120% and overall RSD below 30%. In exceptional cases, average recovery outside 70–120% could be accepted if they are consistent (RSD ≤ 30%) and are ≥30% or ≤140%. In such cases, a correction factor as a function of the validation recovery and supported by QC recovery might be applied to the concentrations measured in samples. See **Supplemental Table 3**.
LOD and LOQ	Estimate LOQ and LOD in a water sample (from the same type that will be monitored later) spiked at analyte concentrations near the LOQ; the maximum should be 10 times the LOQ finally proposed. Confirm the identity of the compound at the level tested by acquiring at least 2 transitions (3 ions in single MS methods). Periodically test that at least the LOQ is attainable in daily work, analyzing QCs spiked at a level near (maximum 10 times higher) the LOQ.	Estimate the LOQ and LOD in 5 different samples and calculate the average value finally proposed as LOQ and LOD, also indicating the range. The ion ratio must be accomplished ensuring the reliable identification of the analyte at both the LOQ and LOD levels (maximum deviation 30%).	The estimation must be made from the chromatograms of spiked samples, corresponding to the quantification transition, based on a S/N = 10 (LOQ) or S/N = 3 (LOD). For the LOQ level, identification of the analyte must also be ensured. Thus, at least one qualification/confirmation transition must be also observed and the ion ratio deviation criterion accomplished (maximum deviation with respect to a reference standard ±30%). For the LOD, the chromatographic peak corresponding to the second transition must be observed (minimum) and the ion ratio accomplished (optimal).

(*Continued*)

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Table 3 (*Continued*)

Parameter	Minimal validation requirements	Optimal validation requirements	Acceptability criteria
Calibration	Perform calibration with standards in solvent (including the same ILIS as in samples), with at least one point below the concentration corresponding to the LOQ. The calibration should include at least 5 levels, and the standards' concentrations corresponding to the (two or three) levels should be validated and extended up to concentrations commonly found in the samples.	NA	Report the value of R^2 and either some visual evidence or data on residuals, lack-of-fit, or other tests. Nonlinear calibrations can be used, but they should be clearly mentioned and supported by appropriate information, as in linear calibration. One-point calibration can be used for estimative purposes (semiquantification) as long as this is clearly indicated and the concentration in the samples does not differ more than 30% from the calibrator's.
Evaluation of matrix effects Use of ILIS	Spike 3 different samples (in DI-based methods) or 3 different sample extracts (e.g., in SPE-based methods) at a medium concentration level and compare the measurement with a reference standard in solvent at the same concentration. Inject spiked samples/extracts and standards in quintuplicate and obtain the average response. Pay attention to the blank measurement in order to subtract its response in case the analyte under study is present in the blank sample. Another approach, but less useful in environmental studies, is comparing matrix-match calibration and calibration in solvent. Here, the difference in the slopes will indicate the matrix effects.	NA	Matrix effects are considered significant if they exceed ±20%. Thus, if the matrix effect is less than ±20%, no correction is, in principle, necessary. Nevertheless, the final recovery of the method, considering all aspects affecting the overall procedure, including matrix effects, will indicate whether some correction is required.

(*Continued*)

Table 3 (*Continued*)

Parameter	Minimal validation requirements	Optimal validation requirements	Acceptability criteria
Identification	Acquire at least 3 MS/MS transitions in MS/MS methods and obtain the ion ratios (normally using peak areas). One transition (named Q) will be used for quantification, and the rest (named q1, q2, etc.) will be used as confirmatory transitions. Obtain the average q/Q ratios (q1/Q, q2/Q, etc.) for the standards included in the calibration and use them as a reference when analyzing samples. Compare the ion ratios in samples with those of the reference standard and calculate the deviation. Acquire at least 3 ions in single MS methods, obtain the ion ratios, and compare with the reference standards. Acquire at least 2 accurate mass ions in HRMS methods. Calculate the Rt deviation with respect to the reference standard.	Acquire the maximum number of MS/MS transitions or ions, if feasible, to improve the identification in problematic cases (see remarks in **Supplemental Table 3**).	At least one ion ratio (q1/Q or q2/Q) in the sample must not exceed a deviation of ±30% with respect to the reference standard (e.g., average of the standards included in the calibration). Maximum error for accurate mass measured ions in HRMS methods <5 ppm (<1 mDa for an $m/z < 200$). Identification criteria also include the deviation in the chromatographic Rt, normally ±0.1 min or ±0.5% if relative to an ILIS.
Quality control	Prepare QCs at two concentration levels (low and high) in selected samples to be analyzed later (see **Supplemental Table 3** for the number of QCs to be prepared). When the samples used for QC preparation are not true blank samples (i.e., they contain the analytes at concentrations similar or higher than the spiked ones), the recovery calculation is compromised, and such QCs might be discarded. Subtracting the blank concentration from the spiked QC is compulsory, but this approach may not be successful in such cases. For this reason, only some QCs (normally those at high analyte concentrations) may be useful to support the quality of data.	Include a third concentration level (i.e., low, medium, high) and increase the number of samples used for QC preparation (see **Supplemental Table 3**).	Acceptability proposed for individual QC recovery is 60–140%. When recoveries are out of this range, the quantification is compromised. In such cases, concentration data might be reported as estimated, indicating the QC recoveries obtained for the compound or that the samples should be reanalyzed. When robust and reproducible recoveries are obtained, with low RSD (e.g., <20%), even if they are out of the acceptability range, a correction factor might be applied; this circumstance should be indicated in the report.

Abbreviations: DI, direct injection; HRMS, high-resolution MS; ILIS, isotopically labeled internal standard; LOD, limit of detection; LOQ, limit of quantification; MS, mass spectrometry; MS/MS, tandem MS; NA, not applicable; OMP, organic micropollutant; QC, quality control sample; RSD, relative standard deviation; Rt, retention time; S/N, signal-to-noise ratio; SPE, solid phase extraction.

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This mainly applies to HRMS-based methods, where a careful discussion on the (tentative) identification of the suspect compounds should be included in the publication. The nomenclature proposed by Schymanski et al. (58) is recommended.

- Authors should pay special attention to problematic cases and appropriately discuss these from an analytical perspective.

Some critical situations may occur.

- Only one good transition (product ion) is available using MS/MS methods, e.g., because the compound is minimally fragmented and/or the remaining potential ions are not selective enough (e.g., low *m/z* ions) and have high chemical noise. Only one transition is not sufficient to support identification of the compound; therefore, if no additional analysis is conducted to confirm the identification, this should be noted in the published report.
- The ion ratio deviation exceeds the level of established tolerance. Tolerances (e.g., ±30%) are a guideline, not a strict criterion, and therefore the knowledge and expertise of the analyst are crucial in questionable cases. The previous acquisition of more ions is quite helpful, as the initially selected ions may have interference and/or more chemical noise. This situation may occur at low concentrations in particular. Using the ion ratios obtained for QCs instead of the standards included in the calibration is recommended to assess the deviations in samples.
- The Rt deviation exceeds the level of established tolerance. This is rather common because of the peak shifts due to the sample matrix. Using the Rt of spiked samples (i.e., QCs) and/or relative Rt (e.g., using ILIS) may resolve doubts in most cases.
- Some QCs exceed the tolerance range (e.g., 60–140%). First, make a judicious interpretation of the data in light of the spiking level and analyte concentration in the blank used for QC preparation. Second, try to identify a trend and evaluate the robustness of QC recoveries. Third, consider applying both statistical tests (e.g., Dixon's Q) to discard potential outliers and a correction factor if enough reproducibility and robustness are observed. The data obtained might be reported as estimated or semiquantitative concentrations when QCs are not satisfactory but it is essential to include the necessary information so that other researchers can interpret them.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Errata

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