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以極致液相層析/串聯式質譜儀檢測魚體組織中鄰苯二甲酸二乙酯與個人保健品

Determination of Diethyl Phthalate and Personal Care Products in Fish Tissues with Ultra-performance Liquid Chromatography/tandem Mass Spectrometry

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中文摘要

鄰苯二甲酸酯(phthalate esters)廣泛使用於工業和消費性產品，個人保健品(personal care products)則包含民眾基於日常健康照護、驅蟲、美容等目的而大量使用的各式各樣的化合物。許多研究顯示鄰苯二甲酸酯和一些個人保健品具有發育毒性且為內分泌干擾物質。這兩類化合物普遍存在於環境中；污水處理廠是它們進入環境的主要途徑之一。一些研究顯示這些化合物可能會累積在魚體內，然而少有研究同時分析不同魚體組織器官裡的鄰苯二甲酸酯或個人保健品成分，且能在魚體組織器官同時檢測這兩大類化合物的分析方法目前仍相當有限。因此，本研究開發檢測魚肉和魚肝內的鄰苯二甲酸二乙酯和十一種個人保健品成分之分析方法。樣本前處理採用基質固相分散法(matrix solid-phase dispersion, MSPD)，以 C8 作為分散劑，並依序用 5 毫升甲醇和丙酮沖提樣本管柱和其下所接的矽膠樣本淨化管柱；樣本經濃縮後以極致液相層析串聯式質譜儀，在多重反應監測模式下獲取質荷比資訊，並搭配同位素稀釋技術進行定量分析。鄰苯二甲酸二乙酯和鹼性個人保健品以正離子電灑游離法游離，並使用 Ascentis Express F5 管柱搭配移動相(A) 5 mM 醋酸氨水溶液(pH = 6.40)、(B)甲醇，進行梯度層析；酸性個人保健品則以負離子電灑游離法游離，並使用 Waters CORTECS UPLC C18 管柱搭配移動相(A) 0.04%乙酸(pH = 3.45)、(B)甲醇，進行梯度層析。

儀器方法最佳化方面，正離子電灑游離法比較了兩種有機移動相和四個游離源溫度，負離子電灑游離法則比較了兩種不同的層析條件。前處理最佳化方面，本研究比較了：(1)兩種沖提溶劑組合；(2)兩種沖提溶劑體積；(3)兩種 MSPD 分散劑；(4)兩種淨化吸附劑；(5)不同量的淨化吸附劑；(6)不同體積的最終樣本萃取液。魚肉和魚肝的基質效應因子大部分分別落在 70.3–95.6% 與 24.3–61.9%；魚肉的萃取效率有一半落在 62.1–76.6%，魚肝則大部分落在 31.6–71.2%。待測物在魚肉和魚

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肝的方法偵測極限分別為 0.57–15.0 ng/g（濕重）及 4.37–104 ng/g（濕重）。本分析方法待測物的定量偏差大多低於 30%，相對標準偏差則均低於 20%。

關鍵字：鄰苯二甲酸二乙酯、個人保健品、魚肉、魚肝、基質固相分散法、極致液相層析串聯式質譜儀

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Abstract

Phthalate esters (PAEs) are widely used in industrial and consumer products; personal care products (PCPs) contain diverse chemicals used at a large scale for daily lives or personal hygiene, which include analgesics, insect repellents, UV filters, and so on. Previous studies indicate that PAEs and some PCP ingredients have developmental toxicity and could disrupt endocrine systems. The two groups of compounds are ubiquitous in the environment, and wastewater treatment plants are one of the major emission sources. Some studies show that PAEs and PCPs may accumulate in fish tissues; however, limited studies determined PAEs or PCPs in different fish tissues simultaneously. Furthermore, few methods are available to analyze PAEs and PCPs together in fish tissues. Thus, this study developed and validated a method to simultaneously determine diethyl phthalate (DEP) and 11 PCPs ingredients in fish muscle and liver. Samples were extracted with matrix solid-phase dispersion (MSPD) using C8 adsorbent; 5-mL methanol and acetone were sequentially passed through the tandem system of a MSPD cartridge piggyback on a silica gel cartridge for cleanup. After concentration, the eluents were analyzed using ultra-performance liquid chromatography/tandem mass spectrometry (UPLC-MS/MS) with multiple reaction

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monitoring (MRM) and were quantified with isotope dilution techniques. DEP and the basic PCPs were separated on an Ascentis Express F5 column (30 × 2.1 mm, 2.0 μm) with the mobile phases consisting of (A) 5 mM ammonium $acetate_{(aq)}$ (pH = 6.40) and (B) methanol, and were ionized at positive electrospray ionization mode (ESI+). The acidic analytes were separated on a Waters CORTECS UPLC C18 column (30 × 2.1 mm, 1.6 μm) with the mobile phases consisting of (A) 0.04% acetic $acid_{(aq)}$ (pH = 3.45) and (B) methanol, and were ionized at negative electrospray ionization mode (ESI-).

The optimization of the instrumental analysis included the tests of two organic mobile phases and four source temperatures for ESI+, and two chromatographic conditions for ESI-. The sample preparation method was optimized by testing two elution solvent combinations, two elution volumes of solvents at each portion, two adsorbents for MSPD, two adsorbents for cleanup, the amount of cleanup adsorbents, and volumes of the final residues.

The matrix effect factors of most analytes in fish muscle and liver ranged from 70.3–95.6% and 24.3–61.9%, respectively. The extraction efficiencies of half the analytes in muscle were 62.1–76.6%, and those of most analytes in liver were 31.6–71.2%. The limits of detection (LODs) of analytes were 0.57–15.0 ng/g weight wet (w.w) for muscle and

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4.37−104 ng/g w.w. for liver, respectively. Most of the quantitative biases were below 30%, and all the relative standard deviations were below 20%.

Keywords: diethyl phthalate, personal care products, fish muscle, fish liver, matrix solid-phase dispersion, UPLC-MS/MS

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Chapter 1. Introduction

Phthalates esters (PAEs) are widely used in consumer products manufacturing, and personal care products (PCPs) are extensively used in human daily lives. Some of both groups of the compounds have developmental toxicity and endocrine disrupting properties [1, 2]. PAEs and PCPs are ubiquitous in the environment, and water is one of the major media for their distribution in the environment [3, 4]. Some of these chemicals are relatively lipophilic and have the potential to bioaccumulate in fish [2, 3]. In addition, the continuous exposures of fish to PAEs and PCPs may increase the potential for accumulation of these compounds in fish tissues because effective exposure duration is increased [5, 6]. Therefore, it is important to investigate the levels of the above compounds in fish. However, the existing methods are limited for simultaneously analyzing PAEs and PCPs in fish tissues. It warrants the method development for determining these compounds in fish tissues.

1.1. Phthalate esters

PAEs, a group of di-esters of 1,2-benzenedicarboxylic acid, are used in several consumer products [7]. Lower molecular weight PAEs like dimethyl phthalate (DMP),

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diethyl phthalate (DEP) and Di-*n*-butyl phthalate (DnBP) are mainly applied to adhesives, waxes, insecticides and cosmetics; higher molecular weight PAEs, such as di-(2-ethylhexyl) phthalate (DEHP) and di-isononyl phthalate (DiNP) are primarly used as plasticizers in products of polyvinyl chloride plastics (PVC) [8]. Because PAEs are not chemically bound to the products where they are applied, they constantly leach into the environment during manufacture and from final products. Moreover, PAEs are used extensively; to date, worldwide annual consumption of PAEs is 6−8 million tons. They have been detected in air, water, sediment, soil and biota worldwide [9]. Wastewater treatment plants (WWTP) is one of the important sources of discharging PAEs to the environment because most conventional WWTPs are not designed for removing these micropollutants [4, 10]. The concentrations of PAEs are reported from <LOQ (limits of quantification) to thoudands of μg/L in surface water [9, 11], and from <LOQ to tens of mg/kg (dry weight, d.w.) in sediment, respectively [9, 12, 13]. In fish, the concentrations of PAEs are reported from <LOQ to hundreds of mg/kg (d.w.) in muscle [14], and ranged from 0.39 to 6.90 mg/kg (d.w.) in livers [15].

Regarding the impact of PAEs on aquatic organisms, PAEs are known to have developmental toxicity and endocrine disrupting effects [1, 16]. Furthermore, some

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studies showed that PAEs may bioacumulate in fish muscle and liver [9, 15, 17]. Valton et al. found that the bioaccumulation factors (BAF) of di-*iso*-butyl phthalate (DiBP), DnBP, butylbenzyl phthalate (BBP) and di-*n*-octyl phthalate (DNOP) for fish musle and the BAFs of DMP, DEP, DiBP, DnBP, BBP, DEHP and DNOP for livers were higher than 2,000 [15, 18].

1.2. Personal care products

Personal care products (PCPs) contain various compounds used in large quantities for personal hygiene or daily life including analgesics, stimulating drinks, insect repellants, UV filters, preservatives, to name a few [3]. Many PCPs enter domestic sewage after usage; however, their removal rates in wastewater vary widely depending on their chemical properties, the operating conditions and the treatment technologies of wastewater treatment [19-23]. Although some PCPs can be removed almost completely and the environmental half-lives of most PCPs are short, the extensitve use and continous emission make most PCPs behave as “pseudo-persistent” in the aquatic environment [24].

PCPs usually exist in surface water at ng/L to μg/L levels [2, 25]. Aquatic creatures may have life-cycle and even multigeneration exposure to PCPs, and PCPs may

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potentially bioaccumulate in fish and affect these organisms even at low concentrations in the surface water. In addition, PCPs are designed to be biogially active, which may result in unintended consequences on aquatic organisms [3].

Analgesics give relief from pains without causing anesthesia. They are increasingly used because of population aging, population growth, and their easy accessibility. Concentrations of analgesics are found to range from <LOD to tens of ng/g (d.w.) in fish muscle [26, 27].

Caffeine is the most consumed stimulant in the world [28]. Alvarez-Munoz et al. reported caffeine in fish muscle at tens of ng/g (d.w.) [29]. Concentrations of caffeine are found to range from <LOD to 4.5 ng/g (d.w.) in whole fish [30].

DEET (*N*,*N*-Diethyl-meta-toluamide) is the most common active ingredient of insect repellents [31]; however, it may pose neurotoxicity to humans [32]. There are few research on analyzing DEET in fish tissues. Tanuoe et al. analyzed DEET in Japanese wild fish muscle, liver, brain, and kidney, and there were just few positive samples [33].

UV filters are commonly used in sunscreen and cosmetics [34]. Because of the lipophilic characteristics of these compounds, many UV filters have potential for bioaccumulation and biomagnification through food chain [35]. In addition, some UV

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filters would be estrogenic [36]. Concentrations of UV filters in fish filet and livers were found to range from <LOD to 182 ng/g (d.w.) and range from <20 to 1,037 ng/g (d.w.), respectively [37, 38].

Parabens, the alkyl esters of *p*-hydroxybenzoic acid, are antimicrobial and are used as preservatives in cosmetics, food and pharmaceuticals [3]. The most common parabens are methyl, ethyl, propyl, butyl and benzyl parabens. Some studies reported parabens as endocrine disrupters [39, 40]. The concentrations of parabens found in fish muscle are from <LOD to 18.5 ng/g (d.w.) [29, 41].

1.3. Analytical methods for PAEs and PCPs in fish tissues

PAEs or PCPs in fish tissues are usually extracted using ultrasonic extraction [15, 38, 42-44], pressurized liquid extraction (PLE) [14, 24, 29, 45], and Soxhlet extraction [46-49]. Liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) is the major technique of instrumental analysis because of its good detection sensitivity and selectivity [26, 29, 30, 33, 47, 50-53].

Most of the above sample preparation methods take long time or use a large amount of organic solvents or require expensive apparatus. Matrix solid-phase dispersion

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(MSPD), which was first introduced by Barker et al. in 1989 and was designed to disrupt and extract semi-solid and solid matrices [54], requires less time, organic solvents, and does not need special equipment. The procedure of MSPD basically consists of three main steps as follows: (1) blend the sample with the dispersant material; (2) transfer the homogenized mixture into a solid-phase extraction (SPE) cartridge; (3) elute analytes with an appropriate solvent or sequence of solvents. The main feature of MSPD is the dispersion of matrices over a huge solid sorbent surface, which increases the contact surface area between matrices and adsorbents as well as the elution solvents. Additionally, in MSPD, extraction and cleanup may be performed in one step, which could reduce the used amount of organic solvents and simplifies the procedure. Furthermore, the extraction process in MSPD takes place under ambient conditions and does not require any expensive equipment. Because of the feasibility, flexibility, versatility and low costs of MSPD, it is wildly applied to viscous, semi-solid or solid matrices, and various groups of analytes [55-57]. For example, Ocaña-Rios et al. used MSPD and gas chromatography-mass spectrometry to analyze two polycyclic aromatic musks and five UV filters in fish muscle, and the limit of detections (LODs) were 0.004–0.012 μg/g d.w. Freitas et al. used MSPD and gas chromatography with electron-capture detection (GC-ECD) to determine

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five pesticide residues in tropical fruits, and the LODs were 5.0−25 μg/kg [58].

1.4. Objectives

Most studies of PAEs or PCPs focus on the levels of these contaminants in water or sediment. Although some studies reported the concentrations of these compounds in fish, most of them only focused on muscle [24, 38, 48, 59-62]. It is vital to analyze other tissues such as liver, which is important for metabolism and detoxification, to learn more about the potential health effects of these chemicals on fish.

There are some existing methods for analyzing PAEs or PCPs in fish tissues; however, few studies investigated PAEs and PCPs simultaneously. Furthermore, some sample preparation methods are solvent consuming, tedious, laborious, and in high-cost. Therefore, an analytical assay is desired that is capable of analyzing these compounds in fish tissues efficiently and simultaneously.

The present study aimed to develop and validate an analytical method for simultaneous analysis of diethyl phthalate (DEP) and 11 PCPs (Table 1, page 59) in fish muscle and liver. The 11 PCPs are acetaminophen, caffeine, DEET, benzophenone, oxybenzone, methyl paraben, ethyl paraben, propyl paraben, butyl paraben, ketoprofen,

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and ibuprofen. The 12 analytes were chosen according to three criteria: (1) high usage or production amount, (2) high concentrations and being frequently detected in the aquatic environment, and (3) lacking field-derived information about bioaccumulation. For instance, the selected PCPs in this study has been shown ubiquitous in the aquatic environment in Taiwan [63-66].

This study tested different elution solvent combinations, elution volumes of solvents at each portion, adsorbents for MSPD, adsorbents for cleanup, amounts of cleanup adsorbents, and volumes of the final residues for optimization of sample treatment. The instrumental analysis was conducted on ultra-performance liquid chromatography/tandem mass spectrometry (UPLC-MS/MS) at electrospray ionization (ESI). MS parameters, mobile phases, chromatography columns, and LC conditions were optimized. The method was validated using Tilapia (*Oreochromis niloticus*) because it can be acquired easily from markets in Taiwan and it is one of the most consumed fish in the world. Matrix effect, extraction efficiency, accuracy and precision were evaluated.

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Chapter 2. Methods

2.1. Reagents and materials

Diethyl phthalate (99.9%, 5,000 μg/mL in methanol) was purchased from AccuStandard (New Haven, CT, USA). Acetaminophen (99.6%, powder) was bought from United States Pharmacopeia (Rockville, MD, USA). DEET (*N*,*N*-diethyl-3-methylbenzamide) (purity≧98%, 250 mg), caffeine, oxybenzone, methyl paraben, ethyl paraben, propyl paraben, ketoprofen, ketoprofen-$^{2}D_3$ and ibuprofen (purity ≧ 98%, powder) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Benzophenone and butyl paraben were bought from Alfa Aesar (Heysham, Lancashire, U.K.; purity≧99%, powder). Diethyl phthalate-$^{2}D_4$ (DEP-$^{2}D_4$), benzophenone-$^{2}D_{10}$ (purity ≧ 98%, 100 μg/mL in nonane), caffeine-$^{13}C_3$ (purity≧98%, 100 μg/mL in methanol), DEET-$^{2}D_6$ (purity≧98%, 100 μg/mL in dichloromethane-$^{2}D_6$), oxybenzone-$^{13}C_6$, ibuprofen-$^{13}C_3$ (purity≧98%, 100 μg/mL in acetonitrile), methyl paraben-$^{13}C_6$ and butyl paraben-$^{13}C_6$ (purity ≧ 98%, 1000 μg/mL in methanol) were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Acetaminophen-$^{2}D_4$ (100 μg/mL in methanol) was obtained from LGC Standards (Teddington, Middlesex, England, U.K.).

HPLC-grade acetone and methanol, and LC/MS-grade methanol were provided by

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Merck (Darmstadt, Hesse, Germany). HPLC-grade *n*-heptane and LC/MS-grade acetonitrile were bought from J.T Baker (Philipsburg, NJ, USA). Ammonium acetate (≈5 M in H_2O) and acetic acid (≧99.8%) were purchased from Sigma-Aldrich. Milli-Q water was from a Milli-Q integral water purification system (Merck Millipore, Darmstadt, Germany).

Solid and liquid state standards were dissolved in methanol as stock solutions, and the concentrations were as below: acetaminophen, caffeine, DEET, benzophenone, oxybenzone and ketoprofen-$^2D_3$ at 1 mg/mL; methyl paraben, ethyl paraben, propyl paraben, butyl paraben, ketoprofen and ibuprofen at 4 mg/mL. Regarding the stable isotope-labeled internal standards originally in nonane, because nonane is not miscible with methanol, we used acetone to make their respective two-fold dilutions as stock solutions, then used methanol or acetone to make the subsequent dilutions, which depended on the concentrations we needed. All stock solutions and the commercialized standard solutions of the rest chemicals were stored at 4℃.

2.2. Sample collection

The tilapia (*Oreochromis niloticus*) samples for method development and validation

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were bought from local markets in New Taipei City. The samples were purchased in the morning, stored at 4°C, and extracted on the next day.

2.3. Sample preparation

Fish muscle and liver samples were homogenized first using a blender (Waring Commercial, Stamford, CT, USA) individually. One gram (wet weight, w.w.) of homogenized samples were weighted into an IKA DT-20 homogenization tube (Wilmington, NC, USA) and stable isotope-labeled internal standards (ISTDs) were added. Regarding muscle samples, 25-μL DEP-$^{2}D_{4}$ at 20 μg/mL in acetone and 25 μL of rest ISTDs in methanol were spiked; the spiked concentrations in methanol were as follows: 20 μg/mL of butyl paraben-$^{13}C_{6}$; 5 μg/mL of acetaminophen-$^{2}D_{4}$, caffeine-$^{13}C_{3}$, DEET-$^{2}D_{6}$, benzophenone-$^{2}D_{10}$, oxybenzone-$^{13}C_{6}$, methyl paraben-$^{13}C_{6}$, ketoprofen-$^{2}D_{3}$ and ibuprofen-$^{13}C_{3}$. Liver samples were spiked with 50-μL mixture of benzophenone-$^{2}D_{10}$ and DEP-$^{2}D_{4}$ at 20 μg/mL in acetone, and 40 μL of rest ISTDs in methanol; the spiked concentrations in methanol were as follows: 25 μg/mL of butyl paraben-$^{13}C_{6}$, ketoprofen-$^{2}D_{3}$ and ibuprofen-$^{13}C_{3}$; 10 μg/mL of acetaminophen-$^{2}D_{4}$, caffeine-$^{13}C_{3}$, DEET-$^{2}D_{6}$, oxybenzone-$^{13}C_{6}$ and methyl paraben-$^{13}C_{6}$.

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After spiking, the samples were homogenized with 4 g of C8 adsorbent (particle size 40–63 μm, carbon content ≧ 11.6%, SiliCycle, Quebec City, Quebec, Canada) using an IKA ULTRA-TURRAX Tube Drive. Afterwards, the mixture was transferred into a 12-mL SPE cartridge with a polyethylene frit at the bottom, then it was compressed and covered with another polyethylene frit. A 6-mL silica gel cartridge (bed weight 2 g, PerkinElmer, Waltham, MA, USA) was for cleanup to adsorb amino acids, fatty acids and organic acids, which was washed twice by 4-mL acetone before used. The sample cartridge was piggybacked on a washed silica gel cartridge and analytes were eluted twice with 5-mL methanol and 5-mL acetone, respectively; the first 5-mL methanol was from the solvent for rinsing the used homogenization tube. The elution rate was at 1-2 drops/sec. The eluents were concentrated to approximately 5 mL at 45℃ by a Savant SPD 1010 SpeedVac (Thermo Fisher, Waltham, MA, USA.), and then were centrifuged by a KUBOTA 2010 centrifuge (Kubota, Bunkyo-ku, Tokyo, Japan) at 3,000 rpm (1409 × g) for 5 minutes. The supernatants were transferred to 15-mL deactivated (silanized) glass centrifuge tubes, concentrated to 1 mL, and were filtered through a methanol-washed Millex Samplicity Filter (hydrophilic PTFE, pore size 0.20 μm, diameter 33 mm) with a Samplicity Filtration System (Merck Millipore, Darmstadt, Germany). Regarding muscle

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samples, the filtrates were further concentrated to 250 μL and were refrigerated at 4°C overnight. Afterwards, the filtrates were centrifuged at 3,000 rpm (1107 × g) for 5 minutes and the supernatants were transferred to 300-μL inserts. For liver samples, the filtrates were concentrated to 500 μL and were refrigerated at 4°C overnight. Thereafter, the filtrates were centrifuged by a Centrifuge 5415 R (Eppendorf, Stevenage, Hertfordshire, UK) at 13,200 rpm (16100 × g) at 4°C for 10 minutes. The subnatants were filtered through a methanol-washed hydrophilic PTFE filter (0.20 μm) with a Samplicity Filtration System again. Four microliters of the final residues were injected onto the UPLC-MS/MS for analysis.

2.4. Water content of samples

Fish muscle and liver were weighted, then the tissues were dried in a Circulator Oven DO45 (Deng Yng, Taishan Dist., New Taipei City, Taiwan) at 105°C until their weights remained constant. After drying, the samples were transferred to a desiccator for cooling. Afterwards, the samples were weighted again. The water contents of samples were determined by using the formula below:

$$\text{Water content (\%)} = \frac{W1 - W2}{W1} \times 100$$

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Where,

W1 = Weight (gram) of sample before drying.

W2 = Weight (gram) of sample after drying.

The water contents of fish muscle and liver were 78.5% and 67.3%, respectively.

2.5. Instrumental analysis

2.5.1. Liquid chromatography

The chromatographic separation was performed on a Waters ACQUITY UPLC system (Waters, Milford, MA, USA). DEP and the basic PCPs (acetaminophen, caffeine, DEET, benzophenone and oxybenzone) were separated on an Ascentis Express F5 column (30 × 2.1 mm, 2.0 μm). The mobile phases were composed of (A) 5 mM ammonium $acetate_{(aq)}$ (pH = 6.40) and (B) methanol, and the flow rate was 0.65 mL/min. The column oven temperature and sample chamber temperature were set at 40°C and 20°C, respectively. The injection volume was four microliters. The chromatographic gradient began from 5% B for 0.5 minutes, then increased to 95% B in 4 minutes, held for 1 minute, and back to the initial compositions in 0.3 minutes. The column was re-equilibrated for 2 minutes and the total chromatographic time was 7.8 minutes (Table 2,

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page 61).

A CORTECS UPLC C18 column (30 mm × 2.1 mm, 1.6 μm, Waters) was used for the separation of the acidic PCPs (methyl paraben, ethyl paraben, propyl paraben, butyl paraben, ketoprofen and ibuprofen). The mobile phases consisted of (A) 0.04% acetic $acid_{(aq)}$ (pH = 3.45) and (B) methanol, and the flow rate was 0.5 mL/min. The column oven temperature and sample chamber temperature were 30℃ and 20℃, respectively. The gradient started with 15% B for 0.5 minutes, and was increased to 100% B in 2.5 minutes, with holding for 0.5 minutes before returning to 15% in 0.5 minutes, and the column was re-equilibrated for 1.7 minutes. The total chromatographic time took 5.7 minutes (Table 2, page 61). The chromatograms of the analytes were shown in Figure 1 and Figure 2 (page 47 and 48).

2.5.2. Tandem mass spectrometry

After the chromatographic separation, analytes were detected by a Waters Quattro Premier XE triple-quadrupole mass spectrometer (Waters, Milford, MA, USA) at multiple-reaction monitoring (MRM) mode; the two most abundant ion transitions of each analyte were for quantification and confirmation, respectively. For ketoprofen and

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ibuprofen, only one ion transition was monitored because they only form one intensive and stable product ion. The MRM parameters were optimized by using a syringe pump to inject 1.0 μg/mL standard solutions of individual analyte directly to the mass spectrometer. The MRM transitions and parameters of each analyte are shown in Table 3 (page 62).

DEP and the basic PCPs were ionized by positive electrospray ionization (ESI+) with the capillary voltage 2 kV, extractor voltage 4 V, source temperature 120℃, desolvation temperature 500℃, cone gas flow 150 L/hr, desolvation gas flow 900 L/hr, and collision cell pressure 3.37×10^{-3} mbar. The desolvation and collision gas were nitrogen and argon, respectively.

Acidic PCPs were ionized by negative electrospray ionization (ESI-) with the capillary voltage 3.0 kV, extractor voltage 3 V, source temperature 120℃, desolvation temperature 450℃, cone gas flow 100 L/hr, desolvation gas flow 900 L/hr, and collision cell pressure 3.37×10^{-3} mbar. The desolvation and collision gas were nitrogen and argon, respectively.

2.6. Method validation

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2.6.1. Extraction efficiency and matrix effect

Extraction efficiency was defined as the peak area ratios of pre-spiked samples to those of post-spike samples at the same levels of analytes. Matrix effect factors were calculated as the peak area ratios of post-spiked samples to those of the same concentrations of chemical standards in methanol. The spiked level of each analyte in muscle samples was 200 ng/g w.w. For liver samples, the spiked level of DEP and acetaminophen was 1,000 ng/g w.w., and those of other analytes were 400 ng/g w.w. The samples were done in four duplicates (n = 4). The spiked level of acetaminophen was higher than most of other analytes in liver samples because there was a peak (retention time = 0.62 min) near acetaminophen (retention time = 0.74) and interfered with the quantification (Figure 3, page 48). Regarding DEP, studies found that PAEs tend to accumulate more in fish livers than in muscle [15, 42], and the endogenous high concentrations of DEP in liver samples may influence the quantification. To prevent the quantification of the two analytes from interference, the spiked level in liver samples was elevated.

2.6.2. Accuracy and precision

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Accuracy and precision were evaluated using pre-spiked fish muscle at three spiked levels with four duplicates (n = 4) at each level, and pre-spiked fish liver at 1,000 ng/g w.w. with four duplicates. The spiked levels of muscle samples were 62.5, 200, and 500 ng/g w.w.

2.6.3. Identification, quantification and data analysis

The instrumental detection limits (IDL) and instrumental quantification limits (IQL) were determined by analyzing low concentrations of chemical standards in methanol. IDL and IQL were defined as the signal-to-noise ratio (S/N ratio) of the confirmatory ion at 3 and the S/N ratio of the quantitative ion at 10, respectively. If calculated IDLs were higher than IQLs based on the above definition, the IQLs were reported as the same values of IDLs. The limits of detection (LODs) and limits of quantification (LOQs) were defined as the S/N ratio of confirmatory ion at 3 and the S/N ratio of quantitative ion at 10 in spiked matrix samples, respectively. If LODs were higher than LOQs based on the above definition, the LOQs were reported as the same values of LODs.

The calibration curves were established using MassLynx 4.1 (Waters) by normalizing the peak areas of native analyte standards to those of their individual isotope-

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labeled internal standards. The curves were made by linear regression with the weighting factor of $1/\chi$. There were at least 6 points in each curve which the concentrations of analytes ranged from 1 to 4,000 ng/mL. The r^2 of all analytes were higher than 0.99. Further data analysis were done using Microsoft Excel 2013.

2.6.4. Quality assurance and quality control

All the glassware that would contacted the samples was deactivated (silanized) to prevent the analytes from adsorbing on the glass surface. All glassware, homogenization tubes, and cartridges were rinsed with methanol and acetone before use. C8 adsorbent and cartridge frits were sonicated with methanol and acetone sequentially, and were dried in a chemical fume hood before use. After use, the glassware was washed with detergent and tap water, and then was rinsed with methanol and acetone. The anatomical tools and blender were washed with tap water, and were rinsed with Milli-Q water, acetone, *n*-heptane, acetone and methanol sequentially. Other labware was washed by tap water, and was rinsed with Milli-Q water, methanol and acetone sequentially. All cleaned labware was dried by air flow in a chemical fume hood and was covered with aluminum foil to avoid contamination.

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A Waters Isolator column (50 × 2.1 mm, 3.5 μm) with an extension tube was installed onto the UPLC system to eliminate the influences of the background DEP from the UPLC system and mobile phases. Caffeine, DEET and oxybenzone were detected in reagent blanks at approximately 1 ng/g, and DEP, benzophenone and methyl paraben were found at tens of ng/g.

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Chapter 3. Results and discussion

3.1. Optimization of chromatography

The chromatographic method for ESI+ in the present research was modified from previous methods developed by our team [66, 67]. Different organic mobile phases (acetonitrile and methanol) were tested for better signal intensities. Methanol as the organic mobile phase provided 1.9−40 times higher signal intensities of DEP and the basic PCPs than those of acetonitrile (ACN) (Figure 4, page 49). Furthermore, when 0.25 μg/mL of benzophenone was injected at 4 μL, the signal intensity reached 1×10^5 with methanol but no apparent peak using ACN (Figure 5, page 49). Methanol is a protic solvent and would be able to facilitate the protonation of basic analytes. As a result, methanol was chosen as the organic mobile phase for ESI+.

Regarding the LC gradient for ESI+, initial organic mobile phases at 5% and 10% methanol were tested for better retention of acetaminophen, which was first eluted analyte from the column. 5% of methanol retained acetaminophen (retention time, RT = 0.69 minute) better comparing with 10% of methanol (RT = 0.47 minute) (Figure 6, page 50). Thus, 5% of methanol was adopted.

For ESI-, the chromatographic method was also modified from a previous method

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developed by our team [66]. The combination of 0.04% acetic acid$_{(aq)}$ (pH 3.45) as the aqueous mobile phase, a CORTECS UPLC C18 column (30 × 2.1 mm, 1.6 μm) and Gradient 1 (the details of the gradient was shown in Table 4, page 63) offered 1.6–3.5 times higher of signal intensities of the acidic PCPs compared with the combination of 10 mM *N*-methylmorpholine$_{(aq)}$ (pH 9.60), a Kinetex EVO C18 column (50 × 2.1 mm, 1.7 μm, Phenomenex, Torrance, CA, USA) and Gradient 2 (the details of the gradient was shown in Table 4, page 63; Figure 7, page 50). The faster slope of Gradient 1 (34%/min) than that of Gradient 2 (22.9%/min), plus the shorter column length of the CORTECS UPLC C18 column than that of the Kinetex EVO C18 column, made better ionization and sharper peaks (Figure 8, page 51). Consequently, the former chromatographic condition was chosen for ESI-.

3.2. Optimization of mass spectrometric parameters

Because DEP was added in this study and some compounds ionized at ESI+ were removed from the previous method developed by our team [66], the source temperature for ESI+ was reevaluated. Four source temperatures (120, 130, 140 and 150℃) were tested for signal intensities at ESI+. The signal intensities of most analytes at 120℃ were

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1.0 to 1.3 times higher than those at 130℃, 140℃ and 150℃ (Figure 9, page 51). Moreover, a lower source temperature caused less heating pressure on the ionization source. Thus, 120℃ was chosen as the source temperature for ESI+.

3.3. Optimization of sample preparation

The MSPD procedure employed in this study was modified from a previous validated method developed by our team for analyzing PCPs, NPAHs and OPAHs in sediment, fish muscle, and liver [66]. Because DEP was added in this study and NPAHs, OPAHs and some PCPs were removed, some parameters were reevaluated for better performance: the type and volume of elution solvents, the type of MSPD adsorbents, the type and amount of cleanup adsorbents, and the volume of final residues for instrumental analysis.

Regarding elution solvents of MSPD on C18 non-endcapped adsorbent (particle size 40–63 μm, carbon content 23%, SiliCycle, Quebec City, Quebec, Canada), two types of solvent sequences (methanol and acetone versus methanol and dichloromethane) were passed through the adsorbent containing spiked standards for comparing the elution efficiency. The combination of methanol and acetone gave eight analytes 1.0–2.0 times

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higher elution efficiencies than those of methanol and dichloromethane (DCM) except for acetaminophen, ketoprofen and ibuprofen (1.2−2.4 times lower) (Figure 10, page 52). The elution efficiencies of DEP and benzophenone were partly influenced by backgrounds from reagent blanks. Studies have shown that background contamination is a common problem when analyzing PAEs and UV filters [45, 68]. When background levels were deducted, the elution efficiencies of methanol/acetone and methanol/DCM for DEP were 33.5% and 18.7%, respectively; the elution efficiencies of methanol/acetone and methanol/DCM for benzophenone were 30.7% and 18.7%, respectively. Overall, the combination of methanol and acetone was chosen.

Elution volume is crucial to the elution efficiency. Because the elution efficiencies of some analytes were still not ideal using methanol and acetone, such as acetaminophen (5.05%) and oxybenzone (15.4%), elution volumes of 5 mL and 7.5 mL at each portion were tested. The elution efficiencies of both volumes were similar on most analytes except for the parabens (4.7−15% better) (Figure 11, page 52). The elution efficiencies on DEP and benzophenone were partly influenced by backgrounds; when the background levels were deducted, the elution efficiencies on DEP and benzophenone were about 26% and 33%, respectively. Increase of the elution volume did not improve the elution on most

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analytes, and thus 5 mL aliquots of elution were decided.

A tandem column system was used for cleanup; therefore, the type of elution solvent combinations (methanol/acetone and methanol/DCM) was also tested on the cleanup cartridge. The test was done firstly on 6-mL acidic alumina cartridges (bed weight 2 g, Sigma-Aldrich, St. Louis, MO, USA), which was used as cleanup adsorbent in the previous method [66]. Elution efficiencies of methanol/acetone were 1.1−1.9 times higher on DEP, DEET, benzophenone and methyl paraben than those of methanol/DCM, but were 1.1−2.2 times lower on acetaminophen, caffeine, ethyl paraben, propyl paraben, and butyl paraben than those of methanol/DCM. Both combinations could not elute oxybenzone, ketoprofen and ibuprofen from the alumina adsorbent (Figure 12, page 53). Ketoprofen and ibuprofen contain a carboxyl group, which may tend to bind with alumina. Again, the elution efficiencies of DEP and benzophenone were partly influenced by backgrounds from reagent blanks. When background levels were deducted, the elution efficiencies on DEP using methanol/acetone and methanol/DCM were 53.5% and 31.2%, respectively; those of benzophenone were 40.9% and 17.1%, respectively. In brief, for the most analytes, methanol/DCM did not provide better elution efficiencies than methanol/acetone. Consequently, methanol/acetone were chosen as the elution solvents

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to avoid using chlorinated solvent.

In addition to elution solvent strength and volumes, the retention of adsorbents is also crucial on elution efficiency. Because elution efficiencies were not good on some analytes, the combination of C8 for MSPD and silica gel for cleanup was further investigated. The elution efficiencies on C8 for most analytes were 1.3 to 5.8 times higher than those on C18 (Figure 13, page 53); the elution efficiencies on silica gel for most analytes were 18 to 90% higher than those for alumina (Figure 14, page 54). Besides, it deserves to be mentioned that the elution efficiencies of oxybenzone, ketoprofen and ibuprofen were improved a lot using silica gel, which were not able to be eluted from alumina. Once again, the elution efficiencies of DEP and benzophenone were influenced by backgrounds. When the background levels were deducted, the elution efficiencies for DEP became 26.2%, 54.0%, 53.5%, and 45.6% on C18, C8, alumina and silica gel, respectively. Regarding benzophenone, the elution efficiencies became 33.5%, 59.1%, 40.9% and 48.8% on C18, C8, alumina and silica, respectively. Accordingly, for better elution, C8 and silica gel were selected as MSPD and cleanup adsorbents, respectively.

Regarding the backgrounds of DEP and benzophenone observed in the reagent blank in above test done on silica gel, silica gel cartridges might be one of the sources. The

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background levels of both DEP and benzophenone decreased a lot if silica gel cartridges were pre-washed with acetone (Figure 15, page 54); the concentrations of DEP reduced, and the backgrounds of benzophenone was eliminated.

To test if the activity of silica gel was influenced by the pre-wash step with acetone, we eluted an MSPD cartridge of non-spiked fish muscle sample and collected the methanol portion and acetone portion of the eluent, respectively. Thereafter, chemical standards of the analytes were spiked to the methanol portion of the eluent, and then passed the spiked methanol eluent and acetone portion of eluent sequentially through silica gel cartridges with and without the pre-wash step, respectively. The peak areas of analytes in the final residues of the two groups were similar (Figure 16, page 55), which demonstrated that the activity of silica gel was not affected by the pre-wash step.

The amount of silica gel was increased to 2 g to remove pigment completely from liver samples. As indicated in Figure 17 (page 55), the extract cleaned up by 2-g silica gel was much cleaner than that by 1 g.

The previous method concentrated the eluents to 100 μL by Savant SPD 1010 SpeedVac and then injected four microliters of the samples onto the UPLC-MS/MS for analysis. However, it was difficult to quantify the small volume precisely, which might

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influence the precision of the method. We modified the protocol and evaporated the eluent to nearly dry and then reconstituted it with 100 μL of methanol for better control on the final volume. Nevertheless, some viscous and light yellow material presented when the eluents of muscle samples were concentrated to around 20–50 μL. In addition, the residues were not able to be completely reconstituted with 100 μL of methanol, which looked like lipids. To solve the above problem, muscle samples were not concentrated to nearly dry and the volume of final residues was increased to 250 μL.

Regarding liver samples, the eluents became difficult to be concentrated to lower volume when concentrated to nearly 100–200 μL, and it would take much time to concentrate liver samples to nearly dry, which might cause more analytes to evaporate. In addition, the sample cleanup of liver was worse than that of muscle. Thus, the volume of final residues of liver samples was increased to more than 250 μL to avoid time-consuming concentration and serious matrix effect, and the peak areas of analytes in 500-μL final residues of post-spiked liver samples were compared with those of the two-fold dilution samples of the 500-μL final residues. All the area ratios of the two-fold dilution samples to the original samples were > 0.5 but < 1, indicating that two-fold dilution improved matrix effect limitedly (Figure 18, page 56). For better sensitivity, 500 μL was

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chosen as the volume of final residues of liver samples.

In terms of the 500-μL final residues of liver samples, there were some suspended solids and lipids after storing at 4°C even though the eluents had been filtered by 0.20-μm PTFE syringe filters when the volume was one mL before concentrated to 500 μL. To remove these materials, the 500-μL residues were centrifuged at 13,200 rpm (16100 × g) at 4°C for 10 minutes after refrigerated at 4°C overnight. Some solids precipitated after the centrifugation, but lipids and other solids still suspended on the surface of the final residues. Thus, only the subnatants (not included the precipitates) were taken and were filtered again through PTFE filtered (0.20 μm) before analysis.

Regarding the chromatograms of matrix blank samples of liver (Figure 3, page 48), there was a peak (RT = 0.62 minute) near acetaminophen (RT = 0.74 minute) and interfered with the quantification. The signal intensity of this unknown signal in the chromatogram of the second product ion of acetaminophen was about 21 times lower than that in the chromatogram of the first product ion. To reduce its impact on quantifying acetaminophen in liver, we used the second abundant product ion as the quantitative ion and the most abundant product ion as the confirmatory ion, respectively.

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3.4. Method validation

The matrix effect factors of analytes in fish muscle and liver ranged from 13.4 to 95.6% and ranged from 4.52 to 61.9%, respectively (Figure 19, page 57; Table 5, page 64). All analytes had lower matrix effect factors in liver than in muscle, indicating that the matrix effects of all analytes were more serious in liver than those of muscle. The matrix effect factors of most analytes in liver ranged from 24.3 to 61.9%, and ranged from 70.3 to 95.6% in muscle; therefore, most analytes did not suffer significant matrix effect in muscle. The matrix effect factors of acetaminophen and caffeine in both muscle (13.4% and 44.2%) and liver (4.52% and 19.8%), plus that of methyl paraben in liver (18.9%) were much lower than most of other analytes, which could be attributed to their earlier elution with other polar compounds from the column.

The extraction efficiencies of analytes in fish muscle and liver were 10.9–76.6% and 1.86–71.2%, respectively (Figure 20, page 57; Table 6, page 64). The extraction efficiencies of DEP, benzophenone, oxybenzone, propyl paraben, butyl paraben and ibuprofen were < 40% in both matrices. Most analytes had lower extraction efficiencies in liver than those of muscle, especially on DEP, ethyl paraben and propyl paraben. The lower extraction efficiencies of most analytes in liver might be partly because the

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concentration time of liver samples was longer than that of muscle samples (about two extra hours), which might cause more analytes to evaporate. In general, extraction efficiencies of half of the analytes in muscle ranged from 62.1 to 76.6%, and those of most analyes in liver ranged from 31.6 to 71.2%.

The IDLs of DEP, analgesics, caffeine, DEET, UV filters and parabens were 4.04 pg, 2.86−8.52 pg (except for ketoprofen at 47.7 pg), 12.5 pg, 0.43 pg, 0.64−17.3 pg, 1.29−7.63 pg, respectively. The IQLs of DEP, analgesics, caffeine, DEET, UV filters and parabens were 10.6 pg, 9.53−9.57 pg (except for ketoprofen at 159 pg), 22.9 pg, 1.03 pg, 1.95−37.1 pg, 2.14−7.63 pg, respectively. (Table 7, page 65)

The LODs of analytes were 0.57 to 15.0 ng/g w.w. (2.65 to 69.9 ng/g d.w.) for fish muscle and 4.37 to 104 ng/g w.w. (13.4 to 319 ng/g d.w.) for fish liver, respectively (Table 8, page 66); the LOQs of analytes ranged from 1.04 to 34.9 ng/g w.w. (4.86 to 163 ng/g d.w.) for fish muscle and ranged from 10.6 to 281 ng/g w.w. (32.4 to 861 ng/g d.w.) for fish liver, respectively. Both the LODs and LOQs of analytes in liver were higher than those of muscle, which might be explained by higher ion suppression on all analytes and lower extraction efficiencies on most analytes in liver. Some analytes had lower LODs or LOQs than those of most other analytes, which might be attributed to their higher IDLs

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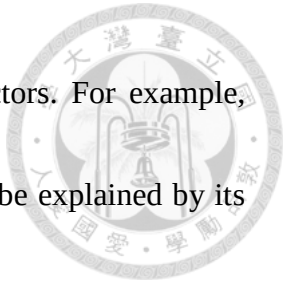

or IQLs, lower extraction efficiencies, and lower matrix effect factors. For example, acetaminophen had highest LOD and LOQ in muscle, which might be explained by its lowest matrix effect factor (13.4%) and slightly higher IDL and IQL than most of other analytes; the lower extraction efficiencies of ethyl paraben (3.29%) and butyl paraben (2.72%) in liver than most of other analytes (14.4%−71.2%) might result in their higher LODs and LOQs in liver than many of other analytes.

Our LODs or LOQs of some analytes were higher than those of some previous reports, but some were similar to or lower than those of some previous reports. Kwon et al. determined pharmaceuticals and PCPs in fish livers using LLE with LC-MS, and the LOQ of oxybenzone was 2.0 times lower than that in our study [69]. Our LOQ of DEP was similar to that of Cheng et al. (5 ng/g w.w.) who analyzed PAEs in fish muscle using Soxhlet extraction with GC-MS [48], and slightly higher than that of Guo et al. (2 ng/g w.w.) who investigated PAEs in seafood using liquid-liquid extraction (LLE) with GC-MS [70]. The LODs of DEET and methyl paraben in liver, and LODs of ethyl parabens, propyl paraben and butyl paraben in muscle in our study were 1.4−2.8 times higher than those of Tanoue et al., and LODs of ethyl parabens, propyl paraben and butyl paraben in liver in our study were 24−74 times higher than those of theirs; however, our LODs of

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DEET and methyl paraben in fish muscle were 5.6 times and 2.7 times lower than those of theirs, respectively [33]. Carmona et al, reported a method analyzing multiple organic compounds in fish muscle using solid-liquid extraction (SLE) and ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC–MS/MS), and the LOQs of parabens and ibuprofen were 1.4−7.5 times higher than those in our study [52]. Ramirez et al. determined pharmaceuticals and PCPs in fish fillet and livers using ultrasonic extraction with HPLC-MS/MS and GC-MS/MS; their LOD of acetaminophen in muscle was 3.4 times lower than that in our study while their LOD of acetaminophen in liver and LODs of caffeine in both matrices were 1.6−3.2 times higher than ours. Furthermore, their LODs of ibuprofen in muscle and liver were 38 times and 25 times higher than ours, respectively [5].

Different LODs and LOQs might result from different extraction techniques, instrumental methods, injection volumes, and definitions of LODs and LOQs. For instance, we calculated LODs based on the confirmatory ions rather than the most intensive product ions. Furthermore, this study dealt with analtytes at a wide range of physical and chemical properties, so we had to comprise parameters of sample treatment and instrumental analysis.

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The relative standard deviations (%RSD) of all analytes were below 20%, and the %RSDs of most analytes were below 8%, which showed that the method provided good precision for all analytes. Most of the quantitative biases (%bias) were below 30% in fish muscle at three spiked levels (62.5, 200, 500 ng/g) and in liver spiked at 1,000 ng/g (Table 9, page 67), indicating that the method offered good accuracy for most analytes. The %bias of propyl paraben was higher than those of most analytes would result from the use of methyl paraben-$^{13}C_6$ as its isotope-labeled internal standard rather than its own isotope internal standard.

The accuracy of some analytes was influenced by the backgrounds from labware or endogenous amount in matrices. The %bias of DEP at the lowest spiked level and %bias of methyl paraben at all spiked levels in muscle were higher than those of most analytes might be due to both backgrounds from labware and endogenous amount in matrices. The concentrations of DEP and methyl paraben in the reagent blank were 35.5 and 23.6 ng/g, respectively; the concentrations of DEP and methyl paraben in the matrix blank were 64.3 and 34.6 ng/g, respectively. The %bias of benzophenone at the lowest and medium spiked level in muscle were higher than those of most analytes, which might be explained by backgrounds from labware because the concentrations of benzophenone in the reagent

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blank and the matrix blank were similar (78.7 ng/g and 71.3 ng/g). When the background levels were deducted, the %biases of these analytes were below 30%.

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Chapter 4. Conclusions

This study developed and validated a method for simultaneously determining DEP and 11 PCPs in fish muscle and livers. Although the cleanup was not so effective on liver samples, the method offered reproducible analytical results on all analytes with %RSD below 20% and accurate analytical results on most of the analytes with quantitative biases below 30% by using isotope dilution techniques. In addition, the LODs of most analytes ranged from to sub-ng/g to tens of ng/g w.w., and some LODs were lower than those in other previous reports, indicating that better sensitivity was acquired for some analytes. Furthermore, the MSPD method consumed only small volumes of organic solvents and did not need expensive devices for extraction. The developed method is able to be applied to the determination of these compounds in wild fish samples to acquire more information about the levels of these chemicals in fish tissues and possible health effects on fish.

Since the cleanup effect on liver samples was not ideal with silica gel, the protocol for preparing liver samples was more complicated than that for muscle samples. Further studies are desired to improve the cleanup, for example, elimination of lipids by freezing them in, or tests of other adsorbents such as Enhanced Matrix Removal-Lipid (EMR-Lipid).

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Reference

1. Mankidy, R., Wiseman, S., Ma, H., and Giesy, J.P., Biological impact of phthalates. *Toxicol Lett*, 2013. **217**(1): p. 50-58.
2. Brausch, J.M. and Rand, G.M., A review of personal care products in the aquatic environment: environmental concentrations and toxicity. *Chemosphere*, 2011. **82**(11): p. 1518-1532.
3. Daughton, C.G. and Ternes, T.A., Pharmaceuticals and personal care products in the environment: Agents of subtle change? *Environ Health Persp*, 1999. **107**: p. 906-938.
4. Jackson, J. and Sutton, R., Sources of endocrine-disrupting chemicals in urban wastewater, Oakland, CA. *Sci Total Environ*, 2008. **405**(1-3): p. 153-160.
5. Ramirez, A.J., Brain, R.A., Usenko, S., Mottaleb, M.A., O'Donnell, J.G., Stahl, L.L., Wathen, J.B., Snyder, B.D., Pitt, J.L., Perez-Hurtado, P., Dobbins, L.L., Brooks, B.W., and Chambliss, C.K., Occurrence of pharmaceuticals and personal care products in fish: results of national pilot study in the United States. *Environ Toxicol Chem*, 2009. **28**(12): p. 2587-2597.
6. Du, B., Haddad, S.P., Luek, A., Scott, W.C., Saari, G.N., Kristofco, L.A., Connors, K.A., Rash, C., Rasmussen, J.B., Chambliss, C.K., and Brooks, B.W., Bioaccumulation and trophic dilution of human pharmaceuticals across trophic positions of an effluent-dependent wadeable stream. *Philos Trans R Soc Lond B Biol Sci*, 2014. **369**(1656): p. 10.
7. Feng, Y.L., Liao, X., Grenier, G., Nguyen, N., and Chan, P., Determination of 18 phthalate metabolites in human urine using a liquid chromatography-tandem mass spectrometer equipped with a core–shell column for rapid separation. *Anal Methods-UK*, 2015. **7**(19): p. 8048-8059.
8. Schettler, T., Human exposure to phthalates via consumer products. *Int J Androl*, 2006. **29**(1): p. 134-139.
9. Net, S., Sempere, R., Delmont, A., Paluselli, A., and Ouddane, B., Occurrence, fate, behavior and ecotoxicological state of phthalates in different environmental matrices. *Environ Sci Technol*, 2015. **49**(7): p. 4019-4035.
10. Kong, M., Song, Y., Zhang, Y., Liu, R., Wei, J., and Zheng, L., Fate of phthalate esters in municipal wastewater treatment plant and their environmental impact. *Water Sci Technol*, 2016. **73**(6): p. 1395-400.
11. Fatoki, O.S., Bornman, M., Ravandhalala, L., Chimuka, L., Genthe, B., and

doi:10.6342/NTU201802422

Adeniyi, A., Phthalate ester plasticizers in freshwater systems of Venda, South Africa and potential health effects. *Water Sa*, 2010. **36**(1): p. 117-125.

12. Yuan, S.Y., Liu, C., Liao, C.S., and Chang, B.V., Occurrence and microbial degradation of phthalate esters in Taiwan river sediments. *Chemosphere*, 2002. **49**(10): p. 1295-1299.
13. Zeng, F., Cui, K., Xie, Z., Liu, M., Li, Y., Lin, Y., Zeng, Z., and Li, F., Occurrence of phthalate esters in water and sediment of urban lakes in a subtropical city, Guangzhou, South China. *Environ Int*, 2008. **34**(3): p. 372-380.
14. Huang, P.C., Tien, C.J., Sun, Y.M., Hsieh, C.Y., and Lee, C.C., Occurrence of phthalates in sediment and biota: relationship to aquatic factors and the biota-sediment accumulation factor. *Chemosphere*, 2008. **73**(4): p. 539-544.
15. Valton, A.S., Serre-Dargnat, C., Blanchard, M., Alliot, F., Chevreuil, M., and Teil, M.J., Determination of phthalates and their by-products in tissues of roach (Rutilus rutilus) from the Orge river (France). *Environ Sci Pollut Res Int*, 2014. **21**(22): p. 12723-12730.
16. Chen, X., Xu, S., Tan, T., Lee, S.T., Cheng, S.H., Lee, F.W., Xu, S.J., and Ho, K.C., Toxicity and estrogenic endocrine disrupting activity of phthalates and their mixtures. *Int J Environ Res Public Health*, 2014. **11**(3): p. 3156-3168.
17. Staples, C.A., Peterson, D.R., Parkerton, T.F., and Adams, W.J., The environmental fate of phthalate esters: A literature review. *Chemosphere*, 1997. **35**(4): p. 667-749.
18. Arnot, J.A. and Gobas, F.A.P.C., A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. *Environ Rev*, 2006. **14**(4): p. 257-297.
19. Miege, C., Choubert, J.M., Ribeiro, L., Eusebe, M., and Coquery, M., Fate of pharmaceuticals and personal care products in wastewater treatment plants--conception of a database and first results. *Environ Pollut*, 2009. **157**(5): p. 1721-1726.
20. Oulton, R.L., Kohn, T., and Cwiertny, D.M., Pharmaceuticals and personal care products in effluent matrices: A survey of transformation and removal during wastewater treatment and implications for wastewater management. *J Environ Monit*, 2010. **12**(11): p. 1956-1978.
21. Verlicchi, P., Al Aukidy, M., and Zambello, E., Occurrence of pharmaceutical compounds in urban wastewater: removal, mass load and environmental risk after a secondary treatment--a review. *Sci Total Environ*, 2012. **429**: p. 123-155.

doi:10.6342/NTU201802422

22. Blair, B.D., Crago, J.P., Hedman, C.J., Treguer, R.J., Magruder, C., Royer, L.S., and Klaper, R.D., Evaluation of a model for the removal of pharmaceuticals, personal care products, and hormones from wastewater. *Sci Total Environ*, 2013. **444**: p. 515-521.
23. Blair, B.D., Crago, J.P., Hedman, C.J., and Klaper, R.D., Pharmaceuticals and personal care products found in the Great Lakes above concentrations of environmental concern. *Chemosphere*, 2013. **93**(9): p. 2116-2123.
24. Subedi, B., Du, B., Chambliss, C.K., Koschorreck, J., Rudel, H., Quack, M., Brooks, B.W., and Usenko, S., Occurrence of pharmaceuticals and personal care products in German fish tissue: A national study. *Environ Sci Technol*, 2012. **46**(16): p. 9047-9054.
25. Yang, Y., Ok, Y.S., Kim, K.H., Kwon, E.E., and Tsang, Y.F., Occurrences and removal of pharmaceuticals and personal care products (PPCPs) in drinking water and water/sewage treatment plants: A review. *Sci Total Environ*, 2017. **596-597**: p. 303-320.
26. Xie, Z., Lu, G., Liu, J., Yan, Z., Ma, B., Zhang, Z., and Chen, W., Occurrence, bioaccumulation, and trophic magnification of pharmaceutically active compounds in Taihu Lake, China. *Chemosphere*, 2015. **138**: p. 140-147.
27. Moreno-Gonzalez, R., Rodriguez-Mozaz, S., Huerta, B., Barcelo, D., and Leon, V.M., Do pharmaceuticals bioaccumulate in marine molluscs and fish from a coastal lagoon? *Environ Res*, 2016. **146**: p. 282-298.
28. Beydoun, M.A., Gamaldo, A.A., Beydoun, H.A., Tanaka, T., Tucker, K.L., Talegawkar, S.A., Ferrucci, L., and Zonderman, A.B., Caffeine and alcohol intakes and overall nutrient adequacy are associated with longitudinal cognitive performance among U.S. adults. *J Nutr*, 2014. **144**(6): p. 890-901.
29. Alvarez-Munoz, D., Rodriguez-Mozaz, S., Maulvault, A.L., Tediosi, A., Fernandez-Tejedor, M., Van den Heuvel, F., Kotterman, M., Marques, A., and Barcelo, D., Occurrence of pharmaceuticals and endocrine disrupting compounds in macroalgaes, bivalves, and fish from coastal areas in Europe. *Environ Res*, 2015. **143**(Pt B): p. 56-64.
30. Wang, J. and Gardinali, P.R., Analysis of selected pharmaceuticals in fish and the fresh water bodies directly affected by reclaimed water using liquid chromatography-tandem mass spectrometry. *Anal Bioanal Chem*, 2012. **404**(9): p. 2711-2720.
31. Costanzo, S.D., Watkinson, A.J., Murby, E.J., Kolpin, D.W., and Sandstrom,

doi:10.6342/NTU201802422

M.W., Is there a risk associated with the insect repellent DEET (N,N-diethyl-m-toluamide) commonly found in aquatic environments? *Sci Total Environ*, 2007. **384**(1-3): p. 214-220.

32. Swale, D.R., Sun, B., Tong, F., and Bloomquist, J.R., Neurotoxicity and mode of action of N, N-diethyl-meta-toluamide (DEET). *PLoS One*, 2014. **9**(8): p. e103713.
33. Tanoue, R., Nomiyama, K., Nakamura, H., Kim, J.W., Isobe, T., Shinohara, R., Kunisue, T., and Tanabe, S., Uptake and tissue distribution of pharmaceuticals and personal care products in wild fish from treated-wastewater-impacted streams. *Environ Sci Technol*, 2015. **49**(19): p. 11649-11658.
34. Giokas, D.L., Salvador, A., and Chisvert, A., UV filters: From sunscreens to human body and the environment. *TrAC-Trend Anal Chem*, 2007. **26**(5): p. 360-374.
35. Gago-Ferrero, P., Diaz-Cruz, M.S., and Barcelo, D., An overview of UV-absorbing compounds (organic UV filters) in aquatic biota. *Anal Bioanal Chem*, 2012. **404**(9): p. 2597-2610.
36. Schlumpf, M., Cotton, B., Conscience, M., Haller, V., Steinmann, B., and Lichtensteiger, W., In vitro and in vivo estrogenicity of UV screens. *Environ Health Persp*, 2001. **109**: p. 239-244.
37. Langford, K.H., Reid, M.J., Fjeld, E., Oxnevad, S., and Thomas, K.V., Environmental occurrence and risk of organic UV filters and stabilizers in multiple matrices in Norway. *Environ Int*, 2015. **80**: p. 1-7.
38. Peng, X., Jin, J., Wang, C., Ou, W., and Tang, C., Multi-target determination of organic ultraviolet absorbents in organism tissues by ultrasonic assisted extraction and ultra-high performance liquid chromatography-tandem mass spectrometry. *J Chromatogr A*, 2015. **1384**: p. 97-106.
39. Soni, M.G., Carabin, I.G., and Burdock, G.A., Safety assessment of esters of p-hydroxybenzoic acid (parabens). *Food Chem Toxicol*, 2005. **43**(7): p. 985-1015.
40. Chen, J., Ahn, K.C., Gee, N.A., Gee, S.J., Hammock, B.D., and Lasley, B.L., Antiandrogenic properties of parabens and other phenolic containing small molecules in personal care products. *Toxicol Appl Pharmacol*, 2007. **221**(3): p. 278-284.
41. Djatmika, R., Hsieh, C.C., Chen, J.M., and Ding, W.H., Determination of paraben preservatives in seafood using matrix solid-phase dispersion and on-line acetylation gas chromatography-mass spectrometry. *J Chromatogr B*, 2016. **1036-**

doi:10.6342/NTU201802422

1037: p. 93-99.

42. Teil, M.J., Tlili, K., Blanchard, M., Chevreuil, M., Alliot, F., and Labadie, P., Occurrence of polybrominated diphenyl ethers, polychlorinated biphenyls, and phthalates in freshwater fish from the Orge river (Ile-de France). *Arch Environ Contam Toxicol*, 2012. **63**(1): p. 101-113.
43. Ji, Y., Wang, F., Zhang, L., Shan, C., Bai, Z., Sun, Z., Liu, L., and Shen, B., A comprehensive assessment of human exposure to phthalates from environmental media and food in Tianjin, China. *J Hazard Mater*, 2014. **279**: p. 133-140.
44. Chen, C.L., Löfstrand, K., Adolfsson-Erici, M., and MacLeod, M., Determination of fragrance ingredients in fish by ultrasound-assisted extraction followed by purge & trap. *Anal Methods-UK*, 2017. **9**(15): p. 2237-2245.
45. Gago-Ferrero, P., Diaz-Cruz, M.S., and Barcelo, D., Multi-residue method for trace level determination of UV filters in fish based on pressurized liquid extraction and liquid chromatography-quadrupole-linear ion trap-mass spectrometry. *J Chromatogr A*, 2013. **1286**: p. 93-101.
46. Kannan, K., Reiner, J.L., Yun, S.H., Perrotta, E.E., Tao, L., Johnson-Restrepo, B., and Rodan, B.D., Polycyclic musk compounds in higher trophic level aquatic organisms and humans from the United States. *Chemosphere*, 2005. **61**(5): p. 693-700.
47. Meinerling, M. and Daniels, M., A validated method for the determination of traces of UV filters in fish using LC-MS/MS. *Anal Bioanal Chem*, 2006. **386**(5): p. 1465-1473.
48. Cheng, Z., Nie, X.P., Wang, H.S., and Wong, M.H., Risk assessments of human exposure to bioaccessible phthalate esters through market fish consumption. *Environ Int*, 2013. **57-58**: p. 75-80.
49. Adeogun, A.O., Ibor, O.R., Omogbemi, E.D., Chukwuka, A.V., Adegbola, R.A., Adewuyi, G.A., and Arukwe, A., Environmental occurrence and biota concentration of phthalate esters in Epe and Lagos Lagoons, Nigeria. *Mar Environ Res*, 2015. **108**: p. 24-32.
50. Sorensen, L.K., Determination of phthalates in milk and milk products by liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom*, 2006. **20**(7): p. 1135-1143.
51. Xu, D., Deng, X., Fang, E., Zheng, X., Zhou, Y., Lin, L., Chen, L., Wu, M., and Huang, Z., Determination of 23 phthalic acid esters in food by liquid chromatography tandem mass spectrometry. *J Chromatogr A*, 2014. **1324**: p. 49-

doi:10.6342/NTU201802422

56.

52. Carmona, E., Andreu, V., and Pico, Y., Multi-residue determination of 47 organic compounds in water, soil, sediment and fish-Turia River as case study. *J Pharm Biomed Anal*, 2017. **146**: p. 117-125.
53. Liu, Y.Y., Hu, X.L., Bao, Y.F., and Yin, D.Q., Simultaneous determination of 29 pharmaceuticals in fish muscle and plasma by ultrasonic extraction followed by SPE-UHPLC-MS/MS. *J Sep Sci*, 2018. **41**(10): p. 2139-2150.
54. Barker, S.A., Long, A.R., and Short, C.R., Isolation of drug residues from tissues by solid-phase dispersion *J Chromatogr*, 1989. **475**: p. 353-361.
55. Barker, S.A., Matrix solid phase dispersion (MSPD). *J Biochem Biophys Methods*, 2007. **70**(2): p. 151-162.
56. Capriotti, A.L., Cavaliere, C., Laganà, A., Piovesana, S., and Samperi, R., Recent trends in matrix solid-phase dispersion. *TrAC-Trend Anal Chem*, 2013. **43**: p. 53-66.
57. Capriotti, A.L., Cavaliere, C., Foglia, P., Samperi, R., Stampachiacchiere, S., Ventura, S., and Laganà, A., Recent advances and developments in matrix solid-phase dispersion. *TrAC-Trend Anal Chem*, 2015. **71**: p. 186-193.
58. Freitas, S., Serafim, F., and Lanças, F., Determination of target pesticide residues in tropical fruits employing matrix solid-phase dispersion (MSPD) extraction followed by high resolution gas chromatography. *J Brazil Chem Soc*, 2018. **29**(5): p. 1140-1148.
59. Ramirez, A.J., Mottaleb, M.A., Brooks, B.W., and Chambliss, C.K., Analysis of pharmaceuticals in fish using liquid chromatography-tandem mass spectrometry. *Anal Chem*, 2007. **79**(8): p. 3155-3163.
60. Mottaleb, M.A., Usenko, S., O'Donnell, J.G., Ramirez, A.J., Brooks, B.W., and Chambliss, C.K., Gas chromatography-mass spectrometry screening methods for select UV filters, synthetic musks, alkylphenols, an antimicrobial agent, and an insect repellent in fish. *J Chromatogr A*, 2009. **1216**(5): p. 815-823.
61. Cao, X.L., Zhao, W., Churchill, R., and Hilts, C., Occurrence of Di-(2-ethylhexyl) adipate and phthalate plasticizers in samples of meat, fish, and cheese and their packaging films. *J Food Prot*, 2014. **77**(4): p. 610-620.
62. Guerranti, C., Cau, A., Renzi, M., Badini, S., Grazioli, E., Perra, G., and Focardi, S.E., Phthalates and perfluorinated alkylated substances in Atlantic bluefin tuna (Thunnus thynnus) specimens from Mediterranean Sea (Sardinia, Italy): Levels and risks for human consumption. *J Environ Sci Health B*, 2016. **51**(10): p. 661-

doi:10.6342/NTU201802422

667.
63. 陳家揚、龍世俊。生活污水中個人保健品殘留化學物質之檢測技術建立研究（2/4）。2008。行政院環保署報告編號 EPA-97-E3S4-02-02。
64. 陳家揚、龍世俊。生活污水中個人保健品殘留化學物質之檢測技術建立研究（3/4）。2009。行政院環保署報告編號 EPA-98-E3-S4-02-03。
65. 陳家揚。生活污水中個人保健品殘留化學物質之檢測技術建立研究(4/4)。2010。行政院環保署報告編號 EPA-99-E3-S4-02-02。
66. Tang, T.C., Determination of nitrated/oxygenated polycyclic aromatic hydrocarbons and personal care products in river water, sediment, fish muscle and liver with ultra-performance liquid chromatography/tandem mass spectrometry. 2015, Nationl Taiwan University.
67. 陳家揚。土壤底泥中農藥多環芳香烴酚類塑化劑有機錫調查計畫(2/2)。2014。行政院環保署報告編號 EPA-103-1604-02-02。
68. Guo, Y. and Kannan, K., Challenges encountered in the analysis of phthalate esters in foodstuffs and other biological matrices. *Anal Bioanal Chem*, 2012. **404**(9): p. 2539-2554.
69. Kwon, J.W., Armbrust, K.L., Vidal-Dorsch, D., Bay, S.M., and Xia, K., Determination of 17 alpha-ethynylestradiol, carbamazepine, diazepam, simvastatin, and oxybenzone in fish livers. *J AOAC Int*, 2009. **92**(1): p. 359-369.
70. Guo, Y., Zhang, Z., Liu, L., Li, Y., Ren, N., and Kannan, K., Occurrence and profiles of phthalates in foodstuffs from China and their implications for human exposure. *J Agric Food Chem*, 2012. **60**(27): p. 6913-6919.

doi:10.6342/NTU201802422

doi:10.6342/NTU201802422

Figures

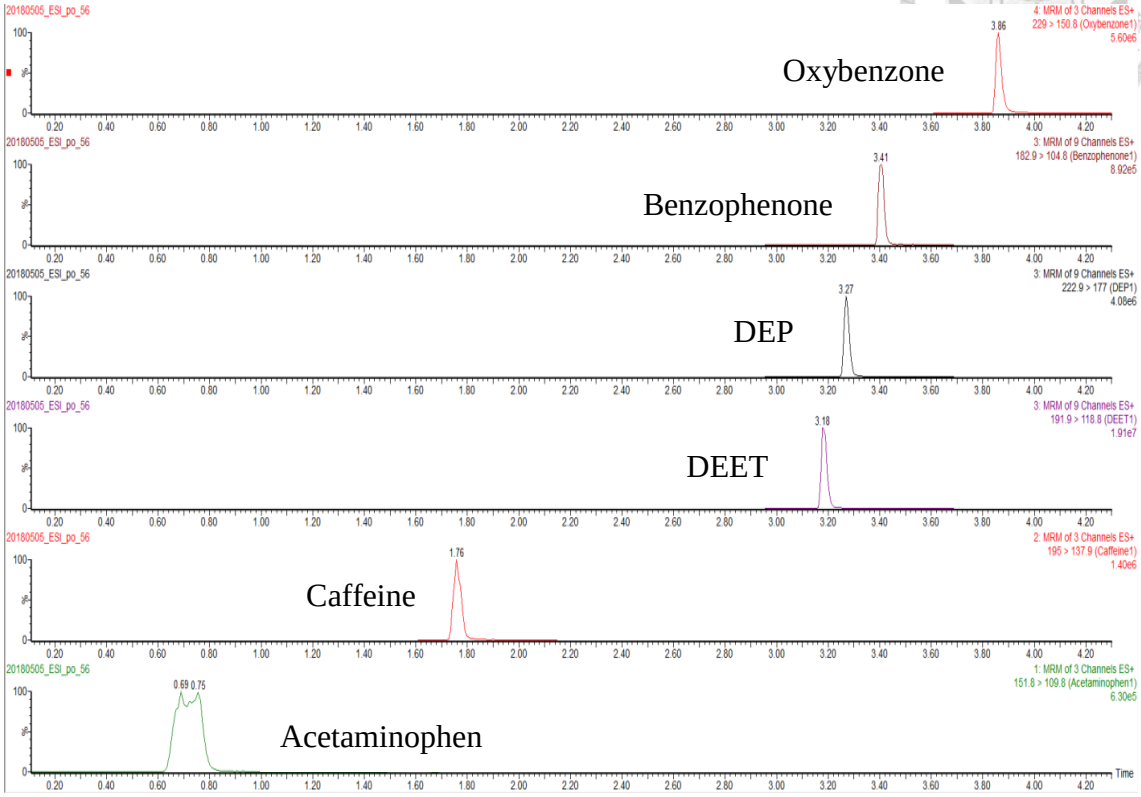

Figure 1. Chromatograms of ESI+ of a chemical standard solution (0.5 μg/mL in methanol standard solution, injection 4 μL)

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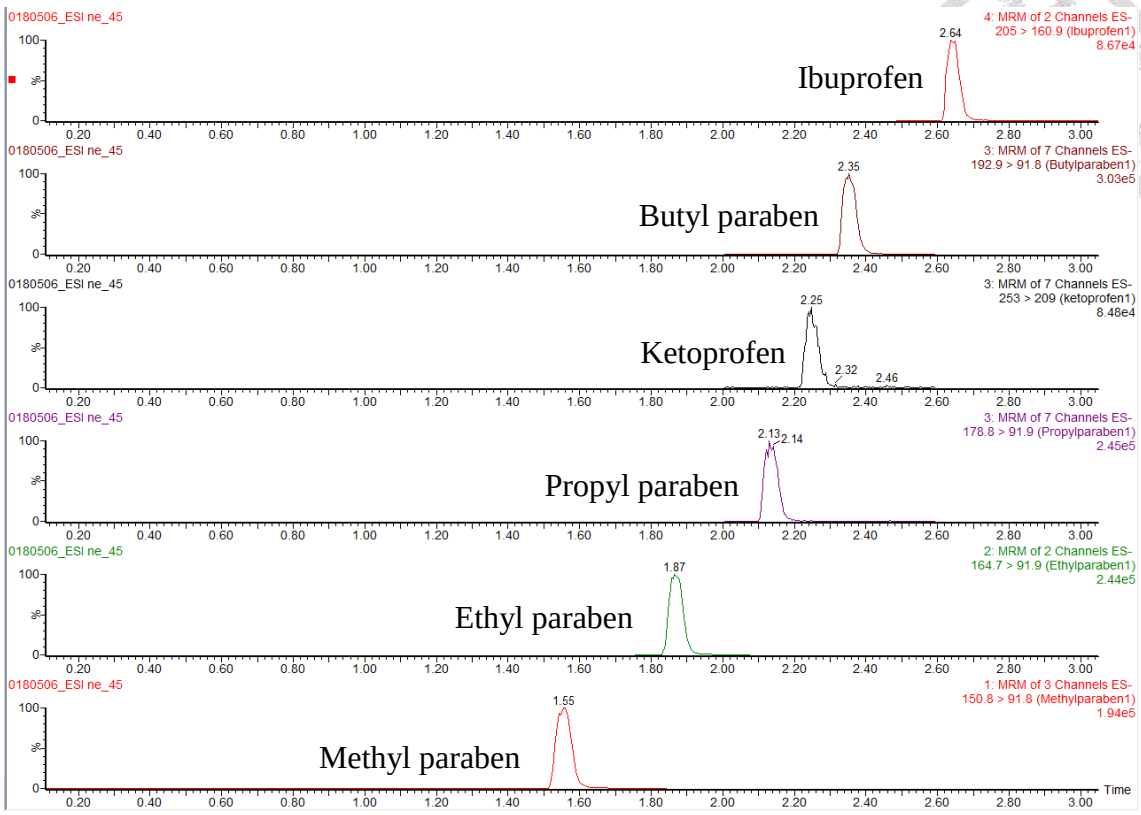

Figure 2. Chromatograms of ESI- of a chemical standard solution (0.5 μg/mL in methanol standard solution, injection 4 μL)

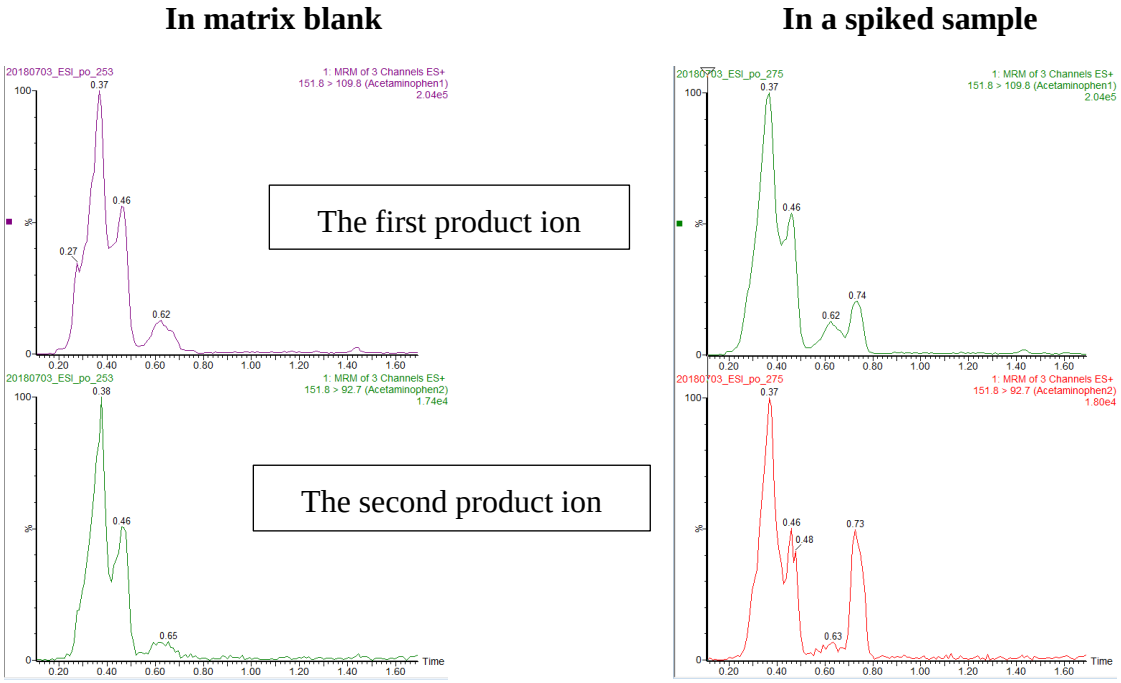

Figure 3. Chromatograms of the first and second product ions of acetaminophen in liver samples (the sample was pre-spiked at 1,000 ng/g w.w. level, injection 4 μL)

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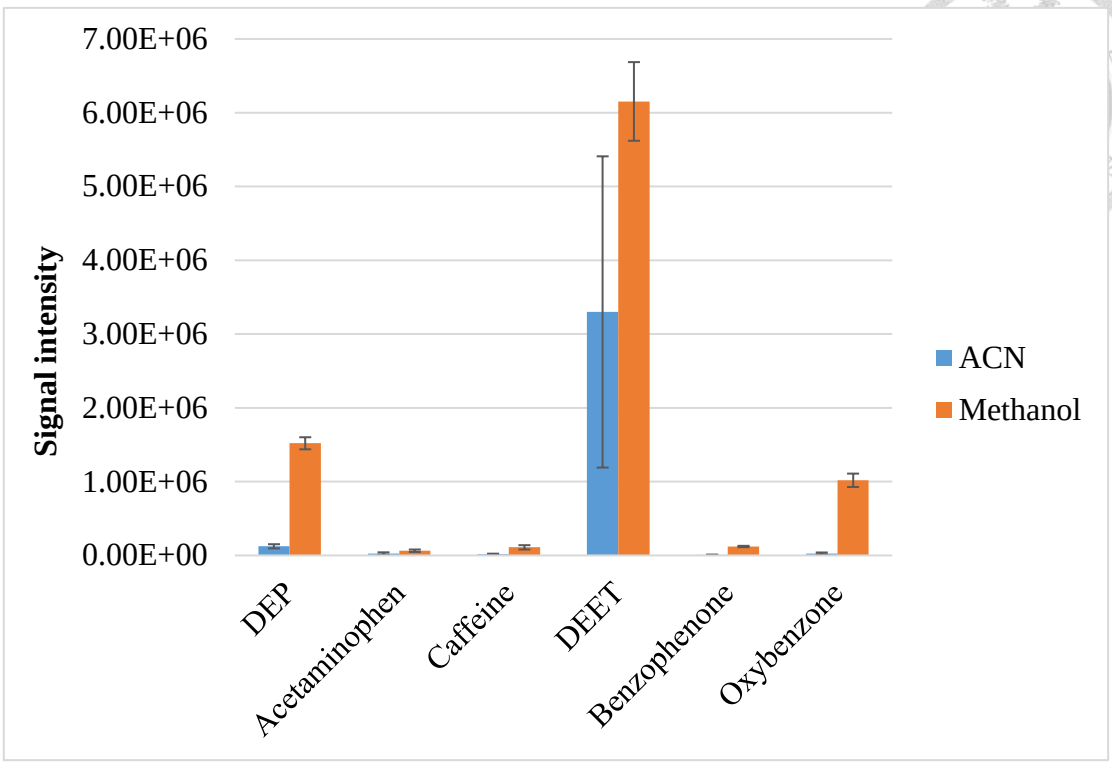

Figure 4. Signal intensities of DEP and basic PCPs with different organic mobile phases (0.25 μg/mL in methanol standard solution, injection 4 μL, n = 3)

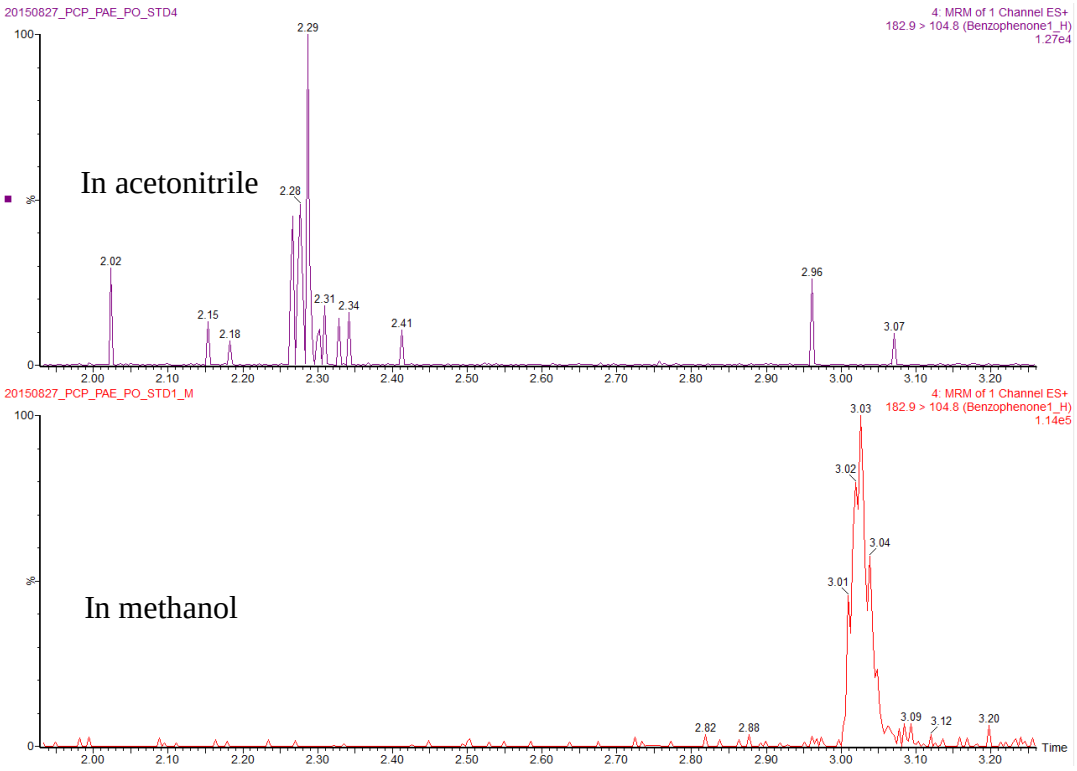

Figure 5. Chromatograms of benzophenone with different organic mobile phases (0.25 μg/mL in methanol standard solution, injection 4 μL)

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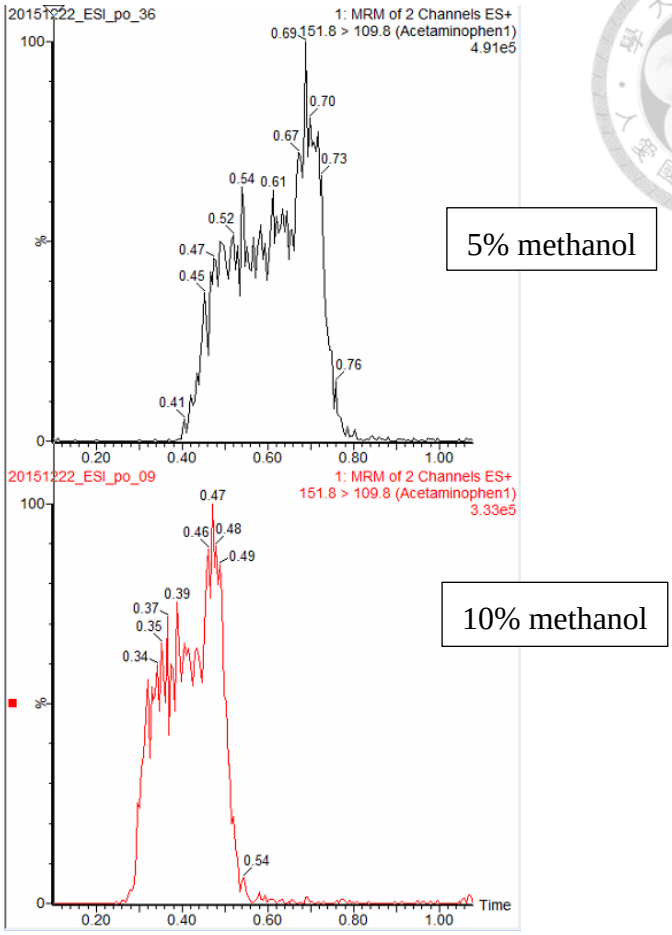

Figure 6. Chromatograms of acetaminophen with different initial organic mobile phase proportions (0.25 μg/mL in methanol standard solution, injection 4 μL)

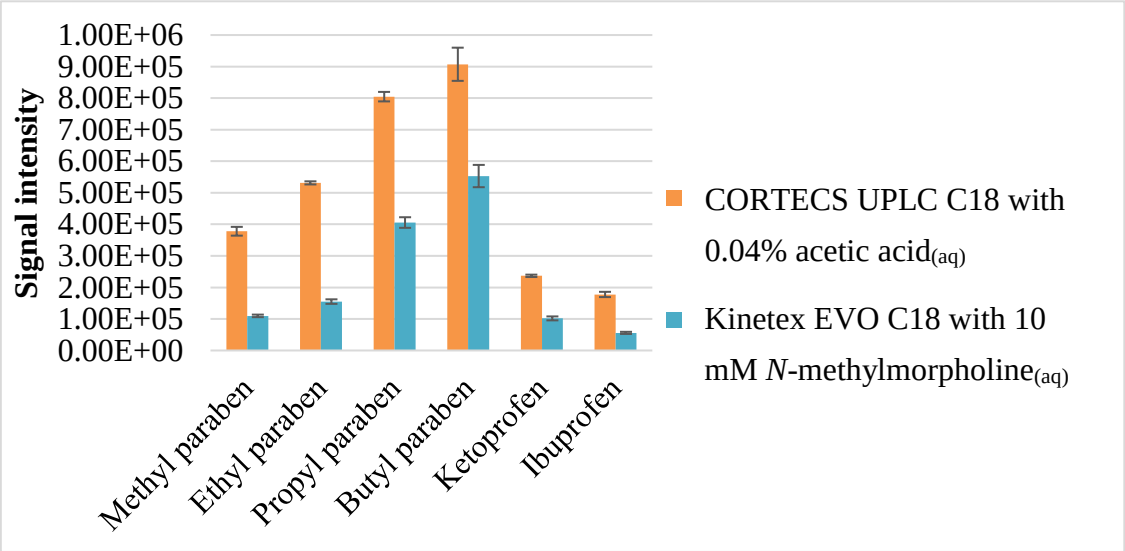

Figure 7. Signal intensities of acidic PCPs under different chromatographic conditions (1 μg/mL in methanol standard solution, injection 4 μL, n = 3)

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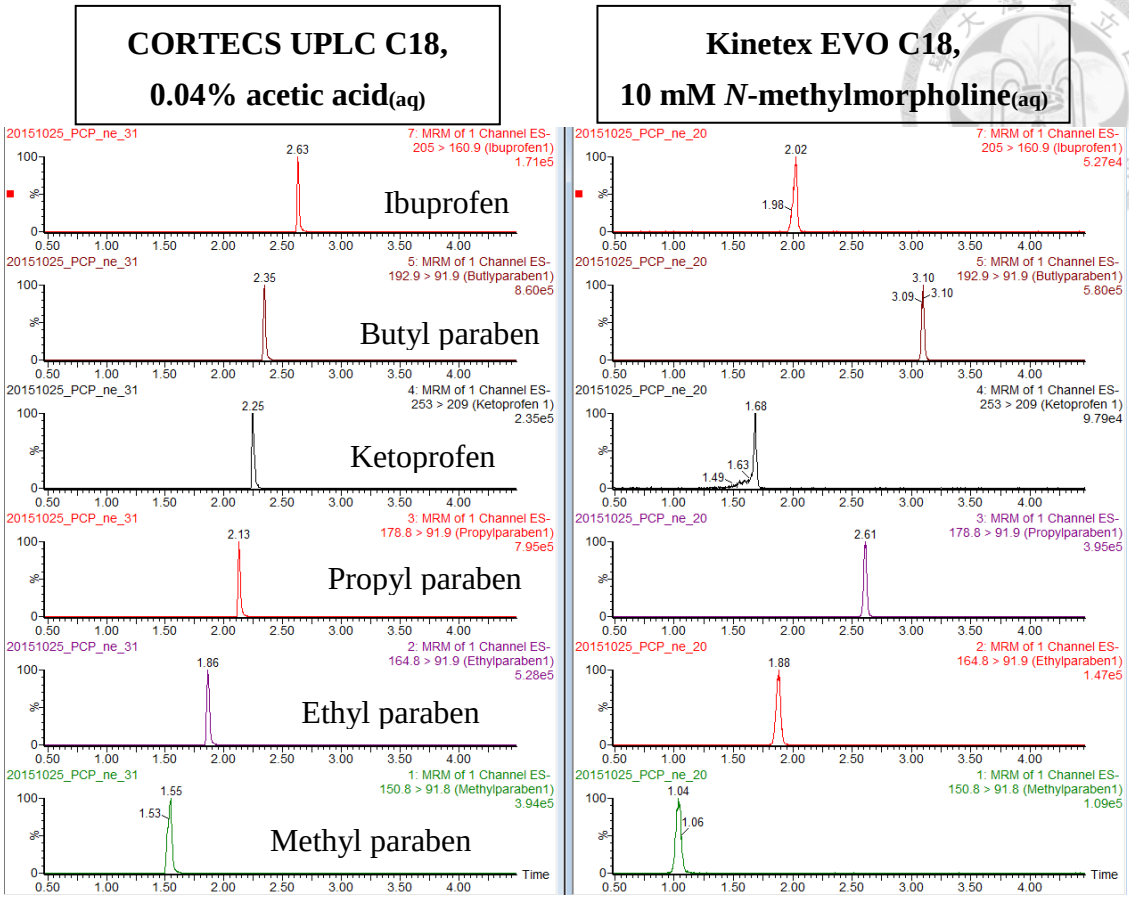

Figure 8. Chromatograms of ESI- with different chromatographic conditions (1 μg/mL in methanol standard solution, injection 4 μL)

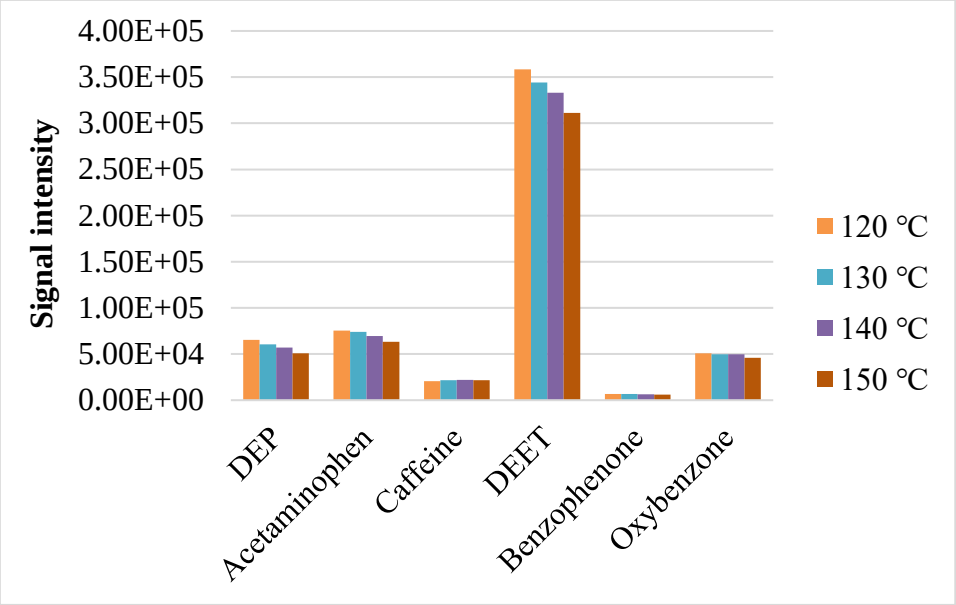

Figure 9. Signal intensities of DEP and basic PCPs with different ionization source temperatures (0.25 μg/mL in methanol standard solution, injection 4 μL, n= 2)

doi:10.6342/NTU201802422

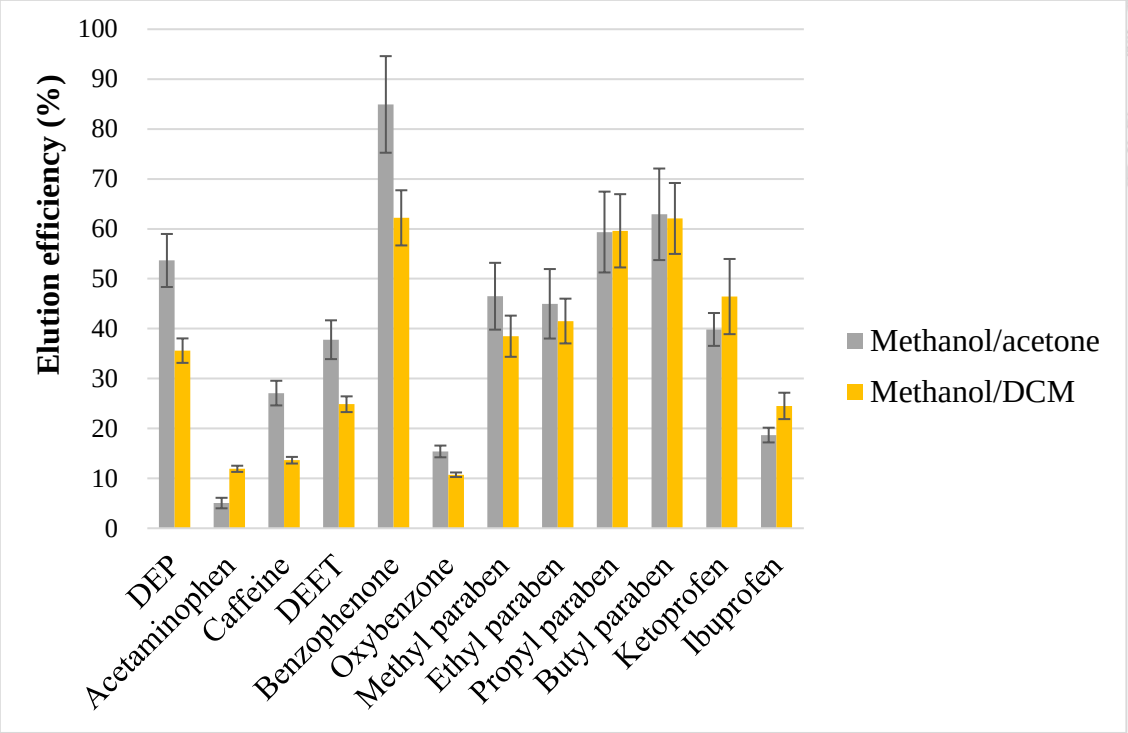

Figure 10. Elution efficiencies (%) of analytes with different combinations of elution solvents on C18 non-endcapped adsorbent (n = 4)

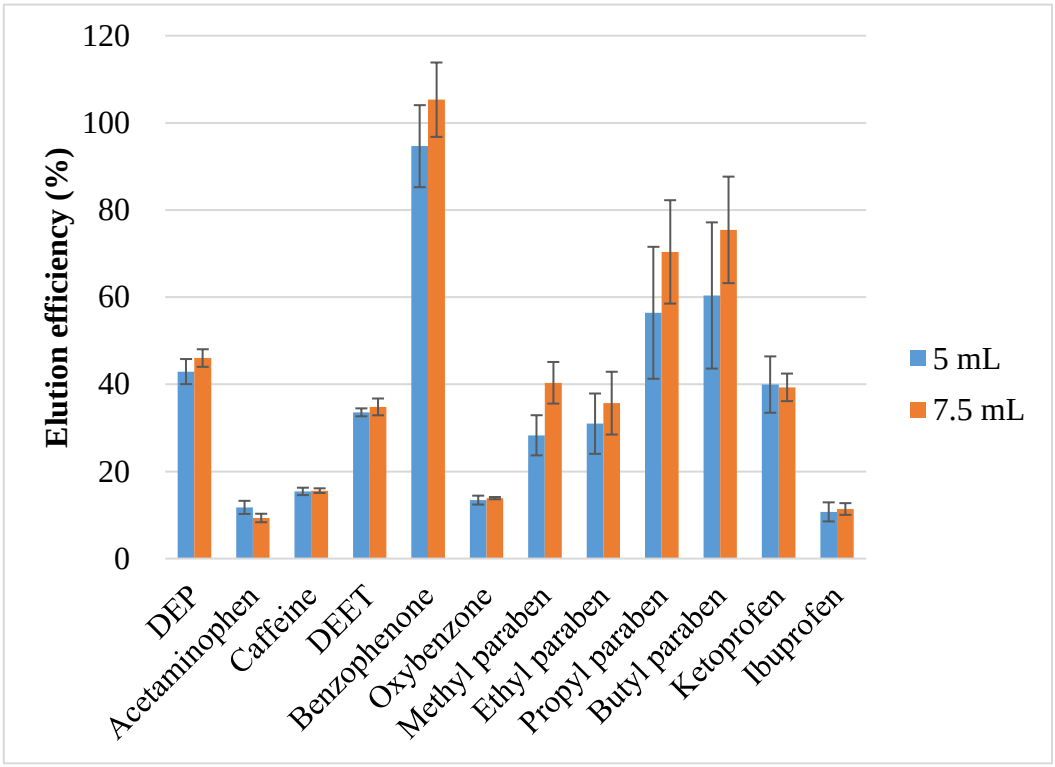

Figure 11. Elution efficiencies (%) of analytes with different elution volumes at each portion (n = 4)

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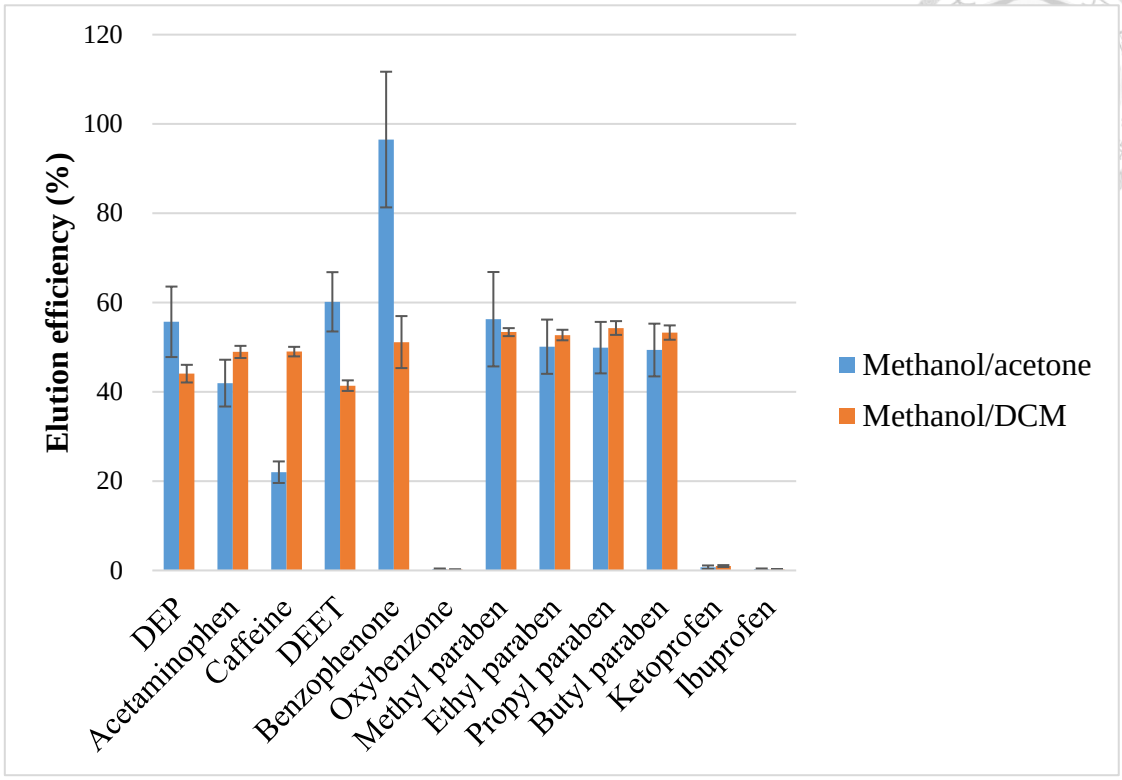

Figure 12. Elution efficiencies (%) of analytes with different combinations of elution solvents on alumina cartridge (n = 4)

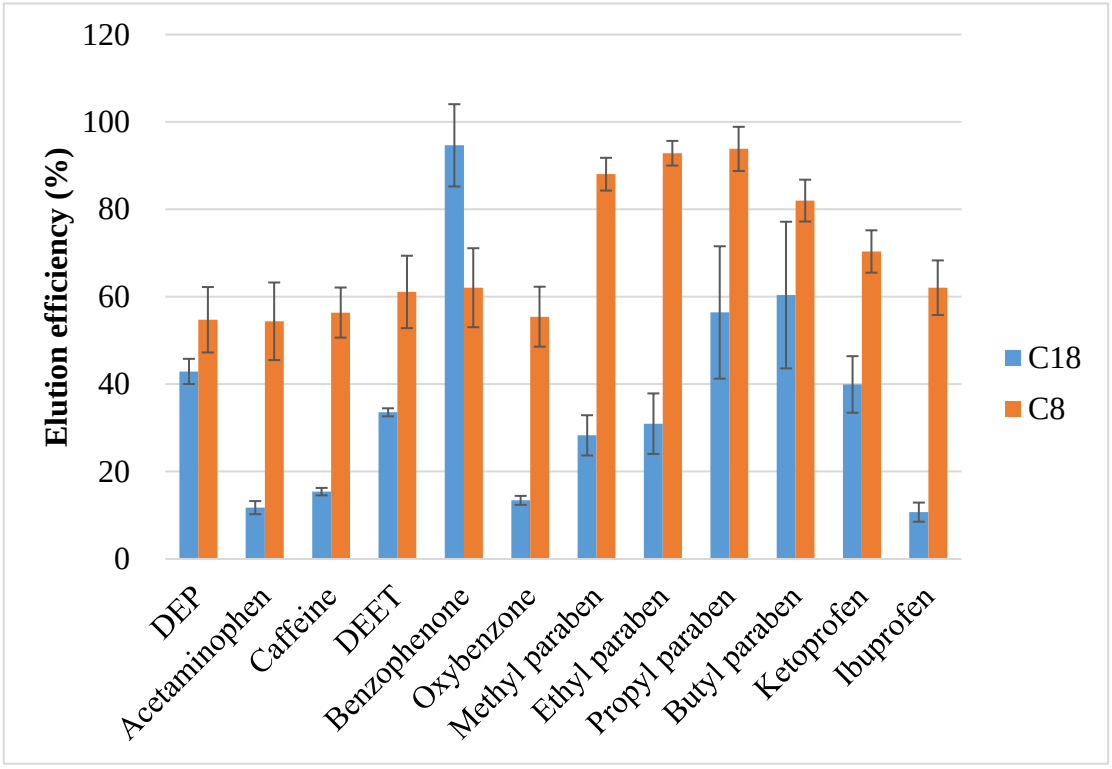

Figure 13. Elution efficiency (%) of analytes from different adsorbents (n = 4)

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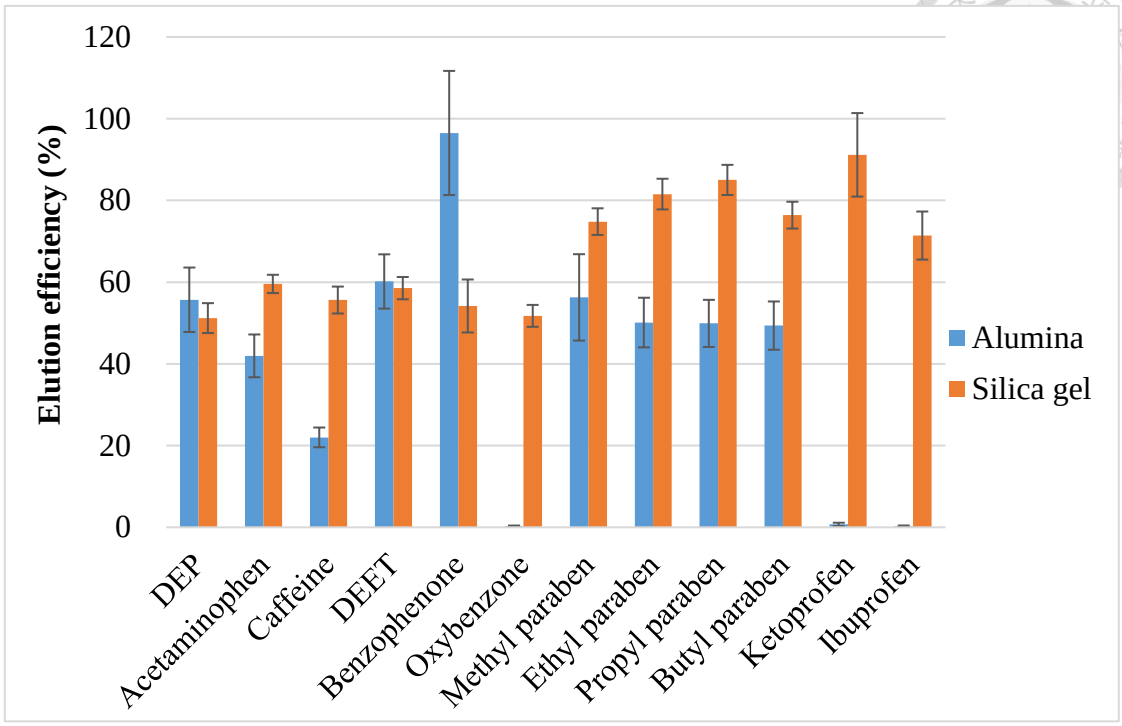

Figure 14. Elution efficiency (%) of analytes from different cleanup adsorbents (n = 4)

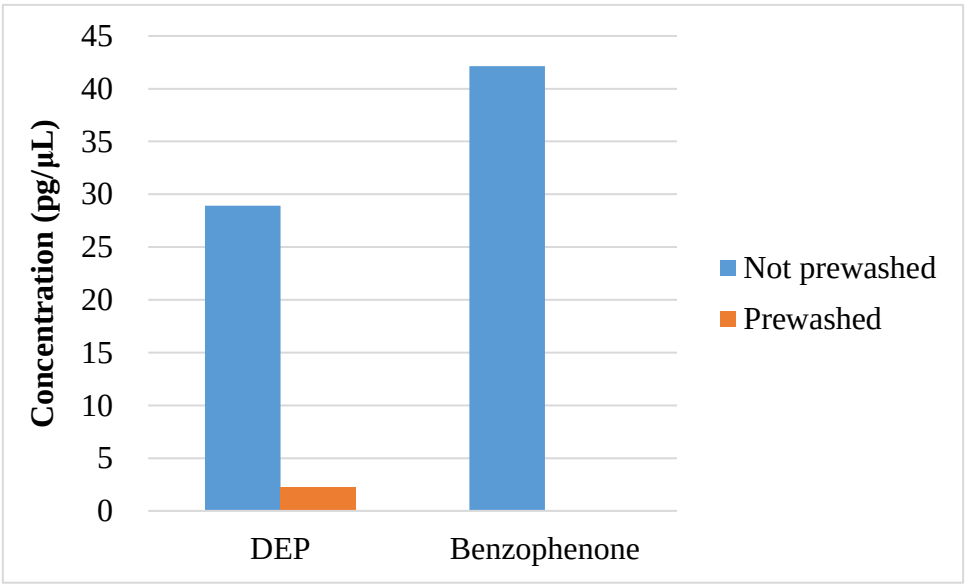

Figure 15. Background levels of DEP and benzophenone using the silica gel with and without the pre-wash step (n = 2)

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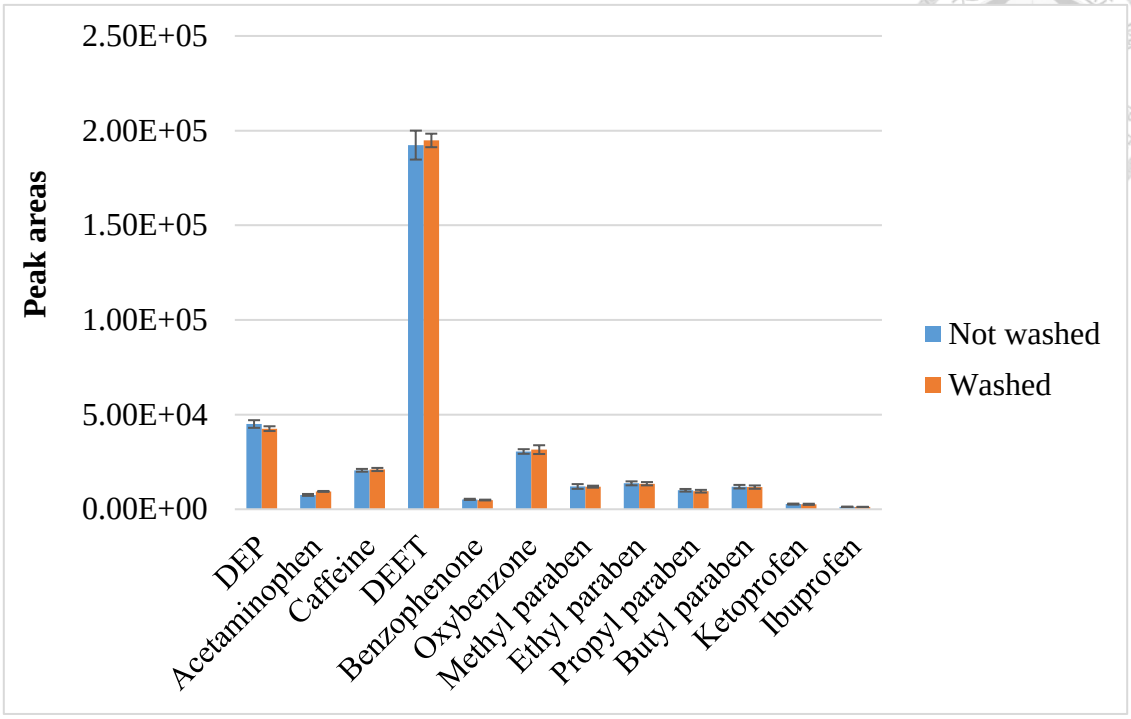

Figure 16. Peak areas of analytes using the silica gel with and without pre-wash step (n = 4)

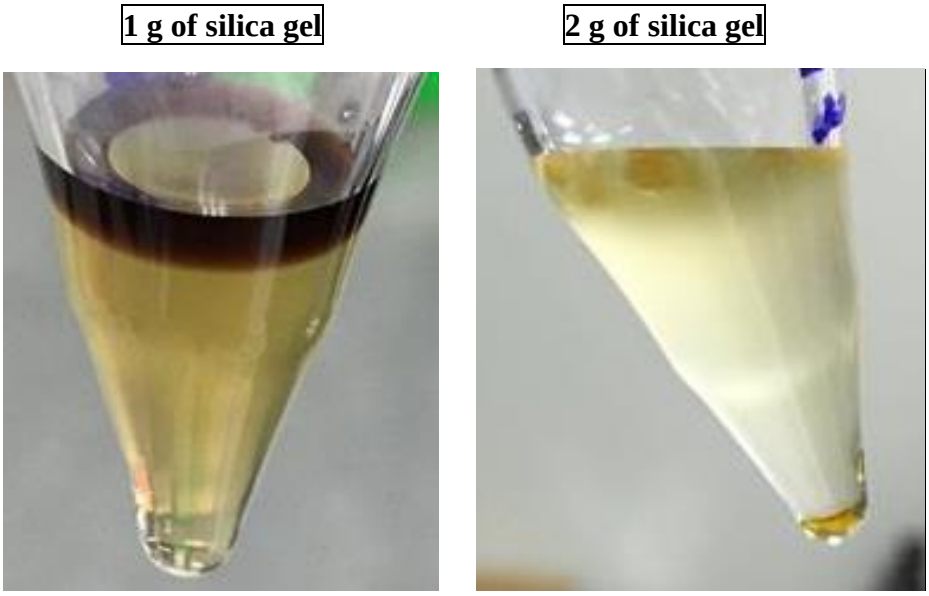

Figure 17. Appearance of eluents of liver samples using different amount of silica gel for cleanup

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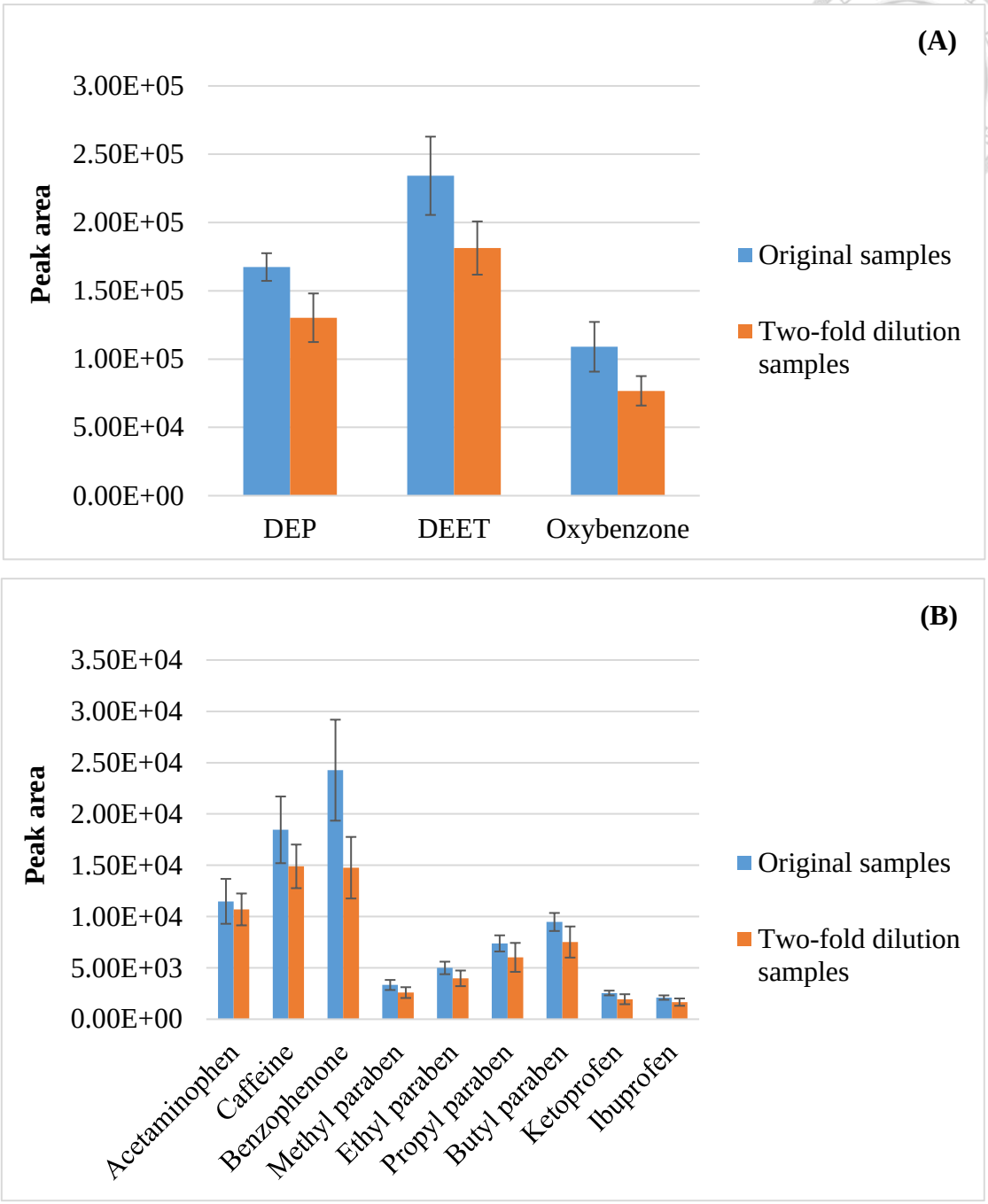

Figure 18. Peak areas of analytes in the 500-μL final residues of liver samples (original samples) and their two-fold dilution samples (n = 4) ((A): the analytes with areas over 50,000; (B): the analytes with areas below 50,000)

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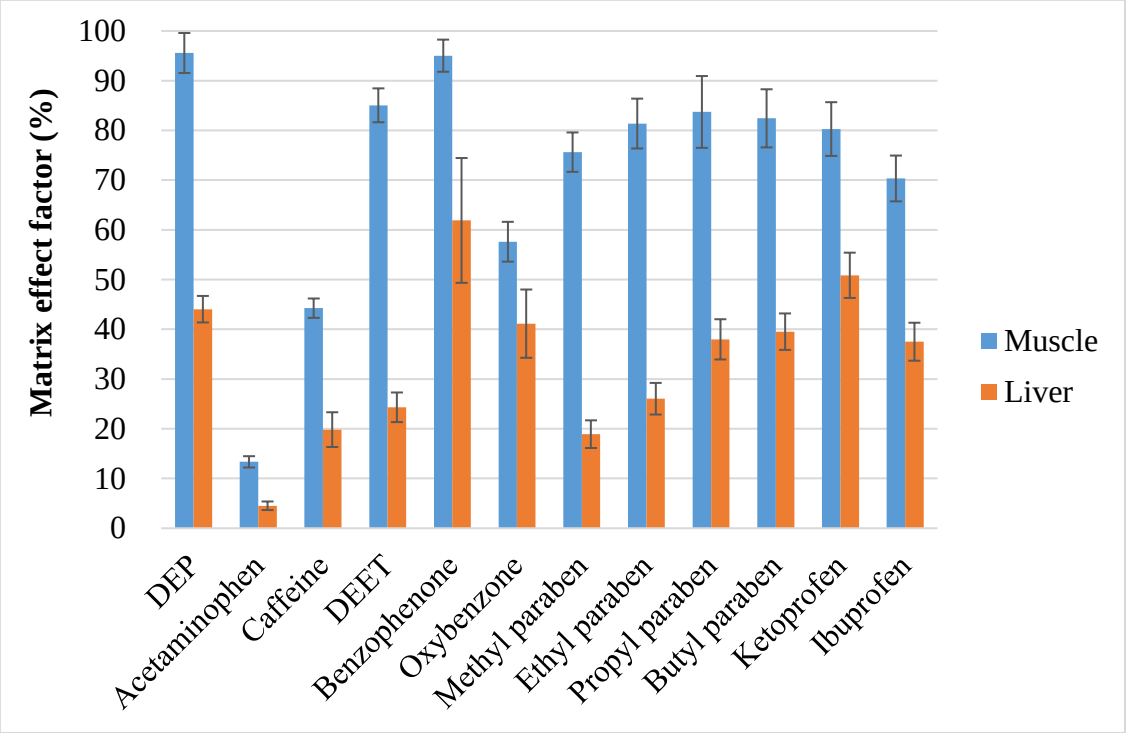

Figure 19. Matrix effect factors (%) of analytes in matrices (n = 4)

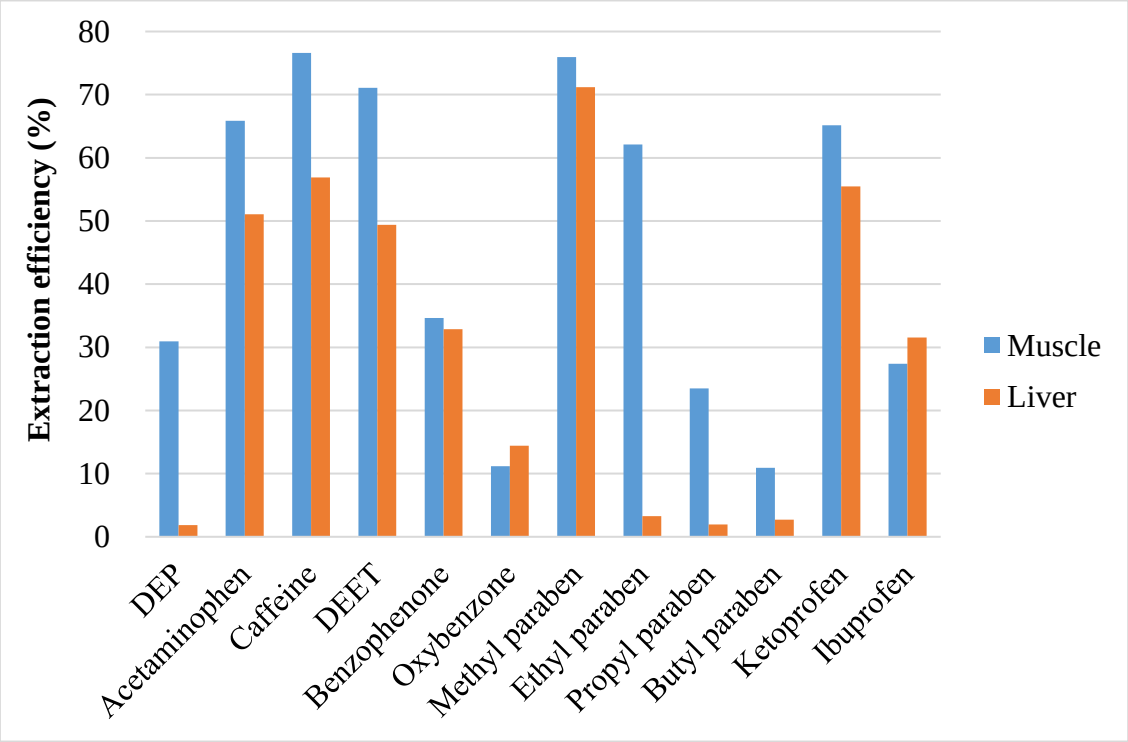

Figure 20. Extraction efficiencies (%) of analytes in matrices (n = 4)

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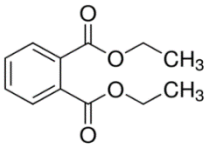
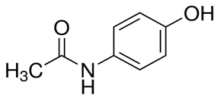
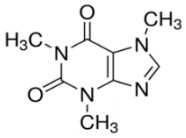
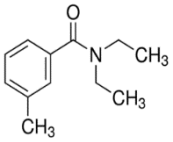
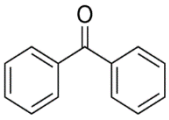
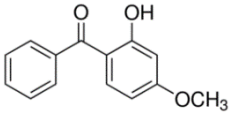
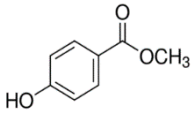
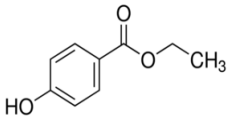
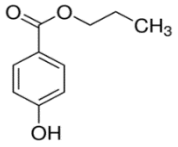
Tables

Table 1. Chemical structures and molecular weights of analytes

Compounds	Molecular weight	Structure
Diethyl phthalate	222.2	
Acetaminophen	151.16	
Caffeine	194.19	
DEET	191.27	
Benzophenone	182.22	
Oxybenzone	228.24	
Methyl paraben	152.15	
Ethyl paraben	166.17	
Propyl paraben	180.2	

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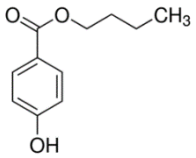
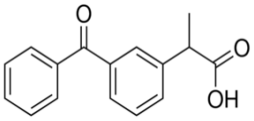
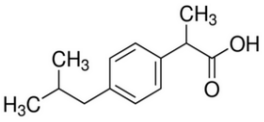
Butyl paraben	194.23	
Ketoprofen	254.28	
Ibuprofen	206.28	

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Table 2. The LC conditions of separating analytes

ESI+			**ESI-**		
Column	Ascentis Express F5 (30 × 2.1 mm, 2 μm)		**Column**	CORTECS UPLC C18 (30 mm × 2.1 mm, 1.6 μm)	
Column temperature (℃)	40		**Column temperature (℃)**	30	
Flow rate (mL/min)	0.65		**Flow rate (mL/min)**	0.5	
Injection volume (μL)	4		**Injection volume (μL)**	4	
Mobile phase / **Gradient (min)**	5 mM ammonium $acetate_{(aq)}$ (pH = 6.40)	MeOH	**Mobile phase** / **Gradient (min)**	0.04% acetic $acid_{(aq)}$ (pH = 3.45)	MeOH
Initial	95	5	Initial	85	15
0.5	95	5	0.5	85	15
4.5	5	95	3.0	0	100
5.5	5	95	3.5	0	100
5.8	95	5	4.0	85	15
7.8	95	5	5.7	85	15

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Table 3. Tandem mass parameters

Compounds	Cone voltages (V)	Precursor ion > product ion I (collision energy, V), product ion II (collision energy, V)
Diethyl phthalate	15	(+) 222.9 > 177.0 (8), 149.1 (17)
ISTD: Diethyl phthalate $^{2}D_{4}$	13	(+) 227.0 > 152.9 (17)
Acetaminophen	27	(+) 151.8 > 109.8 (15), 92.7 (22)
ISTD: Acetaminophen $^{2}D_{4}$	27	(+) 155.8 > 113.8 (15)
Caffeine	35	(+) 195.0 > 137.9 (18), 109.9 (24)
ISTD: Caffeine $^{13}C_{3}$	38	(+) 197.9 > 140.0 (20)
DEET	32	(+) 191.9 > 118.8 (18), 90.8 (30)
ISTD: DEET $^{2}D_{6}$	32	(+) 198.1 > 118.9 (18)
Benzophenone	25	(+) 182.9 > 104.8 (15), 76.9 (27)
ISTD: Benzophenone $^{2}D_{10}$	25	(+) 192.9 > 109.8 (18)
Oxybenzone	33	(+) 229.0 > 150.8 (20), 104.7 (20)
ISTD: Oxybenzone $^{13}C_{6}$	30	(+) 235.1 > 150.9 (18)
Methyl paraben	28	(-) 150.8 > 91.8 (20), 135.7 (15)
ISTD: Methyl paraben $^{13}C_{6}$	27	(-) 156.8 > 97.8 (18)
Ethyl paraben	30	(-) 164.7 > 91.9 (23), 135.9 (15)
ISTD: Methyl paraben $^{13}C_{6}$	27	(-) 156.8 > 97.8 (18)
Propyl paraben	30	(-) 178.8 > 91.9 (20), 135.9 (15)
ISTD: Methyl paraben $^{13}C_{6}$	27	(-) 156.8 > 97.8 (18)
Butyl paraben	35	(-) 192.9 > 91.8 (28), 136.0 (20)
ISTD: Butyl paraben $^{13}C_{6}$	35	(-) 198.9 > 97.8 (28)
Ketoprofen	15	(-) 253.0 > 209.0 (10)
ISTD: Ketoprofen $^{2}D_{3}$	8	(-) 256.0 > 212.0 (7)
Ibuprofen	14	(-) 205.0 > 160.9 (7)
ISTD: Ibuprofen $^{13}C_{3}$	14	(-) 208.0 > 162.9 (7)

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Table 4. Different chromatographic conditions for ESI-

ESI-					
Column	CORTECS UPLC C18 (30 mm × 2.1 mm, 1.6 μm)		**Column**	Kinetex EVO C18 (50 × 2.1 mm, 1.7 μm)	
Column temperature (℃)	30		**Column temperature (℃)**	30	
Flow rate (mL/min)	0.5		**Flow rate (mL/min)**	0.5	
Injection volume (μL)	4.0		**Injection volume (μL)**	4.0	
Mobile phase / **Gradient 1 (min)**	0.04% acetic $acid_{(aq)}$ (pH = 6.40)	MeOH	**Mobile phase** / **Gradient 2 (min)**	10 mM *N*-$methylmorpholine_{(aq)}$ (pH = 9.60)	MeOH
Initial	85	15	Initial	90	10
0.5	85	15	0.5	90	10
3.0	0	100	4.0	10	90
3.5	0	100	5.5	10	90
4.0	85	15	5.8	90	10
6.0	85	15	7.8	90	10
Reference	[65]		**Reference**	[66]	

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Table 5. Matrix effect factors (%) of analytes in matrices (mean ± SD, n = 4)

Compounds	Mucle	Liver
DEP	95.6 ± 4.03	44.1 ± 2.67
Acetaminophen	13.4 ± 1.14	4.52 ± 0.86
Caffeine	44.3 ± 1.95	19.8 ± 3.49
DEET	85.1 ± 3.41	24.3 ± 2.98
Benzophenone	95.0 ± 3.23	61.9 ± 12.6
Oxybenzone	57.6 ± 4.00	41.1 ± 6.87
Methyl paraben	75.6 ± 3.96	18.9 ± 2.78
Ethyl paraben	81.4 ± 5.01	26.0 ± 3.18
Propyl paraben	83.7 ± 7.22	38.0 ± 4.04
Butyl paraben	82.4 ± 5.85	39.5 ± 3.67
Ketoprofen	80.3 ± 5.40	50.9 ± 4.55
Ibuprofen	70.3 ± 4.60	37.5 ± 3.82

Table 6. Extraction efficiencies (%) of analytes in matrices (n = 4)

Compounds	Muscle	Liver
DEP	30.9	1.86
Acetaminophen	65.9	51.1
Caffeine	76.6	56.9
DEET	71.1	49.4
Benzophenone	34.7	32.9
Oxybenzone	11.2	14.4
Methyl paraben	75.9	71.2
Ethyl paraben	62.1	3.29
Propyl paraben	23.5	1.94
Butyl paraben	10.9	2.72
Ketoprofen	65.2	55.5
Ibuprofen	27.4	31.6

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Table 7. IDLs, IQLs, linear ranges and r^2 of calibration curves

Compounds	IDL (pg)	IQL (pg)	Linear range (ng/mL)	r^2
DEP	4.04	10.6	5-4000	0.998
Acetaminophen	8.52	9.57	5-4000	0.998
Caffeine	12.5	22.9	10-4000	0.999
DEET	0.43	1.03	5-4000	0.993
Benzophenone	17.3	37.1	10-4000	0.999
Oxybenzone	0.64	1.95	1-4000	0.999
Methyl paraben	7.63	7.63	5-4000	0.999
Ethyl paraben	2.54	2.54	1-4000	0.999
Propyl paraben	1.29	2.14	5-4000	0.999
Butyl paraben	5.07	5.07	1-4000	0.999
Ketoprofen	47.7	159	50-4000	0.996
Ibuprofen	2.86	9.53	5-4000	0.999

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Table 8. The limits of detection (LODs) and limits of quantification (LOQs) (mean ± SD, ng/g w.w. (ng/g d.w.), n = 4)

	Muscle		Liver	
	LOD	LOQ	LOD	LOQ
DEP	1.40 ± 0.59 (6.51 ± 2.73)	5.52 ± 0.71 (25.7 ± 3.30)	14.8 ± 4.13 (45.4 ± 12.6)	46.2 ± 7.49 (141 ± 22.9)
Acetaminophen	15.0 ± 3.59 (69.9 ± 16.7)	34.9 ± 9.27 (163 ± 43.1)	20.8 ± 10.8 (63.6 ± 32.9)	108 ± 27.9 (331 ± 85.3)
Caffeine	1.22 ± 0.27 (5.68 ± 1.26)	2.18 ± 0.47 (10.1 ± 2.19)	14.7 ± 2.62 (45.0 ± 8.00)	21.1 ± 7.79 (64.6 ± 23.8)
DEET	0.57 ± 0.17 (2.65 ± 0.79)	1.04 ± 0.04 (4.86 ± 0.17)	4.37 ± 1.43 (13.4 ± 4.37)	10.6 ± 3.08 (32.5 ± 9.43)
Benzophenone	5.66 ± 1.45 (26.3 ± 6.75)	9.18 ± 3.23 (42.7 ± 15.0)	104 ± 19.1 (319 ± 58.3)	281 ± 104 (861 ± 318)
Oxybenzone	1.26 ± 0.74 (5.88 ± 3.45)	1.26 ± 0.58 (5.87 ± 2.71)	6.79 ± 1.73 (20.8 ± 5.29)	16.6 ± 5.78 (50.7 ± 17.7)
Methyl paraben	1.24 ± 0.23 (5.79 ± 1.08)	1.57 ± 0.61 (7.29 ± 2.86)	8.18 ± 2.06 (25.0 ± 6.32)	10.6 ± 5.29 (32.4 ± 16.2)
Ethyl paraben	0.85 ± 0.23 (3.94 ± 1.08)	2.58 ± 0.90 (12.0 ± 4.20)	25.2 ± 13.1 (77.0 ± 40.1)	38.4 ± 9.12 (118 ± 27.9)
Propyl paraben	1.90 ± 0.63 (8.84 ± 2.91)	4.91 ± 0.45 (22.8 ± 2.10)	19.3 ± 12.5 (59.0 ± 38.2)	19.5 ± 7.42 (59.7 ± 22.7)
Butyl paraben	1.63 ± 0.36 (7.60 ± 1.67)	3.55 ± 2.28 (16.5 ± 10.6)	43.0 ± 16.1 (132 ± 49.3)	43.0 ± 16.1 (132 ± 49.3)
Ketoprofen	4.48 ± 0.61 (20.8 ± 2.86)	14.9 ± 2.05 (69.4 ± 9.54)	57.2 ± 20.9 (175 ± 64.0)	191 ± 69.7 (584 ± 213)
Ibuprofen	1.20 ± 0.14 (5.57 ± 0.67)	3.99 ± 0.48 (18.6 ± 2.25)	6.81 ± 2.29 (20.8 ± 7.00)	22.7 ± 7.63 (69.4 ± 23.3)

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Table 9. Accuracy and precision in fish muscle and liver (n = 4)

	Muscle						Liver	
Spiked level (ng/g)	**62.5**		**200**		**500**		**1000**	
Compounds	% RSD	% Bias	% RSD	% Bias	% RSD	% Bias	% RSD	% Bias
DEP	16.9%	73.2%	5.69%	-6.43%	3.91%	-25.3%	3.00%	-12.2%
Acetaminophen	2.80%	-7.12%	1.33%	-4.81%	2.31%	-8.70%	6.98%	6.64%
Caffeine	4.30%	-3.88%	2.38%	-0.47%	3.12%	-7.13%	2.82%	4.62%
DEET	3.60%	11.4%	0.86%	11.4%	3.64%	4.80%	1.05%	11.0%
Benzophenone	6.71%	99.8%	4.60%	27.2%	10.7%	0.78%	2.89%	4.98%
Oxybenzone	5.52%	4.75%	5.36%	2.64%	4.82%	-6.29%	6.39%	-13.3%
Methyl paraben	2.38%	33.6%	1.74%	37.1%	2.19%	27.1%	7.94%	1.12%
Ethyl paraben	4.46%	2.20%	2.36%	-5.37%	2.27%	-13.8%	2.34%	-0.46%
Propyl paraben	6.87%	-34.4%	3.13%	-42.4%	3.72%	-34.1%	4.97%	7.20%
Butyl paraben	12.2%	-14.0%	3.84%	-23.9%	2.05%	-27.6%	5.10%	-6.72%
Ketoprofen	4.82%	7.41%	5.22%	3.93%	3.90%	7.77%	14.8%	-21.1%
Ibuprofen	2.75%	-1.47%	1.93%	-1.86%	1.44%	-6.79%	1.84%	-2.18%

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