New extraction method prior to screening of organic micropollutants in various biota matrices using liquid chromatography coupled to high-resolution time-of-flight mass spectrometry

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ABSTRACT

A new extraction method with limited clean-up requirements prior to screening various matrices for organic micropollutants using liquid chromatography-high resolution mass spectrometry (LC-HRMS) for analysis was developed. First, the performance of three extraction methods (QuEChERS with SPE clean-up, ultrasonication with SPE clean-up, extraction without SPE clean-up) was tested, optimized, and compared using > 200 contaminants of emerging concern (CECs) together covering a wide range of physicochemical properties applicable for suspect and non-target screening in biota. White-tailed sea eagle (*Haliaeetus albicilla*) muscle tissue was used in method development and optimization. The method without SPE clean-up was then applied to European perch (*Perca fluviatilis*) muscle, heart, and liver tissues. The optimization and application of the method demonstrated a wide applicable domain of the novel extraction method regarding species, tissues, and chemicals. For future applications, the suitability of the method for suspect and non-target screening was tested. Overall, our extraction method appears to be sufficiently simple and broad (relatively non-discriminant) for use prior to analysis of CECs in various biota.

1. Introduction

There is increasing awareness and anxiety about contaminants of emerging concern (CEC), which can be bioaccumulative and toxic to humans and wildlife [1]. Environmental monitoring programs for biota using e.g., environmental specimen banks aim to assess and monitor the presence of potentially hazardous chemicals in the environment. However, current lists of monitored organic micropollutants only represent a small fraction of hazardous compounds potentially present in wildlife [2]. CECs include a broad range of chemical classes, such as pharmaceuticals, personal care products, pesticides, per- and polyfluoroalkyl substances (PFASs), and industrial chemicals [1]. Most multi-residue sample preparation methods for biota are targeted at specific species or tissues [3–6]. Gas chromatography (GC) approaches are commonly applied when investigating biota samples, since hydrophobic compounds tend to bioaccumulate and thus can be expected to be detected by GC analysis [7,8]. However, more hydrophilic substances such as pharmaceuticals, pesticides, and PFASs, which are typically separated using liquid chromatography (LC) approaches, are often more mobile and can also be bioaccumulative and harmful to

biota [9]. Thus, GC and LC are complementary approaches to separate for subsequent detection of organic micropollutants in biota.

Suspect and non-target screening techniques are able to detect known unknowns and unknown unknowns in diverse matrices [10]. In order to capture a broad range of relevant compounds for suspect and non-target screening, a broad and robust extraction method is needed [11]. Typical sample preparation techniques for suspect and non-target analysis include QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe), ultrasonication, solid-phase extraction (SPE), and accelerated solvent extraction (ASE) for soil and sediment [12–14] and water samples [15–21], whereas methods for biotic matrices are less well studied [22–25]. Baduel et al. [11] developed a sample preparation method for target and non-target screening of biotic samples using QuEChERS extraction and Captiva® cartridge clean-up, validated the method with 77 target analytes, and applied it successfully to fish and breast milk samples. However, few methods have been validated for analyzing biota for a broad range of chemicals, while also aiming for optimized simplicity and cost-efficiency.

The overall aim of the present work was to develop a simple extraction method for broad (relatively non-discriminant) analysis of

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CECs in biota, without including time-consuming clean-up procedures. Three extraction (and sample preparation) methods were tested, optimized, and compared using > 200 CECs together covering a wide range of physicochemical properties (octanol-water partition coefficient (K_{OW}) ranging from −2 to 13) applicable for suspect and non-target screening in biota. Specific objectives were to: i) compare the performance on biota samples of the three extraction methods, which were QuEChERS with SPE clean-up, ultrasonication with SPE clean-up, and extraction without SPE clean-up, ii) calculate detection limits for the target compounds in tissues from white-tailed sea eagle (*Haliaeetus albicilla*) and European perch (*Perca fluviatilis*), iii) apply the method to samples from white-tailed sea eagle and European perch, and iv) test the suitability of the method for suspect and non-target screening in future applications.

2. Materials and methods

2.1. Biota samples

Muscle tissue from white-tailed sea eagle (*Haliaeetus albicilla*) was kindly provided by the environmental specimen bank (ESB) at the Swedish Museum of Natural History (SMNH). The tissue samples were collected by SMNH during 2014 and 2015, mainly from birds killed by traffic (Table SI1 in Supplementary Information (SI)). After collection, the samples were stored frozen at −20 °C until analysis. These samples were used for the extraction experiments and method optimization with different solvents (see sections 3.1 to 3.4). To assess the range of application of the final method, heart, liver, and muscle tissues from 10 individual European perch (*Perca fluviatilis*) were obtained (Table SI2 in SI) and analyzed individually for selected target compounds (see sections 3.3 to 3.4).

2.2. Chemicals and solvents

A total of 217 organic micropollutants and 52 isotopically labeled internal standards (IS) were included in the target method (Table SI3 in SI). The target compounds were selected based on environmental relevance and availability. Together, they represented a broad range of physicochemical properties, including benzotriazoles ($n = 7$), pharmaceuticals ($n = 74$), pesticides ($n = 89$), flame retardants ($n = 16$), food additives ($n = 3$), drugs ($n = 3$), stimulants ($n = 2$), personal care products ($n = 4$), phthalates ($n = 2$), isoflavones ($n = 2$), an industrial chemical ($n = 1$), and PFASs ($n = 14$).

The standards were obtained from Sigma-Aldrich (Steinheim, Germany), European Pharmacopeia Reference Standard (Strasbourg, France), Teknolab Sorbent (Kungsbacka, Sweden), USP Reference standard (USA), BOC Sciences (Shirley, NY), and Supelco (Bellefonte, PA), and were of high purity (> 85%). Standard stock solutions (1 mg mL^{-1}) were prepared by dissolving each compound in methanol or acetonitrile. Working solutions (a mixture of each compound at a concentration of 1 µg mL^{-1}) were prepared by diluting stock solutions in acetonitrile. All standards were stored in darkness at −20 °C.

Acetonitrile, isopropanol, and methanol (all LC/MS grade) were obtained from VWR International (Fontenay-sous-Bois, France). Formic acid (LC/MS grade), ammonium formate, and ammonium acetate were obtained from Sigma-Aldrich (Steinheim, Germany). Deionized water was purified using a Milli-Q system (Millipore, Co, Bedford, MA, USA) filtered through a LC-PAK filter (Darmstadt, Germany) to remove PFASs in system. A QuEChERS mixture of 900 mg MgSO$_4$ + 300 mg Z-sep$^+$ was purchased from Supelco (Bellefonte, PA). Oasis PRiME HLB 6 cc cartridges were supplied by Waters Corporation (Milford, MA).

2.3. Sample preparation

The three extraction methods (Fig. 1) were tested for their ability to extract the 217 target compounds from muscle tissue from white-tailed

sea eagle. For extraction with QuEChERS with SPE clean-up and ultrasonication with SPE clean-up, the tissue was cut up with a solvent-rinsed scalpel (Martor KG, Solingen, Germany) and 1.0 ± 0.1 g portions were transferred to homogenization tubes (15 mL), together with ceramic beads (Precellys, Bertin Technologies, France). To test the extraction efficiency and matrix effects, a spiking solution containing all target compounds ($n = 217$) in acetonitrile was prepared. For extraction efficiency, 50 ng of each compound were added directly onto the muscle tissue ($n = 3$; Fig. 2), and the solvent was then evaporated for approximately 30 min by gentle drying in a fume hood. To test matrix effects, the final muscle extracts ($n = 3$; Fig. 2) were fortified with 50 ng of target compounds ($n = 217$). For all samples, 50 ng of the isotopically labeled standards ($n = 52$) were added before extraction. All QuEChERS and ultrasonicated samples were homogenized with 5 mL acetonitrile in a Precellys tissue homogenizer (Bertin Technologies). For QuEChERS analysis, 3 mL aliquots were extracted with 900 mg MgSO$_4$ and 300 mg Z-sep$^+$ QuEChERS salts. For ultrasonication extraction, the samples were ultrasonicated for 30 min, aliquots were transferred to new vials, and ultrasonication was repeated twice more. The QuEChERS and ultrasonication extracts were frozen at −20 °C for at least 16 h to denature the proteins. The extracts were then cleaned with SPE by Oasis PRiME HLB cartridges, using 5 mL methanol. The clean extracts were blown down to dryness and taken up in 50/50 H$_2$O/MeOH.

In the method involving extraction without SPE clean-up (Fig. 1), 0.5 ± 0.1 g portions of white-tailed sea eagle muscle tissue were weighed into 15 mL homogenization tubes and homogenized with 1 mL acetonitrile + 0.1% formic acid in a Precyells tissue homogenizer (Bertin Technologies). The extracts were then filtered through a 0.2 µm regenerated cellulose syringe filter (Thermo Scientific, Rockwood, USA) into 2 mL Eppendorf safe-lock tubes (Eppendorf AG, Hamburg, Germany). After centrifugation, the aliquots were frozen at −20 °C for at least 16 h to denature the proteins and then 200 µL were taken for analysis. For optimization, the extraction procedure without SPE clean-up was tested using acetonitrile with 0.1% formic acid, H$_2$O/acetonitrile 50/50 with 0.1% formic acid, and isopropanol/acetonitrile 50/50 with 0.1% formic acid.

Laboratory blanks were prepared in the same way as the natural samples, but without biota. For calculation of matrix effects, biota blank samples were prepared in the same way as the samples, but without adding the target analytes and IS.

Calibration curve solutions were prepared using acetonitrile or sample extracts (i.e., matrix-matched calibration extracts from pooled fish muscle samples) at concentrations of 0.5, 1, 2.5, 5, 10, 50, 100, and 300 ng mL^{-1}. Linearity of the signal in both matrix-matched and solvent calibration curves [26] was calculated for all analytes (data not reported).

2.4. Validation of the method

The main analytical challenge in detection of organic micropollutants in biota samples is their occurrence at trace levels and the complexity of biota samples. Thus, it is necessary to optimize analytical procedures to reach low limits of detection and quantification. Instrument detection limit (IDL), instrument quantification limit (IQL), method detection limit (MDL), method quantification limit (MQL), precision, absolute recovery, and matrix effect were determined for all organic micropollutants in the biota samples analyzed in this study. IDL and IQL were calculated based on the standard deviation (SD) of the lowest detectable point in solvent calibration curves. MDLs and MQLs were calculated based on the lowest detectable point in matrix-matched calibration curves (e.g., white-tailed sea eagle or perch) as follows:

$$IDL = 3 * SD \; Concentration_{Lowest \; in \; solvent \; calibration} \tag{1}$$

$$IQL = 10 * SD \; Concentration_{Lowest \; in \; solvent \; calibration} \tag{2}$$
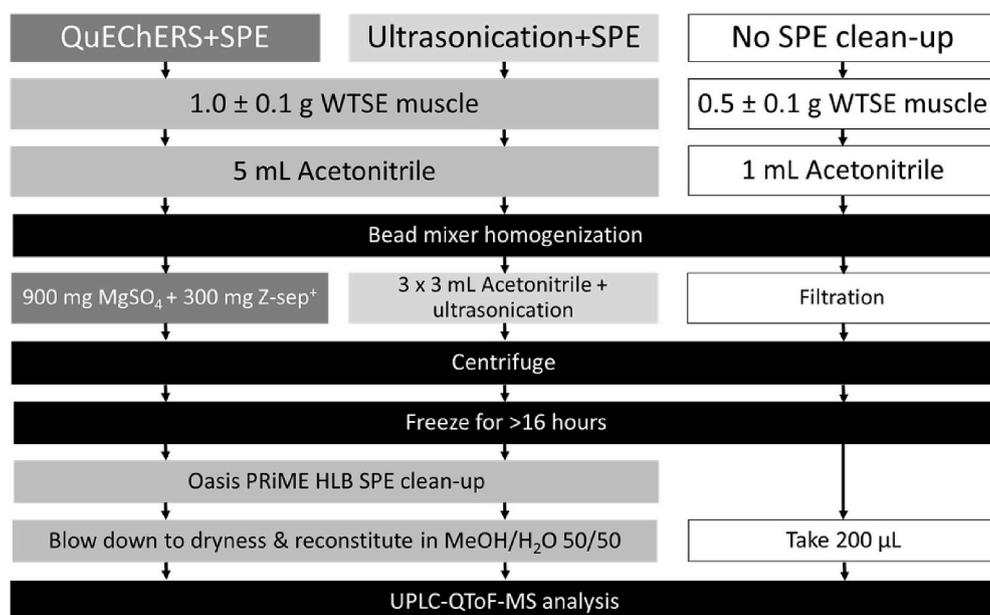
Fig. 1. Sample preparation scheme comparing three different methods for analysis of organic micropollutants: QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) with solid-phase extraction (SPE) clean-up; ultrasonication with SPE clean-up; and extraction with no SPE clean-up. WTSE = white-tailed sea eagle; UPLC-QToF-MS = ultra performance liquid chromatography coupled to quadrupole time of flight mass spectrometry.

Fig. 2. Boxplots comparing different extraction and clean-up methods in terms of: a) matrix effect and b) absolute recovery for pesticides ($n = 89$), pharmaceuticals ($n = 74$), and other compounds ($n = 54$). QuEChERS = Quick, Easy, Cheap, Effective, Rugged and Safe method; SPE = solid-phase extraction.

$$MDL = 3 * SD(Concentration_{Lowest\ in\ MM\ calibration} - Concentration_{\ Biota\ blank}) \qquad (3)$$

$$MQL = 10 * SD(Concentration_{Lowest\ in\ MM\ calibration} - Concentration_{\ Biota\ blank}) \qquad (4)$$

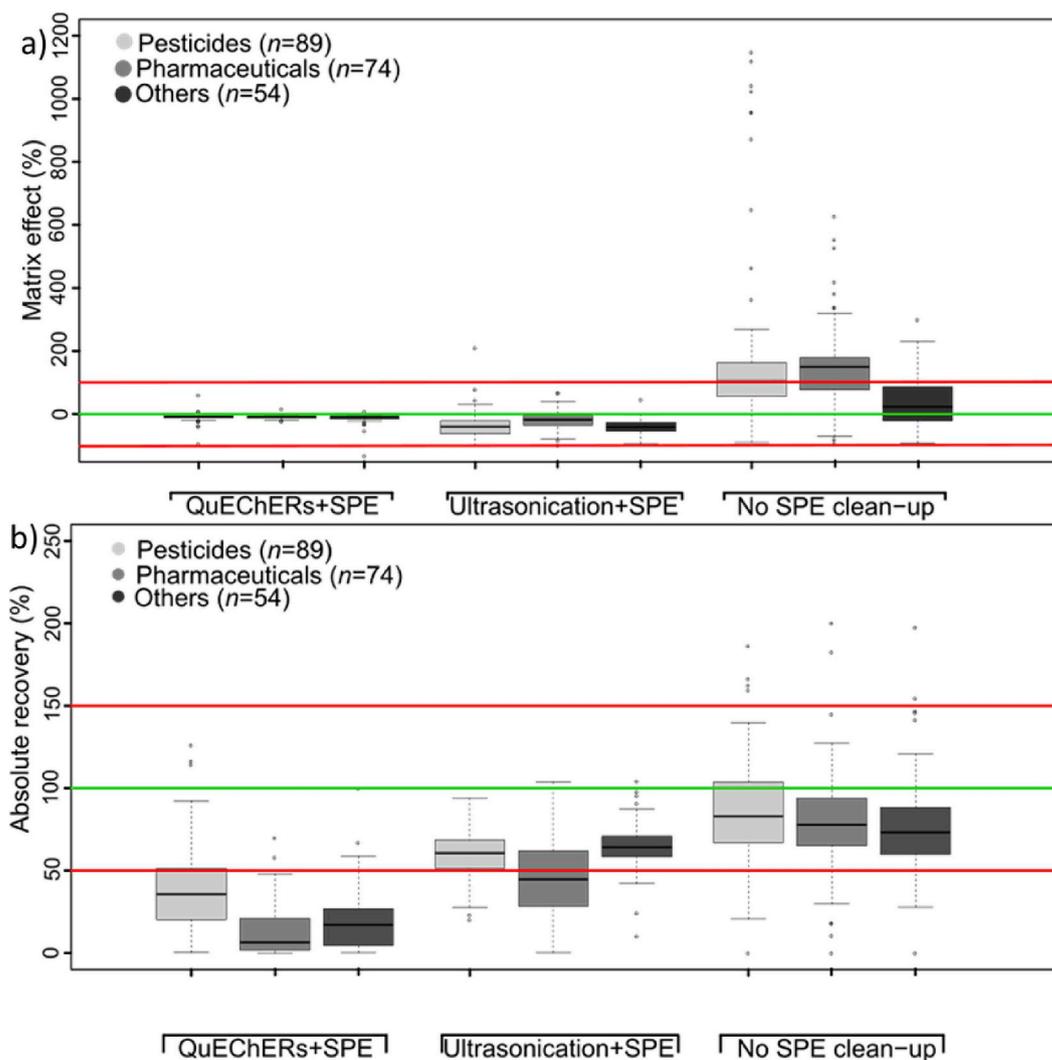Precision was expressed as relative standard deviation (RSD) of triplicate samples. Precision and reproducibility was on average 26 and 13%, respectively (see Table SI4 and Figure SI1 in SI for absolute recovery, matrix effect, precision, reproducibility, MDL, and MQL of each compound).

Absolute recovery and matrix effects were calculated as follows:

$$Absolute\ recovery\ (\%) = \frac{Area_{Post-spiked}}{Area_{Pre-spiked}} * 100 \qquad (5)$$

$$Matrix\ effect\ (\%) = ((\frac{Area_{Post-spiked} - Biota\ blank}{Area_{Solvent-spiked}}) - 1) * 100 \qquad (6)$$

where $Area_{Post-spiked}$ and $Area_{Pre-spiked}$ in (5) refers to the peak area of the target compound in the extract spiked after and before extraction, respectively, and $Area_{Solvent-spiked}$ in (6) refers to the peak area of the target compound in solvent.

2.5. Analytical method for UPLC-QTOF-MS

The instrumental analysis of extracts was carried out using same conditions as reported by Tröger et al. [27]. In brief, the analytes were separated using a Waters Acquity I-Class ultra performance liquid chromatography (UPLC) system equipped with a quaternary pump. For chromatographic separation in positive ionization mode, a reversed-phase ACQUITY UPLC HSS T3-C18 column (2.1 mm × 100 mm and 1.8 μm) (Waters Corporation, Milford, MA) was used, while for negative ionization mode an ACQUITY UPLC BEH-C18 column (2.1 mm × 100 mm and 1.7 μm (Waters Corporation, Milford, MA) was used (detailed information about the UPLC gradient is shown in Table SI5 in SI). The UPLC was coupled to a quadrupole time-of-flight (QToF) mass spectrometer, Xevo G2-S (Waters Corporation, Manchester, UK) with electrospray ionization (ESI) interface working in positive and negative ionization modes. All data were collected in data-independent resolution mode (MS^E-resolution) with a mass range of 100–1200 (m/z), searching for H^+ and H^- adducts with one absolute charge for adduct combinations and 15 ppm mass tolerance for target compounds. Capillary voltage was set to 0.4 kV. Leucine enkephalin was continuously infused for lock mass correction (for details, see Table SI6 in SI). The software UNIFI Waters Scientific Information System (v1.8) was used for instrument control and identification and quantification of compounds.

2.6. Statistical analysis

The data shown in figures are plotted in boxplot diagrams with median, 25th percentile, 75th percentile, minimum and maximum using R software. For comparison of groups, analysis of variance (ANOVA) was performed in Microsoft Excel at significance level $\alpha = 0.05$. Correlations between physicochemical properties (molecular weight, retention time, and log K_{OW}) and recoveries were analyzed by Pearson correlation and tested with t-test in Microsoft Excel at significance level $\alpha = 0.05$.

3. Results and discussion

3.1. Comparison and performance of the three extraction methods for biota

The three extraction methods (Fig. 1) performed differently in terms of absolute recovery and matrix effect for the 217 target compounds (Fig. 2). The two methods including SPE clean-up (ultrasonication and QuEChERS) yielded relatively low matrix effects (on average −31 ± 37% and −11 ± 13%, respectively), whereas the method with no SPE clean-up showed on average matrix enhancement of 134 ± 201% for the target

compounds. Thus, the extraction methods that included a clean-up step helped to reduce the matrix effect, which is in agreement with previous studies [11]. On the other hand, absolute recovery for the QuEChERS and ultrasonication methods with SPE clean-up was on average below 56% (27 ± 25% and 56 ± 20%, respectively), whereas the method without SPE clean-up outperformed the other two methods, with average absolute recovery of 82 ± 32%. High extraction efficiency is important to obtain accurate and reproducible results [28].

As the aim of the study was to keep the extraction method as simple as possible while still recovering at least 75% of the target compounds ($n = 217$), we aimed for target recovery between 50% and 120%. A recovery range of 50–120% was achieved for 27 of 217 of the target analytes using QuEChERS + SPE, for 141 of 217 using ultrasonication + SPE, and for 177 of 217 using no SPE clean-up. For non-target screening analysis, it is generally preferable to have higher recovery over a broad range of compounds rather than low matrix effects, since false negative results (Type II error) are more likely be avoided by not losing possible important compounds [29]. Type I error results (false positives) can be excluded by e.g. blank subtraction, reference/contaminated comparison, or other types of prioritization [29]. Therefore, the method without SPE clean-up was selected as the optimal method and tested further with different extraction solvents.

3.2. Comparison of three extraction solvents for the extraction method without SPE clean-up

Based on previous findings by Grabicova et al. [30], three different extraction solvents/solvent mixtures (i-iii) were selected and tested for optimization of the method without SPE clean-up: i) acetonitrile + 0.1% formic acid, ii) acetonitrile + isopropanol (1:1) + 0.1% formic acid, and iii) acetonitrile + H_2O (1:1) + 0.1% formic acid. Grabicova et al. [30] selected acetonitrile + isopropanol (3:1) + 0.1% formic acid for method validation, because they obtained the highest recovery of target pharmaceuticals in fish using that extraction solvent mixture. Acetonitrile as extraction solvent, combined with freezing out, is a good choice since proteins are denatured within the matrix and since it has been shown that acetonitrile is more selective than methanol for a broad range of compounds [31,32]. In the present study, it was evident from the results that acetonitrile + H_2O (1:1) + 0.1% formic acid achieved on average lower recoveries (66 ± 35%) than acetonitrile + isopropanol (1:1) + 0.1% formic acid (79 ± 27%) and acetonitrile + 0.1% formic acid (82 ± 32%) (Fig. 3). This lower recovery could be explained by the presence of H_2O in the extraction solvent mixture, which can result in loss of substance due to the polarity of the solvent. The matrix effect was similar for all three extraction solvents/solvent mixtures: 135 ± 201% for acetonitrile + 0.1% formic acid, 105 ± 192% for acetonitrile + isopropanol (1:1) + 0.1% formic acid, and 121 ± 187% for acetonitrile + H_2O (1:1) + 0.1% formic acid. A previous study found lower recovery/matrix effects using acetonitrile + isopropanol (3:1) + 0.1% formic acid for selected pharmaceuticals [30]. In the present study, there was no significant difference between the extraction solvents acetonitrile + 0.1% formic acid and acetonitrile + isopropanol (1:1) + 0.1% formic acid ($p > 0.05$). There was no significant correlation between log K_{OW}, molecular weight, or UPLC retention time and absolute recoveries ($p > 0.05$) (Figure SI2 in SI). Although acetonitrile + isopropanol (1:1) + 0.1% formic acid and acetonitrile + 0.1% formic acid achieved similar recoveries with the extraction method without SPE clean-up, the solvent mixture acetonitrile + 0.1% formic acid was selected for application to white-tailed sea eagle and European perch tissue samples (see section 3.4) because it is a slightly less time-consuming method.

3.3. Sample detection limits for quantification and precision of target compounds in white-tailed sea eagle and European perch samples

Relative standard deviation of precision was generally < 20%, with a few exceptions in the pharmaceuticals and pesticides category (Table SI4
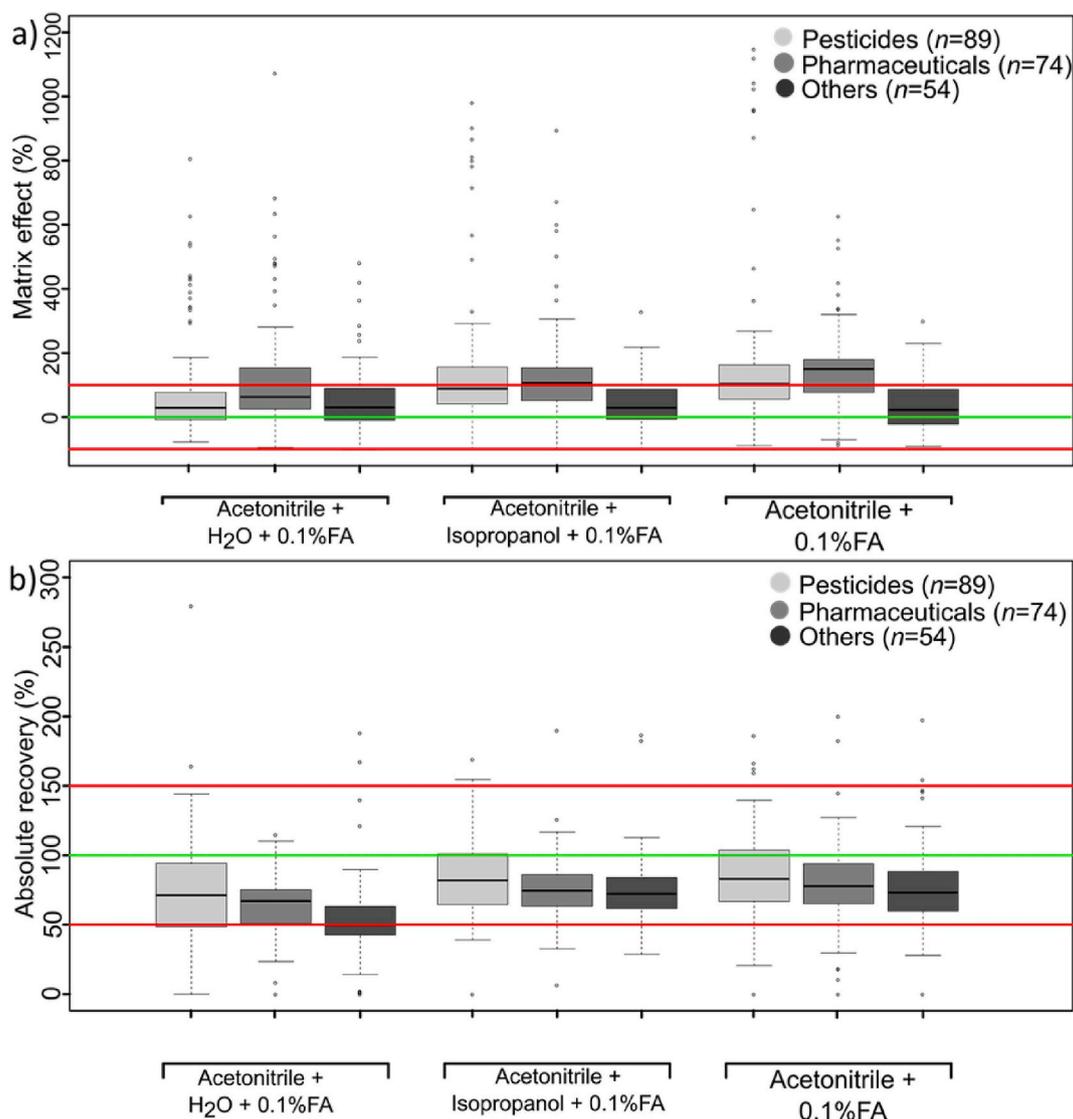
Fig. 3. Boxplots comparing different extraction solvents in terms of: a) matrix effect and b) absolute recovery with the extraction method without solid phase extraction (SPE) clean-up for pesticides ($n = 89$), pharmaceuticals ($n = 74$) and other compounds ($n = 54$). FA = formic acid.

and Figure SI1 in SI). Values of IDL, IQL, MDL, and MQL for muscle tissue of white-tailed sea eagle and European perch are shown in Fig. 4 and Table SI4 in SI. The median IDL and IQL values were generally lower for pesticides and pharmaceuticals (0.20 ng g^{-1} ww and 0.20 ng g^{-1} ww, respectively) than for other compounds (1.0 ng g^{-1} ww). This might be because different compound classes (benzotriazoles, flame retardants, food additives, drugs, stimulants, personal care products, phthalates, isoflavones, industrial chemicals, and PFASs) with very different properties were grouped together. The IDL and IQL values showed high compound-specific variation, which can be explained by differences in the ability of the compounds to ionize in the MS source and other physicochemical properties [33]. The IDL values were comparable to those reported in the literature for multi-residue methods in analysis of pharmaceuticals, ranging from 1 to 100 pg on columns using time-of-flight and triple-quadrupole mass spectrometers [34,35].

Median MDL and MQL values for white-tailed sea eagle muscle were generally lower for pesticides and pharmaceuticals (1.8 ng g^{-1} ww and 1.2 ng g^{-1} ww, respectively) than for other compounds (5.4 ng g^{-1} ww) (Fig. 4). The MDL and MQL values showed similar high variation to the IDLs and IQLs depending on the compound, and the variation was higher for European perch muscle than for white-tailed sea eagle muscle. In target analysis, the goal is generally to achieve as low

detection limits as possible, in particular for known endocrine disruptive compounds [36]. For non-target screening analysis, it is important to determine as many compounds as possible via low detection limits to avoid false negative results [29].

3.4. Method application to white-tailed sea eagle and European perch tissues

Average absolute recovery using acetonitrile $+0.1\%$ formic acid was slightly higher for European perch (103 $\pm$ 24%) than for white-tailed sea eagle (82 $\pm$ 32%) muscle tissue (Figure SI3 in SI). This can be explained by the sample composition, which influences the extraction efficiency for certain compounds, and hence absolute recovery, due to differences in lipid and fat content of the tissue analyzed (0.76 $\pm$ 0.12% for perch) [37]. Nonetheless, our method showed equally good results within acceptable limits (50–120% recovery range) for both eagle and perch muscle tissue.

Muscle, heart, and liver tissue ($n = 10$ for each tissue) from European perch from a reference lake in Sweden were analyzed for the 217 target compounds following extraction without SPE clean-up. The standard deviation of duplicate samples in fish muscle tissue was generally low indicating good reproducibility of the developed method

Fig. 4. Instrument detection and quantification limit (IDL, IQL) and method detection and quantification limit (MDL, MQL), all in log-transformed ng g-1 wet weight, for white-tailed sea eagle (WTSE) and European perch muscle tissue samples.

(Table SI7 in SI). In addition, the overall average relative standard deviation (RSD) for the muscle tissue samples ranged from 8.5 to 29%. In at least 90% of the muscle, heart, and liver samples, 15, 17, and 16 compounds, respectively, were detected (Fig. 5, Table SI7 in SI). The results for the 10 individual fish samples showed that most of these compounds were detected in all tissues, but the concentrations varied between tissue types (e.g., atrazine: 2.0 ± 0.6 ng g^{-1} ww in muscle, 780 ± 490 ng g^{-1} ww in heart, and 160 ± 260 ng g^{-1} ww in liver). This demonstrates that the developed method can successfully be used for different tissue samples from naturally contaminated fish. Overall, the dominant compound in all tissues was 2-hydroxybenzothiazole (OHBT), which has previously been identified as a high production volume chemical in a study by Baduel et al. [38]. The results from our study show that, regardless of tissue composition, it is possible to extract micropollutants using our extraction method without SPE clean-up.

4. Suitability of the method for suspect and non-target screening in future applications

In future applications, the method developed here, with acetonitrile as extraction solvent and without SPE clean-up, is intended for use in multi-compound and multi-class target screening as well as in suspect and non-target screening studies of biota [39]. In these types of studies, it is crucial to capture as many relevant peaks as possible, while limiting the amount of data that needs to be handled. The method was tested in a suspect screening approach by applying a suspect list (target analytes without retention time) to the data obtained for white-tailed sea eagle muscle extract fortified with 50 ng of target compounds (n = 217) before extraction (Fig. 6). In the workflow, filters were applied for data reduction (through a response threshold, retention time threshold, m/z threshold, mass accuracy threshold, and combination of these). In tests with a non-target screening approach, all detected peaks were included

Fig. 5. Bar chart showing composition profile (%) of detected compounds in > 90% of samples in different muscle, heart, and liver tissues from European perch (*Perca fluviatilis*). CECs = contaminants of emerging concern; n = number of detected compounds in > 90% of samples.

Suspect screening approach: Target compounds as Suspect list			**Non-target screening approach:**	
ESI − (n=61)	ESI + (n= 154)		ESI −	ESI +
65	246	No filters	12 982	32 325
47	231	Response threshold (> 4000)	1 563	7 067
63	234	Retention time threshold (< 14 min)	8 749	17 270
65	245	Observed m/z threshold (50 < m/z < 800)	10 241	23 799
59	215	Mass accuracy threshold (-2 < mDa < 2)	NA	NA
45	219	Counts + RT	1 258	3 388
45	218	Counts + RT + Observed m/z	1 193	2 721
44	191	Counts + RT + Observed m/z + Mass accuracy	NA	NA

Fig. 6. Number of peaks detected in white-tailed sea eagle muscle tissue extract fortified with contaminants (n = 217), without and with applying different filters in a suspect screening approach (with the target analytes as suspects) and a non-target approach. RT = retention time; ESI = electron spray ionization; NA = not available, since mass accuracy is determined for non-target analysis at a later stage.

(Fig. 6). The results showed that for suspect screening, a mass accuracy threshold is important for limiting the possible suspects (in our case, 59 of 65 peaks remained in negative ionization mode and 215 of 246 peaks in positive ionization mode). In non-target screening, a mass accuracy threshold cannot be applied, as exact masses have to be compared with reference masses at a later stage. On the other hand, a response threshold drastically limits the number of peaks detected (Fig. 6) and makes data handling and prioritization easier. The non-target screening results showed that further prioritization is required for feasible data handling. The high number of detected features was expected, based on previous studies [15,24].

5. Conclusions

A relatively non-discriminant and simple extraction method would be valuable for suspect and non-target screening. We developed a broad extraction method with no time-consuming clean-up procedure. The extraction method was tested and optimized using > 200 CECs to make it applicable for suspect and non-target screening of biota. This novel method outperformed QuEChERS and ultrasonication with SPE clean-up in terms of absolute recovery. Calculated detection limits for the target compounds in muscle samples from white-tailed sea eagle (*Haliaeetus albicilla)* and European perch (*Perca fluviatilis*) were in the low ng g^{-1} ww range and the method was successfully applied to heart, liver, and muscle tissue samples from European perch. The method is able to capture many peaks relevant in suspect and non-target screening workflows. In future work, the method should be applied to biota in order to extract peaks relevant for identification by suspect and non-target screening.

Credit author statement

Wiebke Dürig: conceptualization, methodology, validation, analysis, writing - original draft, review and editing. Aaron Kintzi: methodology, validation, analysis. Oksana Golovko: conceptualization, writing, review and editing. Karin Wiberg: conceptualization, supervision, funding acquisition, writing and review. Lutz Ahrens: conceptualization, supervision, project administration, funding acquisition, writing, review and editing.

Declaration of competing interest

The authors declare no competing financial interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2020.121294.

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