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# Multiresidue analytical method for the determination of antimicrobials, preservatives, benzotriazole UV stabilizers, flame retardants and plasticizers in fish using ultra high performance liquid chromatography coupled with tandem mass spectrometry

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ABSTRACT

A multiresidue analytical method for the determination of emerging pollutants belonging to personal care products (PCPs) (antimicrobials, preservatives), benzotriazole UV stabilizers (BUVSs) and organophosphorus compounds (OPCs) in fish has been developed using high speed solvent extraction (HSSE) followed by silica gel clean up and ultra fast liquid chromatography coupled with tandem mass spectrometry (UFLC–MS/MS) analysis. Developed extraction and clean up method yielded good recovery (>70%) for all the four groups of emerging pollutants, i.e. antimicrobials (78.5–85.6%), preservatives (85.0–89.4%), BUVSs (70.9–112%) and OPCs (81.6–114%; except for TEP – 68.9% and TPeP – 58.1%) with RSDs ranging from 0.7 to 15.4%. Intra- and inter-day repeatabilities were less than 19.8% and 19.0%, respectively at three spiked levels. The concentrations were given in lipid weight (lw) basis, and the method detection limits were achieved in the lowest range of 0.001–0.006 ng $g^{-1}$ for two antimicrobials, 0.001–0.015 ng $g^{-1}$ for four preservatives, 0.0002–0.009 ng $g^{-1}$ for eight BUVSs and 0.001–0.014 ng $g^{-1}$ for nine OPCs. Finally, the method was successfully validated as a simple and fast extraction method for the determination of 23 compounds belonging to PCPs, BUVSs and OPCs and applied to the analysis of three species of fish from Manila Bay, the Philippines. Concentrations ranged from 27 to 278 ng $g^{-1}$ for antimicrobials, 6.61 to 1580 ng $g^{-1}$ for paraben preservatives, <MDL (method detection limit) to 179 ng $g^{-1}$ for BUVSs and ND (not detected) to 266 ng $g^{-1}$ for OPCs suggesting the ubiquitous contamination by these emerging pollutants in Manila Bay. This is the first method developed for the determination of triclocarban, four paraben preservatives and four BUVSs, in fish.



## 1. Introduction

The presence of emerging pollutants in the aquatic environment and their deleterious effects in resident organisms is an increasing public concern. In the environment, the organisms often encounter and are exposed to myriad synthetic chemicals. The emerging pollutants, personal care products belonging to antimicrobial agents (triclocarban and triclosan), paraben preservatives (methyl-, ethyl-, propyl- and butylparabens) and benzotriazole ultraviolet (UV) stabilizers are used in personal-hygiene products such as toothpastes, soaps, shampoos, body lotions and sunscreen cosmetics. Organophosphorus compounds are used in a large variety of consumer products such as flame retardants and plasticizers, antifoaming agents and additives. These compounds are continuously released into the aquatic environment from point sources such as wastewater treatment plants (WWTP) and effluents [1,2]. Continuous input of chemicals, with short half-lives, through anthropogenic activities make them as pseudo-persistents in the environment [3]. Aquatic organisms have the tendency to accumulate these chemical contaminants in their body when exposed continuously.

Several studies have shown their (chemicals) occurrence in the aquatic, terrestrial and atmospheric environment as a result of anthropogenic activities [4]. To date, numerous commercial UV

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**Table 1**
Details of CAS number, molecular formula and log $K_{ow}$ of the target compounds.

| Abbreviation | Name | CAS number | Formula | Log $K_{ow}$[a] |
|---|---|---|---|---|
| Antimicrobials (2) | | | | |
| TCS | Triclosan | 3380-34-5 | $C_{12}H_7Cl_3O_2$ | 4.76 |
| TCC | Triclocarban | 101-20-2 | $C_{13}H_9Cl_3N_2O$ | 4.90 |
| Preservatives (4) | | | | |
| MeP | Methyl paraben | 99-76-3 | $C_8H_8O_3$ | 1.96 |
| EtP | Ethyl paraben | 120-47-8 | $C_9H_{10}O_3$ | 2.47 |
| PrP | Propyl parben | 94-13-3 | $C_{10}H_{12}O_3$ | 3.04 |
| BuP | Butyl parben | 94-26-8 | $C_{11}H_{14}O_3$ | 3.57 |
| Benzotriazole UV stabilizers (8) | | | | |
| UV-P | 2-(2*H*-5-methylphenyl)benzotriazole | 2440-22-4 | $C_{13}H_{11}N_3O$ | 4.31 |
| UV-9 | 2-(2*H*-benzotriazol-2-yl)-4-methyl-6-(2-propenyl)phenol | 2170-39-0 | $C_{16}H_{15}N_3O$ | |
| UV-234 | 2-(2*H*-benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl)phenol | 70321-86-7 | $C_{30}H_{29}N_3O$ | 7.67 |
| UV-320 | 2-(2H-benzotriazol-2-yl)-4,6-di-tert-butylphenol | 3846-71-7 | $C_{20}H_{25}N_3O$ | 7.21[b] |
| UV-326 | 2-*tert*-Butyl-6-(5-chloro-2*H*-benzotriazol-2-yl)-4-methylphenol | 3896-11-5 | $C_{17}H_{18}ClN_3O$ | 6.58[b] |
| UV-327 | 2,4-Di-*tert*-butyl-6-(5-chloro-2*H*-benzotriazol-2-yl)phenol | 3864-99-1 | $C_{20}H_{24}ClN_3O$ | 7.81[b] |
| UV-328 | 2-(2*H*-benzotriazol-2-yl)-4,6-di-*tert*-pentylphenol | 25973-55-1 | $C_{22}H_{29}N_3O$ | 7.25[b] |
| UV-329 | 2-(2*H*-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol | 3147-75-9 | $C_{20}H_{25}N_3O$ | 6.21 |
| Organophosphorus compounds (9) | | | | |
| TCP | Tritolyl phosphate (*o*-, *m*-, *p*-tricresyl phosphate) | 1330-78-5 | $C_{21}H_{21}O_4P$ | 5.11 |
| TEP | Triethyl phosphate | 78-40-0 | $C_6H_{15}O_4P$ | 0.80 |
| TEHP | Tris(2-ethylhexyl) phosphate | 78-42-2 | $C_{24}H_{51}O_4P$ | 9.49 |
| TBEP | Tris(2-butoxyethyl) phosphate | 78-51-3 | $C_{18}H_{39}O_7P$ | 3.75 |
| TPhP | Triphenyl phosphate | 115-86-6 | $C_{18}H_{15}O_4P$ | 4.59 |
| TBP | Tributyl phosphate | 126-73-8 | $C_{12}H_{27}O_4P$ | 4.00 |
| TPrP | Tripropyl phosphate | 513-08-6 | $C_9H_{21}O_4P$ | 1.87 |
| EHDPP | 2-Ethylhexyl diphenyl phosphate | 1241-94-7 | $C_{20}H_{27}O_4P$ | 5.73 |
| TPeP | Tripentyl phosphate | 2528-38-3 | $C_{15}H_{33}O_4P$ | 5.29 |

[a] Experimental values, from database of physico-chemical properties. Syracuse Research Corporation: http://www.syrres.com/esc/physdemo.htm.
[b] Nakata et al. [14].

filter formulations with varying compositions are marketed and afford protection against sunlight radiation. In terms of production and use, the 2-hydroxyphenyl derivatives of benzotriazole constitute one of the most important families of UV stabilizers [5]. Apart from many beneficial properties, some UV filters are suspected to disrupt the endocrine system of the organisms, leading to adverse effects on reproduction and development [6,7]. Danovaro et al. [8] found that parabens and UV filters in sunscreen products can cause abrupt and complete bleaching of hard corals, even at extremely low ($10\,\mu L\,L^{-1}$) concentrations.

Trace analysis of emerging pollutants in various matrices is achieved with high end instrumentation such as liquid chromatography (LC)/gas chromatography (GC) coupled with single or tandem mass spectrometry [1]. The use of tandem mass spectrometry (MS/MS) in multiple reaction monitoring (MRM) has become popular for quantitative environmental analysis with increased selectivity and sensitivity, and LC–MS/MS allows the determination of highly polar organic pollutants without derivatization, down to nanogram levels [1]. These advanced analytical techniques have been used in the determination of PCPs [9–12] and UV filters/stabilizers [9,10,12–14] in biota. Very few studies are available on OPCs in human plasma [15], urine [16] and fish tissues [17]. Appropriate sample treatment (extraction, clean up, etc.) is a prerequisite for trace level determination of emerging chemicals with low detection limits. Sample treatment processes often affect the recovery and precision of the analysis even if advanced instrumentation is used.

Multiresidue analytical methods were developed successfully for number of EDCs, pesticides, PPCPs and phenolic compounds from sewage [18–20], water [21,22], soil/sediment [23], and vegetables [11] but scantly developed for fauna (fish) [12,24]. Nevertheless, methods to determine individual or a group of compounds are available in plenty. Adolfsson-Erici et al. [25] determined triclosan in fish bile using GC–MS after extraction with hexane/methyl-t-butyl-ether and hydrolyzing enzymatically. Balmer et al. [9] extracted methyltriclosan in lake fish using column and accelerated solvent extraction (ASE) followed by gel permeation chromatography (GPC) and GC–MS quantification. Canosa et al. [26] reported matrix solid-phase dispersion (MSPD) extraction and GC–MS/MS analysis of triclosan and methyltriclosan in fish samples. Extraction of UV filters from fish tissues were done by ASE with cyclohexane/dichloromethane (1:1) at room temperature [27] and soxhlet extraction with petroleum ether/ethyl acetate (2:1) [7] followed by GPC and silica gel clean up. Buser et al. [10] determined UV filters (4-methylbenzylidene camphor and octocrylene) in fish by GC–MS after homogenization and extraction using separatory funnel.

Although number of studies reported the levels of OPCs, especially flame retardants in water and wastewater samples using LLE [28–30], SPE [31–33], etc., reports on biological matrices were scantly carried out. Sundkvist et al. [17] determined OPCs in fish by GC–HR (high resolution) MS after ASE and clean up with GPC. In fact, there is a dearth of analytical methods for the determination of parabens in biota except for human samples [34,35].

With regard to the consumption of chemical of anthropogenic use, worldwide demand for PCPs, flame retardants and plasticizers are increasing exponentially. At the same time, there is lack of information about environmental occurrence, behavior and eventual fate of these emerging contaminants all over the globe, except from some developed countries. The objective of this study was to develop a suitable multi-residue analytical method of high speed solvent extraction followed by LC–ESI-MS/MS to determine antimicrobials (triclocarban and triclosan), preservatives (parabens), UV stabilizers (benzotriazole), and OPCs (flame retardants and plasticizers) in biological (fish) samples.

## 2. Experimental

### 2.1. Chemicals and reagents

All standards (detailed names with CAS number are furnished in Table 1) were obtained with the highest available purity. UV-P, -326, -328, -329, TEHP, TBEP, TPhP, TBP, TPrP and EHDPP were

**Table 2**
UFLC–MS/MS experimental parameters.

| Compound | tR (min) | MRM 1 (*m*/*z*) (quantification) | DP[a] | CE[b] | MRM 2 (*m*/*z*) (qualification) | DP | CE |
|---|---|---|---|---|---|---|---|
| **ESI−** | | | | | | | |
| TCS | 3.90 | 286.8 > 35.0 | −40 | −38 | – | – | – |
| $^{13}C_{12}$-TCS | 3.89 | 298.9 > 34.8 | −35 | −46 | – | – | – |
| TCC | 3.87 | 312.8 > 125.8 | −90 | −32 | 312.8 > 159.9 | −90 | −18 |
| MeP | 3.28 | 151.2 > 92.0 | −50 | −26 | 151.2 > 136.0 | −50 | −16 |
| $^{13}C_6$-MeP | 3.28 | 156.9 > 97.9 | −115 | −28 | – | – | – |
| EtP | 3.47 | 164.9 > 91.8 | −55 | −30 | 164.9 > 135.8 | −55 | −20 |
| PrP | 3.59 | 178.9 > 91.9 | −45 | −32 | 178.9 > 136.0 | −45 | −22 |
| BuP | 3.67 | 192.9 > 92.0 | −55 | −32 | 192.9 > 136.0 | −55 | −22 |
| $^{13}C_3$-IBU | 3.79 | 208.0 > 163.1 | −30 | −10 | – | – | – |
| **ESI+** | | | | | | | |
| UV-P | 5.57 | 226.1 > 98.9 | 81 | 25 | – | – | – |
| UV-9 | 7.66 | 266.0 > 119.1 | 51 | 29 | – | – | – |
| UV-234 | 10.23 | 448.2 > 370.1 | 51 | 31 | 448.2 > 91.1 | 51 | 85 |
| UV-320 | 10.13 | 324.0 > 268.1 | 101 | 33 | 324.0 > 212.1 | 101 | 39 |
| UV-326 | 10.69 | 316.0 > 260.0 | 66 | 27 | 316.0 > 154.0 | 66 | 37 |
| UV-327 | 11.70 | 358.0 > 302.0 | 21 | 33 | – | – | – |
| UV-328 | 11.41 | 352.1 > 282.1 | 96 | 33 | – | – | – |
| UV-329 | 8.60 | 324.0 > 212.0 | 56 | 33 | – | – | – |
| TCP | 6.06 | 369.0 > 165.0 | 161 | 69 | 369.0 > 65.0 | 161 | 89 |
| TEP | 5.08 | 183.0 > 127.0 | 36 | 15 | 183.0 > 99.0 | 36 | 25 |
| TEHP | 9.95 | 435.1 > 71.1 | 81 | 31 | 435.1 > 99.0 | 81 | 29 |
| TBEP | 5.92 | 399.1 > 299.1 | 66 | 19 | 399.1 > 199.1 | 66 | 25 |
| TPhP | 5.72 | 327.0 > 51.1 | 91 | 113 | 327.0 > 152.0 | 91 | 59 |
| TPhP-$d_{15}$ | 5.70 | 342.2 > 82.0 | 166 | 73 | – | – | – |
| TBP | 5.85 | 267.1 > 155.0 | 81 | 15 | 267.1 > 99.0 | 81 | 31 |
| TBP-$d_{27}$ | 5.83 | 294.2 > 101.9 | 71 | 31 | – | – | – |
| TPrP | 5.57 | 225.0 > 98.9 | 51 | 25 | 225.0 > 81.0 | 51 | 67 |
| EHDPP | 6.19 | 363.0 > 251.0 | 71 | 25 | 363.0 > 76.9 | 71 | 76 |
| TPeP | 6.23 | 309.0 > 81.0 | 146 | 87 | 309.0 > 98.9 | 146 | 29 |

[a] Declustering potential (V).
[b] Collision energy (eV).

procured from Wako Chemicals (Osaka, Japan). TCS, TCC, MeP, EtP, PrP and BuP were purchased from Kanto Chemical (Tokyo, Japan). UV-9, -234, -320, -327 and TCP were obtained from Sigma–Aldrich (St. Louis, MO, USA). TEP and TPeP were from Tokyo Chemical (Tokyo, Japan). Isotope-labeled standards (ISs), $^{13}C_3$-IBU, $^{13}C_{12}$-TCS, and $^{13}C_6$-MeP were obtained from Otsuka (Tokyo, Japan). TPhP-$d_{15}$ and TBP-$d_{27}$ were procured from Tokyo Chemical Industry (Tokyo, Japan) and Sigma–Aldrich (St. Louis, MO, USA), respectively. LC–MS grade methanol, dichloromethane, acetonitrile, formic acid and ammonium acetate, and silica gel (Wako gel) were from Wako Chemicals (Osaka, Japan). Anhydrous sodium sulphate (Extra pure) was procured from Nacalai Tesque Inc. (Kyoto, Japan). Ultrapure water was delivered by Direct-Q3 (Millipore, Japan) water purification system. Individual stock solutions of each compound were prepared in acetonitrile at 1 mg mL$^{-1}$ (BUVSs 0.1 mg mL$^{-1}$) and stored in amber glass vials at −20 °C. Working solutions were freshly prepared in methanol from the stock and stored at 4 °C in the dark. Mixed standards were prepared and used for fortification in recovery experiments and for standards calibration.

### 2.2. Extraction and clean up

Five grams of fresh fish muscle tissues was freeze-dried and homogenized well with 100 g anhydrous sodium sulphate ($Na_2SO_4$), spiked with ISs ($^{13}C_{12}$-TCS, $^{13}C_6$-MeP, TPhP-$d_{15}$ and TBP-$d_{27}$) and standard mixture, and extracted with a mixture (1:1, v/v) of hexane and acetone, using a High Speed Solvent Extractor (SE-100, Mitsubishi Chemical Analytechs, Japan) at 30 °C at a 10 mL min$^{-1}$ flow rate for 30 min. After extraction, the extract was concentrated to <10 mL using a rotary evaporator (EYELA, Japan) and make up to 10 mL using n-hexane. A portion of the extract (2 mL) was used for lipid measurement gravimetrically and 1 mL of the extract was employed for clean up. Four grams of 5% $H_2O$ deactivated silica gel was stirred with 20 mL of hexane, and the slurry was transferred to a glass column (200 × 10 mm i.d.) having a glass wool plug, then 1 g of $Na_2SO_4$ was layered above the silica gel and conditioned with 25 mL of hexane. The extract was loaded onto the column and analytes were eluted with 100 mL of dichloromethane. Then the dichloromethane fraction was evaporated using rotary evaporator until about 1 mL, and transferred into glass vials and dried under a gentle stream of nitrogen gas. After complete solvent evaporation the residue was reconstituted in 1 mL of methanol and 1 ng each of $^{13}C_3$-IBU (ibuprofen) and TPhP-$d_{15}$ were added as syringe standards for negative ionization (NI) and positive ionization (PI) modes, respectively and analyzed by LC–MS/MS. Parallely, a blank (only $Na_2SO_4$) was processed as described above for each batch of 7 fish samples.

### 2.3. Instrumentation

Identification and quantification were performed on an UFLC-XR system (Shimadzu, Japan) coupled to a triple quadrupole mass spectrometer (AB Sciex, Tokyo, Japan). The chromatographic separation was achieved with a Asentis express $C_{18}$ analytical column (2.7 μm, 100 × 2.1 mm; Supelco, Bellefonte, USA) at a flow rate of 0.2 mL min$^{-1}$ for PI and 0.3 mL min$^{-1}$ for NI using 0.1% (v/v) formic acid in Milli-Q water (A) and 10 mM ammonium acetate in methanol (B), as mobile phase. The gradient conditions were as follows: (A) 80%, (B) 20% for initial and hold for 2 min; (A) 5%, (B) 95% at 3 min and hold for 8 min; (A) 0%, (B) 100% at 9 min and hold for 13 min for PI and (A) 90%, (B) 10% for initial and hold for 1 min; (A) 5%, (B) 95% at 2 min and hold for 5 min for NI. The column and sample tray temperatures were kept at 10 °C and 40 °C, respectively. The sample injection volume was set as 10 μL. Analytes were determined using an AB Sciex Triple Quad 5500 (AB Sciex, Tokyo, Japan) with a turbo-spray ion source. Quantification was performed in either PI or NI of multiple reaction monitoring

**Table 3**
Validation parameters for determination of selected PCPs, BUVSs and OPCs in fish samples.

| Compound | Linearity[a] | Precision[b] | Recovery (%)[c] | Intra-day (%RSD) | | | Inter-day (%RSD) | | | MDL[d] (pg g$^{-1}$) | MQL[e] (pg g$^{-1}$) | ME[f] (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ($R^2$) | (%RSD) | (%RSD) | 0.1 ng g$^{-1}$ | 0.5 ng g$^{-1}$ | 1.0 ng g$^{-1}$ | 0.1 ng g$^{-1}$ | 0.5 ng g$^{-1}$ | 1.0 ng g$^{-1}$ | | | |
| TCS | 0.9997 | 7.6 | 78.5 (5.5) | 16.7 | 16.4 | 19.8 | 14.7 | 13.3 | 15.8 | 6 | 21 | 78.5 |
| TCC | 0.9994 | 4.5 | 85.6 (6.8) | 15.5 | 9.5 | 4.8 | 11.0 | 10.7 | 5.7 | 1 | 4 | 90.0 |
| MeP | 0.9992 | 5.7 | 86.5 (3.7) | 13.1 | 8.5 | 6.3 | 17.0 | 6.6 | 4.1 | 15 | 49 | 94.2 |
| EtP | 1.0000 | 4.3 | 89.4 (3.6) | 9.4 | 8.9 | 4.4 | 5.3 | 0.5 | 1.2 | 3 | 11 | 105 |
| PrP | 0.9998 | 5.4 | 85.0 (2.0) | 11.8 | 8.3 | 6.6 | 13.9 | 13.6 | 10.7 | 7 | 24 | 100 |
| BuP | 1.0000 | 2.9 | 86.7 (4.4) | 15.6 | 16.1 | 7.3 | 8.7 | 14.0 | 6.7 | 1 | 3 | 96.4 |
| UV-P | 0.9997 | 5.6 | 70.9 (0.7) | 9.1 | 14.2 | 13.4 | 5.8 | 6.2 | 4.5 | 8 | 26 | 100 |
| UV-9 | 0.9990 | 4.1 | 103 (5.2) | 4.7 | 2.4 | 1.3 | 8.4 | 4.7 | 0.9 | 0.2 | 1 | 76.8 |
| UV-234 | 0.9997 | 3.1 | 111 (6.5) | 17.2 | 12.7 | 15.4 | 15.6 | 7.8 | 8.9 | 2 | 5 | 95.2 |
| UV-320 | 0.9998 | 7.2 | 96.0 (7.9) | 16.8 | 12.9 | 15.1 | 15.7 | 12.4 | 10.9 | 0.3 | 5 | 117 |
| UV-326 | 0.9997 | 8.1 | 102 (11.7) | 17.9 | 6.6 | 16.1 | 13.2 | 3.5 | 12.4 | 8 | 1 | 121 |
| UV-327 | 0.9998 | 7.6 | 104 (6.7) | 17.3 | 10.0 | 14.1 | 19.0 | 11.7 | 6.5 | 8 | 27 | 106 |
| UV-328 | 0.9998 | 5.6 | 112 (6.8) | 16.3 | 9.4 | 15.8 | 16.8 | 4.0 | 11.2 | 9 | 26 | 97.9 |
| UV-329 | 0.9986 | 4.2 | 108 (4.7) | 13.8 | 18.1 | 17.0 | 14.4 | 10.8 | 15.2 | 2 | 7 | 106 |
| TCP | 0.9968 | 4.9 | 93.9 (5.3) | 10.8 | 16.9 | 10.4 | 3.5 | 2.6 | 4.7 | 2 | 8 | 97.9 |
| TEP | 0.9987 | 9.7 | 68.9 (1.1) | 19.0 | 16.4 | 16.0 | 10.8 | 14.5 | 9.8 | 4 | 13 | 84.6 |
| TEHP | 1.0000 | 4.8 | 114 (9.7) | 17.6 | 10.7 | 14.7 | 9.3 | 4.4 | 6.6 | 5 | 16 | 109 |
| TBEP | 0.9999 | 2.9 | 109 (8.6) | 5.5 | 3.4 | 5.2 | 3.1 | 1.8 | 2.4 | 4 | 12 | 110 |
| TPhP | 0.9980 | 7.8 | 107 (5.7) | 3.3 | 6.2 | 6.4 | 10.9 | 7.4 | 2.2 | 14 | 45 | 103 |
| TBP | 0.9996 | 4.6 | 94.6 (15.4) | 9.8 | 11.2 | 7.7 | 4.6 | 5.8 | 3.5 | 2 | 7 | 105 |
| TPrP | 0.9941 | 2.8 | 81.6 (8.8) | 8.4 | 11.4 | 5.4 | 8.6 | 6.6 | 6.2 | 1 | 3 | 104 |
| EHDPP | 0.9987 | 9.4 | 95.9 (2.8) | 16.2 | 15.1 | 19.1 | 16.0 | 13.3 | 15.8 | 8 | 28 | 101 |
| TPeP | 0.9998 | 9.7 | 58.1 (12.3) | 11.4 | 16.2 | 16.1 | 5.1 | 6.8 | 6.9 | 6 | 19 | 80.5 |

[a] Determination coefficient for 5 points analytical curves in the 0.01, 0.05, 0.1, 1, and 10 ng mL$^{-1}$ range.
[b] RSD: relative standard deviation ($n$ = 7).
[c] Average recovery and RSD at three concentrations: 0.1, 0.5 and 1.0 ng g$^{-1}$.
[d] MDL: method detection limit ($3SD_{blank}$).
[e] MQL: method quantitation limit ($10SD_{blank}$).
[f] ME: matrix effect.

analysis. The respective MS parameters for PI and NI were set as follows, curtain gas: 30 and 10 psi; collision gas: 9 and 7 psi; ion spray voltage: 5500 and −4500 V; temperature: 700 °C for both; ion source gas 1: 80 and 50 psi and ion source gas 2: 70 and 60 psi. The analytes were confirmed with the product/precursor ions and also based on the retention time (RT) of each peak corresponding to the standard. MRM transitions and other operating parameters are summarized in Table 2. The mass spectrometer was controlled by Analyst 1.5.1 software of AB Sciex.

### 2.4. Quality assurance and quality control

Working standards (from 0.01 to 10 ng mL$^{-1}$) of antimicrobials, preservatives, BUVSs and OPCs were prepared and stored at 4 °C in dark. Standard calibration was performed by plotting analyte concentrations versus peak areas and excellent linearity with the correlation coefficient ($R^2$ > 0.994) was displayed for all the analytes and are listed in Table 3. The method detection limits (MDLs) and method quantification limits (MQLs) were determined as the amount which would give three and 10 times, respectively the standard deviation of the peak area for seven replicates of the blank analysis. Recoveries for all the analytes in fish muscle tissue samples were assessed by spiking of standard mixture at three concentrations of 0.1, 0.5 and 1.0 ng g$^{-1}$. Precision was evaluated as the relative standard deviation (RSD) of replicate measurements. Both intra- and inter-day reproducibilities of the analytical method were assessed. Intra-day precision was evaluated over a short period of time under the same instrumental conditions with nine determinations covered three replicates each of three concentrations (0.1, 0.5 and 1.0 ng g$^{-1}$) of fish tissue spiked. Inter-day precision of the analytical method was verified by determinations that covered three concentrations (0.1, 0.5 and 1.0 ng g$^{-1}$) of fish tissue spiked, three replicates each analyzed on three different days. The concentrations were calculated by comparing their peak areas relative to the standards and the final concentrations were not corrected for recovery. Both method blank and method spike were used for each sample batch to ensure no laboratory contamination was introduced during sample preparation and analysis, and to maintain the method performance.

### 2.5. Optimization of sample clean up and chromatographic separation

GPC was performed to remove lipid from the sample extract. Salad oil (0.5 g), was loaded to a gel permeation chromatography (Bio-Beads S-X3, Bio-Rad, CA, 500 mm × 20 mm i.d.) column after spiking with 1 ng g$^{-1}$ of the standard and ISs mixture. Dichloromethane/hexane (1:1, v/v) was used as the mobile phase at a flow rate of 5 mL min$^{-1}$. As reported by Kajiwara et al. [36] for chlorinated compounds the first 120 mL was discarded, and the following fractions (50 mL + 50 mL) were collected and subjected for UFLC–MS/MS to determine the target compounds.

In order to optimize the silica gel clean up, salad oil (0.5 g) was spiked with 1 ng g$^{-1}$ concentrations of the standards mixture of target analytes and ISs ($^{13}C_{12}$-TCS, $^{13}C_6$-MeP and TBP-$d_{27}$). To see the efficiency of clean up solvents, hexane and dichloromethane (100 mL) were used at different ratios, i.e. 5, 25 and 50% dichloromethane in hexane, and 100% dichloromethane. Further, to optimize the volume of dichloromethane, we have used four different volumes (i.e. 25, 50, 75 and 100 mL) to elute the target compounds for their recovery.

### 2.6. Matrix effect

To find the matrix effect on signal responses, all analytes (1 ng g$^{-1}$) were spiked in neat solution (A) and spiked after extraction of fish extracts (B) and compared their peak areas, and the matrix effect (ME) ratio was calculated as: ME (%) = B/A × 100 in accordance with Matuszewski et al. [37]. The absence of absolute matrix effect is indicated by a value of 100%. The values of less

or greater than 100% indicate signal suppression or enhancement, respectively.

### 2.7. Application to biological samples

Muscle tissue of three different fish species, bluetail mullet (*Valamugil buchanani*, $n = 1$), coral grouper (*Epinephelus corallicola*, $n = 1$), and flathead grey mullet (*Mugil cephalus*, $n = 3$), which were collected from Manila Bay, the Philippines during June 2008 were analyzed. The fish were frozen, transported to Japan and stored in the Environmental Specimen Bank (*es*-BANK) of Ehime University [38] at −25 °C until chemical analysis.

## 3. Results and discussion

### 3.1. MRM parameters

The precursor and product ions of individual target compound were obtained by tuning after direct injection of 1 ng mL$^{-1}$. The optimized MS/MS parameters for MRM analysis (declustering potential, collision energy, etc.) for the analytes (antimicrobials, preservatives, BUVSs and OPCs) and the resulting precursor and product ions are given in Table 2. The multi-class characteristics of 17 compounds (UV-P, -9, -234, -320, -326, -327, -328, -329, TCP, TEP, TEHP, TBEP, TPhP, TBP, TPrP, EHDPP and TPeP) out of 23 target compounds belonging to BUVSs and OPCs were sensitive in PI mode, whereas rest of the six antimicrobials and preservatives (TCS, TCC, MeP, EtP, PrP, and BuP) were sensitive in NI mode. Protonated molecule $[M+H]^+$ for PI and deprotonated molecule $[M-H]^-$ for NI, were used as the precursor ions for all the analytes. In the present study, $m/z$ 126 was selected as product ion for TCC due to its high intensity. Sapkota et al. [39] reported that dominant fragmentation pattern was observed for deprotonated TCC consists of cleavage of the nitrogen–carbon bond closest to the aromatic ring carrying two chlorine atoms, yielding a product ion at $m/z$ 160, and $m/z$ 162. Monochlorinated aniline is also produced but to a minor extent ($m/z$ 126 and $m/z$128).

### 3.2. Chromatographic separation

To obtain the lowest detection limit for each target compound, the chromatographic separation was optimized. Two LC columns and three different conditions of mobile phase were tested to obtain maximum peak separation with symmetric shape. A $C_{18}$ column was used for separation of OPCs or PPCPs in the previous studies [40–42]. Two commercially available octadeclsilica-based reversed-phase LC columns, Zorbax Extend-$C_{18}$ (1.8 μm, 100 × 2.1 mm) and Asentis express $C_{18}$ (2.7 μm, 100 × 2.1 mm), were examined for response and separation. As a result, Asentis express $C_{18}$ column seemed to be more appropriate for the separation of 23 compounds, because of its high efficiency and low back pressure. Therefore, Asentis express $C_{18}$ was used as the LC column for real sample analysis.

In order to achieve good separation with sensitivity of those target compounds under both PI and NI, formic acid in Milli-Q water

**Table 4**
Recoveries of target compounds in salad oil by GPC ($n = 3$).

| Target compound | Recovery (%)[a] | | Total recovery (%) (first + second) |
|---|---|---|---|
| | First 50 mL | Second 50 mL | |
| TCS | 98.1 | 16.2 | 114 |
| $^{13}C_{12}$-TCS | 99.8 | 5.58 | 105 |
| TCC | 37.8 | 2.78 | 40.6 |
| MeP | 71.3 | 23.6 | 94.8 |
| $^{13}C_6$-MeP | 77.5 | 8.60 | 86.0 |
| EtP | 76.8 | 6.75 | 83.6 |
| PrP | 83.1 | 3.85 | 86.9 |
| BuP | 84.6 | 3.89 | 88.4 |
| UV-P | 3.16 | 0.429 | 3.6 |
| UV-9 | 92.5 | 0.655 | 93.1 |
| UV-234 | 38.5 | 0.302 | 38.8 |
| UV-320 | 24.3 | 0.093 | 24.3 |
| UV-327 | 21.8 | 0.367 | 22.1 |
| UV-328 | 22.5 | 0.376 | 22.9 |
| UV-329 | 39.5 | 0.287 | 39.8 |
| TCP | 26.7 | 0.103 | 26.8 |
| TEP | 7.80 | ND[b] | 7.80 |
| TEHP | 1.27 | ND | 1.27 |
| TBEP | 1.44 | 0.150 | 1.59 |
| TPhP | 78.4 | 0.075 | 78.4 |
| TPhP-$d_{15}$ | 84.5 | 0.950 | 85.5 |
| TBP | 3.73 | ND | 3.73 |
| TBP-$d_{27}$ | 2.13 | ND | 2.13 |
| TPrP | 6.08 | ND | 6.08 |
| EHDPP | 12.2 | ND | 12.2 |
| TPeP | 2.33 | 0.061 | 2.39 |

[a] Spike level: 1 ng g$^{-1}$.
[b] ND: not detected.

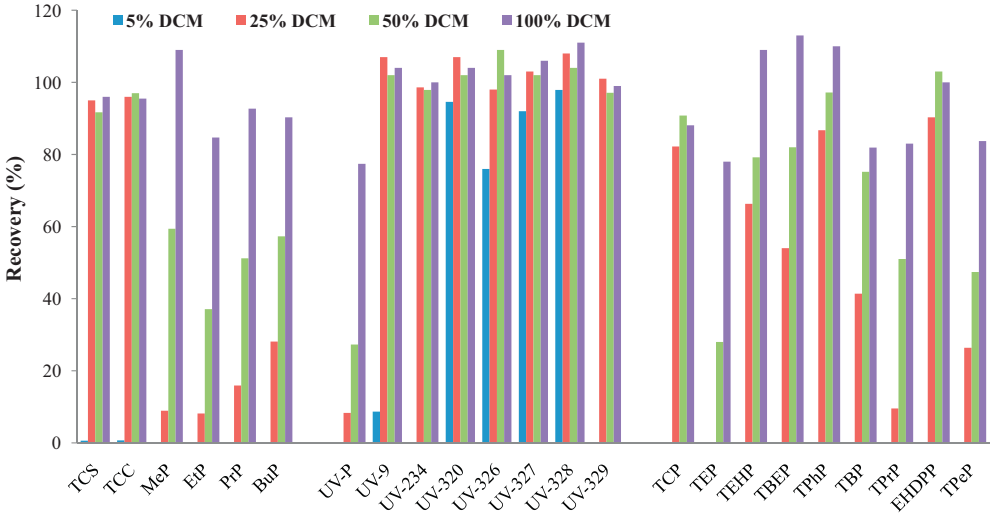


**Fig. 1.** Recoveries obtained for the silica gel clean up of target compounds, spiked at 1 ng g$^{-1}$ using dichloromethane and hexane.

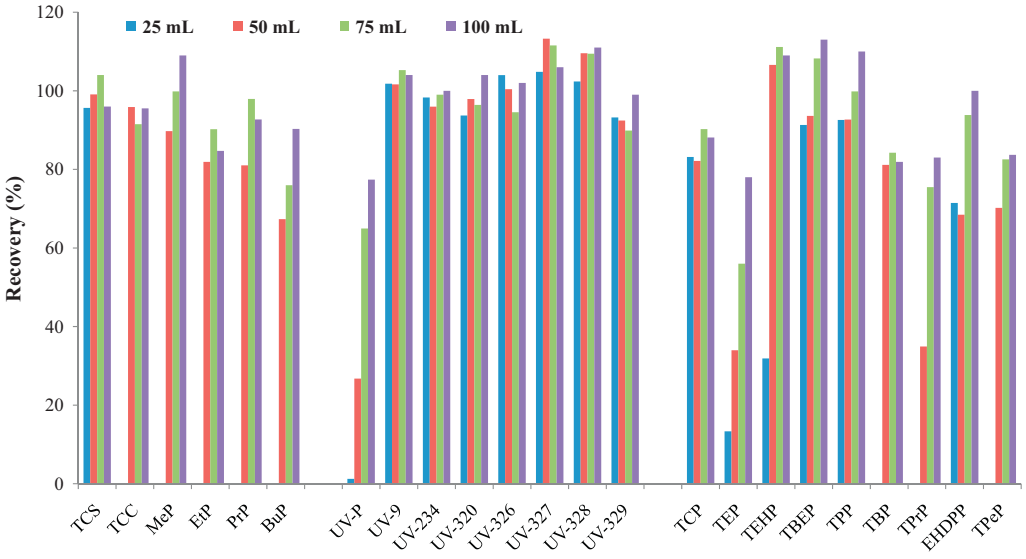


**Fig. 2.** Recoveries of target compounds at different volumes of dichloromethane.

and ammonium acetate in methanol were used as mobile phase additives. We checked the peak intensity using water and methanol as mobile phases and with 0.1% formic acid in Milli-Q water and 10 mM ammonium acetate in methanol as reported by Martinez-Carballo et al. [43]. We observed well resolved symmetrical peaks with good peak intensity for 0.1% formic acid in Milli-Q water and 10 mM ammonium acetate in methanol rather than without additives. Hence, these mobile phases were thus preferred for the simultaneous determination of the target analytes.

### 3.3. Sample clean up

Several methods have been used regarding the purification of BUVSs and OPCs [14,17]. The recovery of PCPs, BUVSs and OPCs from salad oil using GPC is shown in Table 4. The result shows that total recoveries of 5 PCPs (TCS, MeP, EtP, PrP, and BuP) with 100 mL dichloromethane/hexane (1:1, v/v) were good and ranged from 83.6 to 114% except for TCC (40.6%). Among eight BUVSs and nine OPCs, only UV-9 (93.1%) and TPhP (78.4%) showed good

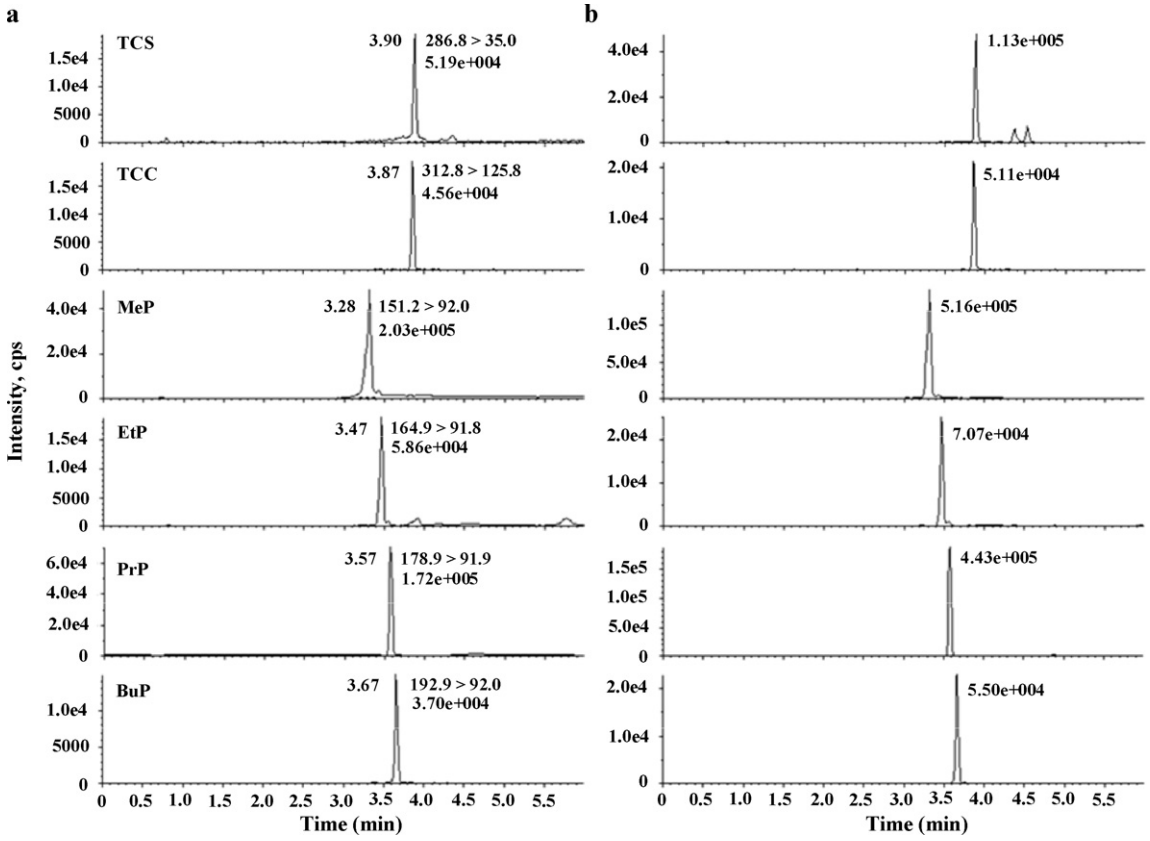


**Fig. 3.** LC–MS/MS chromatograms of the quantitative ions for preservatives and antimicrobials in (a) blank and (b) fish (*E. corallicola*).

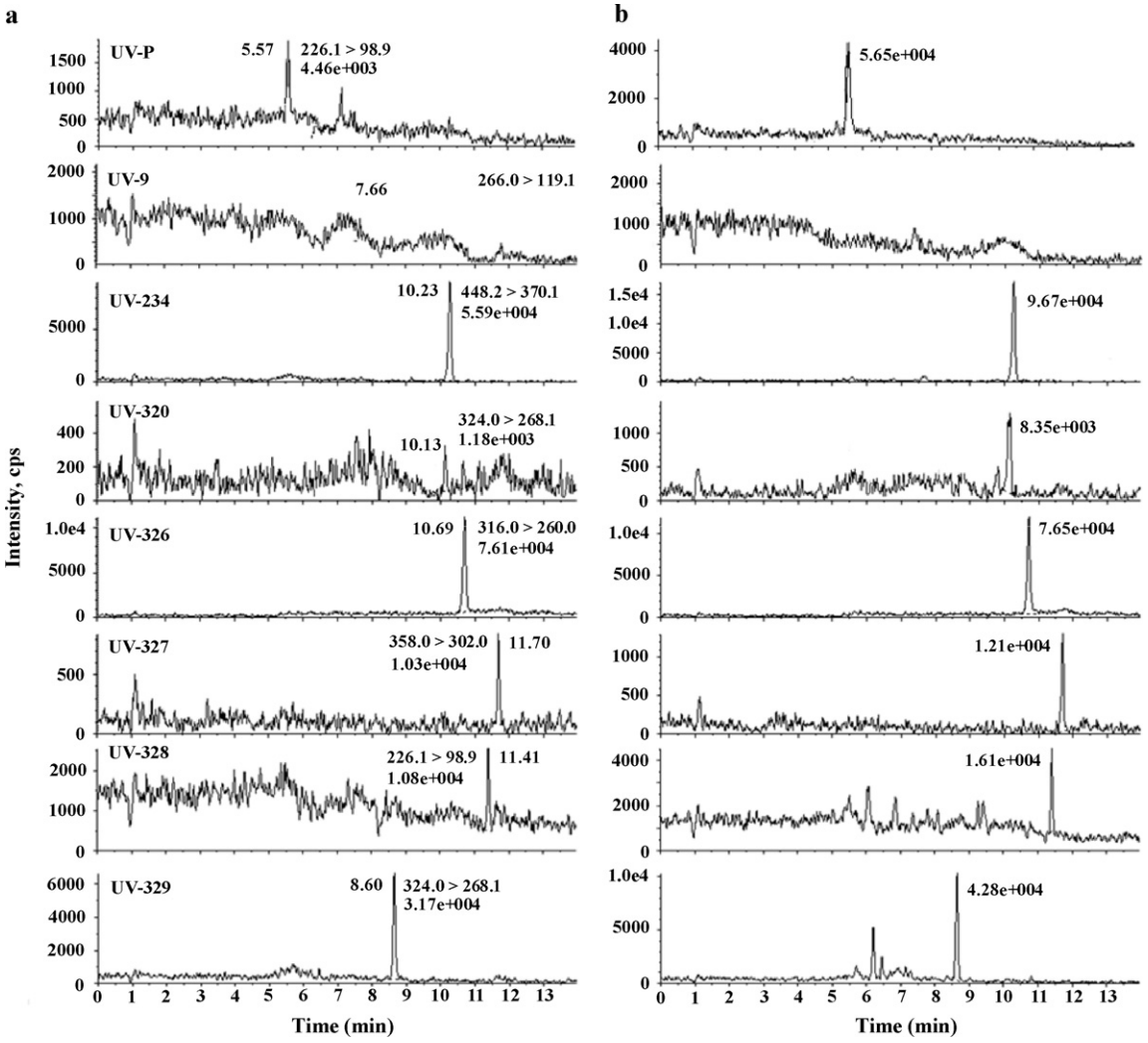


**Fig. 4.** LC–MS/MS chromatograms of the quantitative ions for BUVSs in (a) blank and (b) fish (*E. corallicola*).

recovery than rest of the compounds with recoveries <39.8%. Most of the BUVSs and OPCs were eluted in the first 120 mL fraction. Therefore, to determine all the 23 compounds in single fraction, we used silica gel clean up instead of GPC and optimized the same for recovering all the analytes extracted from fish tissue. The mean recoveries of the analytes for each solvent composition were summarized in Fig. 1. In general, polar aprotic solvents seemed to be appropriate for all the analytes. Among the tested solvent compositions, recovery has increased with the polarity of eluent, i.e. 5% dichloromethane/hexane < 25% dichloromethane/hexane < 50% dichloromethane/hexane < 100% dichloromethane. Therefore, 100% dichloromethane was employed as the eluent. Canosa et al. [26] reported that recoveries of triclosan and methyltriclosan were low with n-hexane as the extraction solvent using matrix solid-phase dispersion with lipid removal in fish sample, however, the recoveries were ~90% and 100%, respectively with dichloromethane, which is consistent with our result.

Further, the volume of dichloromethane was optimized and found that the recovery of all BUVSs except UV-P and TCS was good with 25 ml dichloromethane, however, this volume is not sufficient to elute the preservatives (methyl, ethyl, propyl and butyl parabens) and TCC of personal care products, and TEP, TEHP, TBP, TPrP and TPeP of OPCs. Further, in 50 mL and 75 mL elution most of the compounds showed relatively good recovery, however compounds such as UV-P and TEP showed less recovery (<65%) even with 75 mL. So, considering the maximum recovery, 100 mL dichloromethane was chosen (Fig. 2).

### 3.4. Method performance

Linearity of calibration, good recovery through sample preparation, precision (expressed as repeatability in terms of relative standard deviation (%RSD)) and lower MDL are essential factors for a quantitative analytical method. By using UFLC–MS/MS operated in MRM mode, linearity for all the analytes in the range of 0.01–10 ng $mL^{-1}$ with good correlation coefficient values ($R^2$) from 0.9941 to 1.0000 was obtained with the standard RSD ranged from 2.8 to 9.7% and shown in Table 3. The average recoveries of PCPs, BUVSs and OPCs in spiked fish muscle tissues at three concentrations (i.e. 0.1, 0.5 and 1.0 ng $g^{-1}$) are presented in Table 3. Good recoveries (>70%) for almost all the analytes were obtained, ranged from 78.5 to 89.4%, 70.9 to 112% and 81.6 to 114% for PCPs, BUVSs and OPCs, respectively (except for TPeP – 68.9% and TEP – 58.1%) with RSD values ranged between 0.7% and 15.4%, which are satisfactory for a quantitative analysis. Interestingly, Martinez-Carballo et al. [43] also obtained low recoveries for TEP (63 ± 3.9%) and TCEP (70 ± 3.0%), though got excellent recoveries (80–94 ± 2.0–12%) for the other organophosphorus ethoxylates (OPEs). As the reason for such low recoveries, they too observed about 10% losses of TEP during concentration with $N_2$ gas, which might have occurred in our

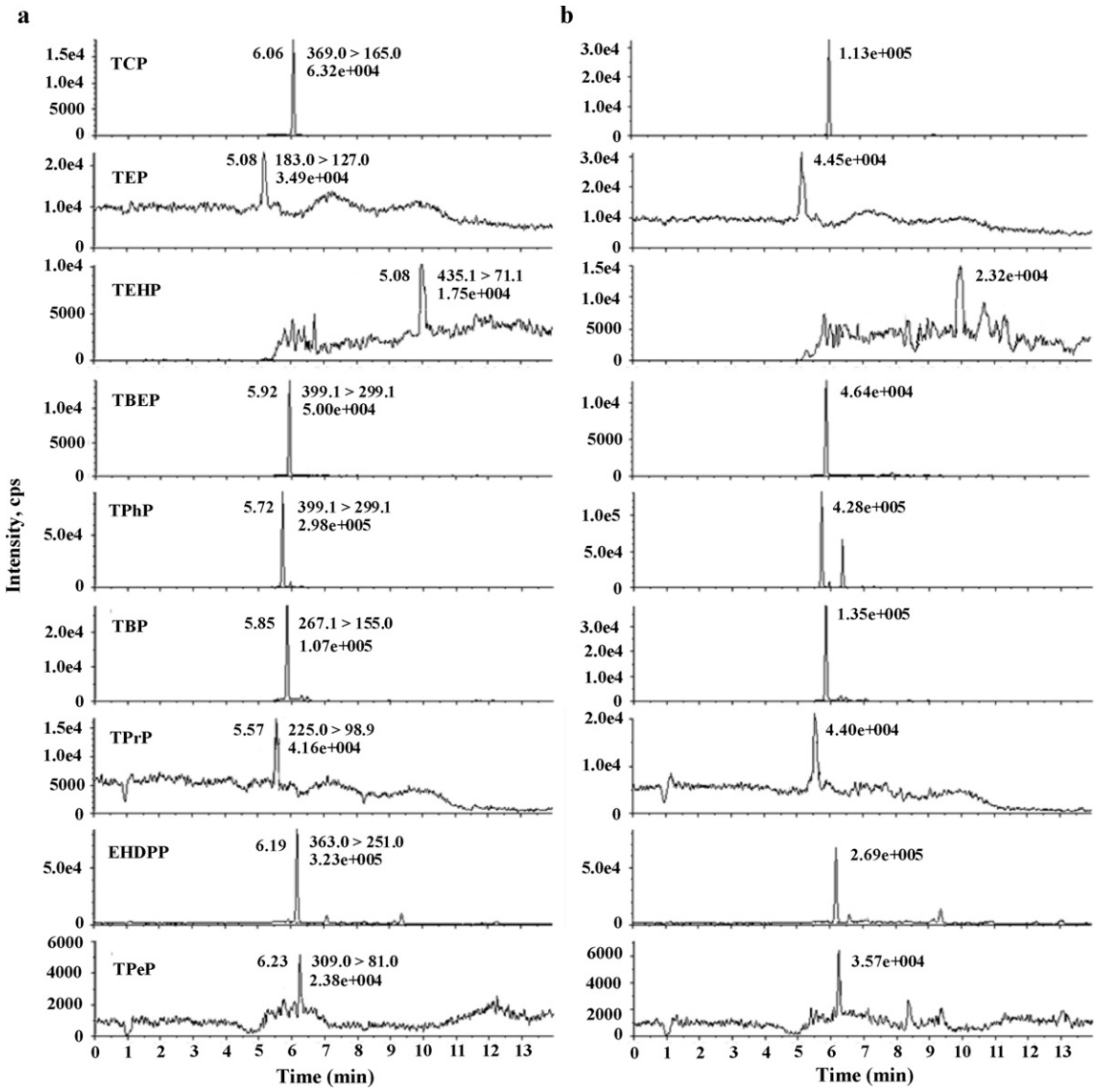


**Fig. 5.** LC–MS/MS chromatograms of the quantitative ions for OPCs in (a) blank and (b) fish (*E. corallicola*).

protocol also. In general, recoveries ranging from 60 to 150% were considered to be acceptable for a quantitative analytical method [24].

The calculated MDL and MQL for fish muscle tissue ranged from 0.0002 to 0.015 ng $g^{-1}$ lw (lipid weight) and from 0.001 to 0.049 ng $g^{-1}$ lw, respectively (Table 3). As a result, a reliable, sensitive and selective analytical method for quantitative determination of antimicrobials, preservatives, BUVSs and OPCs was established and validated. The representative MRM chromatograms of analytes in the blank and fish samples analyzed in negative and positive ionization modes are shown in Figs. 3–5, respectively.

### 3.5. Matrix effect

The main disadvantage of electrospray mass spectrometry is its susceptibility to matrix components. Matrix effect as percent for each analyte was determined with fish samples by post-extraction approach and the results demonstrated that the signal response of the compounds was affected by the ion suppression/enhancement by the matrix, but not significantly. The matrix components in fish samples decreased signal responses in 10 compounds (i.e. four PCPs, three BUVSs and three OPCs) of 23 analytes with matrix effects of 78.5–97.9% and increased signal responses of >110% was observed with UV-320, UV-326 and TBEP (Table 3). No signal response was observed for PrP and UV-P. TEP and TPeP experienced higher matrix suppression, so their recovery was lower (68.9% and 58.1%, respectively) than the other compounds, this may also be due to evaporation of analytes during concentration by purging with $N_2$ as suggested by Martinez-Carballo et al. [43].

### 3.6. Application to fish tissue

The developed method was applied to fish samples. Five individual fish samples belonging to three species from Manila Bay, the Philippines were analyzed. The concentrations of the target compounds in fishes are summarized in Table 5. Most of the compounds were found at high ng $g^{-1}$ range (0.78–3450 ng $g^{-1}$), whereas, UV-9 was found below the method detection limits (<0.0002 ng $g^{-1}$) in all fish samples (Table 5).

Bactericides, TCS and TCC, were found in the range of 13.5–278 ng $g^{-1}$. TCS was predominant than TCC in all fish muscle. Higher concentrations of MeP observed, with the highest concentrations of 2770 ng $g^{-1}$ in bluetail mullet (*V. buchanani*) and 3450 ng $g^{-1}$ in coral grouper (*E. corallicola*), and a mean

**Table 5**
Concentrations of target analytes (ng g$^{-1}$ lw) in fish muscle tissues.

| Target compound | *V. buchanani* | *E. corallicola* | *M. cephalus* ($n$ = 3) | |
|---|---|---|---|---|
| | ($n$ = 1) | ($n$ = 1) | Mean | (Min–Max) |
| TCS | 130 | 157 | 123 | (39.9–278) |
| TCC | 39.9 | 13.5 | 28.2 | (27–28.8) |
| MeP | 2770 | 3450 | 1000 | (605–1580) |
| EtP | 183 | 129 | 105 | (46.6–195) |
| PrP | 311 | 1140 | 74.5 | (46.6–129) |
| BuP | 22.9 | 20.9 | 18.3 | (6.61–37.3) |
| UV-P | 57.4 | 160 | 9.07 | |
| UV-9 | <MDL[a] | ND[b] | <MDL | |
| UV-234 | ND | 14.3 | 34.6 | (22–47.1) |
| UV-320 | 9.60 | 0.78 | 6.88 | (4.11–9.15) |
| UV-326 | 211 | <MDL | 18.9 | |
| UV-327 | 2.57 | 18.5 | 14.6 | (10.6–18.5) |
| UV-328 | 18.4 | 21.1 | 105 | (30.2–179) |
| UV-329 | ND | 39.4 | 7.29 | (6.69–7.89) |
| TCP | ND | 45.4 | 2.14 | |
| TEP | 152 | <MDL | 180 | (139–231) |
| TEHP | ND | 117 | 153 | (117–189) |
| TBEP | ND | ND | 11.6 | (7.15–14.1) |
| TPhP | 108 | 351 | 23.9 | |
| TBP | ND | 143 | 137 | (63.2–266) |
| TPrP | ND | <MDL | 1.76 | (0.99–2.88) |
| EHDPP | 739 | ND | ND | |
| TPeP | <MDL | 21 | 16.3 | (4.67–24.8) |
| Lipid (%) | 0.23 | 0.13 | 0.72 | |

[a] MDL: method detection limit.
[b] ND: not detected.

concentration of 1000 ng g$^{-1}$ (605–1580 ng g$^{-1}$) in flathead grey mullet (*M. cephalus*) are indicating the ubiquitous use of MeP in cosmetic formulations and other preservative applications, and consequent contamination of aquatic habitat. The concentrations of PrP in coral grouper were high (1140 ng g$^{-1}$) among target compounds, and EtP and BuP were also present in all fish samples. The removal of paraben in WWTP was higher than 90% [44]. However, high concentrations of MeP and PrP quantified in this study, may be attributed to high production/usage volume of parabens or direct discharge of wastewater into Manila Bay without treatment. Among BUVSs, UV-328 was found at the highest concentration (18.4–179 ng g$^{-1}$). The level of UV-328 in fish was similar to those previously reported in various organisms from Ariake Sea, Japan [14]. Among OPCs, flame retardants (TCP, TBEP, TPrP and TPeP) were found at relatively low concentrations (0.99–45.4 ng g$^{-1}$), whereas, plasticizers (TEP, TEHP, TPhP, TBP and EHDPP) were detected in higher concentrations (23.9–739 ng g$^{-1}$) (Table 5). Most of the phosphoric acid triesters are stable against hydrolysis at neutral pH and half-lives of TMP, TEP and TPhP at pH 7 are in the range 1.2–5.5 years [2]; the ubiquitous presence may have resulted in higher levels in fish.

This analytical method was developed for simultaneous analysis of two antimicrobials, four preservatives, eight BUVSs and nine OPCs in fish tissue with the lowest detection limits ever reported (see Table 6). Mottaleb et al. [12] developed a screening method for UV filters, musks, alkylphenols, insect repellent and antimicrobials in fish and found low recovery in Sonara sucker with high lipid content (~4.9%) using GPC and GC–MS/MS than the bluegill with low

**Table 6**
A comparison of method reproducibility and sensitivity for PCPs and OPCs in biological matrices.

| Compound ($n$) | Matrix | Extraction method | Recovery (%) | MDL/LOD (ng g$^{-1}$ lw) | Analytical method | Reference |
|---|---|---|---|---|---|---|
| Antimicrobials | | | | | | |
| TCS and TCC | Fish | HSSE | 84–88.9 | 0.001–0.006 | UFLC–MS–MS | Present study |
| TCS | Fish | Solvent Homogenization | | 2.5 | GC–SIM–MS | [45] |
| TCS | Snail | Soxhlet | 85.2–143 | 10 | GC–MS | [46] |
| TCC | Snail | Soxhlet | 96.2–108 | 10 | GC–MS | [46] |
| TCS | Fish | Rotary extractor | | 5.5 | GC–SIM–MS | [12] |
| TCS | Fish | Sonication | | 38 | GC–MS–MS | [24] |
| TCS | Dolphin Plasma | Solvent Extraction | 51 ± 23 | 0.033 | HRGC/HRMS | [47] |
| TCS | Vegetable | PFE | 70 | 6.6 | GC–MS | [11] |
| Paraben preservatives | | | | | | |
| MeP, EtP, PrP and BuP | Fish | HSSE | 83–89.5 | 0.001–0.015 | UFLC–MS–MS | Present study |
| MeP, EtP, PrP and BuP | Breast Tissue | Mechanical Shaking | 96 ± 4.4–113 ± 13 | 1.05–3.75[a] | GC–MS | [35] |
| BUV stabilizers (8) | Fish | HSSE | 70.5–120 | 0.0002–0.009 | UFLC–MS–MS | Present study |
| BUV stabilizers (5) | Fish | Soxhlet | 93 ± 4.4–122 ± 11 | 0.02–0.15 | GC–MS–MS | [14] |
| UV-filters | Fish | Homogenization/Shaking | 70–105 | 6–50 | LC–MS–MS | [13] |
| OPCs (9) | Fish | HSSE | 66.3–126 | 0.001–0.014 | UFLC–MS–MS | Present study |
| OPEs | Plasma | SPE-HPLC | 60–92 | 0.2–1.8 | LC–MS | [15] |
| OPFRs | Fish | ASE | 64–110 | 0.05–11 | GC–HRMS | [17] |

[a] Wet weight basis-breast cancerous tissue.

lipid content (~0.4%) without GPC and GC–MS. Calderon-Preciado et al. [11] developed a multi-residue method for the determination of emerging pollutants in leafy vegetable by matrix solid-phase dispersion with pressurized fluid extraction using acetone:hexane (1:1) mixture and GC–MS analysis, in which they achieved a LOD of 6.6 ng $g^{-1}$ and the mean recovery of 70% (46–91%) for triclosan. HSSE needs only 30 min to extract four samples at a time than the conventional soxhlet extraction, which is time and labor intensive. The developed method can also avoid GPC, which is another time and solvent consuming step. The results therefore demonstrate that the PCPs, BUVSs and OPCs can be simultaneously extracted from biological tissues and determined with good sensitivity and precision.

## 4. Conclusions

The novelty of this study is the simultaneous determination of six PCPs (preservatives and antimicrobials), eight BUVSs and nine OPCs (flame retardants and plasticizers) in fish by a single method with simple and rapid extraction and clean up followed by UFLC–MS/MS determination. This sensitive method gave MDLs in pg $g^{-1}$ (lw) range for biota sample. Recoveries were good for PCPs (78.5–89.4%), BUVSs (70.9–112%) and OPCs (81.6–114%) with RSD from 0.7 to 15.4%. The repeatability (precision) is also good with RSDs < 19.8% for intra-day and <19.0% for inter-day. The performance of this multi-residue method for PCPs, BUVSs and OPCs in fish samples was extremely good with low detection limits than the previously reported methods.

As far as we know, this is the first analytical method for TCC, paraben preservatives and four BUVSs (UV-P, -9, -234 and -329) in biota (fish) samples. This multi-residue procedure minimizes the sample preparation time in screening and monitoring applications with low volume of sample (0.5 g) compared to 2–20 g and 30 g used for BUVSs [14] and OPCs [17], respectively. Furthermore, this method can be applied for the routine analysis of multi-class emerging chemicals with ease using sentinel organisms which reflect the fate of the environment.

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