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Trace analysis of acidic pharmaceutical residues in waters with isotope dilution gas chromatography–mass spectrometry via methylation derivatization

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ABSTRACT

Acidic pharmaceutical residues are pollutants of emerging concern and are generally monitored by HPLC–MS/MS. However, due to the limited separation efficiency of HPLC column and lack of suitable mass transition for confirmation analysis, some interference may not be separated completely and differentiated from ibuprofen, which may cause the results with interference, especially in sample with complex matrix. The objective of this study is to develop a sensitive and reliable method for the determination of acidic pharmaceutical residues in water samples by GC–MS with better resolution by using methylation derivatization and isotope dilution techniques. TMSDM, a mild reagent, was used as the derivatization reagent coupling with the isotope dilution technique, for the first time, to improve the precision and accuracy of the analytical method to determine the pharmaceutical residues in water. The MDLs for the five acidic organic compounds: ibuprofen, gemfibrozil, naproxen, ketoprofen and diclofenac were from 0.7 to 1.1 ng/L, with recoveries ranging from 93 to 110%. Alternative to the HPLC–MS/MS method, the developed GC–MS protocols provides an additional option for the analysis of acidic pharmaceutical residues in water, with better separation efficiency in reducing interferences from complicated sample matrix, for determination of ibuprofen residues.

1. Introduction

Chemicals of emerging concerns are a large class of chemical pollutants, including pharmaceutical and personal care products (PPCPs), endocrine disrupting compounds (EDCs), perfluorinated compounds (PFCs) and others. They are disposed or discharged to the environment through domestic and industrial wastewater, such as septic sewage, landfills and wet weather runoff. In the past decades, the studies on the occurrence, transport and fate of PPCP and EDCs in the environmental system increased. A variety of pharmaceutical pollutants have been investigated and reported, such as hormones, contraceptives, anti-neoplastics, beta-blockers, anti-cancer drugs, lipid regulators and anti-inflammatories [1,2]. An U.S. Geological Survey's (USGS) study indicated that at least one organic wastewater contaminants (OWCs) was detected in 80% of the streams sampled, with 82 of the 95 analyzed OWCs detected in at least one sample in the studied sampling sites [3].

The main path for pharmaceuticals to enter the environment is disposal via wastewater [4]. As the concerns of these compounds increasing, most EU and US national water pollution control programs have been devoted to the identification and quantification of various PPCPs during the last few decades [5,6]. Non-steroidal anti-inflammatory drugs (NSAID), such as ibuprofen, naproxen, ketoprofen and diclofenac, are widely used in the treatment of inflammation and arthritis. Gemfibrozil is used to help reduce cholesterol and triglycerides in the blood. These drugs are among the most abundant pharmaceutical trace pollutants in rivers and its tributaries [7]. These compounds are difficult to detect due to their low concentration in the samples and their high polarity and hydrophilic structures. The chemical structure and physical property of these drugs are listed in Table 1.

With the fast development of high sensitive mass spectrometry technique, high performance liquid chromatography coupled with triple quadrupole mass spectrometry (HPLC–MS/MS) has been applied to the determination of PPCP and EDCs, especially for the polar and non-volatile organic compounds [8–13]. In our past studies, a HPLC–MS/MS method had been developed and applied in the detection of the acidic pharmaceutical residues in water. However, a certain interference to the analysis of ibuprofen in surface water samples was suspected, due to the limited separation capability of HPLC column and lack of qualitative mass transition.

Gas chromatography–mass spectrometry (GC–MS) is another commonly used analytical technique for trace analysis of organic compounds. It is still a challenge to analyze organic compounds containing functional groups, such as –COOH, –OH, –NH and –SH (i.e. sugars, steroids and cholesterol) due to

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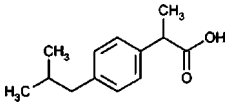
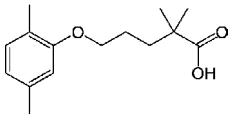
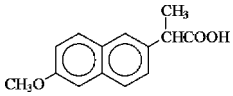
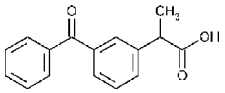
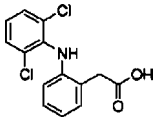
Table 1
Target compounds and their labeled compounds.

Target compounds	Molecular weight	Molecular formula	GC–MS monitored ion at SIM mode
Ibuprofen		$C_{13}H_{18}O_2$	161[a], 177, 220
		Arthritis, antipyretic, analgesic	164[b], 180[b], 223[b]
Gemfibrozil		$C_{15}H_{22}O_3$	83[a], 143, 264
		Lowers lipid levels	89[b], 169[b], 270[b]
Naproxen		$C_{14}H_{14}O_3$	185[a], 170, 244
		Reduction of pain, fever, inflammation and stiffness	188[b], 247[b]
Ketoprofen		$C_{16}H_{14}O_3$	191[a], 209, 268
		Analgesic, antipyretic	194[b], 212[b], 271[b]
Diclofenac		$C_{14}H_{11}Cl_2NO_2$	214[a], 179, 242
		Reduce inflammation, analgesic	218[b], 246[b], 309[b]

[a] Quantitation ion.
[b] Monitoring ion for labeled compounds.

their weak volatility, high polarity and high susceptibility in forming inter-molecular hydrogen bonding [14,15]. Derivatization is commonly used to improve chromatographic performance [16,17], as reported in the determination of the acidic compounds using trifluoroacetic anhydride (TFAA), pentafluoroacetic anhydride (PFAA), N-methy-N-tert-butyldimethylsilyl-trifluoroacetamide (MTBSTFA), N,O-Bis(trimethylsilyl) trifluoroacetamide (BSTFA), BSTFA/trimethylchlorosilane (TMCS) and hexamethyldisilazane (HMDS)/trifluoroacetic acid (TFA), as the derivatization reagents [18–22]. MTBSTFA, BSTFA and BSTFA/TMCS are the most commonly used derivatization reagents for the detection of compounds containing active carboxyl acid or hydroxyl groups. It was reported that MTBSTFA reacted with analytes and yielded tert-butyl dimethylsilyl (TBDMS) derivatives, with more thermo stability and less moisture sensitivity than that from BSTFA [23]. Compare to the above derivization reagents, trimethylsilyldiazomethane (TMSDM) is a non-explosive, non-mutagenic and moderate derivatization reagent, which can react with alcohol in aqueous solution [24]. It is an alternative to diazomethane, to react with carboxylic acid group to yield methyl ester, and has been reported in the determination of anti-inflammatory drugs, salicylic acid, clofibric acid and alcohol [25–28]. However, there is no report before to apply the isotope dilution technique to control the precision and accuracy of the analytical method for determination of the pharmaceutical residues in water with TMSDM as the derivatization reagent.

The objective of this study is: (1) to develop an isotope dilution GC–MS method via derivatization reaction, for the quantitative analysis of acidic pharmaceutical residues as listed in Table 1; (2) to provide an alternative approach with high separation efficiency for the confirmation analysis of results obtained by HPLC–MS/MS.

2. Experiments

2.1. Chemicals and reagents

The standard compounds of ketoprofen, ibuprofen, naproxen, diclofenac sodium and gemfibrozil were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The isotope standard 2,4,5-trichlorophenoxy-3,6-d_2-acetic-d_2 acid (99 atom % D) (2,4,5-TCPAA-d_4), ibuprofen-d_3 (α-methyl-d_3, 99 atom % D), naproxen-d_3 (α-methyl-d_3, 99 atom % D), ketoprofen-d_3 (α-methyl-d_3, 98 atom % D), diclofenac-d_4 (phenyl-d_4, 98 atom % D), gemfibrozil-d_6 (2,2-dimethyl-d_6, 99 atom % D) were purchased from C/D/N Isotopes Inc. (Quebec, Canada). All native standards were prepared in methanol at 500 μg/mL and isotope standards were prepared in methanol at 200 μg/mL. All standard stock solutions were stored in a freezer at temperature of −20 °C up to three months. HPLC grade methanol, tetrahydrofuran, ethyl acetate, acetonitrile, BSTFA/1% TMCS and MTBSTFA were obtained from Fluka (Buchs SG, Switzerland). 2.0 M TMSDM solution in hexane was purchased from Sigma–Aldrich Co. (Missouri, USA). The stock solution of 10 μg/mL native and isotope mixture standards were prepared in acetone. The calibration standards were prepared by further dilution with acetone/methanol mixture (9/1, v/v) for derivatization reaction with TMSDM. Deionized water was obtained by passing tap water through a Milli-Q integral 5 system (Millipore, Singapore) with the resistivity greater than 18.2 MΩ cm^{-1} and on-line TOC of less than 5 ppb.

2.2. Equipment and materials

2.2.1. Equipment and instrumentation

GC–MS analysis was performed on Agilent 7890 GC system with split/splitless inlet coupled with 5975C inert XL MSD. A DB-5 MS (30 m × 0.25 mm × 0.25 μm) column was connected between the GC inlet and MS interface. The carrier gas was helium with constant flow at 1.5 mL/min. GC inlet temperature was set at 280 °C, interface temperature at 250 °C and MS Quad at 150 °C. The monitored ions were collected at selective ion mode with the dwell times at 50, as listed in Table 1. The initial oven temperature was set at 40 °C for 2 min, with GC oven temperature ramp as follows: 20 °C/min to 120 °C, 10 °C/min to 280 °C and hold for 5 min, 20 °C/min to 300 °C and hold for 5 min. GC injection volume was 2 μL at splitless mode. As some of fragments may be produced from both native and isotope labeled compounds, the ions selected for quantitative analysis might not be the ions with highest abundance, e.g. the dominant ions for ketoprofen and gemfibrozil were *m*/*z* 209 and

m/z 143, respectively, but *m/z* 191 and m/z 83 were selected for the quantitative analysis of ketoprofen and gemfibrozil in the GC–MS analysis.

The LC–MS/MS system used in this study is Quattro Premier XE MS/MS (Manchester, UK), consisting of Acquity™ UPLC system (MA, USA) coupled with Quattro Premier™ XE tandem quadrupole mass spectrometer. The analytes were separated on an Acquity™ UPLC HSS T3 Column (2.1 mm × 100 mm, 1.8 μm) and detected by electrospray tandem mass spectrometer in both positive and negative ionization mode. The MS condition was listed as follow: source temperature 120 °C, desolvation temperature 380 °C, cone gas flow 28 L/h, capillary voltage 3.50 kV, extractor 3 V, RF lens 0.8 V, source temperature 120 °C, injection volume 10 μL. The mobile phase consisted of solvent A (90%) (0.1% ammonium acetate and 0.1% acetic acid in water) and solvent B (10%) (acetonitrile/methanol = 50/50, v/v). The solvent B was increased to 90% from 1.5 min to 3 min at flow rate of 0.35 mL/min. The details of instrument conditions for each compound are shown in Table 2. Under multiple reaction monitoring (MRM) mode, there is only one mass transition found for ibuprofen, and at least two mass transitions for all other analytes.

2.2.2. Sample extraction

Liquid Liquid Extraction (LLE): After filtering with 0.45 μm cellulose acetate membrane filter and adjusting the pH to 2 with 6 M HCL, 1 litre of water sample was spiked with 100 ng of each isotope labeled compound standard and extracted with dichloromethane (60 mL× 3). The extract was dried over anhydrous sodium sulphate and further evaporated to 2 ~5 mL, followed by a solvent exchange process using 10 mL of solvent (acetone/methanol = 90/10 (v/v) for GC–MS or methanol for HPLC–MS/MS) and further blown down to 1 mL by a TurboVap® Concentration Workstation (Hopkinton, MA, USA). 10 μL of 10 μg/L of 2,4,4-TCPAA-d_4 was spiked into the extract as the internal standard prior to HPLC–MS/MS injection.

Solid Phase Extraction (SPE): 1 litre of water sample was filtered through 0.45 μm cellulose acetate membrane filter before SPE. After adjusting the pH to 2 and spiked with 100 ng of each isotope labeled chemical standard, the water sample was passed through the SPE cartridge (pre-conditioned with 5 mL × 2 of methanol and equilibrated with 10 mL of DI water) via Autotrace SPE workstation (Sunnyvale, CA, USA) at flow rate of 10 mL/min. After washing with 10 mL of DI water, the SPE cartridge was eluted with 5 mL × 2 of acetontrile containing 1% acetic acid, followed by 5 mL of acetone and methanol mixture (1:1). The eluent was concentrated to less than 1 mL with TurboVap® Evaporation system and followed by addition of 3 mL 10% methanol in acetone (for GC–MS analysis) or methanol (for HPLC–MS/MS analysis) for solvent exchange and further nitrogen blowdown to 1 mL. 10 μL × 10 μg/L of 2,4,4-TCPAA-d_4 was spiked into the extract as the internal standard before derivatization reaction and HPLC–MS/MS analysis.

2.2.3. Derivatization process

2.2.3.1. Derivatization with TMSDM. Derivatization with TMSDM was carried out in the commercially silanized glass vials. Standard mixture or water sample extract was prepared in the matrix of acetone/methanol (90/10, v/v) [25]. 20 μL of 2.0 M TMSDM reagent was spiked into 1 mL of standard or samples extract. After thorough mixing, the homogenous reaction mixtures were kept at ambient temperature for about 70 min before injection. The chromatogram of analytes' TMSDM-derivitives is showed in Fig. 1.

2.2.3.2. Derivatization with MTBSTFA or BSTFA/TMCS. 1 mL of standard mixture or water sample extract was prepared in acetone, THF, hexane, ethyl acetate or dichloromethane matrix, respectively. After mixing with 50 μL of MTBSTFA or BSTFA/TMCS, the reaction mixtures were kept at ambient temperature for 2 hrs before GC–MS injection.

3. Results and discussion

3.1. Selection of derivatization reagent

Different derivatization reagents yield different derivatives, resulting in different selectivity and sensitivity on GC–MS detection. Derivatization with a selective reagent can improve the detectability and minimize the interferences in complex matrix [29]. To find the suitable derivatization reagent for the targeted acidic pharmaceutical compounds, three reagents: MTBTSFA, TMSDM and BSTFA/TMCS, were used to test their feasibilities as derivatization reagents for GC–MS analysis in terms of sensitivity, selectivity and stability. The mixture standards of the target analytes, each at 10 μg/mL, were reacted with different reagents and analyzed on the GC–MS. It was found that *m/z* of M^+ (molecular ion of precursor compound) and $[M+57]^+$ were present in the mass spectrum of MTBSTFA-derivatives, with the dominant fragment ion being $[M+57]^+$. The $[M+57]^+$ ion was formed by adding *t*-butyldimethylsilyl (TBDMS) groups onto the precursor molecule [30]. However, in the mass spectrum of BSTFA/TMCS-derivatives, the $[M]^+$ was presented as the dominant fragment. Different solvents, such as acetone, THF, ethyl acetate, hexane and dichloromethane, were used to prepare standards for the derivatization with BSTFA/TMCS. THF was found to be the best solvent that gave the best sensitivity in all targeted compounds. Methanol shall be avoided during the reaction, as BSTFA/TMCS could react with its hydroxyl group and result in low derivatization reaction yield. In TMSDM derivatization reaction, the *m/z* of M^+ and $[M+15]^+$ were present in the GC–MS spectrum. The presence of *m/z* of $[M+15]^+$ proved the expected methylation reaction. The mass spectra of the derivatives of ibuprofen reacted with different dirivatization reagents are showed in Fig. 2.

The GC–MS sensitivity to the respective products of the above mentioned derivatization reaction and their stability were investigated with the derivatization reaction time controlled as 2 h. It was found that the response intensities of the derived products of ibuprofen, gemfibrozil, naproxen and ketoprofen were 1.4–3.7 times higher using MTBSTFA as derivatization reagent than those using TMSDM, but the response intensity of diclofenac was about 75% using MTBSTFA than TMSDM. Comparing BSTFA/TMCS derivatives and TMSDM derivatives, similar response intensities for ibuprofen, gemfibrozil and naproxen were observed, while the response intensities of BSTFA/TMCS derivatives for ketoprofen and diclofenac were about 25% and 50% of that from TMSDM derivatives, respectively. However, if the derivatization reaction lasted for more than 12 h, the response intensities of all MTBSTFA derivatives declined more than 20%. For example, the response of ketoprofen and diclofenac dropped to 33% and 25%, respectively, while that of naproxen dropped to 56%. The response intensities of all BSTFA/TMCS derivatives dropped from 31% to 44%. The response intensities of TMSDM derivatives reamined almost unchanged, ranging from 95% to 102%. There was not much change in response intensities of the TMSDM derivatives even when the reaction lasted for more than 2 days. These results showed that the TMSDM-derived product is more stable than the derivatives from the other two reagents. Thus, TMSDM was chosen in further studies (Fig. 3).

3.2. Derivatization time

To optimize the reaction time, each 1 mL of 10 μg/mL standard mixture was spiked with 20 μL of TMSDM reagent and kept at room temperature for 10 min, 40 min, 70 min, 100 min, 130 min, 160 min

Table 2
MRM transitions of target compounds at both positive and negative electrospray ionization mode by UPLC–MS/MS.

Native compounds	Monitoring transition	Cone voltage	Collision energy	Labeled compounds	Monitoring transition	Cone voltage	Collision energy
Ibuprofen	204.8 > 161	18	6	Ibuprofen-d_3	208 > 164	18	8
Gemfibrozil	248.9 > 121	17	16	Gemfibrozil-d_6	255 > 121.2	23	15
	248.9 > 127	17	10		255 > 133	23	12
Naproxen	228.8 > 170	14	15	Naproxen-d_3	232.0 > 173	16	15
	228.8 > 185	14	6		232.0 > 188	16	7
Ketoprofen[a]	255.2 > 105	28	23	Ketoprefen-d_3	258.3 > 211.9	26	15
	255.2 > 209	28	44		258.3 > 105.0	26	25
Diclofenac	293.7 > 250	18	12	Diclofenac-d_4	297.8 > 254	19	11
	293.7 > 214	18	20		297.8 > 217	19	20
				2,4,5-TCPAA-d_4	258.8 > 161.9	19	29
					258.8 > 198.8	19	16

[a] Ketoprofen was analyzed in positive mode, other compounds were analyzed in negative mode.

and 190 min, respectively, before GC–MS injection. It was found that the reaction of all target compounds with TMSDM could not complete until 70 min (Fig. 3). Therefore, in further experiments, all derivatization reactions were conducted for 70 min (Fig. 4).

3.3. Selection of solvent as reaction media

Selection of the suitable organic solvent is critical for the derivatization reaction as it is the media for the reaction. To find the appropriate solvent for the derivatization reaction with TMSDM, 1 mL of 100 ng/mL of standard mixture was prepared in dichloromethane, methanol, acetone and acetone/methanol (90:10), respectively, and reacted with 20 μL 2.0 M TMSDM for 70 min before GC–MS analysis. It was found that acetone/methanol as reaction media gave the highest response to all five compounds (Fig. 5). It is because the analytes and derivatization reagent are highly soluble in acetone and the presence of methanol in the reaction media acted as a catalyst to yield respective methyl esters. It was reported that the reaction of TMSDM with carboxylic acids in methanol gave good yields of the desired methyl ester [31–33]. Thus, the acetone/methanol mixture solvent as reaction media showed better performance to all analytes than acetone or methanol alone. The reaction in dichloromethane produced the lowest response intensities which are possibly due to the low solubility of TMSDM in dichloromethane.

3.4. Optimization of GC inlet temperature

The GC inlet temperature is known to affect the derivatization reaction yield and system sensitivity. The GC inlet temperature was set at 180 °C, 200 °C, 220 °C, 250 °C, 280 °C and 300 °C respectively, to investigate the effect of inlet temperature to the GC–MS detection response intensity (Fig. 6). It was found that lower inlet temperatures generated lower response intensities. When the inlet temperature increased from 180 °C to 280 °C, the response intensity increased about 30% for ibuprofen and gemfibrozil, 70% for naproxen and ketoprofen, and 50% for diclofenac. There were no significant changes in response intensities when the inlet temperature increased from 280 °C to 300 °C. Thus 280 °C is the optimal GC inlet temperature for the reaction and used in the subsequent studies.

3.5. Extraction methods

During method development, it was found that the pH value of water sample was very important for both LLE and SPE processes. The recovery of ketoprofen, naproxen, diclofenac and 2,4,4-TCPAA-d_4 could be as low as less than 5%, especially if the pH of the water samples was not adjusted properly. Quenching the residual chlorine in treated water by sodium thiosulfate could improve the recovery of analytes, especially for ketoprofen and diclofenac. Isotope dilution technique was adopted in this study to reduce the

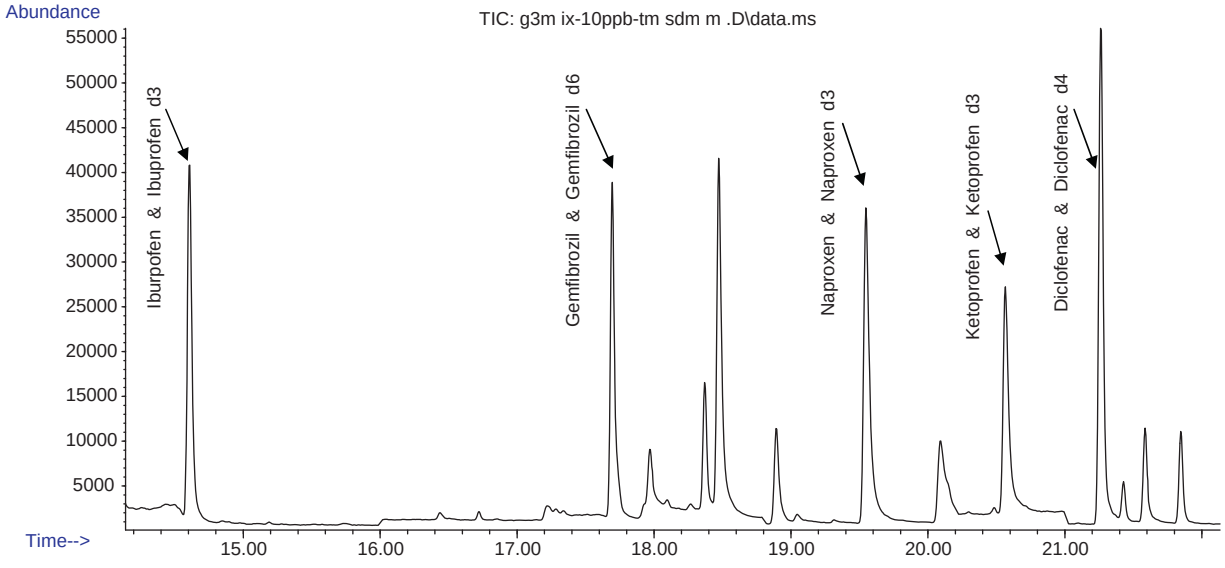


Fig. 1. GC–MS chromatogram at SIM mode of TMSDM derived mixture of 10 ng/mL native compounds and 100 ng/mL isotope labeled compounds.

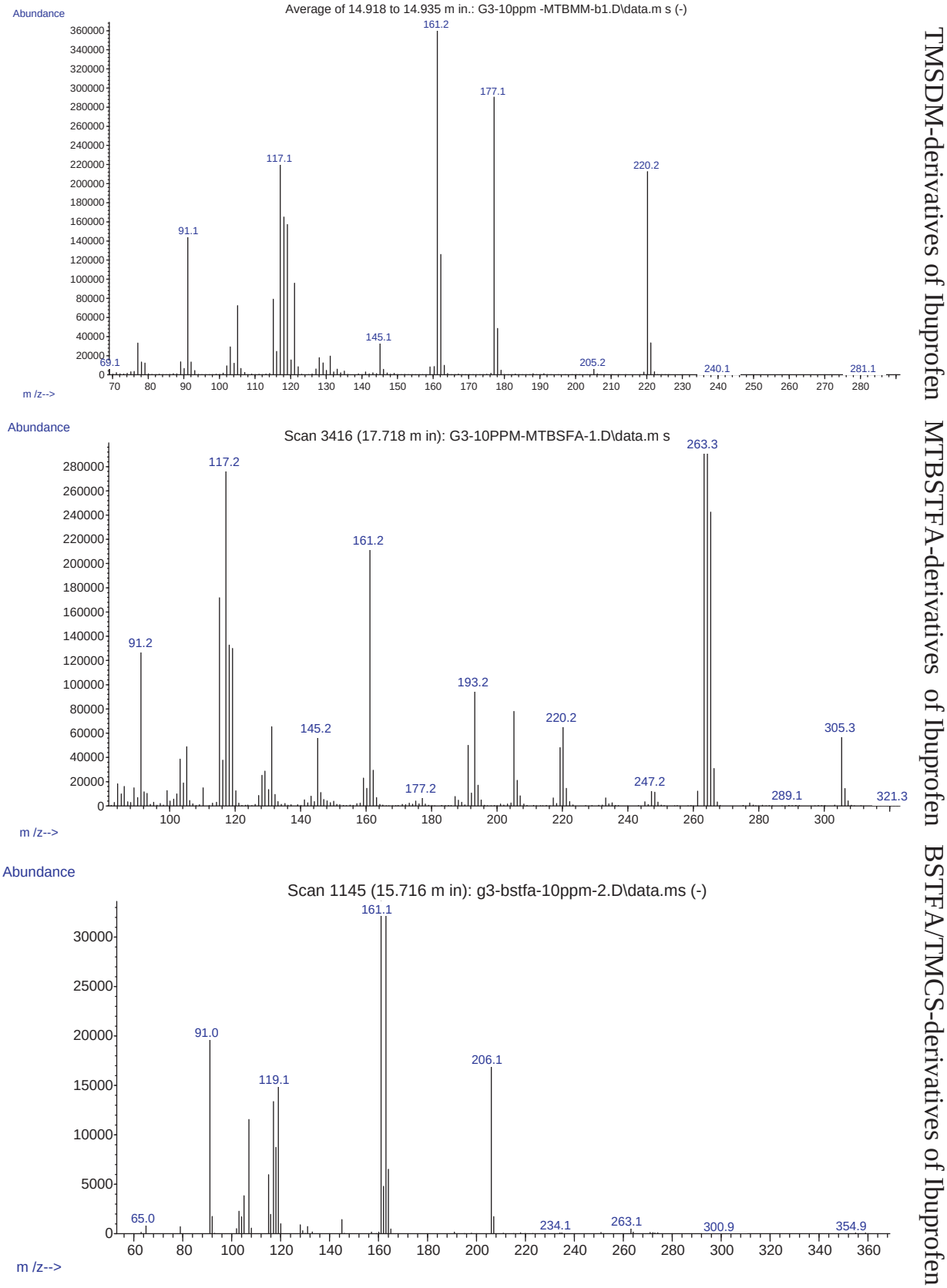


Fig. 2. Mass spectra of the derivatives from ibuprofen reacting with different reagents.

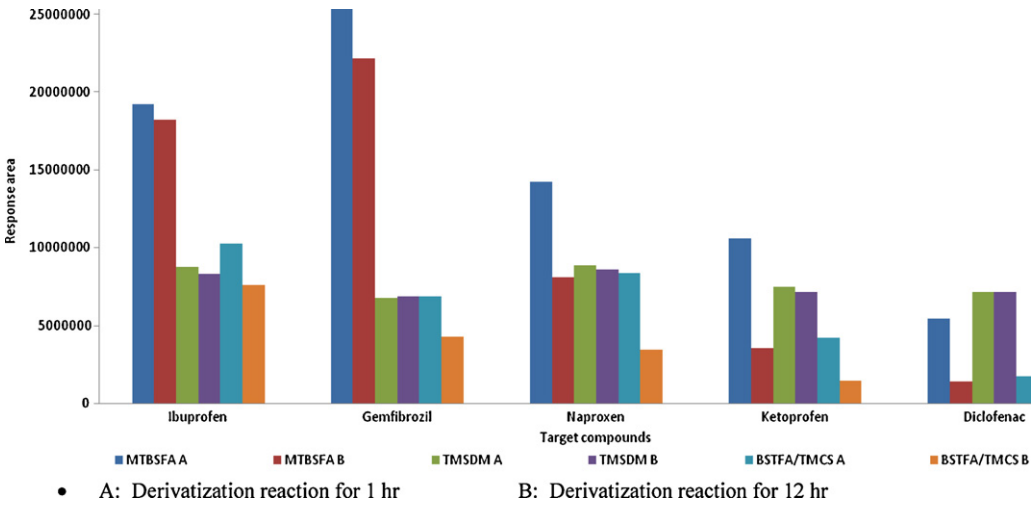


Fig. 3. Effect of derivatization reagents and reaction time versus the response intensities of GC–MS detection.

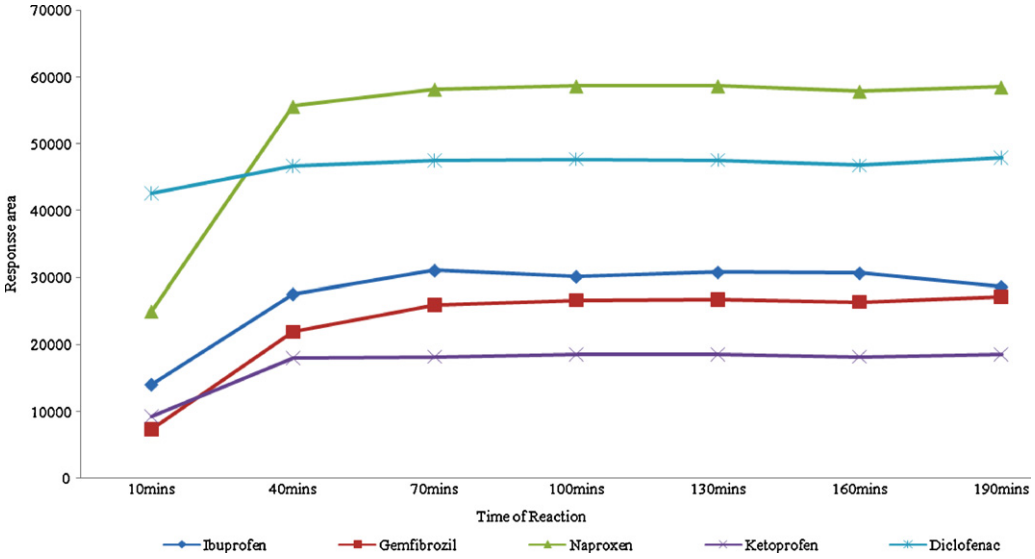


Fig. 4. Effect of reaction time versus the GC–MS response intensities of derivatives of target compounds reacting with TMSDM.

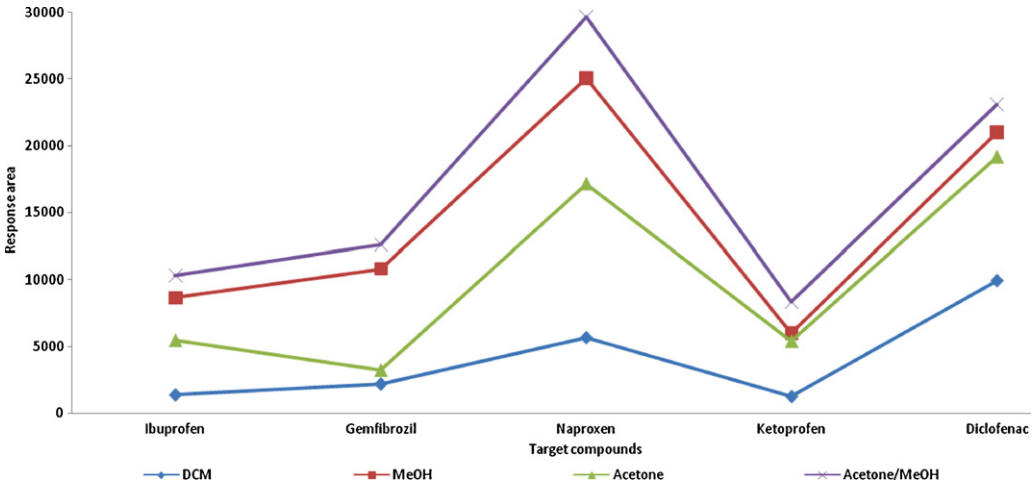


Fig. 5. Effect of solvent versus the GC–MS response intensities of derivative of target compounds reacting with TMSDM.

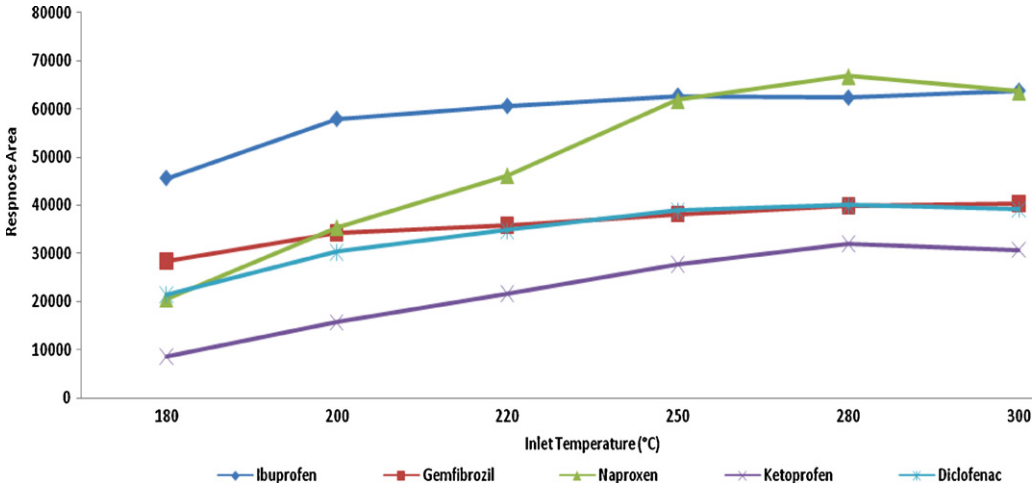


Fig. 6. Effect of GC inlet temperature versus the GC–MS response intensities of derivative of target compounds reacting with TMSDM.

matrix effect. It was found that both LLE and SPE could achieve good recoveries to all target compounds, ranging from 80% to 120% (Table 3). For the SPE process, the SPE cartridges of ENV (Varian), strata-X (Phenomenex) and HLB (Waters) produced similar recoveries.

3.6. Method validation and real sample applications

The developed GC–MS method was validated with linearity, precision, recovery and method detection limit (MDL) [34].

3.6.1. Calibration and linearity

To each of the calibration standard mixtures and water samples, a fixed amount of 100 ng of each isotope labeled standard was spiked. The concentration of the target compound was calculated over its related isotope standard, to minimize the sample matrix effect. In the calibration range of 2 ng/mL to 200 ng/mL, the coefficients of linear regression (r^2) of calibration curve were 0.997, 0.997, 0.996, 0.993 and 0.993 for ibuprofen, gemfibrozil, naproxen, ketoprofen and diclofenac, respectively (Table 3).

3.6.2. Precision, recovery and method detection limit

Instrument precision was measured by calculation of the relative standard deviation (RSD) of the response intensities of the triplicate injections of 2 μL × 50 ng/mL standard solution. The within-batch RSDs were from 1.9% to 4.6%, while the between batch RSDs were from 2.4% to 11.3% (Table 3).

The accuracy was determined by the calculation of the RSD of analyte recoveries, processed by the SPE or LLE, with triplicate 1 L deionized water samples spiked with 50 ng of each native standard mixture and 100 ng of each isotope standard. The mean recoveries by SPE were from 89% to 108%, while that by LLE were from 93% to 110%, as indicated in Table 3. Both SPE and LLE methods generated satisfactory recoveries. Of course, compared with LLE, the SPE process is more environment friendly.

3.6.3. MDL

MDL of acidic pharmaceutical compounds was determined over seven replicates of deionized water samples spiked with native standard chemicals at 5 ng/L and extracted by LLE and SPE, respectively, and calculated with MDL = $t_{(n-1,\alpha=0.99)}$ (S). S is the standard deviation of the replicate analyses. When the number of replicates = 7, $t_{(n-1,\alpha=0.99)}$ = 3.14 [34]. MDLs of analytes by LLE for target compound were found to be 1.0 ng/L for ibuprofen, 1.1 ng/L for gemfibrozil, 0.7 ng/L for naproxen, 0.7 ng/L for ketoprofen and 1.1 ng/L for diclofenac (Table 3). Similar MDLs of each compound by SPE were found to be from 0.8 ng/L to 2.1 ng/L (Table 3). To simplify the reporting results, the practical quantitation limit to all compounds were around 3–5 folds of their MDLs.

3.7. Comparison of analytical results of real water samples analyzed from GC–MS and HPLC–MS/MS detection

Tandem MS/MS at MRM mode can produce excellent sensitivity and selectivity by reducing the background noise and interference, especially when multiple daughter fragment ions being monitored and mass ratio being applied for confirmative analysis. In general, a minimum of one ion ratio must be measured and all measured ion ratios must meet the criteria. Mass transition, the ratio of mass transitions, and retention time have been used to identify and differentiate the ananlytes and the impurities. However, to certain compounds, such as ibuprofen, only one daughter fragment ion (m/z 161) was generated and one transition reaction (m/z 204.8 → 161) could be monitored under MRM mode, no mass ratio could be applied for qualitative confirmation of the analytical results in case of interference occurs. Therefore, to compound like ibuprofen, with single fragmentation at MRM, the selectivity of

Table 3
Protocols of method validation.

	Ibuprofen	Gemfibrozil	Naproxen	Ketoprofen	Diclofenac
Linear range (ng/mL)	2–200	2–200	2–200	2–200	2–200
Coefficient of linear regression (r^2)	0.997	0.997	0.996	0.993	0.993
Within-batch RSD (%)	3.8	2.9	3.3	4.6	1.9
Between-batch RSD (%)	9.0	4.5	11.3	2.4	4.3
Recovery (%) (LLE)	110	103	93	95	98
Recovery (%) (SPE)	108	89	94	102	97
MDL (ng/L) (LLE)	1.0	1.1	0.7	0.7	1.1
MDL (ng/L) (SPE)	1.5	1.9	1.0	2.1	0.8

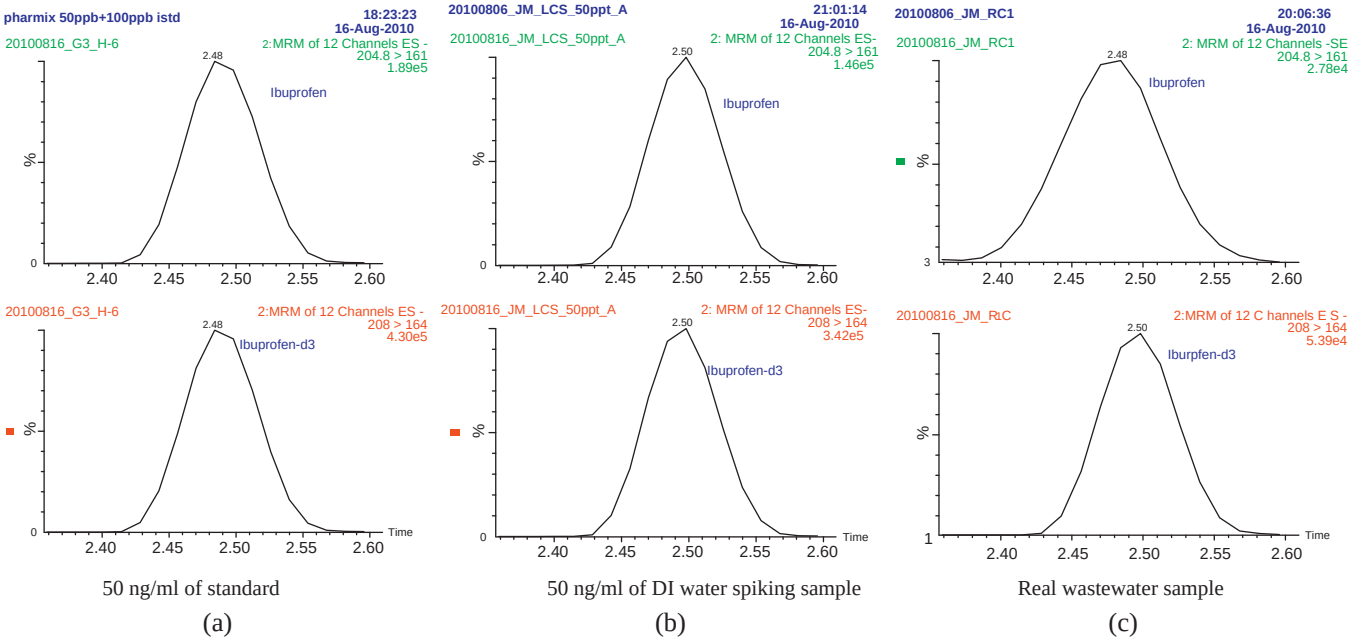


Fig. 7. Chromatograms of ibuprofen and ibuprofen-d_3 by HPLC–MS/MS.

Table 4
Real samples analyzed by GC–MS and UPLC–MS/MS.

Sample	Ibuprofen		Gemfibrozil		Naproxen		Ketoprofen		Diclofenac	
	GC–MS	UPLC–MS/MS	GC–MS	UPLC–MS/MS	GC–MS	UPLC–MS/MS	GC–MS	UPLC–MS/MS	GC–MS	UPLC–MS/MS
1	<MDL	5.6	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
2	3.2	61.9	3.5	3.8	7.7	8.2	<MDL	<MDL	<MDL	<MDL
3	<MDL	21.3	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
4	3.4	10.1	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
5	<MDL	12	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
6	3.7	47.3	2.6	3.2	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
7	10.7	53.8	2.7	2.0	9.5	10.1	<MDL	<MDL	2.7	2.1
8	<MDL	20.5	<MDL	<MDL	4.7	3.7	<MDL	<MDL	<MDL	<MDL
9	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
10	3.3	8.5	<MDL	<MDL	2.1	3.4	<MDL	<MDL	<MDL	<MDL
11	4.8	123.1	126.3	144.5	266.1	243.5	81.6	67.3	151.2	144.6
12	59.1	136.7	418.3	512.2	450.4	424.9	248.7	271.1	198.4	234.1

Note: Samples 1–8: surface water; samples 9 and 10: treated water; samples: 11 and 12: wastewater.

LC–MS/MS will be compromised. This may cause the results with interference for ibuprofen. To ibuprofen analysis by LC–MS/MS, the retention time was the only factor used to identify the analytes and impurities (Fig. 7), the positive results were suspected to be caused by interferences co-eluting with ibuprofen (Fig. 7c, peak broadening and slight retention time shift were found in some samples). These interferences were not able to be removed or separated completely by different HPLC columns like C-18, phenyl and HSS T3 and HILIC etc. Types of cleanup processes have been attempted but failed in solving this problem. More than 100 different kinds of water samples (e.g. wastewater, surface water and treated water) have been analyzed both by GC–MS and HPLC–MS/MS methods, after SPE process, with some of the results shown in Table 4. The results of the GC–MS and HPLC–MS/MS analyses of ibuprofen were significantly different from each other. However, the other four compounds tested in both methods gave comparable results. These results suggest that, to analyze the compound like ibuprofen, with single fragmentation at MRM, the selectivity of LC–MS/MS will be compromised. This may cause the results containing interference for ibuprofen on LC–MS/MS analysis, due to the limitation of the HPLC column separation efficiency and lack of second mass transition for confirmative analysis. Similarly, false positive result from LC–MS/MS under MRM to analysis of sebuthylazine residues was also reported by Andreas Schurmann, even when the retention time and intensity ratio of the two MRM transitions matched perfectly [35].

4. Conclusion

GC–MS coupled with TMSDM derivatization reaction and isotope dilution technique has been proven, for the first time, to be a sensitive, selective, stable and reliable approach in the determination of acidic pharmaceutical residues in water. Different derivatization reagents, derivatization solvent media, derivatization reaction time, GC inlet temperature and sample extraction methods have been studied in detailed and optimized. Both SPE and LLE showed satisfactory recoveries to all target compounds. The method has been validated with good linearity, precision, accuracy and MDLs. Although HPLC–MS/MS is the most sensitive technique in the detection of residual organic compounds, it may produce results with interference in the detection of certain compound with only one mass transition, e.g. ibuprofen, in water sample with complex matrix, due to the limited separation efficiency of HPLC column and lack of a second mass transition to

differentiate analyte and impurity. The developed GC–MS protocols have been successfully applied in the detection of ibuprofen and other acidic pharmaceutical residues in real water sample monitoring.

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