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A new analytical method to determine non-steroidal anti-inflammatory drugs in surface water using *in situ* derivatization combined with ultrasound-assisted emulsification microextraction followed by gas chromatography–mass spectrometry

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ABSTRACT

Because of the high stability and potential toxic effects of non-steroidal anti-inflammatory drugs (NSAIDs), it is important to closely monitor their concentrations in the environment using a sensitive analytical method. In this study, a simple, rapid, efficient, and sensitive analytical method based on gas chromatography–mass spectrometry (GC–MS) was developed to determine the levels of seven common NSAIDs in various types of surface water. To simplify sample preparation, *in situ* derivatization using methyl chloroformate was combined with ultrasound-assisted emulsification microextraction. For selection and optimization of significant variables, experiments were statistically designed using Plackett–Burman design and central composite design. The resulting optimal conditions for derivatization and extraction were 100 μL of chloroform (extraction solvent), 10.0 mL of sample, and 240 μL of pyridine (catalyst as a base in derivatization). The optimized sample preparation coupled with optimized GC–MS analysis in selected ion monitoring mode provided good linearity from 0.010 to 5.0 ng mL^{-1}, and a limit of detection between 0.0050 and 0.010 ng mL^{-1}, good intra-day and inter-day precision (0.30–6.3% and 5.1–9.5%, respectively), and good accuracy (relative recovery; 91–117% at 0.20 ng mL^{-1} and 77–105% at 2.5 ng mL^{-1}). Compared with previously reported methods, the current method requires a small volume of sample and simple sample preparation steps for sensitive determination of NSAID levels using a conventional GC–MS system. The method was successfully applied to determine the levels of seven common NSAIDs in various types of surface water.

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1. Introduction

Widespread use of certain pharmaceuticals has resulted in their continuous release into the environment through various routes including excreta and improper disposal of unused drugs. The presence of pharmaceutical residues in the environment, especially in aquatic systems, is therefore an emerging concern. Non-steroidal anti-inflammatory drugs (NSAIDs), which are used in human and veterinary medicine for their analgesic, antipyretic, and anti-inflammatory effects due to inhibition of cyclooxygenases such as COX-1 and COX-2 in the prostaglandin formation pathway [1,2], are one of the most commonly used pharmaceutical products. With pK_a values generally ranging between 3.0 and 5.0, these acidic compounds are not extensively degraded and are often stable in water after excretion or disposal [3]. Because of their potential toxic effects [4–6] it is important to closely monitor their concentrations in the environment using a sensitive analytical method. A number of studies have reported detection and determination of levels of NSAID residues in various aquatic samples including surface water, ground water, and drinking water.

Many different analytical instruments have been used for the quantitation of NSAIDs [7]. Liquid chromatography (LC) has been combined with various detection methods including fluorescence detection (FL) [8], ultraviolet detection (UV) [3,8–10], mass spectrometry (MS) [10,11], and tandem mass spectrometry (MS–MS) [9,12]. Gas chromatography (GC) coupled to MS [13–15] and MS–MS [16] are also widely used as sensitive and selective methods. However, NSAIDs need to be derivatized prior to GC analysis to obtain analytes with increased volatility and selectivity, along with enhanced detectability [17]. This is achieved using various derivatization methods including silylation [4,13,14], alkylation [18,19], and acylation [20]. Use of alkyl chloroformate (methyl, ethyl, propyl,

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and isobutyl chloroformate) is beneficial for the determination of acidic NSAIDs because chemical reactions between active functional groups (primary amines or carboxylic acids) and alkyl chloroformate can occur at room temperature and be completed within a few minutes [17,21]. Moreover, alkylation can progress in aqueous conditions, and thus can be applied directly to aqueous samples [17,21,22].

A variety of extraction methods have been applied to increase the concentration of NSAIDs existing at very low levels in environmental samples prior to the derivatization. Solid phase extraction (SPE) [16,23,24] is widely used for environmental samples of large volumes. Despite the use of smaller volumes of potentially toxic solvents in SPE than liquid–liquid extraction (LLE), SPE still requires addition of a considerable amount of organic solvents; plus, disposable cartridges or discs with a special manifold are required. Recently, microextraction-based methods, which are considered relatively green, have been employed, including solid phase microextraction (SPME) [3,25], single-drop microextraction (SDME) [26], liquid–liquid–liquid microextraction (LLLME) [27], hollow-fiber liquid-phase microextraction (HF-LPME) [8,28,29]. Since its introduction in 2006 [30], dispersive liquid–liquid microextraction (DLLME) has been extensively employed as a simple and effective method for the extraction and pre-concentration of a wide variety of compounds from aqueous samples [31]. Modified DLLME techniques have also recently been developed, including ultrasound-assisted emulsification microextraction (USAEME). In USAEME, only a small volume of extraction solvent is used without any water-miscible organic solvents that are needed as dispersers in DLLME, because the extraction solvent is emulsified by use of ultrasound waves that boost mass-transfer from the aqueous phase to the organic phase by promoting the formation of a large surface area [32]. Therefore, USAEME has been suggested as an alternative to DLLME in the analysis of various target analytes in aqueous samples [21,33–36].

There is a growing interest in coupling derivatization methods with microextraction [21,37,38]. In this study, a new analytical method to determine the concentrations of seven acidic NSAIDs prevalent in surface water was developed by combining *in situ* methyl chloroformate derivatization with USAEME followed by GC–MS. Important variables were screened and optimized by experimental design including Plackett–Burman design (PBD) [39–41] and central composite design (CCD) [42,43] instead of the traditional "one-variable-at-a-time (OVAT)" approach because the experimental design allows a depiction of the interactive effects among variables using less time, expenses, and reagents [44,45]. As a result, a convenient, simple, yet efficient analytical method with high sensitivity was established and successfully applied to real surface water samples.

2. Materials and methods

2.1. Chemicals, apparatus, and sample collection

Analytical standards including ibuprofen sodium (IBU), naproxen sodium (NAP), ketoprofen (KET), diclofenac sodium (DIC), indomethacin (IND), and 2-naphthoic acid (2-NPA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Flurbiprofen (FLUR) and etodolac (ETO) were obtained from TCI (Tokyo, Japan). Structure, molecular weight, and pK_a values of these target compounds are shown in Table 1. Pyridine, methyl chloroformate (MCF), ethyl chloroformate (ECF), *i*-butyl chloroformate (*i*BuCF), chloroform, dichloroethane, tetrachloroethane, toluene, diethyl ether, ethyl acetate, and cyclohexane were also purchased from Sigma-Aldrich. Dichloromethane was of HPLC-grade from Burdick & Jackson (Muskegon, MI, USA). HPLC-grade methanol (MeOH) was purchased from Duksan (Ansan, Korea). All other chemicals were analytical-grade reagent or higher.

Doubly distilled water was obtained using a Milli-Q water purification system from Millipore (Bedford, MA, USA). A centrifuge (model 1580MGR) from Gyrozen (Incheon, Korea) was used for phase separation. An ultrasonic bath (PowerSonic 410, Hwashin Technology, Yeongcheon, Korea) was used for emulsification during extraction. Volume of the sedimented phase was measured using a 100 μL syringe (Hamilton Company, Reno, NV, USA).

Standard stock solutions were prepared in MeOH by dissolving each NSAID to obtain a final concentration of 10 mg mL^{-1} except for IND (5.0 mg mL^{-1}). Standard working solutions were diluted serially with MeOH from stock solutions. All standard stock and working solutions were stored at −20 °C and 4 °C, respectively.

Surface water samples were collected from several locations near our laboratory in Suwon, Korea and the collected volume for each sample was about 500 mL. Tap water was collected from our laboratory. Pond water was collected from a pond within the Sungkyunkwan University campus; this pond is filled with effluent water from a Sungkyunkwan University wastewater treatment plant. Reservoir water was collected from Ilwol reservoir, which is located close to a residential area near from Sungkyunkwan University.

2.2. GC–MS conditions

Derivatized compounds were analyzed using a Hewlett–Packard (HP) GC system 6890 Series equipped with a 5973 Mass Selective Detector (MSD) system. The system was controlled by the Enhanced ChemStation Version B.01.00 program. The capillary column used for GC was an Agilent J&W HP-5MS UI (30.0 m × 0.25 mm i.d., 0.25 μm film thickness coated 5% diphenyl/95% dimethylpolysiloxane). Helium (purity 99.999%) was used as a carrier gas at a constant flow rate of 1.0 mL min^{-1} and injections were made in split mode with the ratio set to 10:1. GC oven temperature programming was as follows: initial temperature was held at 150 °C for 3 min, increased to 230 °C at the rate of 20 °C min^{-1}, held for 5 min, and then further increased to 280 °C at the rate of 25 °C min^{-1}, where it was held for 6 min. Total chromatographic time was 20 min. Inlet temperature, ion source, and MS transfer line temperature were adjusted to 260 °C, 230 °C, and 280 °C, respectively. Ionization was performed in electron ionization (EI) mode at 70 eV. All injected samples were operated in selected ion monitoring (SIM) mode for quantitation. Retention times, major fragment ions, and quantification ions of the analytes are displayed in Table 2.

2.3. Optimized in situ derivatization coupled to USAEME conditions

The collected samples were filtered through filter papers (Hyundai Micro, Seoul, Korea), followed by 0.45 μm-pore-size membrane filters (Millipore, Tullagreen, Ireland) to remove particulate matter. The samples were then stored in 250 mL DURAN laboratory bottles with a cap at 4 °C until analysis. A 10-mL aliquot of filtered water sample was spiked with 2-NPA (internal standard, IS) at 0.50 ng mL^{-1} in a 15-mL screw cap glass test tube with a conical bottom. A mixture of 400 μL of MeOH and 240 μL of pyridine was added to the aqueous sample solution using a 1,000 μL micropipette. After briefly vortexing the sample, a mixture of 100 μL of chloroform (extraction solvent) and 100 μL MCF (derivatization reagent) was added to the glass test tube, which was then vortexed for 30 s. The tube was immersed in an ultrasonic bath, and derivatization and extraction were performed using ultrasonic radiation at an ultrasonic frequency of 40 kHz and power of 500 W for 3 min at room temperature. During this process, the clear aqueous sample became cloudy because of dispersion of fine droplets of chloroform throughout the sample. The cloudy solution was then centrifuged at 2,898 × *g* for 5 min. The sedimented organic phase, which had an

Table 1
Structures, molecular weights, and pK_a values of the target NSAIDs.

Analyte	Structure	MW (g/mol)	pK_a	Classification
IBU		206.3	4.91	Propionic acid derivatives
FLUR		244.3	4.22	Propionic acid derivatives
NAP		252.2	4.15	Propionic acid derivatives
KET		254.3	4.45	Propionic acid derivatives
DIC		296.2	4.15	Acetic acid derivatives
ETO		287.4	4.65	Acetic acid derivatives
IND		357.8	4.50	Acetic acid derivatives
2-NPA		172.2	4.17	IS

Table 2
Retention times and mass fragmentation results of the target NSAIDs.

Analyte	Retention time (min)	Major fragment ions (*m*/*z*)	Quantification ion (*m*/*z*)
IBU	5.30	161, 177, 220, 117	161
2-NPA (IS)	6.16	155, 127, 186	155
FLUR	8.07	199, 258, 178, 183	199
NAP	8.86	185, 244, 141, 170	185
KET	10.05	209, 105, 268, 77	209
DIC	11.17	214, 242, 309, 179	214
ETO	11.72	228, 272, 301, 198	228
IND	18.38	139, 371, 111, 312	139

average volume of 18.8 ± 1.3 μL, was removed using a 100 μL Hamilton syringe and transferred to a 250 μL glass insert equipped with a 2.5 mL GC vial. One microliter of the extracted phase was injected into the GC–MS system. A schematic overview of the experiment is provided in Fig. 1.

2.4. Software for response surface methodology

Experimental design was performed using Design-Expert 8 (Stat-Ease Inc., Minneapolis, MN, USA). The total sum of peak areas of all target analytes was used as the response.

3. Results and discussion

3.1. Selection and optimization of critical, qualitative variables

To establish an efficient sample preparation method combining *in situ* derivatization using alkyl chloroformate with LPME, a large number of variables that could affect derivatization and/or micro-extraction efficiencies had to be optimized. Several critical variables including derivatization reagent and organic solvent for derivative extraction, are qualitative variables that assume discrete levels and these variables were optimized before applying response surface methodology (RSM) to optimize quantitative variables for further experimental design as usually performed [46]. First, the most appropriate derivatization reagent among alkyl chloroformates was screened. According to the known mechanisms of derivatization using alkyl chloroformate, the carboxylic acid group of NSAIDs can react with various kinds of alkyl chloroformate [47]. In this work, reaction efficiency was compared among three reagents, namely MCF, ECF, and *i*BuCF, using the proper alcohol to dissolve the NSAIDs, *i.e.*, MeOH, ethanol, and *i*-butanol for derivatization with MCF, ECF, and *i*BuCF, respectively, to prevent the formation of different kinds of alkyl derivative by-products. To compare derivatization yields, derivatized products were extracted using a conventional LLE method. MCF yielded the largest peak areas for all tested NSAIDs (Fig. 2, Supplementary

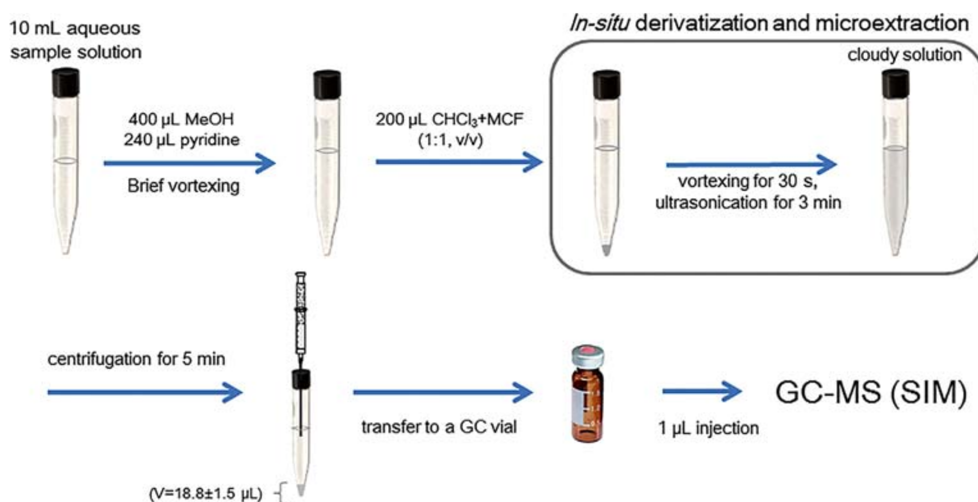

Fig. 1. A schematic of the overall experimental procedure.

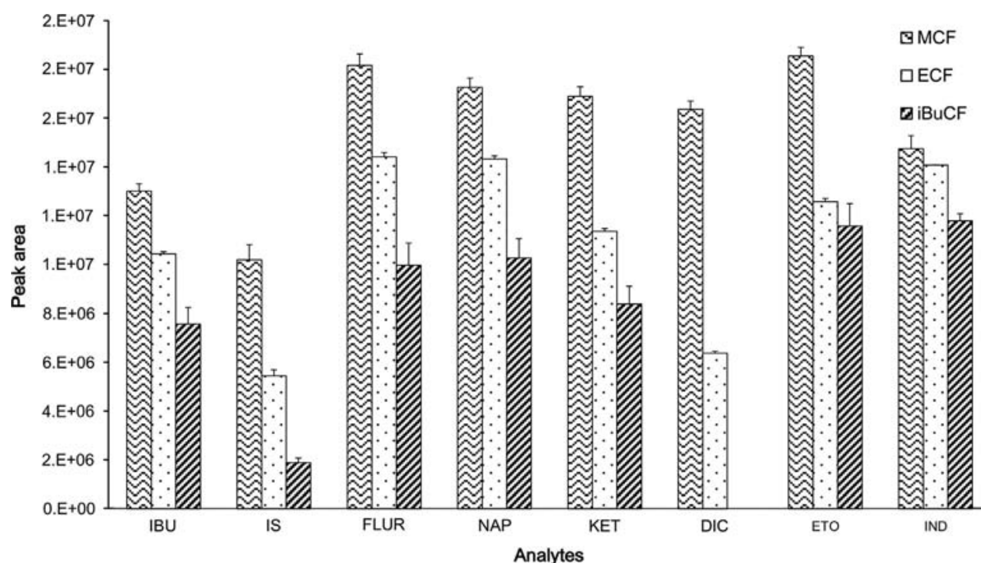

Fig. 2. Selection of type of derivatization reaction ($n=3$). Experimental conditions: sample volume, 100 μL; analyte concentration, 1.0 μg mL^{-1}; alcohol volume, 60 μL; pyridine volume, 20 μL; volume of extraction solvent (chloroform), 100 μL; volume of alkyl chloroformate, 20 μL. After the derivatization, chloroform was added and the sample was vortexed for 1 min for extraction.

Fig. S1). Accordingly, MCF was selected as the *in situ* derivatization reagent.

Because samples derivatized using MCF must be extracted into organic phase before GC–MS analysis, the next was to investigate the most appropriate organic solvent for extraction of derivatives. Some properties including water solubility, density, extraction capacity, and chromatographic compatibility [35] were taken into consideration, resulting in evaluation of the following solvents: toluene and cyclohexane (lighter than water); dichloromethane, chloroform, dichloroethane, and tetrachloroethane (heavier than water). To compare extraction yields, the derivatized products were extracted using the USAEME method and the used conditions were roughly selected based on our preliminary experiments. As shown in Fig. 3, chloroform exhibited the highest extraction efficiency among the tested solvents. Therefore, chloroform was chosen as the extraction solvent for subsequent experiments.

Besides the variables above, some minor qualitative variables were also considered. Chemical derivatization using MCF in the presence of methanol and pyridine in aqueous solution yield the transformation of the carboxylic acids of NSAIDs into esters and could be affected by the reaction pH of the aqueous solution [48–50]. Thus, the effect of derivatization pH was investigated by varying the pH from 3 to 11. As expected by the reaction chemistry, the derivatization yield of all NSAIDs was significantly reduced in acidic conditions (pH 3 and 5) while similar yields were achieved in neutral to basic conditions except for IND, which exhibited the best yield at pH 7 (Supplementary Fig. S2). Because the pH of all tested samples was close to 7, we chose to perform derivatization without any pH adjustment thereafter.

When performing USAEME, the extraction solvent can be emulsified by ultrasonic radiation without the use of a dispersive solvent. Nonetheless, vortexing or incorporation of air can assist dispersion of the extraction solvent, which is referred to as vortex-assisted liquid–liquid microextraction (VALLME) [51] and air-assisted liquid–liquid microextraction (AALLME) [52], respectively. In this study, the effect of vortexing on the extraction efficiency of USAEME was investigated (Supplementary Fig. S3). Though the power and time of ultrasonic radiation are quantitative variables, implying that they could be included for experimental design, their optimal conditions were selected beforehand in order to have the number of variables to be screened adequate for PBD. As a result, it was determined that vortexing for 30 s followed by ultrasonic radiation at 500 W for

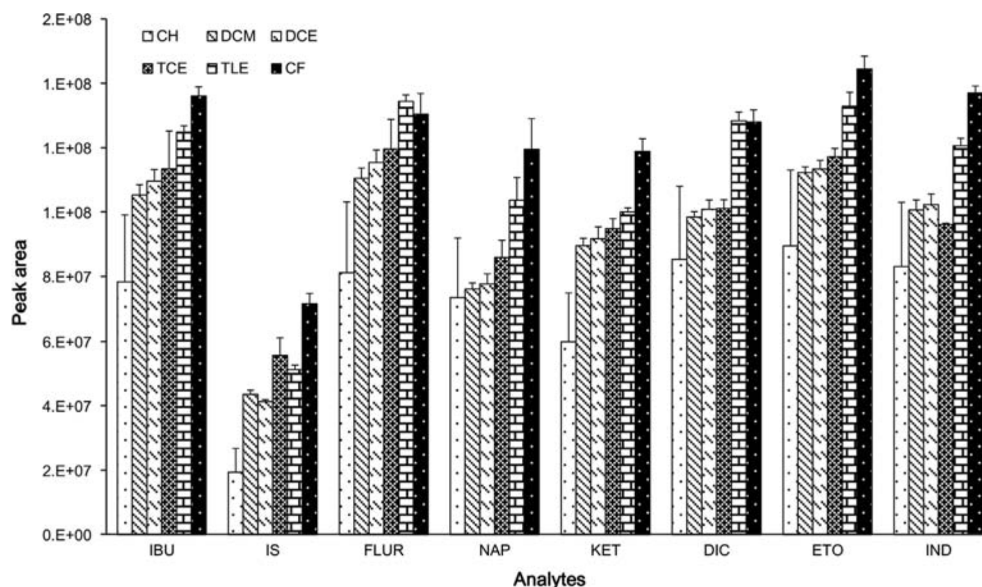

Fig. 3. Selection of extraction solvent for extraction of NSAID derivatives ($n=3$). CH, cyclohexane; DCM, dichloromethane; DCE, dichloroethane; TCE, tetrachloroethane; TLE, toluene; CF, chloroform. Experimental conditions: sample volume, 5.0 mL; analyte concentration, 1.0 μg mL^{-1}; volume of MeOH, 400 μL; volume of pyridine, 100 μL; volume of extraction solvent, 100 μL; volume of MCF, 100 μL; ultrasonic radiation time, 90 s; ultrasonic radiation power, 500 W.

3 min was optimal for emulsification and these conditions were used in subsequent experiments (Supplementary Figs. S4 and S5).

3.2. *Experimental design for screening and optimization of significant variables*

3.2.1. *Screening of the most significant variables using PBD*

Experimental design was adopted for screening and optimization of significant variables. A first-order design based on PBD was applied to determine the most significant variables affecting the response, because PBD allows fast screening with mathematically computed significance of a large number of variables [39,41]. All analytes exhibited similar peak areas at the same concentrations (please see Fig. 4b and c) and were similarly affected by variable changes (please see Supplementary Figs. S3, S4, and S5 for example). Accordingly, the sum of all NSAID peak areas was input as response for modelling. Seven quantitative variables that were likely to affect derivatization and/or extraction efficiencies were tested at two levels as follows: (1) volume of MCF, 100 and 300 μL; (2) volume of pyridine (catalyst as a base in derivatization), 50 and 500 μL; (3) volume of MeOH (catalyst and probable disperser of chloroform), 50 and 500 μL; (4) extraction temperature, 10 and 50 °C; (5) volume of chloroform (extraction solvent), 100 and 300 μL; (6) sample volume, 5 and 10 mL; (7) ionic strength (NaCl concentration), 0 and 20% w/v. PBD produced 12 experimental runs consisting of eight design points and four central points and the run order was randomized to nullify the effects of extraneous or nuisance variables.

The *t*-values of tested factors from the statistical analysis were plotted in a Pareto chart, displaying two limit lines, Bonferroni limit line and *t* limit line (Supplementary Fig. S6). Bonferroni correction conservatively adjusts the standard *t*-value by lowering the alpha value with the number of comparisons (α/n). Accordingly, variables with *t*-value above the Bonferroni limit line are regarded almost certainly significant, while variables with *t*-value between Bonferroni limit and *t* limit line are termed as possibly significant variables [53,54]. The volume of extraction solvent was the most significant variable above the Bonferroni limit with a negative effect, which means that responses decreased with increased factor levels. Sample volume, pyridine volume, and MCF volume were probably significant variables ranging between the *t* limit and Bonferroni limit. The MCF volume that was just above the *t*-value limit was excluded for simple experimental design. Accordingly, the three variables of chloroform volume, sample volume, and pyridine volume were employed for the optimization procedure below. For optimization, the levels of the other variables were fixed based on the results of the PBD and preliminary experiments as follows: MCF volume, 100 μL; extraction temperature, room temperature; MeOH volume, 400 μL; NaCl concentration, 0% w/v.

3.2.2. *Optimization of the selected variables using CCD*

In the next step, the three selected variables were optimized using a second-order design. In this study, the CCD was employed and this is one of the most widely used RSM designs for optimizing analysis conditions [44]. The number of experimental trials, orders, and levels are summarized in Supplementary Table S2. The quality of fit of a second-order polynomial model was evaluated by the coefficient of determination (R^2), which was higher than 0.93. *P*-values for all three variables were less than 0.05 by analysis of variance (ANOVA), indicating that the variables assessed by CCD were statistically significant model terms at the 95% confidence level. The resulting model was expressed as a second-order polynomial quadratic equation of the response (sum of target peak areas):

$$\text{Peak area} = 6.910E^{008} - 4.891E^{008} \times A + 5.639E^{008} \times B - 1.976E^{008} \times C - 3.996E^{008} \times A \times B + 1.857E^{008} \times A \times C - 2.010E^{008} \times B \times C + 1.857E^{008} \times A^2 + 1215E^{008} \times B^2 + 5.331E^{007} \times C^2$$

in which the terms, *A*, *B*, and *C* were coded factors for the volumes of extraction solvent (μL), sample (mL), and pyridine (μL), respectively.

In response surface plots relating the response to the variables (Supplementary Fig. S7), the response was found to increase with decreased extractant volume and pyridine volume, and this trend was previously observed as negative effects in PBD-based screening, whereas the response increased with higher sample volume. A lower extraction solvent volume resulted in elevated concentrations of analytes in the sedimented phase and therefore increased peak areas, while a larger sample volume at the same analyte concentration likely resulted in movement of more analytes to the sedimented phase. In the case of pyridine, which can affect derivatization and/or extraction efficiency, the volume of the

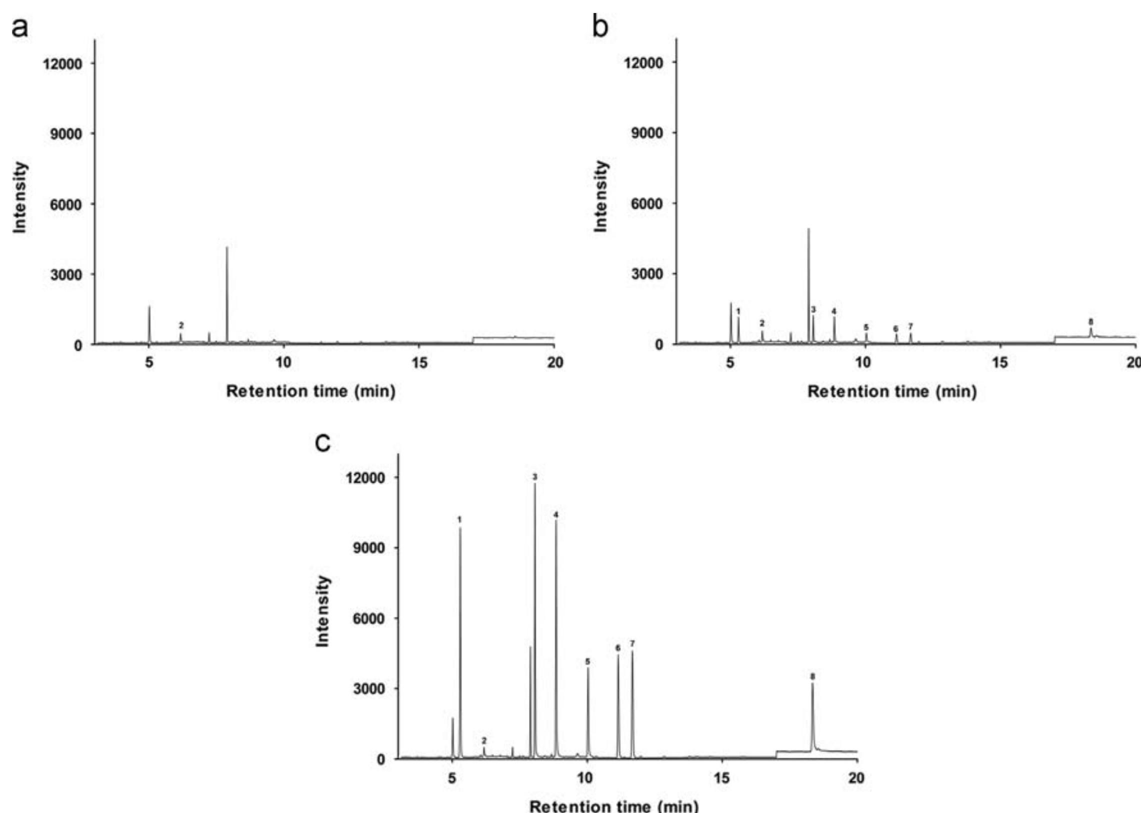

Fig. 4. Chromatograms of pond water samples (a) unspiked; (b) spiked at 0.20 ng mL^{-1}; (c) spiked at 2.5 ng mL^{-1}) analyzed by GC–MS in SIM mode. Peak identification: (1) IBU; (2) IS; (3) FLUR; (4) NAP; (5) KET; (6) DIC; (7) ETO; (8) IND. Experimental conditions are described in Section 2.

organic phase was found to increase with a larger volume of pyridine. This implies the pyridine negatively affected extraction efficiency probably by diluting the extracted phase.

Using the model above, the optimal conditions for the three variables were a chloroform volume of 99.20 µL, sample volume of 10.45 mL, and pyridine volume of 233.9 µL. However, for convenience, the actual values for the final optimal conditions were adjusted as follows: volume of extraction solvent, 100 µL; sample volume, 10.0 mL; volume of pyridine, 240 µL. The results reproduced using the final optimal conditions were fairly close to those predicted from the model.

3.3. *Validation of the established method and application to real surface water samples*

The optimized sample preparation method was applied to standard working solutions. NSAIDs simultaneously derivatized and extracted were analyzed by the GC–MS in SIM mode optimized for sensitive and selective detection. The established method was validated in terms of linearity, limit of detection (LOD), limit of quantification (LOQ), and intra- and inter-day precisions; results are summarized in Table 3. All validation parameters were calculated using the peak area ratio of the target analyte to the IS. Linear range was 0.010–5.0 ng mL^{-1} for IBU, FLUR, and NAP, and 0.020–5.0 ng mL^{-1} for KET, DIC, ETO, and IND, with the correlation coefficients (r^2) higher than 0.999 for all analytes, and ANOVA assured no significant lack of fit to the regression equations. LOD, which was estimated as the analyte concentration at which the signal-to-noise ratio (S/N) was at least 3, ranged between 0.0050 and 0.010 ng mL^{-1}. Analyte concentration at which the S/N was larger than 10 and the RSD% was no larger than 15% was determined as the LOQ, and was found to range from 0.010–0.020 ng mL^{-1} and included as the lowest concentration of the linear curve. Intra-day precision was calculated by analyzing standard working solutions at three different concentration levels within a single day. Inter-day precision was assessed by taking measurements on three successive days. Intra- and inter-day precision values (expressed as RSD%) ranged from 0.30–6.3% and 5.1–9.5%, respectively.

The accuracy of the developed method was also evaluated using several types of real surface water samples, including tap water, pond water, and reservoir water. Accuracy was estimated as relative recovery (RR%) in samples spiked with standards at two different concentrations (0.20 and 2.5 ng mL^{-1}) using the following equation: RR%=[(concentration of the spiked sample−concentration of unspiked sample)/added concentration] × 100 [38]. As shown in Fig. 4 and Table 4, no NSAIDs were detected in the unspiked samples and the ranges of measured relative recovery values were reasonable: 77–109%, 87–117%, and 99–109% for tap water, pond water, and reservoir water, respectively.

A large number of analytical methods including those based on microextraction have been developed to determine the concentrations of NSAIDs in environmental water samples (Supplementary Table S1). Various sample preparation methods such as conventional SPE, SPME, and the newer LPME methods have been coupled to the chromatographic separation by LC or GC with UV, FL, MS, or MS–MS detection. SPE–GC–MS methods reported in [15,19,20] yielded similar LOD values and linear ranges to those measured in the current study. However, these methods usually required a large sample volume of between 500 and 1000 mL to achieve a similar sensitivity to our method, which only required 10 mL of sample. LC–MS–MS yielded slightly better sensitivity for sample volumes less than 10 mL than our method [11]; other LC-based methods that do not use MS–MS detection for small sample volumes tend to have lower sensitivity [3,26]. Though a much smaller sample volume similar to our method was used in SPME–GC–MS methods, the

Table 3
Analytical validation results—linear range, correlation coefficient, LOD, LOQ, and precision.

Analytes	Linear range (ng mL^{-1})	Correlation coefficient (r^2)	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	Intra-day precision (RSD%, n=3)			Inter-day precision (RSD%, $n=3\times3$)		
					Low [a]	Med [b]	High [c]	Low [a]	Med [b]	High [c]
IBU	0.010–5.0	0.9993	0.0050	0.010	1.9	2.8	3.4	5.1	7.5	9.0
FLUR	0.010–5.0	0.9985	0.0050	0.010	4.7	2.9	3.4	6.3	6.6	8.3
NAP	0.010–5.0	0.9990	0.0050	0.010	0.30	3.5	5.2	9.4	5.2	8.7
KET	0.020–5.0	0.9997	0.010	0.020	2.5	3.3	5.1	9.5	5.4	8.3
DIC	0.020–5.0	0.9998	0.010	0.020	3.2	4.4	4.1	6.1	8.3	7.2
ETO	0.020–5.0	0.9998	0.010	0.020	1.8	4.0	5.1	6.7	9.2	8.7
IND	0.020–5.0	0.9998	0.010	0.020	6.3	3.1	3.5	8.1	5.3	7.7

[a] 0.20 ng mL^{-1}.
[b] 1.0 ng mL^{-1}.
[c] 2.5 ng mL^{-1}.

Table 4
Relative recovery (RRRSD%) measured in three different types of surface water samples.

Analyte	Tap water recovery (RSD%) [a]			Pond water recovery (RSD%)			Reservoir water recovery (RSD%)		
	Blank	0.20 ng mL^{-1}	2.5 ng mL^{-1}	Blank	0.20 ng mL^{-1}	2.5 ng mL^{-1}	Blank	0.20 ng mL^{-1}	2.5 ng mL^{-1}
IBU	ND[b]	109 (1.3)	88 (1.9)	ND	117 (7.6)	90 (5.7)	ND	109 (3.2)	102 (2.8)
FLUR	ND	103 (5.2)	85 (1.5)	ND	106 (5.2)	91 (7.9)	ND	107 (2.8)	100 (2.6)
NAP	ND	91 (2.9)	87 (1.9)	ND	106 (5.3)	95 (8.1)	ND	99 (6.9)	103 (1.5)
KET	ND	94 (5.9)	83 (1.5)	ND	106 (3.6)	91 (8.1)	ND	105 (1.4)	101 (2.7)
DIC	ND	99 (2.3)	85 (3.9)	ND	103 (7.8)	91 (8.5)	ND	104 (2.6)	100 (2.0)
ETO	ND	97 (3.5)	87 (1.7)	ND	100 (5.3)	95 (9.1)	ND	99 (1.2)	105 (1.8)
IND	ND	94 (13)	77 (8.2)	ND	106 (3.3)	87 (6.3)	ND	109 (9.7)	102 (3.6)

[a] n=3.
[b] Not detected.

procedures were longer due to long extraction and/or derivatization time [55,56] or the method sensitivity was relatively low [50] in comparison with the current method. Moreover, our USAEME method requires no special equipment and avoids the possible fiber degradation or carry-over. Our method provided very low LOD and LOQ values using a relatively simple and low-cost analytical platform, GC–MS. Though the use of GC requires the derivatization of analytes in addition to extraction and/or pre-concentration, which could make GC less preferable to LC for NSAID analysis in water samples, the combination of *in situ* MCF derivatization of NSAIDs and USAEME of derivatives simplified the sample preparation steps and helped improve method sensitivity when combined with GC–MS analysis in SIM mode. Though no compounds were detected in the samples tested in this study, the developed method appears useful considering the reported levels of NSAIDs in surface water [57].

4. Conclusions

In this work, a novel analytical method was established to determine levels of seven widely-used NSAIDs in various types of surface water samples. Sample preparation was fast, simple, yet efficient due to combination of *in situ* MCF derivatization with USAEME and GC–MS analysis of derivatives. MCF enabled rapid *in situ* derivatization in aqueous samples, while USAEME facilitated effective and efficient extraction and concentration of derivatives of target analytes using a very small volume of organic solvent. Experimental design allowed the following: (1) identification of significant variables (volumes of extraction solvent, sample, and base catalyst) affecting extraction and/or derivatization efficiency, (2) assessment of individual and interactive effects of the significant variables, and (3) optimization of variables to achieve maximal sample preparation efficiency. As demonstrated in our method validation for linearity, LOD, LOQ, intra- and inter-day precisions, and recovery, the developed method was sensitive and reproducible over the established calibration ranges. Compared with previously reported methods, the current method requires a small volume of sample and simple sample preparation steps for sensitive determination of NSAID levels using a conventional GC–MS system.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.06.027.

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