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RESEARCH ARTICLE

Reliability-Corrected Green Suitability Mapping for Materials-Enabled Microextraction of Veterinary Antibiotics in Environmental Water

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Abstract

The environmental surveillance of veterinary antibiotics goes beyond the sensitive response of a chromatographic method, but also involves the logical integration of validation behavior, material-friendly sample preparation, and laboratory application possibilities. In this paper, we describe the Reliability-Corrected Green Suitability Mapping (RCGSM) of six veterinary antibiotics in environmental waters: trimethoprim, oxytetracycline, tetracycline, sulfamethoxazole, chlortetracycline, and doxycycline. The key research question explored herein is whether validation properties and green and practical features can be harmonized into a decision hierarchy that would discriminate the analytical readiness of each analyte from the overall greenness of the procedure. The former one is expressed by the Validation Readiness Score (VRS) derived from linear range, detection limit, quantitation limit, recovery, and precision; the latter one by the Operational Green Index (OGI) defined in terms of extraction time, sample consumption, detection period, recovery period, eco-scale, AGREE, and BAGI. The highest VRS corresponds to oxytetracycline (0.890), followed by trimethoprim (0.690), sulfamethoxazole (0.683), tetracycline (0.650), doxycycline (0.385), and chlortetracycline (0.278). The combination of µSPEed and UPLC-PDA analysis delivers the maximal OGI score of 0.878. It is due to the combined presence of several key properties: simultaneous detection of six analytes, fast extraction, small amount of sample required, and good eco-sustainability. Therefore, the answer to the research question formulated is yes, yet the hierarchy constructed remains analyte-dependent. Thus, it is possible to report results with confidence for OTC and TMP, with caution regarding recovery for SMX and TC, and with reservation for DC and CTC at the quantification limit.

Keywords: veterinary antibiotics, environmental water, green analytical chemistry, microextraction, UPLC-PDA, analytical validation, materials-enabled sample preparation

1 Introduction

Antibiotics for animals are critical in animal farming, aquaculture, companion pet treatment, and disease control strategies. However, the potential discharge of antibiotic residues from these practices into the environment poses analytical and technological challenges. Discharge routes may include animal excretion, manure application, farm runoff, fishery discharge, municipal effluents, and diffuse agricultural drainage. Once in aquatic environments, these chemicals are not passive tracers, and they experience hydrolysis, photolysis, sorption to suspended matter, complexation with organic molecules or metals, and pH-induced variations in charge states. This makes surveillance difficult because the detection approach needs to measure low concentrations while handling highly variable aqueous matrices. Early investigations of the environmental impacts of pharmaceuticals demonstrated that antibiotic residues are part of a larger class of biological contaminants that require reliable analytical methods [1–3].

This challenge is compounded by the structural diversity of the selected multi-residue antibiotics, which includes four tetracyclines (TC, OTC, CTC, and DC) with ionisable groups, preference for metal binding, and varied extraction characteristics depending on matrix conditions. Additionally, SMX is a sulfonamide antibiotic with different acidic, polar,

and chromatographic properties compared to the other tetracyclines, while TMP is a basic antibiotic. In this sense, any extraction technique applied must address various extraction principles rather than relying on one molecular family, thereby explaining why absolute linearity of calibration curves may not translate into comparable confidence in reporting the concentrations of all analytes. Investigations of antibiotic concentrations in water indicate the importance of assessing the overall detection capabilities in terms of sensitivity, recovery, selectivity, and matrix tolerance [4–6].

The relevance of antibiotic surveillance is also evident within the context of antimicrobial resistance (AMR). The presence of antibiotic residues in wastewaters, sediments, and agricultural runoff can coexist with AMR bacteria and genes, and the mere presence of a residue does not imply ecological selection of AMR phenotypes or genotypes. However, the environmental microbiology field has underscored the necessity of developing monitoring schemes based on reliable and repeatable procedures that can be performed in realistic laboratory settings. Methods that are too complex, solvents-intensive, or costly to perform are useful for generating accurate individual measurements but inadequate for routine surveillance campaigns. Rapid methods may be desirable, yet they are limited to specific applications, particularly if the recovery or quantification capability differs between compounds [7–9].

Moreover, literature on pharmaceutical occurrences in rivers and effluents indicates that datasets of pharmaceutical residues have significant geographic biases and are more common in areas with better analytical capacity. Effluent studies further reveal that discharge facilities often have complex and varying pharmaceutical composition, requiring a suitable balance between sensitivity and sample throughput. These findings are applicable to veterinary antibiotic surveillance, as the analysis programme may require the screening of numerous water samples from irrigation channels, agricultural watersheds, wastewater-impacted streams, and seasonal runoff events. Here, the best method is not necessarily the one with the lowest single limit of detection (LOD). Instead, it is the method capable of producing reliable results in relation to the target analytes while keeping sample and solvent volumes low and allowing for high throughput [10, 11].

Such an assessment requires the principles of green analytical chemistry. The tenets of green chemistry encourage reducing hazardous components, waste generation, and energy consumption in chemical synthesis. Similarly, the principles of green analytical chemistry were applied in optimizing sample preparation techniques, instruments, and measurements. Several methodologies have been proposed to evaluate environmental impact, procedural greenness, life-cycle assessment, and practical applicability of a new analytical approach. They include Analytical Eco-Scale, AGREE, ComplexGAPI, and BAGI, among others. It is important to emphasize that green and miniaturized procedures are only useful if they are feasible in ordinary laboratories and require low energy input. Therefore, a comprehensive assessment is needed to ensure that a method optimized for water analysis has good analytical features and also offers practical advantages [12–14]. Such assessments are essential since routine analyses of environmental water quality cannot rely on single LODs or LOD/LOQ ratios but must be validated for deployment in realistic laboratory conditions [15, 16].

Miniaturization represents a valuable option within the above discussion. For instance, liquid–liquid microextraction, ultrasonication-assisted dispersive microextraction, magnetic solid-phase extraction, electrochemically controlled solid-phase microextraction, and μSPEed have been used to minimize sample and solvent volumes. All such extraction methods have different material-based functionality. While the first two methods rely on phase dispersion techniques, the second two involve magnetically driven sorbent recovery or electrochemically controlled adsorption of the extractable compounds. The latter procedure employs cartridge-based sorption of the target molecules. Therefore, the differences between extraction procedures include not only the extraction rate but also the amount of labor required, reproducibility, sample volume, cartridge longevity, and practical aspects of implementation. Comparative assessment requires an approach that combines the above factors to produce a valid assessment of the selected approach [17–19].

The current manuscript attempts to address a unique research question. Namely, can validation descriptors and green descriptors be converted into a reproducible rank-ordering system for the readiness of environmental water surveillance of veterinary antibiotics? Specifically, the manuscript introduces the method known as reliability-corrected green suitability mapping (RCGSM). This approach enables one to determine the Validation Readiness Scores (VRS) for all selected compounds in a particular procedure, while the Operational Green Index (OGI) provides a global assessment of the chosen extraction and chromatography setup. Both scores are derived from the descriptors collected in the paper and assessed via equation normalization and weighting. The results of the calculation are provided below.

Unlike other works published in scientific journals, the current paper proposes an analysis methodology rather than providing greenness assessments for a range of methods. Moreover, the paper presents a systematic approach to converting numerical descriptors into a valid hierarchy that evaluates both compound-specific and procedure-specific features. In this context, the current study has unique contributions since it answers a unique research question using a dataset of specific interest. It evaluates both validation and operational characteristics using specific descriptors, applies a specific calculation (RCGSM), reports VRS and OGI hierarchies, and derives an overall surveillance readyness strategy from this information. Such contributions make the manuscript suitable for the Journal of New Technology and Materials. In other words, the material nature of the technique used is addressed not through sorbent choice alone. Rather, it is evaluated in terms of operational parameters such as sample and solvent minimization, throughput, and detection and recovery capabilities.

The following sections present the numerical descriptors of antibiotic analysis in environmental water and the equation to derive the scores. Further, the influence of each major parameter on the ranking of methods will be discussed, followed

by the ranking of antibiotic surveillance procedures.

2 Analysis Framework and Scoring Criteria

The numerical matrix follows the established protocol for veterinary antibiotic residues in environmental water samples using six-analyte μSPEed–UPLC-PDA by Antos et al. [20]. This citation is required to cite the source of both validation and comparative descriptors, although the current calculation organizes these descriptors in the context of an RCGSM-based decision mapping study. The analyte matrix consists of retention time, linear dynamic range, calibration equation, coefficient of determination (r^2), limit of detection (LOD), limit of quantification (LOQ), recovery, intra-day precision, inter-day precision, and real-sample outcome. The procedure matrix includes the mode of sample preparation, detection type, antibiotic coverage, sample mass (amount), extraction time, recovery range, LOD range, Analytical Eco-Scale score, AGREE score, and BAGI score. All descriptors are considered fixed numerical descriptors in the RCGSM calculation.

Each descriptor has been given a specific purpose for scoring. Positive descriptors include linearity, recovery, greenness, applicability, and precision, meaning that a higher score indicates more desirable performance. Inverse-benefit descriptors include LOD, LOQ, sample volume, and extraction time because lower values represent a higher surveillance capability. This distinction is essential since environmental laboratories do not typically evaluate methods based on detection limit alone. The method must also offer good turnaround time, sample efficiency, reliability, and accuracy for all target analytes.

The first step in the analysis considers the reporting readiness of each antibiotic under the validated procedure. For each compound, an overall precision penalty was obtained through an arithmetic average of the intra-day and inter-day RSD values at medium and high fortification:

$$P_i = \frac{\mathrm{RSD}_{\mathrm{intra,ML}} + \mathrm{RSD}_{\mathrm{inter,ML}} + \mathrm{RSD}_{\mathrm{intra,HL}} + \mathrm{RSD}_{\mathrm{inter,HL}}}{4}. \tag{1}$$

Through Eq. (1), four precision measures were reduced to one descriptor. The lower the value of P_i, the more reliable the analyte, especially at higher concentrations. Such precision is critical to reliable quantification since an antibiotic may exhibit acceptable RSD at the medium level but poor precision near the lower detection threshold. The formula avoids weighting precision at a single measurement level, meaning that each analyte is given equal consideration at medium and high levels of fortification.

Dimensionless scores were then generated for individual validation descriptors:

$$L_i = \min\left[1, \max\left(0, \frac{r_i^2 - 0.9900}{0.0100}\right)\right], \tag{2}$$

$$D_i = 1 - \frac{\mathrm{LOD}_i - \min(\mathrm{LOD})}{\max(\mathrm{LOD}) - \min(\mathrm{LOD})}, \tag{3}$$

$$Q_i = 1 - \frac{\mathrm{LOQ}_i - \min(\mathrm{LOQ})}{\max(\mathrm{LOQ}) - \min(\mathrm{LOQ})}, \tag{4}$$

$$R_i = \frac{\mathrm{Recovery}_i}{100}, \tag{5}$$

$$C_i = 1 - \frac{P_i}{20}. \tag{6}$$

From Eqs. (2) to (6), the heterogeneity of various descriptors was eliminated by placing them on a normalized scale from 0 to 1. While the linearity descriptor rewards high linearity scores above $r^2 > 0.99$, no value will exceed unity as a result of this transformation. Similarly, LOD and LOQ are inverse scores since low values imply greater surveillance capabilities. The recovery rate is transformed to the fractional format, while precision is penalized to a 20% threshold of intra-day and inter-day variability. From a scientific perspective, the purpose of the transformations is to ensure that each descriptor has a proportional impact on the score based on their role in the surveillance process, not according to their actual units. Therefore, LOD, LOQ, precision, and recovery rates are considered to have equal impact despite the heterogeneity of their units.

The Validation Readiness Score was calculated as:

$$\mathrm{VRS}_i = 0.20L_i + 0.25D_i + 0.20Q_i + 0.20R_i + 0.15C_i. \tag{7}$$

From Eq. (7), it is seen that the detection capability receives the highest contribution among all descriptors because veterinary residues may be present near the lower end of the analytical range. Since linearity, quantification capability, and recovery determine the validity of quantitative reporting, they share almost equal contributions. Although precision was considered less significant than other descriptors, it still received a proportional score due to the fact that all analytes lie within the valid validation range. The scientific importance of Eq. (7) is that VRS is a measure of reporting capability, not abundance: it determines the reliability of a method for reporting antibiotic residues and cannot be interpreted in

terms of environmental presence.

In the second part of the analysis, a whole validation procedure has been evaluated in the context of routine surveillance. For the comparative procedure experiment, each analytical procedure was evaluated based on the dimensionless Operational Green Index. Three descriptors (LOD midpoint, extraction time, and sample mass) were considered inverse-benefit variables. Four remaining descriptors (Recovery midpoint, Analytical Eco-Scale, AGREE, and BAGI) were considered direct-benefit variables.

The normalized score for an inverse-benefit descriptor was given as:

$$S_x = 1 - \frac{x - \min(x)}{\max(x) - \min(x)}. \tag{8}$$

Eq. (8) transforms an inverse-benefit descriptor by giving maximum score to the smallest possible value. Such transformations are relevant to routine surveillance because they reward a rapid and efficient method while maintaining sensitivity. As a practical result, the normalized score for a variable of interest is a relative comparison, where lower values receive a higher score.

The normalized score for a direct-benefit descriptor was given as:

$$S_z = \frac{z - \min(z)}{\max(z) - \min(z)}. \tag{9}$$

According to Eq. (9), the larger the direct-benefit descriptor, the higher the score it obtains. This allows comparison of multiple indices (e.g., Eco-Scale points, AGREE, and BAGI) based on different scales, which is very important to the present analysis because applicability and greenness are not auxiliary considerations, but important descriptors for decision making.

The final complete score for the whole procedure is:

$$\begin{aligned} \mathrm{OGI}_m = {} & 0.15S_{\mathrm{Eco}} + 0.15S_{\mathrm{AGREE}} + 0.15S_{\mathrm{BAGI}} \\ & + 0.20S_{\mathrm{time}} + 0.15S_{\mathrm{volume}} + 0.10S_{\mathrm{LOD}} + 0.10S_{\mathrm{recovery}}. \end{aligned} \tag{10}$$

In Eq. (10), greenness and applicability are given the highest total weight, followed by the extraction time and sample volume. This is consistent with routine environmental monitoring because analytical performance is less important than rapid and resource-efficient deployment of the method. From the scientific perspective, the equation suggests that low LOD cannot dominate the ranking of analytical procedures because even a sensitive procedure with long extraction time, large sample volume, and low AGREE and BAGI scores might rank poorly.

3 Analyte-Level Validation Readiness and Reporting Confidence

The validation descriptors used for analyte-level scoring are presented in Table 1. The six compounds elute within a compact retention-time interval from 2.65 to 4.64 min, indicating that the chromatographic segment is well suited to rapid screening. The calibration models show strong linearity, with r^2 values from 0.9914 to 0.9998. LOD values range from 0.30 to 1.23 $\mu g\,L^{-1}$, while LOQ values range from 0.92 to 3.73 $\mu g\,L^{-1}$. The analysed environmental water samples were negative for all six targets, so the interpretation concerns method readiness and surveillance confidence rather than site-specific contamination frequency. This distinction is central to the paper: RCGSM does not convert non-detections into environmental conclusions; it clarifies how much analytical confidence can be attached to each compound if the same method is used for surveillance.

Table 1. Validation descriptors used for reliability-corrected antibiotic readiness mapping.

Antibiotic	RT (min)	LDR ($\mu g\,L^{-1}$)	Calibration equation	r^2	LOD ($\mu g\,L^{-1}$)	LOQ ($\mu g\,L^{-1}$)	Recovery (%)	Mean pre-cision (%)	RSD	Real sam-ples
TMP	2.65	1.06–10	$y = 10.517x + 569.9$	0.9914	0.35	1.06	85.9	11.58		n.d.
OTC	2.87	0.92–10	$y = 9.5258x - 2398.1$	0.9998	0.30	0.92	63.8	4.50		n.d.
TC	3.12	1.96–10	$y = 9.0029x - 4570.8$	0.9991	0.65	1.96	51.3	8.80		n.d.
SMX	3.77	3.40–10	$y = 28.905x - 6989.2$	0.9990	0.61	1.84	64.7	10.38		n.d.
CTC	4.28	3.72–10	$y = 5.6707x - 3702$	0.9960	1.23	3.73	47.2	11.48		n.d.
DC	4.64	3.02–10	$y = 6.2144x - 2855.4$	0.9974	1.00	3.02	45.9	15.68		n.d.

Table 1 shows that the six-antibiotic panel is chromatographically compact but analytically uneven. The retention sequence from TMP to DC occupies less than 2 min, which favours short analytical cycles, yet the validation descriptors

diverge markedly. OTC has the lowest detection and quantification limits, TMP has the highest recovery, and DC has the largest precision penalty. The table therefore establishes the reason for the RCGSM calculation: the method cannot be described accurately by one global validation statement because each antibiotic carries a different balance of sensitivity, recovery, and reproducibility.

Figure 1 provides a visual dashboard of the same analyte-level descriptors. It is included to make the numerical hierarchy in Table 1 easier to interpret: the embedded bars show how sensitivity, recovery, precision, and the final VRS differ between the six antibiotics without replacing the tabulated values used for calculation.

Analyte	RT (min)	r^2	LOD (µg/L)	LOQ (µg/L)	Recovery (%)	RSD (%)	VRS
TMP	2.65	0.9914	0.35	1.06	85.9	11.58	0.690
OTC	2.87	0.9998	0.30	0.92	63.8	4.50	0.890
TC	3.12	0.9991	0.65	1.96	51.3	8.80	0.650
SMX	3.77	0.9990	0.61	1.84	64.7	10.38	0.683
CTC	4.28	0.9960	1.23	3.73	47.2	11.48	0.278
DC	4.64	0.9974	1.00	3.02	45.9	15.68	0.385

VRS = 0.20L + 0.25D + 0.20Q + 0.20R + 0.15C

Figure 1. Analyte-level validation-readiness dashboard for the six veterinary antibiotics. The display combines retention time, coefficient of determination, LOD, LOQ, recovery, mean precision RSD, and the final VRS, with embedded horizontal bars showing the relative contribution of each descriptor to reporting confidence.

Figure 1 condenses the validation matrix into an immediate visual hierarchy. The practical message is that high readiness is not identical to high recovery or early elution; it emerges from the combined pattern of LOD, LOQ, linearity, recovery, and precision. This figure is therefore useful for laboratory communication because it allows analysts and decision-makers to see why OTC is a stronger surveillance target than CTC or DC even when all six compounds are included in the same method.

Figure 2 separates the chromatographic and sensitivity components of the validation matrix. The upper trace shows the compact elution order from TMP to DC, while the lower bars compare LOD and LOQ values; the figure clarifies why OTC is analytically strong and why CTC and DC require more caution near the quantification boundary.

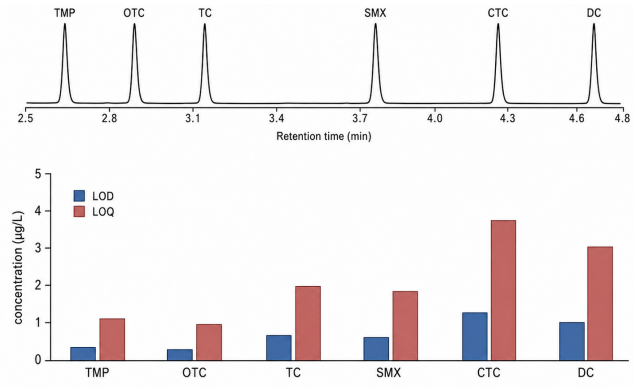

Figure 2. Retention and sensitivity profile of the six-antibiotic method. The chromatographic trace summarizes the retention-time order, and the paired bar chart compares LOD and LOQ values for each analyte.

The interpretation of Figure 2 is that chromatographic compactness does not eliminate analyte-specific detection constraints. OTC benefits from the lowest LOD and LOQ, while CTC and DC occupy the high-LOD/high-LOQ end of the method. In environmental monitoring terms, this means that a short run time can support rapid screening, but lower-confidence analytes still require careful reporting when measured near the quantification limit.

Figure 3 complements the sensitivity view by isolating the recovery and precision descriptors. This visual comparison shows that TMP has the strongest recovery, whereas OTC has the smallest precision penalty; it also shows the combined constraint on DC, where low recovery and the largest mean precision RSD reduce the VRS.

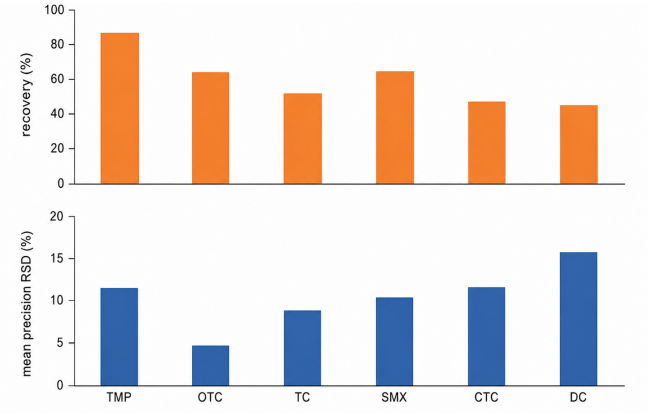

Figure 3. Recovery and precision profile for TMP, OTC, TC, SMX, CTC, and DC. The upper panel compares recovery percentages, and the lower panel compares mean precision RSD values used in the validation-readiness calculation.

Figure 3 explains why recovery and precision must be evaluated together. TMP is the strongest compound by recovery, but OTC is stronger by precision and sensitivity; DC is constrained by both low recovery and the highest RSD average. This figure therefore prevents a misleading recovery-only interpretation and supports the score-based approach used in the next part of the analysis.

The validation matrix shows that analytical confidence is not evenly distributed across the antibiotic panel. OTC has the lowest LOD and LOQ and the strongest mean precision, which makes it a highly stable target even though its recovery is lower than that of TMP. TMP has the strongest recovery and a low detection limit, but its scaled linearity score is weaker because its r^2 value is the lowest in the group, although still acceptable for quantitative work. SMX and TC show intermediate behaviour; SMX benefits from stronger recovery, whereas TC benefits from stronger linearity and precision. CTC and DC are the most constrained compounds because they combine higher detection or quantification limits with recoveries below 50%, and DC also shows the highest mean precision penalty. This pattern demonstrates the practical necessity of compound-specific reliability mapping in multi-residue environmental analysis.

The calculated VRS values are given in Table 2. OTC receives the highest score (0.890), reflecting the best combination of low LOD, low LOQ, strong precision, and excellent linearity. TMP ranks second (0.690), mainly because its recovery is substantially stronger than that of the tetracycline derivatives and its detection limit remains low. SMX and TC occupy the middle region of the decision map, with similar total readiness but different limiting descriptors. DC and CTC form the lower-confidence group, primarily because sensitivity and recovery reduce their score.

Table 2. Validation Readiness Score (VRS) calculated from linearity, sensitivity, quantification capability, recovery, and precision.

Antibiotic	L_i	D_i	Q_i	R_i	C_i	VRS	Rank
OTC	0.980	1.000	1.000	0.638	0.775	0.890	1
TMP	0.140	0.946	0.950	0.859	0.421	0.690	2
SMX	0.900	0.663	0.673	0.647	0.481	0.683	3
TC	0.910	0.620	0.630	0.513	0.560	0.650	4
DC	0.740	0.250	0.253	0.459	0.216	0.385	5
CTC	0.600	0.000	0.000	0.472	0.426	0.278	6

Table 2 answers the analyte-level part of the research question. It demonstrates that the six antibiotics separate into three reporting groups: OTC and TMP provide stronger evidence for high-confidence surveillance, SMX and TC require routine but recovery-aware interpretation, and DC and CTC require conditional interpretation when concentrations are close to LOQ. The table also shows that the score is not controlled by one descriptor alone; OTC is first despite moderate recovery because it performs strongly in sensitivity, quantification, linearity, and precision.

The weighted construction of the VRS is visualized in Figure 4. The stacked bars demonstrate that OTC leads because it combines strong detection, quantification, linearity, and precision terms, whereas CTC and DC lose score mainly through sensitivity and recovery penalties. The figure is especially useful for distinguishing total readiness from the individual descriptors that produce it.

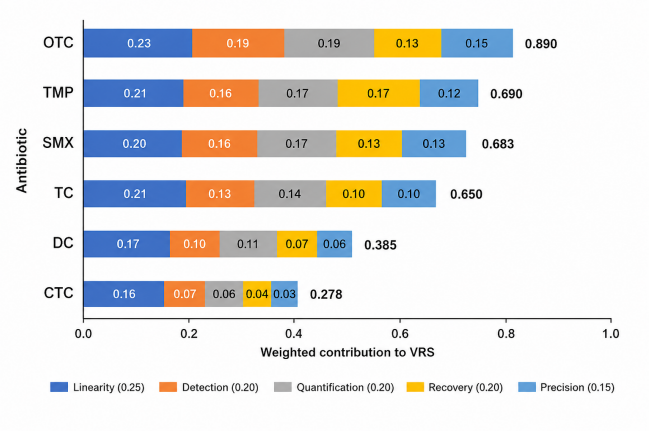

Figure 4. Weighted component contributions to the Validation Readiness Score (VRS). Each segment represents the weighted contribution of linearity, detection capability, quantification capability, recovery, and precision to the final analyte-level score.

Figure 4 provides the diagnostic explanation behind the final VRS values. The stacked segments make clear that the lower scores of CTC and DC are produced mainly by sensitivity and recovery losses, whereas OTC has a broad distribution of strong contributions. This matters for method improvement because it identifies which validation component should be targeted if a future sorbent or chromatographic adjustment is intended to improve a specific antibiotic.

Figure 5 places sensitivity and recovery on the same coordinate system while using marker size to indicate VRS. This map confirms that a strong surveillance target is not defined by recovery alone: OTC combines the lowest LOD with high precision and therefore receives the largest readiness marker even though TMP has higher recovery.

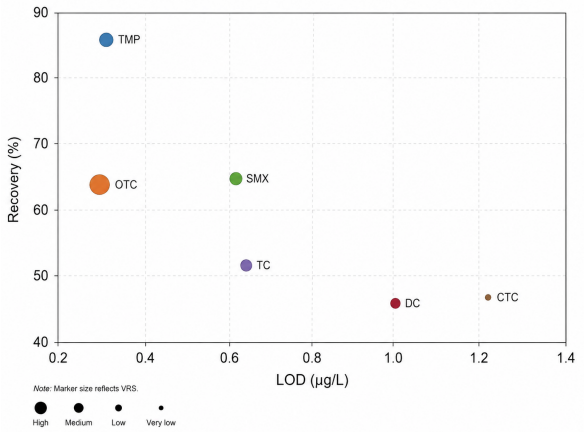

Figure 5. LOD–recovery map with marker size proportional to VRS. The visualization shows how sensitivity and recovery jointly influence the readiness hierarchy, separating high-confidence analytes from conditional targets.

Figure 5 gives a two-variable view of the same conclusion and shows why a single-axis performance claim would be incomplete. TMP is favoured by recovery, OTC is favoured by sensitivity, and the marker sizes reveal how the full VRS balances these competing strengths. The map is therefore an interpretive bridge between raw validation values and the tiered reporting categories used later in the paper.

The analyte-specific hierarchy assigns the greatest confidence to OTC and TMP, routine but recovery-aware confidence to SMX and TC, and conditional confidence to DC and CTC. The lower position of DC and CTC does not invalidate their inclusion in the analytical panel. Instead, it indicates that their quantitative results should be interpreted more carefully near LOQ values or in samples with stronger matrix effects. In practical reporting, OTC and TMP can be considered high-confidence targets, SMX and TC can be considered standard surveillance targets, and DC and CTC can be flagged for recovery correction, confirmatory detection, or matrix-matched calibration when regulatory or risk-screening decisions depend on marginal concentrations.

The numerical contrast between OTC and TMP is particularly informative. OTC has a lower recovery than TMP, but its superior LOD, LOQ, precision, and linearity outweigh that limitation in the weighted score. TMP shows the opposite behaviour: recovery is excellent, but lower scaled linearity and a larger precision penalty prevent it from reaching the OTC score. The VRS calculation therefore does not simply reward the largest recovery value or the lowest detection limit. It

ranks each antibiotic according to the full validation profile, which is more appropriate for multi-class surveillance where different chemical structures respond differently to the same extraction and detection conditions.

4 Procedure-Level Operational-Green Suitability

The procedure-level descriptors are shown in Table 3. The comparison covers liquid–liquid microextraction, ultrasound-assisted dispersive liquid–liquid microextraction, magnetic extraction procedures, electrochemically controlled solid-phase microextraction, and µSPEed coupled to UPLC-PDA. Their operational profiles differ widely. Extraction time ranges from 6 to 80 min, sample amount ranges from 0.5 to 20 mL, and LOD intervals extend across more than two orders of magnitude. Such variation justifies a composite decision score because no single descriptor can represent the whole laboratory burden.

Table 3. Procedure-level descriptors used for operational-green suitability mapping.

Sample preparation / detection	Antibiotic coverage	Sample amount	Extraction time (min)	Recovery (%)	LOD ($\mu g\,L^{-1}$)	Eco	AGREE	BAGI
LLME / HPLC-UV	OTC, TC, CTC	5 mL	6	77.5–87.6	0.5–2.0	79	0.65	60.0
UA-DLLME / HPLC-DAD	SCT, SMR, SPD, SDZ, SMM, SMX, SDM	5 mL	23	80.0–116.0	0.7–7.8	78	0.61	67.5
MSPE-DLLME / HPLC-UV	DOC, OTC, TC	4 mL	35	70.6–121.5	1.8–2.9	72	0.49	60.0
MSPE / UPLC-TUV	OTC, TC, CTC	10 mL	80	91.0–104.6	0.027–0.107	77	0.56	55.0
MNSPE / HPLC-UV	OTC, TC, DMC, CTC, MC, DC	20 mL	15	76.2–98.0	0.0286–0.0519	76	0.56	55.0
EC-SPME / HPLC-UV	DC, OTC, TC	3 mL	25	71–104	2.42–7.59	78	0.57	57.5
µSPEed / UPLC-PDA	TC, OTC, DC, CTC, SMX, TMP	500 µL	6	45.9–85.9	0.30–1.23	76	0.64	67.5

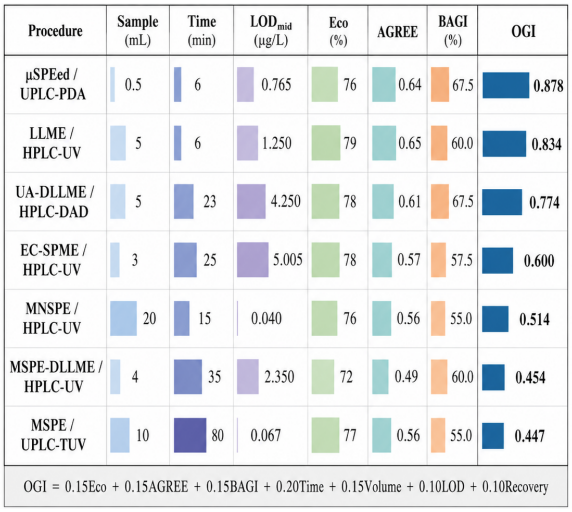

Figure 6. Procedure-level operational-suitability dashboard for the compared extraction and chromatographic procedures. The embedded bars summarize sample volume, extraction time, LOD midpoint, Eco-Scale, AGREE, BAGI, and final OGI.

Table 3 shows that the comparative procedures represent genuinely different laboratory strategies rather than minor variations of the same workflow. The lowest LOD intervals occur for magnetic extraction approaches, but these methods require larger sample volumes or longer extraction times. The µSPEed–UPLC-PDA procedure, in contrast, is distinguished

by 500 µLsample consumption, 6 min extraction, and complete coverage of the six-antibiotic panel. These differences justify a procedure-level OGI because the operational burden is not visible from LOD values alone.

Figure 6 converts the procedure-level descriptors into a compact operational dashboard. It highlights why the µSPEed–UPLC-PDA configuration ranks first: the method has the smallest sample requirement, the shortest extraction time, favourable AGREE and BAGI values, and broad coverage of the antibiotic panel evaluated in this study.

Figure 6 shows that the leading procedure is not the most sensitive method in isolation, but the most balanced method for routine use. Its smallest sample requirement is especially important for surveillance campaigns where repeat sampling, replicate preparation, or limited sample availability can constrain laboratory throughput. The figure therefore connects material-efficient extraction design with a practical environmental-monitoring outcome.

Figure 7 presents the same comparison as a relative-performance heat map. The blue cells emphasize descriptor-level performance, while the green OGI column summarizes the final operational suitability; this format helps identify whether a method ranks highly because of speed, sample economy, sensitivity, or sustainability indicators.

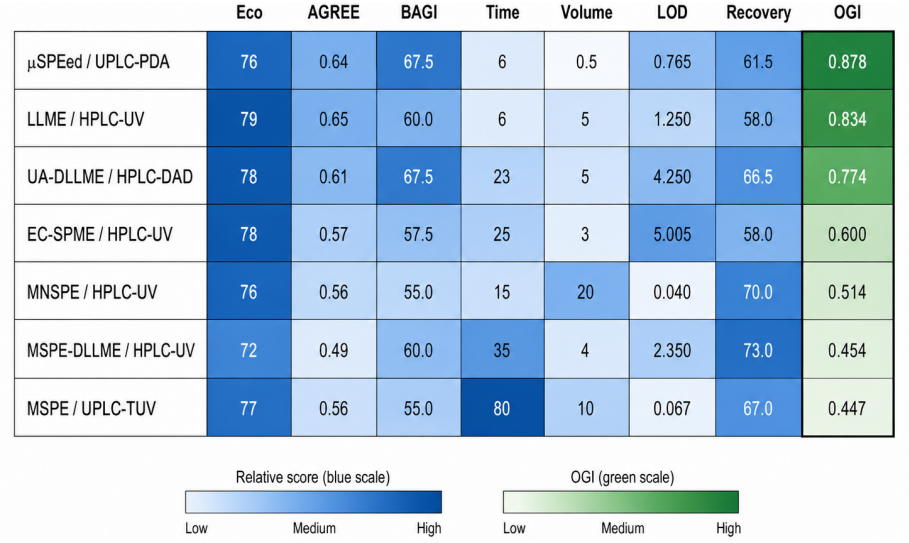

Figure 7. Comparative heat map of procedure-level descriptors and final Operational Green Index (OGI). Blue shading represents relative descriptor performance, and the green column reports the final OGI used for procedure ranking.

Figure 7 identifies the source of each procedure's rank. High heat-map intensity in one descriptor does not guarantee a high OGI because the final column integrates several forms of suitability. The figure is particularly useful for explaining why procedures with excellent detection limits may still rank below µSPEed–UPLC-PDA when extraction time, sample volume, AGREE, BAGI, and recovery behaviour are considered together.

The comparison identifies a clear analytical trade-off. Magnetic solid-phase extraction coupled with UPLC-TUV reaches an exceptionally low LOD interval, but it requires 80 min of extraction and a 10 mL sample. The MNSPE/HPLC-UV method also achieves low detection limits but uses 20 mL of sample. The µSPEed–UPLC-PDA procedure does not have the absolute lowest detection interval, yet it combines the shortest extraction time, the smallest sample amount, and the broadest coverage of the exact six-antibiotic set evaluated in the analyte-level calculation. This combination is decisive for high-frequency monitoring because sample preparation often limits total throughput more strongly than chromatographic runtime.

Table 4. Operational Green Index (OGI) ranking of the compared analytical procedures.

Procedure	Sample (mL)	Time (min)	LOD midpoint ($\mu g\,L^{-1}$)	Eco	AGREE	BAGI	OGI
µSPEed / UPLC-PDA	0.5	6	0.765	76	0.64	67.5	0.878
LLME / HPLC-UV	5	6	1.250	79	0.65	60.0	0.834
UA-DLLME / HPLC-DAD	5	23	4.250	78	0.61	67.5	0.774
EC-SPME / HPLC-UV	3	25	5.005	78	0.57	57.5	0.600
MNSPE / HPLC-UV	20	15	0.040	76	0.56	55.0	0.514
MSPE-DLLME / HPLC-UV	4	35	2.350	72	0.49	60.0	0.454
MSPE / UPLC-TUV	10	80	0.067	77	0.56	55.0	0.447

Table 4 answers the procedure-level part of the research question. The highest OGI belongs to µSPEed–UPLC-PDA, not because every descriptor is individually superior, but because no major operational weakness offsets its speed and

sample economy. LLME/HPLC-UV remains competitive because of speed and greenness, whereas MSPE/UPLC-TUV and MNSPE/HPLC-UV lose position despite low LOD values. The table confirms that operational-green suitability is a balance among sensitivity, practical burden, and sustainability descriptors.

The operating trade-off behind the OGI values is shown in Figure 8. Procedures positioned toward the lower-left region require less sample and shorter extraction time, and larger markers indicate stronger OGI. This view shows why the µSPEed–UPLC-PDA method is preferred for routine monitoring even though MNSPE/HPLC-UV and MSPE/UPLC-TUV achieve lower detection limits.

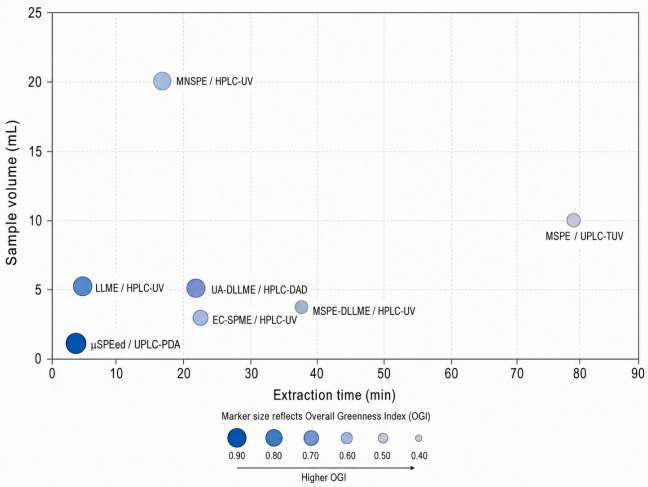

Figure 8. Extraction-time and sample-volume trade-off map for the compared procedures. Marker size reflects OGI, showing that operational suitability depends on speed, sample economy, and green-practical descriptors rather than sensitivity alone.

Figure 8 gives the most direct operational interpretation of the OGI ranking. A method placed near the lower-left region can process samples with less material and time demand, and a large marker indicates that this advantage is supported by the full score rather than by plotting position alone. The figure demonstrates why a surveillance laboratory may rationally select a faster and lower-volume method even when another procedure reports a lower LOD interval.

Figure 9 provides the final rank order of the operational-green comparison. The small separation between µSPEed–UPLC-PDA and LLME/HPLC-UV reflects their similar speed, whereas the lower positions of MSPE-DLLME/HPLC-UV and MSPE/UPLC-TUV reflect longer extraction time and less favourable operational balance.

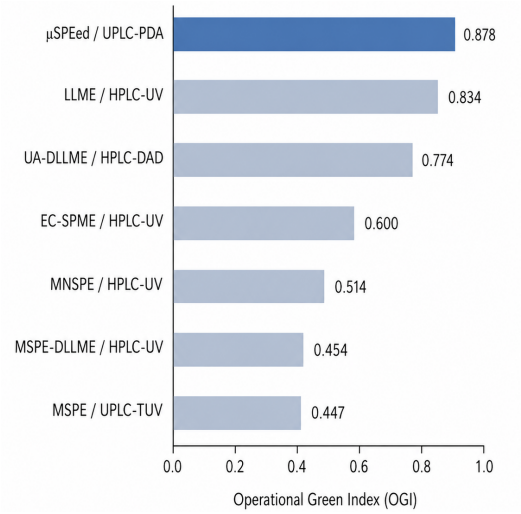

Figure 9. Ranked Operational Green Index (OGI) values for the compared analytical procedures. The figure emphasizes the final procedure-level hierarchy derived from the weighted descriptor matrix.

In conclusion, the final answer on the question about the most optimal methods according to RCGSM criteria is illustrated in Fig. 9. The graph shows that µSPEed–UPLC-PDA and LLME/HPLC-UV belong to the top of operational group because of the combination of their sensitivity advantages with practical feasibility. The magnetic sorbents are

positioned below because of higher costs associated with them despite their sensitivity potential. However, the ranking is not a call for discarding sensitive sorbents as a confirmation tool only. They may become especially useful in confirmation analysis.

5 Comparison of Analytical Suitability, Greenness, and Applicability

As indicated above, the optimal operation-greenness index (OGI) calculated using the OGI equation and validated descriptors shows that a procedure with an optimal balance of factors provides the highest OGI index, but not the lowest LOD midpoint. The highest OGI of all tested approaches belongs to µSPEed–UPLC-PDA (0.878), because this approach is the most economical in terms of material consumption, the fastest in extraction time, maintains the highest AGREE index, and retains high applicability (BAGI). The second rank goes to LLME/HPLC-UV because of rapidity, good eco-score, high AGREE, and good BAGI applicability. Third place belongs to UA-DLLME/HPLC-DAD, which is characterized by high BAGI applicability but slower extraction process and higher LOD midpoint.

Low OGI indices for MNSPE/HPLC-UV and MSPE/UPLC-TUV do not indicate any inconsistency with analytical reality. Although these two procedures provide good LOD intervals, they consume more sample volume, have longer extraction times, and lower BAGI applicability, making them unsuitable for regular work. Laboratory practice does not only focus on the sensitivity; when screening dozens of samples for contaminants in a programme, sample size and extraction time may play even more significant roles.

6 Integrated Environmental Monitoring Decision Map and Limitations

The integrated RCGSM evaluation helps to separate the procedure and compound levels into clear groups of results. The procedure ranking demonstrates that µSPEed–UPLC-PDA is the best approach among the described procedures thanks to its OGI of 0.878. The analyte ranking reveals that OTC, TMP, SMX, and TC are better suited for quantification compared to DC and CTC, which belong to the lower group. Therefore, a laboratory could use this approach, but divide the antibiotics into different groups depending on validation readiness. In terms of water quality control in environmental surveillance, this solution would allow combining the benefits of a rapid and green method with accurate communication of analytical confidence in detected substances.

The proposed decision map for environmental water monitoring consists of three tiers. The first one includes OTC and TMP as they combine high sensitivity with satisfactory validation readiness. The second tier includes SMX and TC as compounds that are acceptable for quantitative detection, but require recovery correction to achieve accurate results. The last tier includes DC and CTC as suitable targets, but with LOQ-related caution in complicated samples. Figure 10 reflects these conclusions and proposes three colour groups corresponding to the three tiers.

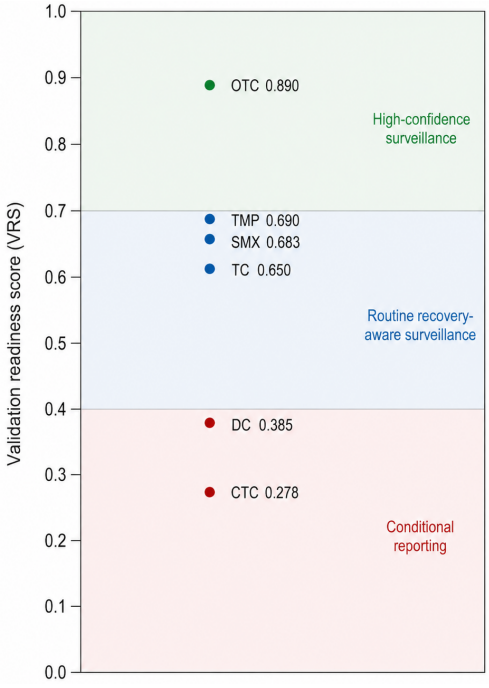

Figure 10. Tiered RCGSM decision map based on analyte-level VRS. The green zone denotes high-confidence surveillance, the blue zone denotes routine recovery-aware surveillance, and the red zone denotes conditional reporting for analytes that require additional caution near LOQ or in complex matrices.

The tiered reporting is more advantageous compared to a simple pass/fail validation decision because it retains multi-analyte character of the survey, indicating which compounds may require special attention in a certain sample matrix. Therefore, Fig. 10 provides an adequate decision framework for laboratory analysis and subsequent actions concerning detected compounds. In addition to the analytical benefits, this technique offers clear material and technological opportunities.

µSPEed–UPLC-PDA is based on a material-economical and fast extraction technique coupled with UPLC. This setup allows reducing the time spent during extraction while providing a short retention period of target contaminants. Moreover, there is no need to use expensive HPLC systems in laboratories focused primarily on screening tasks. Thus, the procedure is very convenient for decentralized environmental laboratories, agricultural surveillance programmes, and preliminary water body surveys involving multiple samples with low solvent volumes and moderate instrumentation.

Non-detected antibiotics are an important indicator of water quality. A negative result may reflect either the fact of a sufficiently small content of the compound or other factors like sampling area, seasonality, and dilution in case of hydrological activity. However, RCGSM technique provides clear guidance on interpreting such results and assigning appropriate analytical confidence to them. A non-detected OTC or TMP is more reliable compared to non-detected CTC or DC due to higher LOQ and lower recovery of these compounds. Thus, this ranking can be helpful in assessing the risk and choosing next steps during further analysis.

The described methodology is most beneficial for those laboratories that have to prioritize analytical capacity and sustainability. Not all laboratories can afford applying the method of high-resolution mass spectrometry for every surveillance program. Therefore, a method based on a PDA setup combined with the efficient procedure of magnetic solid-phase extraction can be an optimal decision if validation limits and compound specificity are clearly identified. As was already mentioned above, the RCGSM technique allows calculating new scores when introducing new matrices and targets.

There are several boundaries for this methodology. First of all, although the proposed weight system is open, another laboratory can assign other weights in favour of sensitivity or recovery. In addition, this technique is based only on the analysis of validation characteristics, while real-world samples can vary in terms of matrix, seasonality, suspended solids, organic matters, and pH. Therefore, an effective way of improving the results is conducting the experiment with additional matrix matching and seasonal changes. Another limitation of this approach is its dependence on the set of applied procedures. If a laboratory starts using some new sorbents or detection instruments, OGI values have to be recalculated again.

7 Conclusion

This study answers its research question through showing that validation descriptors and green-practical descriptors can be integrated into a clear, reproducible, and understandable hierarchy for veterinary-antibiotic surveillance in environmental water. The RCGSM framework produces two complementary metrics, the Validation Readiness Score assessing compound-level validation confidence and the Operational Green Index assessing procedure-level efficiency, feasibility, and reusability. The combined hierarchy is superior to simply saying that an analytical method is validated or green because it highlights which antibiotics can be analyzed with greater confidence as well as the procedure best suited for repeat sampling. On the analyte level, oxytetracycline has the highest VRS at 0.890 since it includes both the lowest LOD and LOQ, along with the best linear and precise performance. The second-ranked antibiotic is trimethoprim with a VRS score of 0.690 due to high recoveries and low LOD despite inferior linearity and precision terms relative to oxytetracycline. Sulfamethoxazole and tetracycline rank third and fourth, with VRS scores of 0.683 and 0.650 respectively, while doxycycline and chlortetracycline rank fifth and sixth with VRS scores of 0.385 and 0.278. This demonstrates the six-antibiotics panel is not homogeneous in regard to their analysis potential. On the procedure level, the highest-ranking operational index belongs to µSPEed-UPLC-PDA, with an OGI score of 0.878. Its high score derives from its small sample consumption, rapid extraction time, analysis of all selected compounds, strong performance of AGREE and BAGI descriptors, and acceptable sensitivity to be used as a routine monitoring approach. Although the LLME/HPLC-UV system ranks second regarding operational readiness, it only supports four selected antibiotics with relatively large consumption. Magnetic extraction procedures, although producing the lowest detection limits among all, have much less optimal OGI scores due to large sample size, longer extraction time, or less practicality. Overall, this study demonstrates that the most operationally green method is not always the most sensitive, but the one where the most robust and defendable combination of sensitivity, material usage, time management, and practical applicability is obtained. The practical consequence of these findings for the use in environmental laboratory studies is a differential reporting hierarchy in which oxytetracycline and trimethoprim should be considered high-confidence analytes; sulfamethoxazole and tetracycline can be reported as routine, yet their near-LOD results will be corrected with respect to their recovery; and doxycycline and chlortetracycline can be maintained in the panel, but the results close to LODs should have confirmations by matrix-matched calibration or additional analysis. Non-detections should also be reported separately, according to specific analyte properties. From the theoretical perspective, RCGSM introduces a hierarchical method selection framework for materials-based analytical chemistry, integrating the principles of sorbent-assisted microextraction, chromatography validation, green analytical chemistry, and practical aspects of the laboratory routine into one algorithm. The similar approach can also be expanded to other classes of antibiotics, various aqueous

samples, novel sorbents, detectors, and automation of sample preparation with corresponding changes to descriptor matrices in each case. The future studies in this topic may include expansion of the decision hierarchy to different seasons, matrix-enriched agricultural runoff, and waste-polluted waters as well as development of an extended confirmatory LC-MS/MS validation to establish links between VRS scores and environmental risk levels.

Data availability

All numerical values used in the decision matrices are presented in Tables 1–4. The graphical summaries are provided in Figures 1–10. The scoring calculations are reproducible from Eqs. (1)–(10).

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