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新店溪中藥物分布之探討:

分析方法之建立及風險評估

Occurrence of Pharmaceuticals in Sin-Dian River: analytical method development and risk assessments

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本論文係陳鍹友君（R95541202）在國立臺灣大學環境工程學研究所完成之碩士學位論文，於民國 97 年 6 月 27 日承下列考試委員審查通過及口試及格，特此證明

口試委員：

林郁真　（簽名）
（指導教授）

系主任、所長　（簽名）

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摘要

近年來，藥物及個人保健用品在環境中造成的汙染漸漸受到重視，且逐漸成為一項專門的議題 ”新興汙染物”。雖然這類的汙染物於環境中被偵測到的濃度非常低（ng/L~μg/L 等級）且不會對人體造成立即的影響，但我們還是不可忽略這類污染物未來可能造成的影響與危害。為了對這類污染物持續監測並思考改正的辦法，建立一套標準且可信賴的分析方法是勢在必行的。在這份研究中，分析方法是建立於以固相萃取法(SPE)濃縮汙染物後再以高效液相層析質譜質譜分析儀(HPLC-MS/MS)來分析新店溪中是否有目標汙染物的存在，而我的目標藥物包括五種非類固醇類消炎止痛藥(acetaminophen, ibuprofen, naproxen, ketoprofen and diclofenac)，三種雌激素(estrone, 17α-ethylnylestradiol and 17β-estradiol)，一種抗癲癇藥(propranolol)及一種降血脂劑(gemfibrozil)。實驗方法的精確度及準確度都被控制在 ±20%以內。Acetaminophen, propranolol 及 gemfibrozil 的最低偵測極限(MDL)是 0.2 ng/L，naproxen 是 2 ng/L，ketoprofen 及 diclofenacand 是 5 ng/L，estrone, 17α-ethylnylestradiol, 17β-estradiol 及 ibuprofen 則是 10 ng/L。對於目標藥物在表面水中的環境風險評估是以評估其風險商數 RQ 值(risk quotient)來決定，預測的環境濃度(PEC)與已偵測的環境濃度(MEC)的比較則是為了評估這套方法的可行性及可信度。結果顯示大部分的藥物 PEC 及 MEC 值都不會差很多，而雌激素(estrone, 17α-ethylnylestradiol and 17β-estradiol)及抗癲癇藥(propranolol)的風險商數>1， 對於環境水體具有潛在的風險。

關鍵字: 高效能液相質譜層析儀(HPLC-MS/MS), 固相萃取法(SPE), 藥物及個人保健用品(PPCPs), 預測環境濃度(PEC), 已偵測到的環境中濃度(MEC), 風險評估。

Abstract

Pharmaceuticals and personal care products (PPCPs) have recently received significant attentions and become emerging chemicals of concern despite the detected environmental concentrations were generally low (in the ng/L to μg/L range). In order to monitor and later remediate this contamination, this study developed an analytical method using solid phase extraction (SPE) followed by liquid-chromatography tandem mass spectrometry (HPLC-MS/MS) to monitor the occurrence of the five non-steroidal anti-inflammatory drugs (acetaminophen, ibuprofen, naproxen, ketoprofen and diclofenac), three estrogens (estrone, 17α-ethylnylestradiol and 17β-estradiol), an anti-hypertensive (propranolol) and a lipid regulator (gemfibrozil) in the Sin-Dian River in Taiwan. Precision and accuracy of the method were evaluated, and the method detection limits (MDLs) were 0.2 ng/L for acetaminophen, propranolol and gemfibrozil, 2 ng/L for naproxen, 5 ng/L for ketoprofen and diclofenac, and 10 ng/L for estrone, 17α-ethylnylestradiol, 17β-estradiol and ibuprofen. The measured concentrations were later compared with the predicted environmental concentrations (PEC) in order to evaluate the validity of the prediction procedures. Environmental risk assessment of the target compounds in surface waters was performed by examining the risk quotient (RQ), and the results indicated the potential risk of estrone, 17α-ethylnylestradiol,

17β-estradiol and propranolol in our aquatic environment.

Keywords: HPLC-MS/MS, Solid Phase Extraction (SPE), Pharmaceuticals and Personal Care Products (PPCPs), Predicted environmental concentration (PEC), Measured Environmental Concentration (MEC), Risk Assessment

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Chapter 1 Introduction

1.1 Background/Problem Statements

Pharmaceuticals and personal care products (PPCPs) have recently been receiving increased attentions and become emerging chemicals of concern. They are broadly used in human medication (both prescribed and over-the-counter), aquaculture, and livestock breeding. They posses possible human health risks problem and are potentially toxic to aqueous environment. News from U.S. showed that 15 PPCPs, including ibuprofen, naproxen and various antibiotics were detected in trace amount in the tap waters (WashingtonPost). Even though the detected concentrations were generally low (in the ng/L to μg/L range), the problem can not be ignored. The current PPCPs usage at Taiwan is high, and they may slowly accumulate to significant concentrations in the environment. According to the investigation of the union of pharmacist association R.O.C, a survey of how to cope with the remaining medicines 61.5% of population (total of 33,000 questionnaires) threw medicines to trash cans directly, and only 15.6% would take the medicines back to hospitals, clinics or pharmacies (Taiwan Environmental Information Center, 2008). It revealed a message that people in Taiwan don't have the concept about how to deal with their unused medicines, and this has the potential to lead to serious environmental problems. In order to monitor and later

remediate this contamination, developing a standard analytical method for measuring these pharmaceuticals in trace concentrations is necessary.

1.2 Aims and objectives

In this thesis, an analytical method using solid phase extraction (SPE) followed by liquid chromatography tandem mass spectroscopy (HPLC-MS/MS) analysis was developed to monitor the occurrence of the five non-steroidal anti-inflammatory drugs (NSAIDs) (acetaminophen, ibuprofen, naproxen, ketoprofen and diclofenac), three estrogens (estrone, 17α-ethylnylestradiol and 17β-estradiol), an anti-hypertensive (propranolol) and a lipid regulator (gemfibrozil) in the Sin-Dian River. Precision and accuracy of the method were evaluated and the method detection limits were determined at ng/L level. Environmental risk assessment of the target pharmaceuticals in surface waters was performed through evaluating the risk quotient (RQ). Predicted environmental concentrations (PEC) were compared with measured environmental concentrations (MEC) in order to evaluate the validity of the prediction procedures.

Chapter 2 Literature Review

In the following literature review section, I reviewed the occurrence data in natural environments and other potential contamination sources such as waste streams of wastewater treatment plants, hospitals, and regional discharges. I then discussed fate of PPCPs in the environment and reason for using LC-MS/MS for analysis. Lastly, I reviewed the currently work for risk assessment of ecological and for human health concern.

In recent years, the occurrence of pharmaceutically active compounds in the aquatic system has been known as part of emerging contaminants issues (Halling-Sorensen, Nors Nielsen et al. 1998; Daughton and Ternes 1999). PPCPs and their metabolites could get into our environment through many source pathways, such as effluent of sewage treatment plants (STPs), industrial wastewaters, regional discharges, and hospital wastewaters (Halling-Sorensen, Nors Nielsen et al. 1998; Gomez, Petrovic et al. 2006; Nakada, Komori et al. 2007). PPCPs were seen in several of surface water such as lakes and rivers (Boyd, Reemtsma et al. 2003; Thomas and Hilton 2004). Acute toxicity data for some PPCPs were reported; however, the studies of the risk of low PPCPs concentration on aquatic systems and human health are lacking. Although some acute toxicity data have been reported, they might not be appropriate to affirm the risk caused

by pharmaceutical compounds in the environment because these kinds of data limited the biological activities and potency of pharmaceutical compounds(Thomas and Hilton 2004).

Occurrence in the natural environments: In South Korea, the samples collected from three rivers receiving effluents from wastewater treatment plants demonstrated that antibiotics were detected at concentrations ranging from 1.7 to 36 ng/L; the estrogens were only sometimes detected and have concentrations up to 5.0 ng/L; the NSAIDs (acetaminophen, naproxen, ibuprofen, diclofenac) were at 1.1-73 ng/L and gemfibrozil at 1.8-9.1 ng/L (Kim, Cho et al. 2007). Boyd et al. had detected PPCPs and EDCs in stormwater canals in USA, and the results from the six month sampling duration showed the following concentrations: naproxen (not detected (ND) – 145 ng/L), ibuprofen (ND – 674 ng/L), and estrone and 17β-estradiol were not detected nor quantifiable (Boyd, Reemtsma et al. 2003). Hernando et al., 2006 had detected naproxen, diclofenac and ibuprofen in the rivers with the concentrations ranging from 70 – 70ng/L, 26 - 72 ng/L and 60 – 152 ng/L, respectively (Hernando, Heath et al. 2006). Kuch and Ballschmiter had detected estrone, 17α-ethylnylestradiol and 17β-estradiol in river waters with concentrations ranging from 0.1 - 4.1 ng/L, 0.1 - 5.1 ng/L and 0.15 – 3.6 ng/L, respectively (Kuch and Ballschmiter 2001).

Occurrence in the potential contamination sources: Hernando et al., 2006 had detected naproxen, diclofenac and ibuprofen in sewage treatment plant (STP) effluents with the concentrations ranging from 625 – 625ng/L, 32 - 1420 ng/L and 18 – 1860 ng/L, respectively(Hernando, Heath et al. 2006). Kuch and Ballschmiter (2001) had detected estrone, 17α-ethylnylestradiol and 17β-estradiol in STP effluents with concentrations ranging from 0.35 - 18 ng/L, 0.1 - 8.9 ng/L and 0.15 – 5.2 ng/L, respectively (Kuch and Ballschmiter 2001). Gomez et al. (2006) had detected ibuprofen, acetaminophen, diclofenac and propranolol in hospital effluent with concentrations ranging from 1.5 – 151 μg/L, 0.5 – 29 μg/L, 0.06 – 1.9 μg/L and 0.2 – 6.5 μg/L (Gomez, Petrovic et al. 2006). Heberer et al. (2004) had reported that diclofenac had been detected in supply water used for artificial ground water recharge with the mean concentration of 35 ng/L (Heberer, Mechlinski et al. 2004).

Fate of PPCPs in the engineered and natural environment: Bendz et al. (2005) had indicated the removal rates of gemfibrozil, ibuprofen, ketoprofen, naproxen, diclofenac and propranolol by STPs were 69%, 90%, 69%, 66%,17-69%, and 96%, respectively(Bendz, Paxeus et al. 2005); Gomez et al. had reported that acetaminophen was removed more than 99%, and ibuprofen and diclofenac were removed 92% and 40% by STPs, respectively(Gomez, Martinez Bueno et al. 2007). Japanese researchers

had done the study of occurrence of 70 pharmaceuticals and personal care products, and 57 of them were detected in Tone river basin which include acetaminophen, diclofenac, ibuprofen, ketoprofen, propranolol etc. Especially, ketoprofen was frequently detected in effluent samples at hundreds ng/L degree, but almost not detected in river water samples. The Japanese researchers attributed this reason to the highly photodegradable character which is found by Lin et al. (Lin and Reinhard 2005; Nakada, Komori et al. 2007). Some studies indicated that the traditional wastewater treatments, like coagulation, flocculation and sedimentation, couldn't remove PPCPs compounds effectively; however, chlorine, ozone, activated carbon, and membrane filtration are feasible in removing those compounds(Adams, Wang et al. 2002; Boyd, Reemtsma et al. 2003). Some pharmaceuticals would be absorbed by human body, or degraded in the body, and became inactive form; there are still some compounds and their metabollites would be executed in active form and enter the waste water system(Roberts and Thomas 2006) Even though the concentrations of PPCPs would be very low in natural water body (ng/L~μg/L), there still are some characters like persistence, bioaccumulation, and toxicity, which would be harmful to the environment (Daughton and Ternes 1999). In Pakistan, diclofenac was used with a big amount in animal husbandary, and it may due to the collapse of vulture nature.(Oaks, Gilbert et al. 2004)

Analytical methods used for quantification: Previously, the majority of analytical methods were performed by gas chromatography-mass spectroscopy (GC-MS), but it often needed derivatization step for acidic compounds. In the end of last century, liquid chromatography-mass spectroscopy (LC-MS) has had a big improvement both in terms of technology and application. LC-MS/MS is suitable to analyze polar pharmaceuticals and their metabolites, and especially good for environmental analysis by its high selectivity (Petrovic, Hernando et al. 2005). Brun et al. (2006) had established analytic methods by GC-MS for acidic compounds and by HPLC-MS for neutral compounds. The method detection limits (MDLs) for acidic compounds such as diclofenac, gemfibrozil, ketoprofen and naproxen were 30 ng/L; for neutral compounds such as acetaminophen and carbamazepine were 10 and 20 ng/L, respectively (Brun, Bernier et al. 2006). Chen et al. (2008) had established an analytical method for clofibric acid, ketoprofen, ibuprofen, diclofenac and carbamazepine by LC-MS/MS, and their limit of detections were 1, 6, 4, 0.5 and 0.2 ng/L, respectively. Gulkowska et al. (2008) reported that the MDLs were different between influent samples and effluent samples, and influent ones were with higher MDLs ranging from 4.0 to 93 ng/L comparing to effluent ones with lower MDLs ranging from 4.0 to 37 ng/L. The reason of the difference might be the matrix effects (Gulkowska, Leung et al. 2008).

Ecological and human health risk assessments: Regulations on entire classes of pharmaceuticals in the environment is impractical. New pharmaceuticals are still producing, and it would be difficult to keep up with changes of the pharmaceutical markets. Consequently, risk assessment procedure is needed to be done to preselectcompounds which have the potential to cause environmental problems (Castiglioni, Fanelli et al. 2004). Risk assessment guidelines for pharmaceuticals were developed in the European Agency for the Evaluation of Medicinal Products (EMEA). The guidelines estimated predicted environmental concentration (PEC) with a formula and evaluated with toxicological data observed by standard toxicity tests (Bound and Voulvoulis 2006). According to the paper of risk assessment of top 25 English prescription pharmaceuticals by Jones et al. (2002), paracetamol, amoxicillin, and oxytetracycline were thought to have potential risk with risk quotient PEC/PNEC ratios bigger than one. However, the risk quotient of paracetamol changed from 0.09 to 1.29 based on applying different PNEC data(Jones, Voulvoulis et al. 2002). Carlsson et al. (2006) selected 27 active pharmaceuticals for environmental hazard and risk assessment. Among them, the RQ (PEC/PNEC) values of ethylnylestradiol, estradiol, estriol and paracetamol were over one and thought to be riskful (Carlsson, Johansson et al. 2006). Santos et al. (2007) indicated that although there was a big decrease of the concentration of ibuprofen, the risk of ibuprofen was both presented in influent samples and effluent

samples of wastewater treatment plants with MEC/PNEC values by 41 and 5.3. However, the risk quotient of naproxen was 1.28 in effluent samples and 0.20 in effluent samples.(Santos, Aparicio et al. 2007)

Chapter 3 Experimental Methods

3.1 Description of the sampling site

Sin-Dian River is the main river in Taipei, and it belongs to Dan-Sui River basin, the biggest basin in the northern Taiwan. It combined with Da-Han River in Banqiao Jiangzicui and then flow into Dan-Sui River. The length of Sin-Dian River is 82 km and the drainage area is 910 km^2. **Figure 3.1** is the map of the environment around Sin-Dian River and the hospitals and regional discharge points are also indicated on the map. Six sampling locations were labeled A1, A2, A3, A4, A5 and A6. **Table 3.1** is the specific sampling information of the sampling programs.

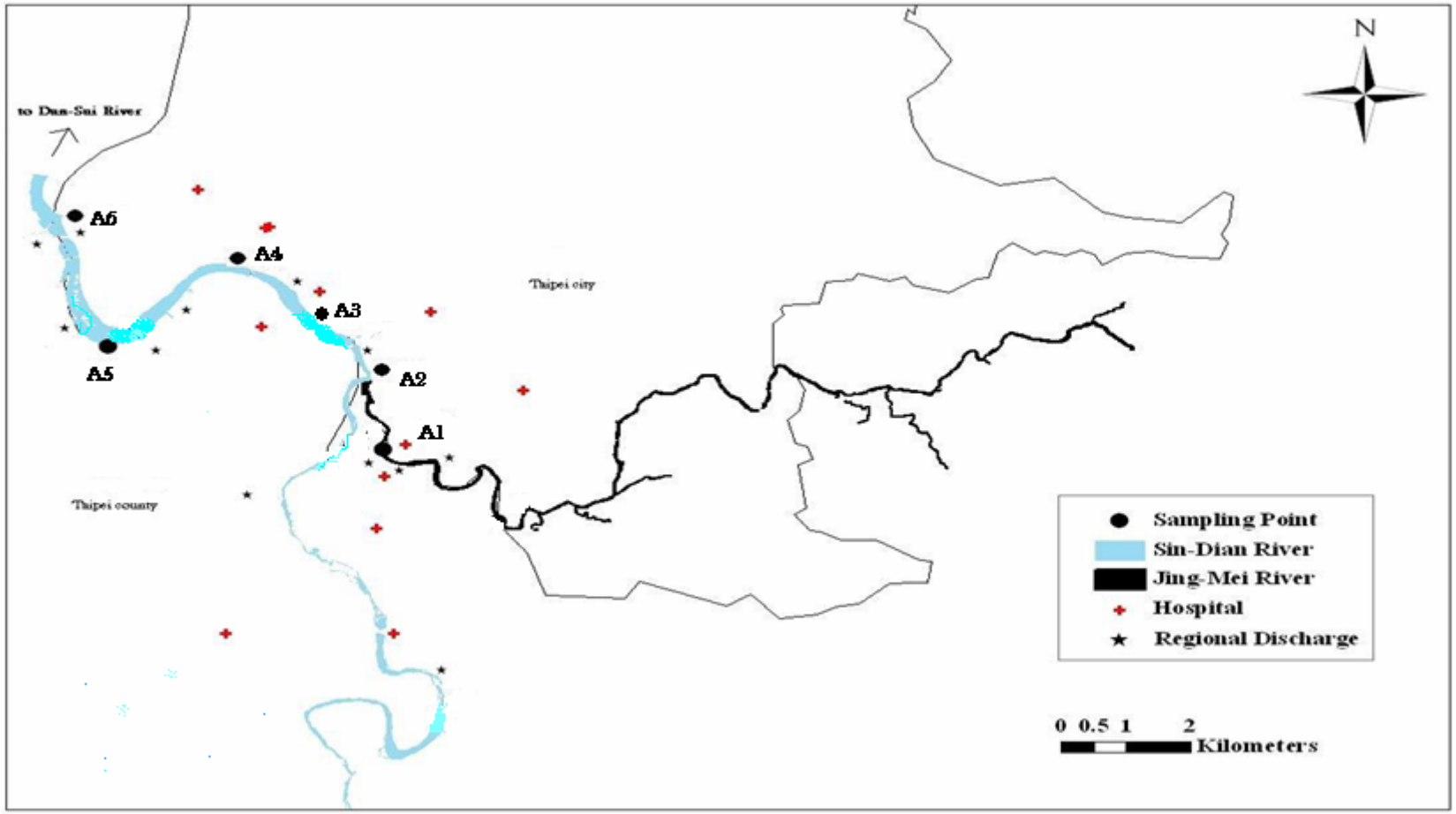


Figure 3.1 Map of sampling points along Sin-Dian River

Table 3.1 Sin-Dian River sampling locations

Site	Location	Position
A1	No.1 water gate of Jing-Mei River	121°32’13” E, 024°59’31” N
A2	Intersection of Sin-Dian and Jing-Mei River	121°32’01” E, 025°00’11” N
A3	Under Ueong-Fu Bridge	121°31’37” E, 025°00’42” N
A4	Near Chung-Cheng Bridge	121°31’09” E, 025°01’13” N
A5	Under Hwa-Zong Bridge	121°29’42” E, 025°00’36” N
A6	Under Hwa-Jiang Bridge	121°29’03” E, 025°02’02” N

3.2 Sample collection and storage

River water samples were collected in 1L brown amber glass bottles. Before sampling, every bottle werewashed 3 times with tap water first, 3 times with D.I. water and then rinsed with river water. There were three bottles of sample every sampling location for triplet experiment. After collection, these samples were stored in an iced box before arriving to the lab for restraining from bacteria growing. Samples were vacuum filtered through 0.45 and 0.22 μm filter paper and stored at 4 ℃ before solid-phase extraction (SPE).

3.3 SPE and HPLC-MS/MS analysis

3.3.1 Materials

Methanol (HPLC-grade) was obtained from Mallinckrodt Baker; formic acid (ACS-grade) was from Riedel-deHaën; sodium hydroxide was from nacalai tesque and sulfuric acid was from Fluka. The purity of standards of target compounds were higher than 99 %.

Acetaminophen, gemfibrozil, ibuprofen, ketoprofen, naproxen and β-estradiol were purchased from Sigma-Aldrich; diclofenac and propranolol were from USP; estrone and 17α-ethinylestradiol were from Riedel-de Haën. The stock standard solutions of individual compounds were prepared by methanol at a concentration of 1000 mg/L and stored in the brown bottles at -20℃. Mixed working solutions (10, 1, 0.1 0.01 mg/L mg/L) were freshly prepared prior to extraction and the solvent was mixed by 50% methanal and 50% D.I. water.

3.3.2 Solid phase extraction

Water samples were concentrated and purified with solid phase extraction (SPE) technique. HLB cartridges were first washed by 5 mL 100% methanol and then 5mL D.I. water. Water samples were adjusted to pH 7.0 by adding 0.5N H_2SO_4. Aliquot of water samples (250mL) were loaded onto the HLB cartridges at the flow rate of 3-6mL/min. Cartridges were then washed with 6mL D.I water, and dried with nitrogen streams. After that, the analytes were eluted by 8mL 50 % MeOH +50 % D.I. water with eight times of 1 mL. The collected extracts were dried by nitrogen and heated to 37℃ and dissolved by 0.5 mL 50% MeOH in D.I. water. Finally, the dissolved samples would be filtered by 0.45 μm and 0.22 μm aperture filters (13mm diameter, material : PTFE), and then run the HPLC-MS/MS analysis.

3.3.3 HPLC-MS/MS analysis

Analyses were carried out using HPLC-MS/MS system (Applied Biosystems API 4000 LC-MS/MS with data processing software, Analyst 1.4.2). HPLC module includes degassor, (Agilent 1100 Series Micro Vacuum Degasser), pump (Agilent 1100 Series Binary Pump) and autosampler equipment (CTC Analytics HTC PAL System). Chromatographic separation was performed by HPLC column：ZORBAX Eclipse XDB-C18 (150 × 4.6 mm, 5 μm). The mobile phase A, 0.1% formic acid in D.I. water and mobile phase B, 0.1% formic acid in 100% methanol were used in both of the positive and negative ion modes. The HPLC solvent gradient is showed in **Table 3.2**. The column is equilibrated for 5 minutes before injection of samples. The injection volume of 20μL was used for analyses and the analysing time was 10 minutes for each sample. Detection was carried out using quadrupole mass spectrometer equipped with

an electrospray ionization (ESI) interface. The analyses were done in positive ion mode for acetaminophen, propranolol, estrone, β-estradiol and 17α-ethinylestradiol and in negative ion mode for ibuprofen, gemfibrozil, ketoprofen, naproxen and diclofenac. In MS/MS system, the parameters of analyses were listed in following **Table 3.3**. Multiple reaction monitoring transition mode (MRM) was applied to obtain individually the optimal declustering potential (DP), collision energy (CE), collision cell exit potential (CXP) and transitions chosen, etc. The physical and chemical characters of compounds are showed in **Table 3.4**, and MRM experimental parameters are summarized in **Table 3.5.**

Table 3.2 The gradient elution program for chromatographic separation

Time (min)	Flow rate (μL/min)	Mobile phase A (%)	Mobile phase B (%)
0	1000	90	10
0.2	1000	90	10
3.0	1000	30	70
4.5	1000	10	90
5.0	1000	5	95
8.0	1000	5	95
8.5	1000	90	10
10.0	1000	90	10

Table 3.3 LC-MS/MS parameters

Parameters	**Positive**	**Negative**
Ionization mode	ESI(+)	ESI(-)
Ion Spray Voltage	5.5 kV	-4.5 kV
Curtain Gas	10	10
Gas1 & Gas2	60 & 50	60 & 50
Temperature	550℃	450℃
Interface Heater	ON	ON
Collisionally activated dissociation	5	5

Table 3.4 The physical and chemical characteristics of compounds

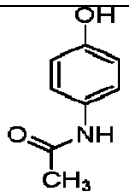
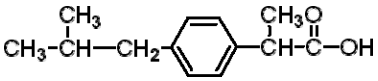
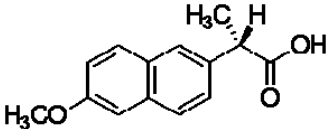
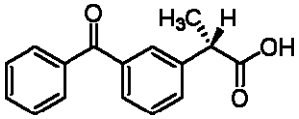
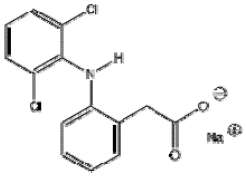
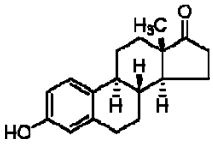
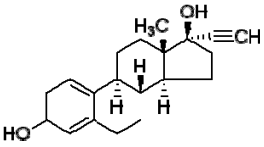
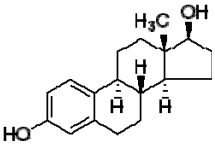
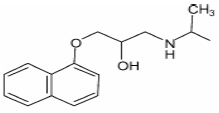
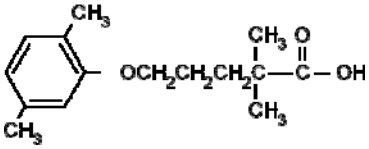
Compound	CAS No.	Log Kow	MW	Structure
Acetaminophen	103-90-2	0.49	151.2	
Ibuprofen	15687-27-1	3.79	206.3	
Naproxen	22204-53-1	3.1	230.3	
Ketoprofen	22071-15-4	3	254.3	
Diclofenac	15307-79-6	0.7	318.13	
Estrone	53-16-7	3.13	270.37	
17α-Ethynylestradiol	57-63-6	3.67	296.4	
17β-Estradiol	50-28-2	4.01	272.39	
Propranolol	525-66-6	1.20-3.48	295.8	
Gemfibrozil	25812-30-0	4.77	250.3	

Table 3.5 MRM pairs and mass spectrum parameters

Compound	m/z	product ion	DP	EP	CE	CXP
Acetaminophen	152	93	54	10	32	4.1
		110[M-CH2-CO+H]+	54	10	24.1	6.8
Ibuprofen	205	161[M-H-CO2]-	38	10	10	8
Naproxen	229	169.8[M-H-C2H3O2]-	33	10	22	13
		184.9[M-H-CO2]	33	10	9.5	15
Ketoprofen	253	208.9[M-H-CO2]-	26	10	9	9
		197[M-H-C2O2]-	26	10	7	10
Diclofenac	294	250[M-H-CO2]-	40	10	15	13
		214[M-H-ClCO2]-	40	10	28	10
Estrone	271	133	68	10	35	11
		253[M-H2O+H]+	68	10	20	6
17α-Ethynylestradiol	279	133	60	10	25	12
		159	60	10	30	12
17β-Estradiol	255	133	66.8	10	25.7	14.7
		159	66.8	10	28.3	7.2
Propranolol	260	116[N-isopropyl-N-2-hydroxypropylamine+H]+	70	10	26.5	8.7
		183[M-H2O-C3H7NH]+	70	10	27	15
Gemfibrozil	249	120.9[M-H-C7H12O2]-	50	10	17	7
		126.9	50	10	14	7

3.4 PEC calculations

The typical formula used to estimate predicted environmental concentrations (PECs) is shown below:

$$\mathrm{PEC}(\mu\mathrm{g/L}) = \frac{\mathrm{A}*(100-\mathrm{R})}{365*\mathrm{P}*\mathrm{V}*\mathrm{D}*100}$$

which is adopted and modified from the European Agency for the Evaluation of Medicinal Products (EMEA) guidelines (Jones, Voulvoulis et al. 2002; Ashton, Hilton et al. 2004; Castiglioni, Fanelli et al. 2004). In the formula, **A** is the amount used per year, **R** is the removal rate in percentage, **P** is the number of inhabitants around the target district, **V** is the volume of wastewater per day per capita, **D** is the dilution factor set to be 10 in the environment and **100** is the conversion factor for percentage.

In this study, the usage amount of pharmaceutical **A** was from database from Bureau of National Health Insurance. Medicine prescribed in 2005 for all the target compounds were used except for that of ketoprofen. Because no data of ketoprofen was found in year 2005, 2002 data was used for the estimation. **P** is the number of inhabitants in Sin-Dian city, Ueong-Ho city, Chung-Ho city, and Wen-Shan district of Taipei city. The data is collected from internet. **V** is the volume of wastewater per day per capita. **D** is the dilution factor set to be 10 in the environment (the data are shown in table 3.7) (Jones, Voulvoulis et al. 2002; Ashton, Hilton et al. 2004). The removal rate, **R**, is the hardest part to be decided in the formula. I assumed there were 2 different source pathways of PPCPs; one is taken by human beings and then excreted and released, and the other is released directly to the environment without digested by human beings (**Fig. 3.2**). Their proportions are assumed to be 80% and 20% respectively based on the reasons that I supposed most people would take large amount of their prescription medicine, however, the charge for health insurance in Taiwan is relative low, many elder

people liked to ask for a lot of medicines just for satisfaction, and it would lead to a bad circumstance that the medicines were easy to be overdue and would be dumped directly to the environment by many pathways. That is why I assumed such proportions as 80% for through human bodies and 20% for directly getting into the environment. Parts of the PPCPs taken by human could be absorbed to body, being transformed to metabolites or directly being excreted to the environment. Castiglione, Fanelli et al. reported that some of the active metabolites which can be hydrolysed and reconverted to their parent compounds must also be considered. However, in this study, I excluded the possibility of reconversion of conjugates and only looks at the residual parent compounds being excreted(Castiglioni, Fanelli et al. 2004).

These residuals entering the natural environment are assumed to undergo some of the natural attenuation processes and degraded. The removal rates of attenuation are decided by the half-lives of their possible natural attenuation mechanisms reported from literatures. Since the rivers at Taiwan flow at relative fast rates and sunlight is not always strong in Taipei, I assume that the removal rate to be 60% if the reported half-life is under 5 hours, 30% for half life between 5 hours to 10 hours; no removal whe half life is over 10 hours. The special case is ketoprofen which half-life is too short (few minutes) and it would be photodegraded very fast when it got into the environment, so the attenuation rate is determined as 99% (**Table 3.6**) (Lin and Reinhard 2005; Nakada, Komori et al. 2007). Additionally, since there is no metabolite data of estrone, I assumed it is 100% released as parent compound. **Table 3.7** summarized all the information used for PEC calculations.

Table 3.6 Data of removal rates

PPCPs	Total removal rate (%)	Removed by transforming to metabolites (%)	Removed through natural attenuation(%)
Acetaminophen	76.8	96[a]	0[b], biodegradable steadily
Ibuprofen	76.8	96[c]	0[e], 14.8±0.7 h
Naproxen	71.2	35[d]	60[e], 1.4±0.10 h
Ketoprofen	99.16	20[d]	99[e], 4.1±0.13 min
Diclofenac	87.2	85[f]	60[c], half-life<1day, photodegradation t1/2=4h
Estrone	60	not available	60[e], 2.3±0.07 h
17α-Ethynylestradiol	83.68	74[a]	60[e], 2.3±0.11 h
17β-Estradiol	90.4	95[a]	60[e], 2.0±0.14 h
Propranolol	91.68	99[f]	60[e], 1.1±0.04 h
Gemfibrozil	40	50[f]	0[e], 14.8±0.70 h

a.(Johnson, Belfroid et al. 2000) b.(Schowanek and Webb 2002) c.(Ashton, Hilton et al. 2004) d.(Bendz, Paxeus et al. 2005) e.(Lin and Reinhard 2005)

f.(Ternes 1998)

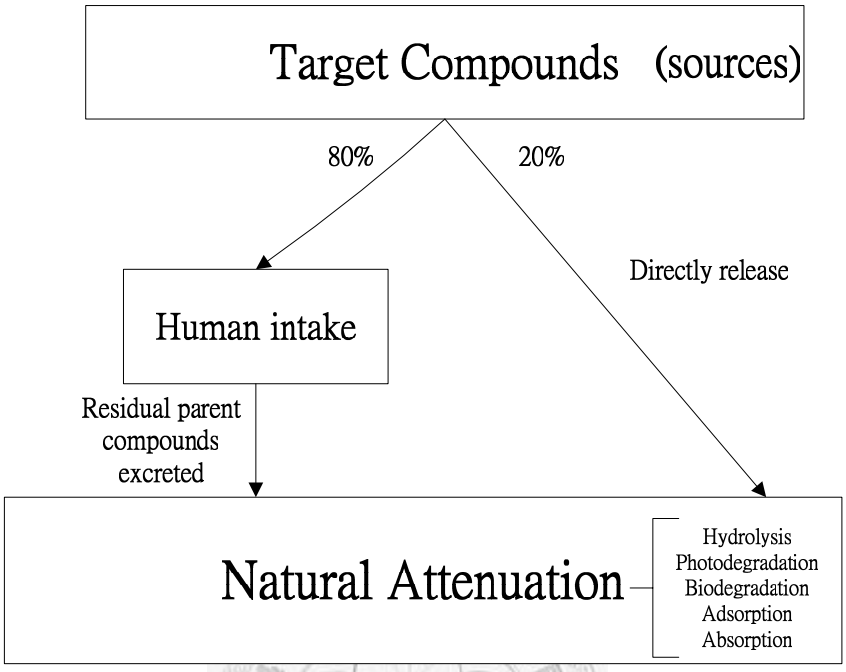


Figure 3.2 Pathways of pharmaceutical compound

Table 3.7 Information for calculating PEC

Compounds	PEC(μg/L)	A-Amount used per year(kg/yr)	R-Removal rate
Acetaminophen	848.1	142278.0	76.8
Ibuprofen	313.2	52543.8	76.8
Naproxen	27.3	3685.9	71.2
Ketoprofen	1.7	*7914.0	99.16
Diclofenac	21.1	6400.9	87.2
Estrone	0.0002	0.0189	60
17α-Ethinylestradiol	0.0001	0.0281	83.68
17β-Estradiol	0.0222	8.9821	90.4
Propranolol	8.4	3916.0	91.68
Gemfibrozil	173.5	11256.9	40
V-Volume of waster per capita and day (lpcd)		560898682.6	
P-Number of inhabitants along the river		1198800	
D-Diluton factor		100	

*The usage data of ketoprofen in 2005 is lack, so I used 4 times of the 2002.1~3 usage quantity to replace it

Ref. (Lin, Lin et al. 2007)Establishing Analytical Methods for Pharmaceuticals in the Aquatic Environments (http://www.ws1hr.taipei.gov.tw/; Wen-Shan) ,
(Agency; http://wuss.wra.gov.tw/livewater.asp; http://www.ris.tpc.gov.tw/_file/1392/SG/24964/38972.html)

Chapter 4 Results and Discussion

4.1 Significance of the sampling points

Dan-Sui River basin is the biggest river basin in northern Taiwan, and comprises three main rivers: Da-han River, Sin-Dian River and Keelung River. Based on the data of Taipei water department (http://www.twd.gov.tw/news/200504/), 97% of more than 4 million people's livelihood water is supplied by Sin-Dian River. The main tap water supplier of Taipei city is Chin-tan weir which is on the upstream of Sin-Dian River. The upstream of Sin-Dian River is the watershed of Fei-Tsui reservoir. Based on these reasons, it is important to monitor the occurrences of PPCPs in Sin-Dian River.

4.2 Optimization of HPLC-MS/MS analysis

In this study, the multi-compounds analysis method was developed to determine the occurrences of PPCPs in Taiwan surface water systems. In HPLC-MSMS analysis, fine chromatographic separation of the target compounds was acquired from optimizing the composition of mobile phases and the gradient elution program. **Figure 4.1** and **figure 4.2** present total ion chromatograms (TIC) for all compounds in solvent (50 % methanol and 50% D.I. water) .**Figure 4.3** and **figure 4.4** demonstrated the retention times of the target compounds in the positive ion and negative ion mode and were in the range of 4 to 7 min and 6 to 8 min, respectively. In tandem-MS mode of this study, based on the peak for all target compounds, the positive model $[M+H]^+$ and negative model $[M-H]^-$ were determined and selected as precursor ions. From multiple reactants monitoring (MRM) mode, it is useful that the analyses are confirmed and quantitatively determined

by precursor ions and their product ions. The selected product ions with the highest intensity in the mass spectra were listed in **figure 4.5** to **figure 4.14**. Identification and quantization of the target pharmaceuticals were by comparing LC retention time and the chosen MRM transitions.

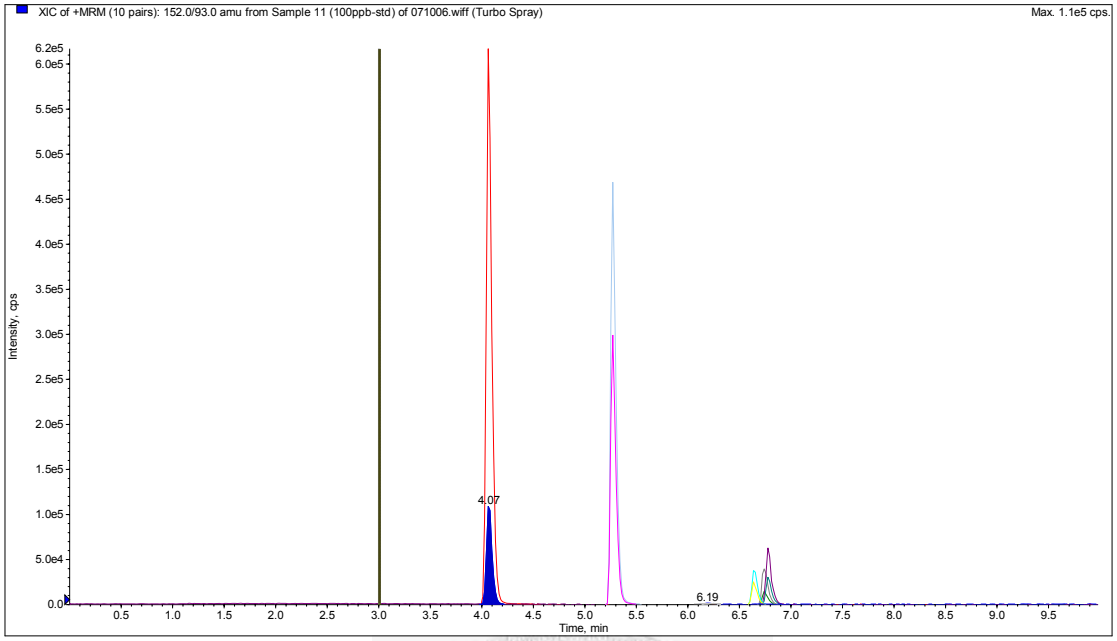


Figure 4.1 The TIC chromate graphs of 10 target compounds in ESI (+) model of LC –MS/MS

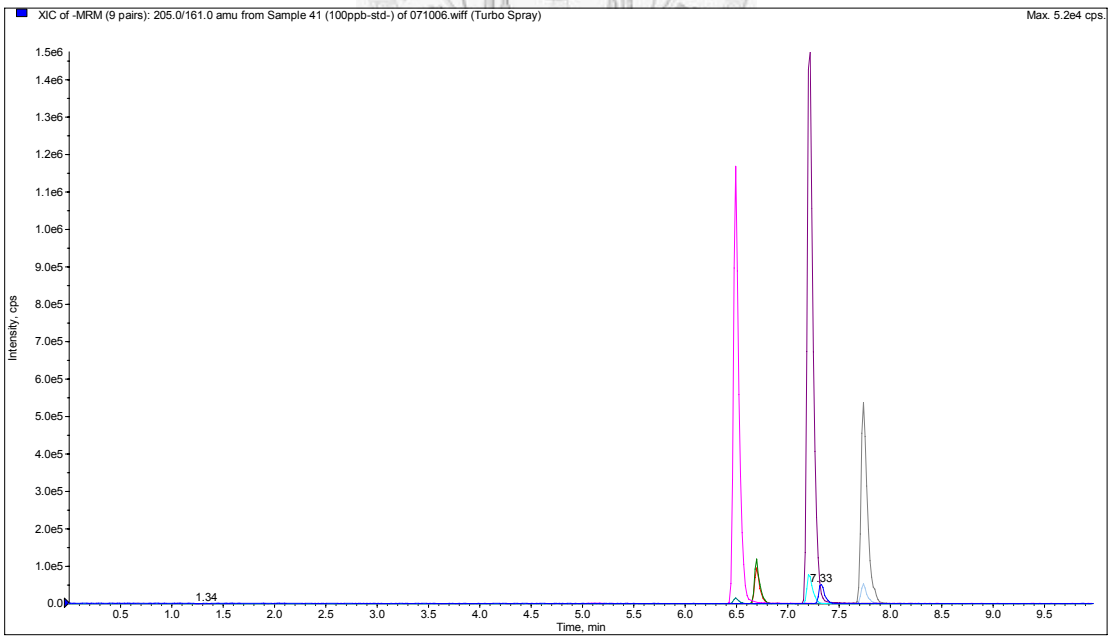


Figure 4.2 The TIC chromate graphs of 10 target compounds in ESI (-) model of LC –MS/MS

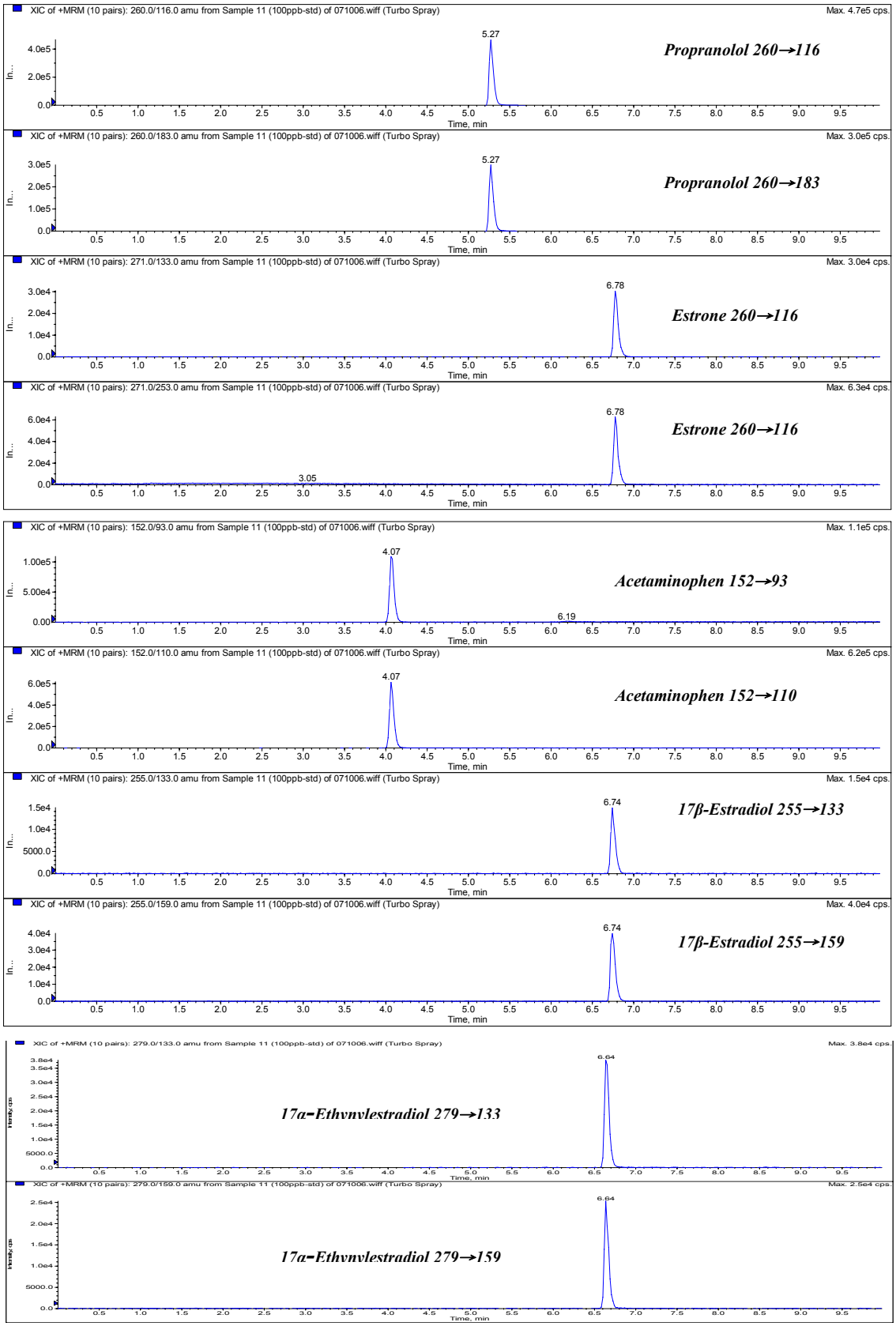


Figure 4.3 The EIC graphs of 5 target compounds under ESI (+) model by LC-MS/MS

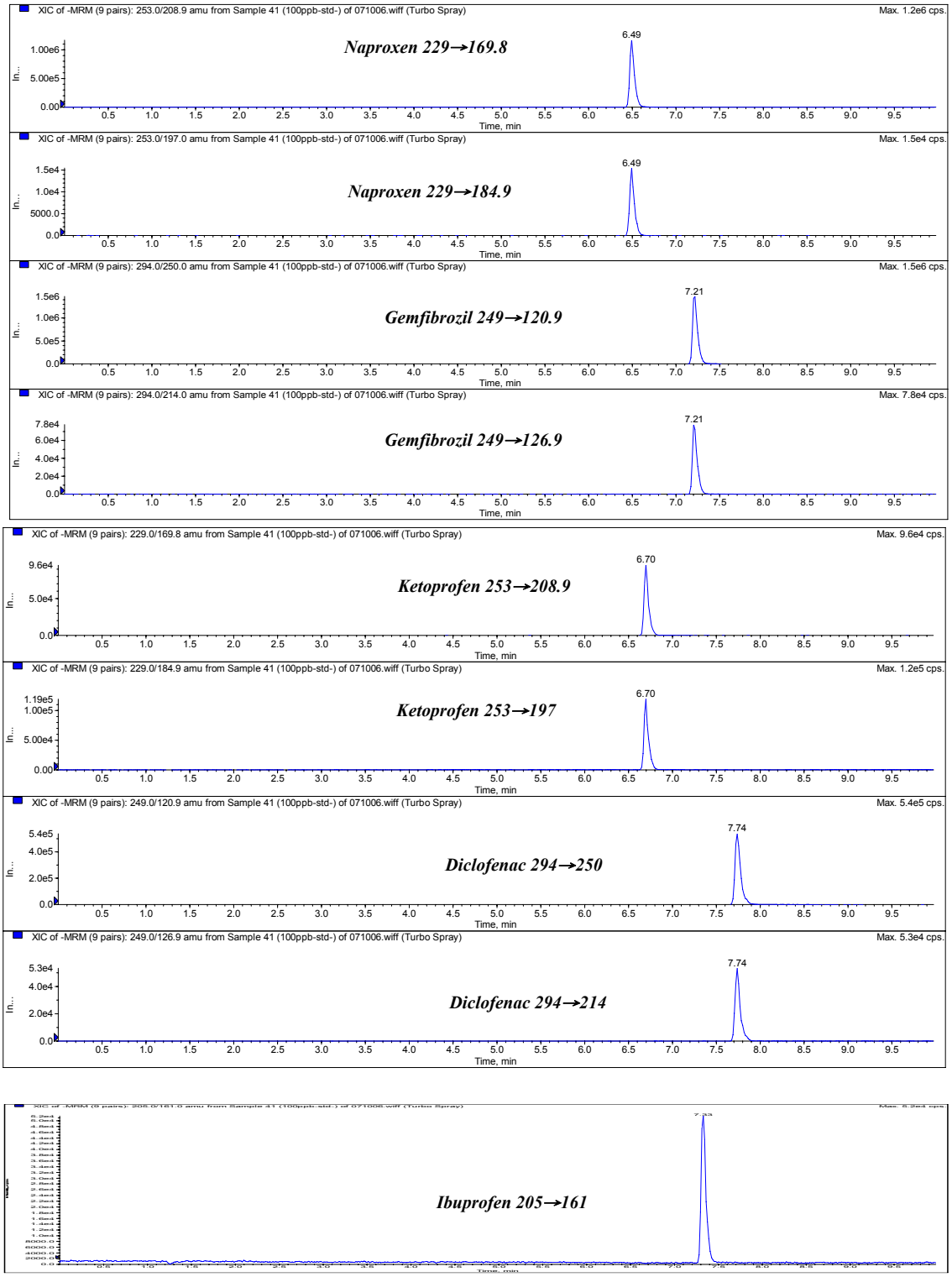


Figure 4.4 the EIC graphs of 5 target compounds under ESI (-) model by LC-MS/MS

■ Positive mode

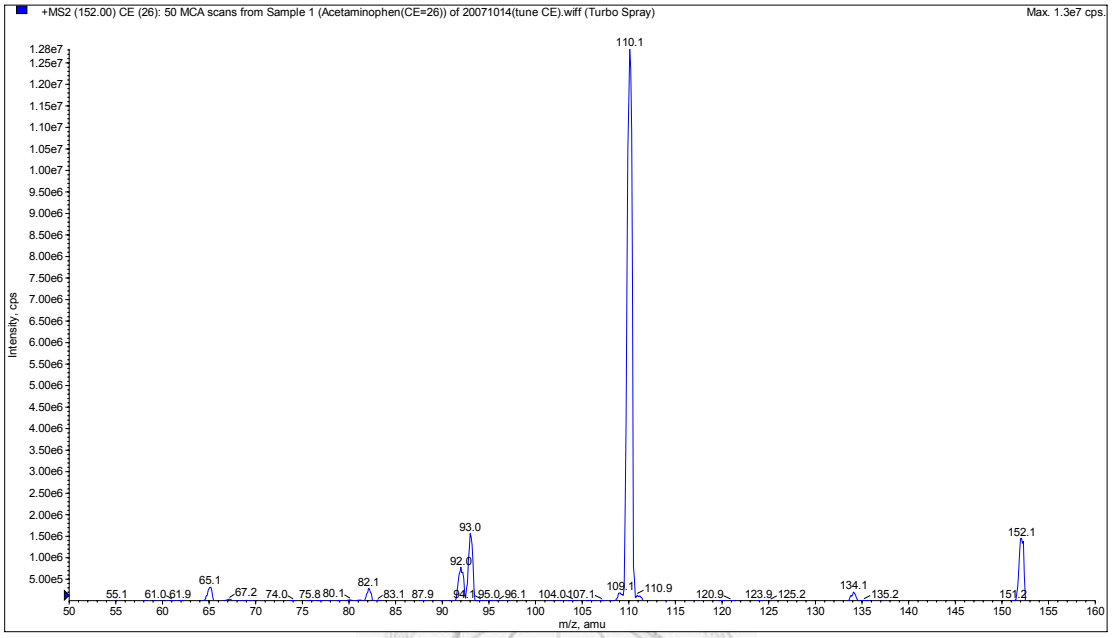


Figure 4.5 The mass spectrogram of Acetaminophen and its product ion on ESI(+) mode

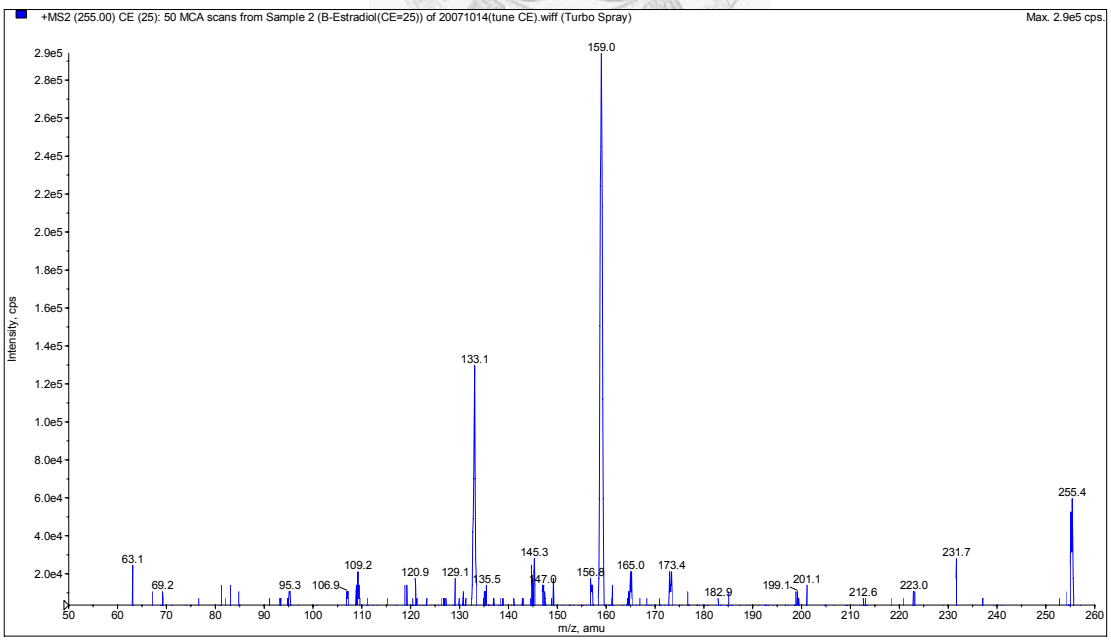


Figure 4.6 The mass spectrogram of 17 β -Estradiol and its product ion on ESI(+) mode

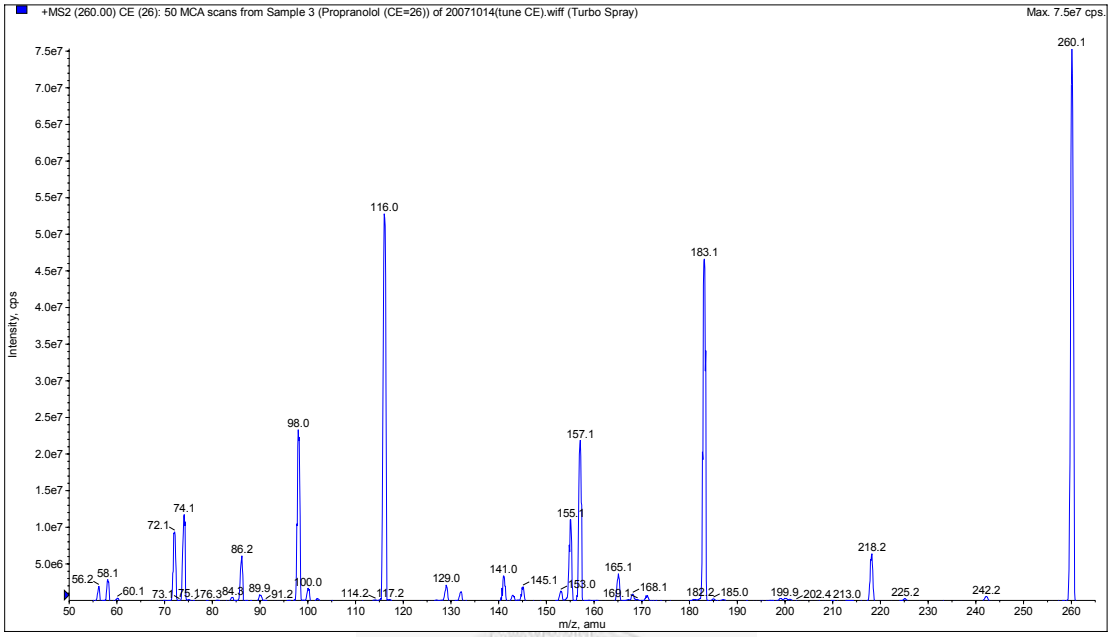


Figure 4.7 The mass spectrogram of Propranolol and ,its product ion on ESI(+) mode

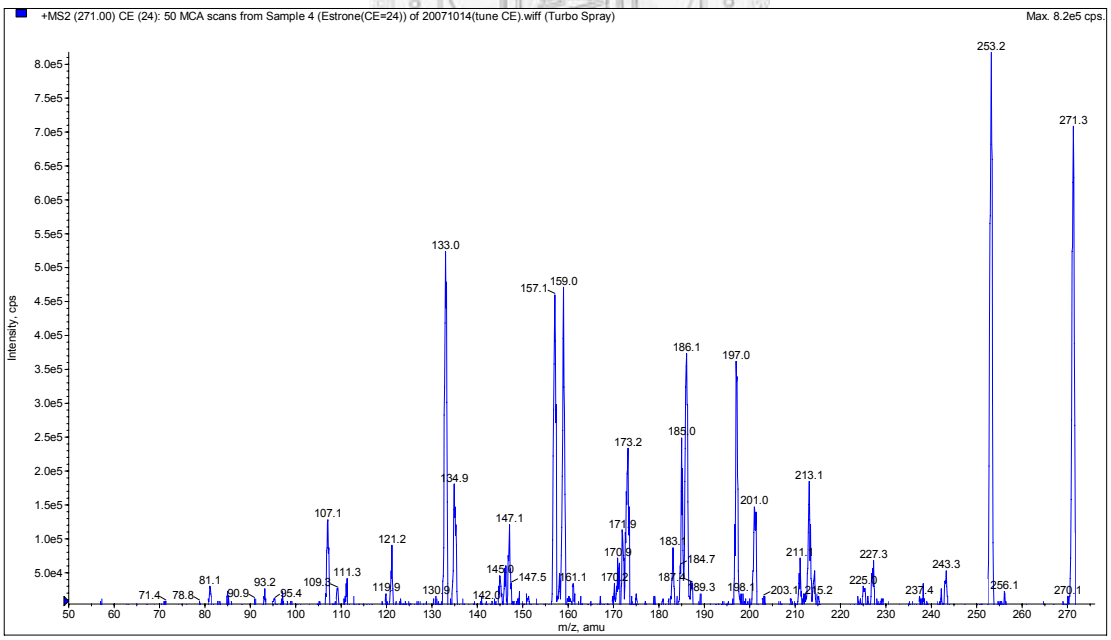


Figure 4.8 The mass spectrogram of Estrone and its product ion on ESI(+) mode

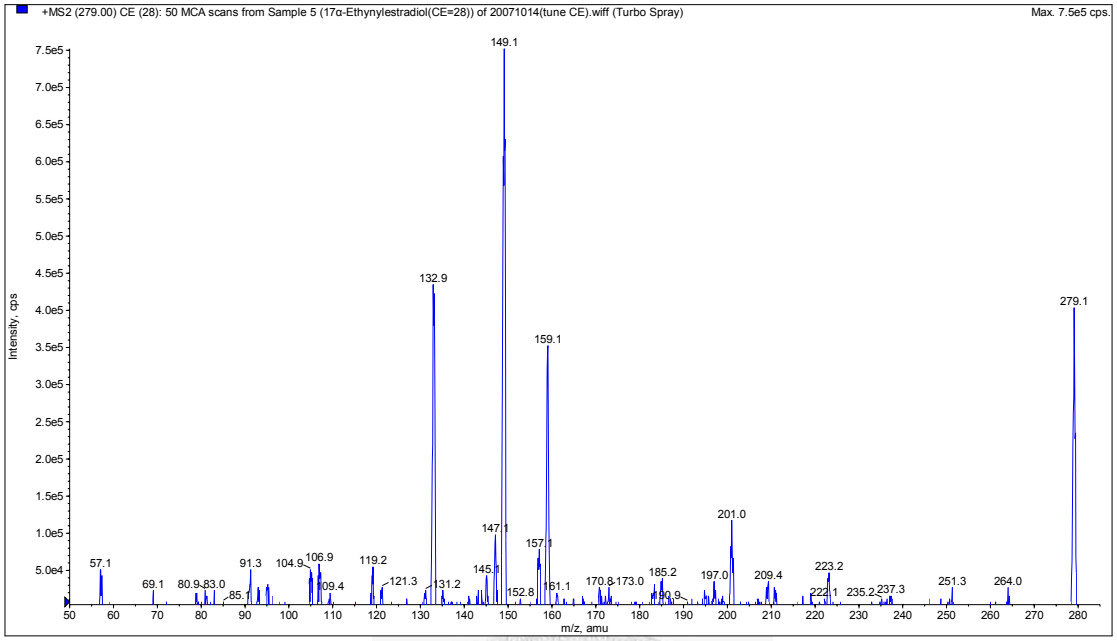


Figure 4.9 The mass spectrogram of 17α-Ethynylestradiol and its product ion on ESI(+) mode

■ Negative mode

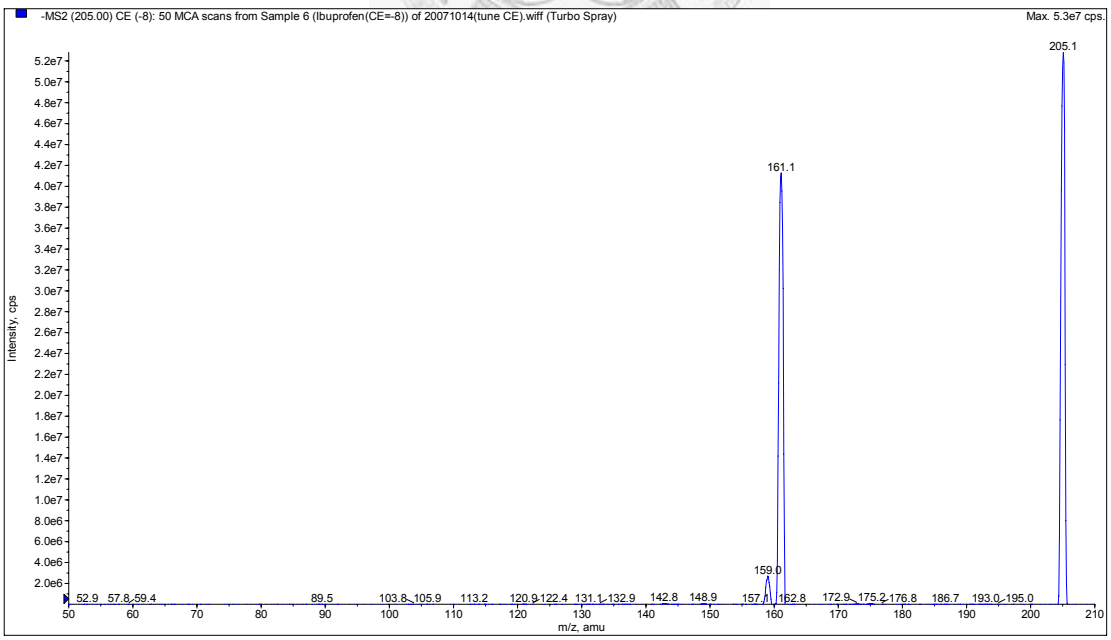


Figure 4.10 The mass spectrogram of Ibuprofen and its product ion on ESI(-) mode

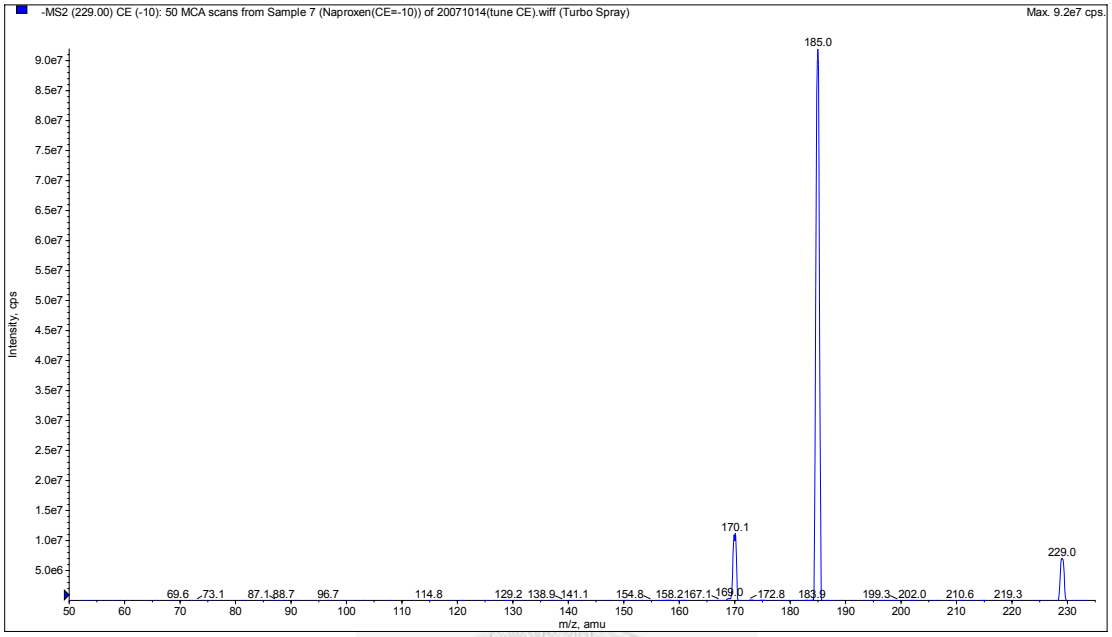


Figure 4.11 The mass spectrogram of Naproxen and its product ion on ESI(-) mode

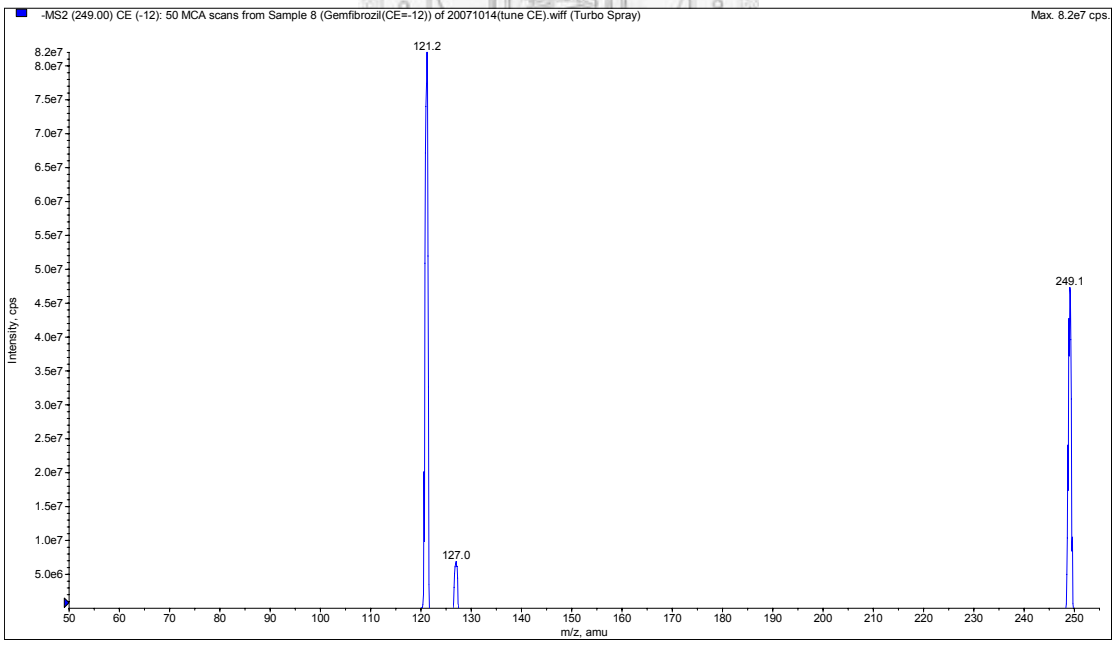


Figure 4.12 The mass spectrogram of Gemfibrozil and its product ion on ESI(-) mode

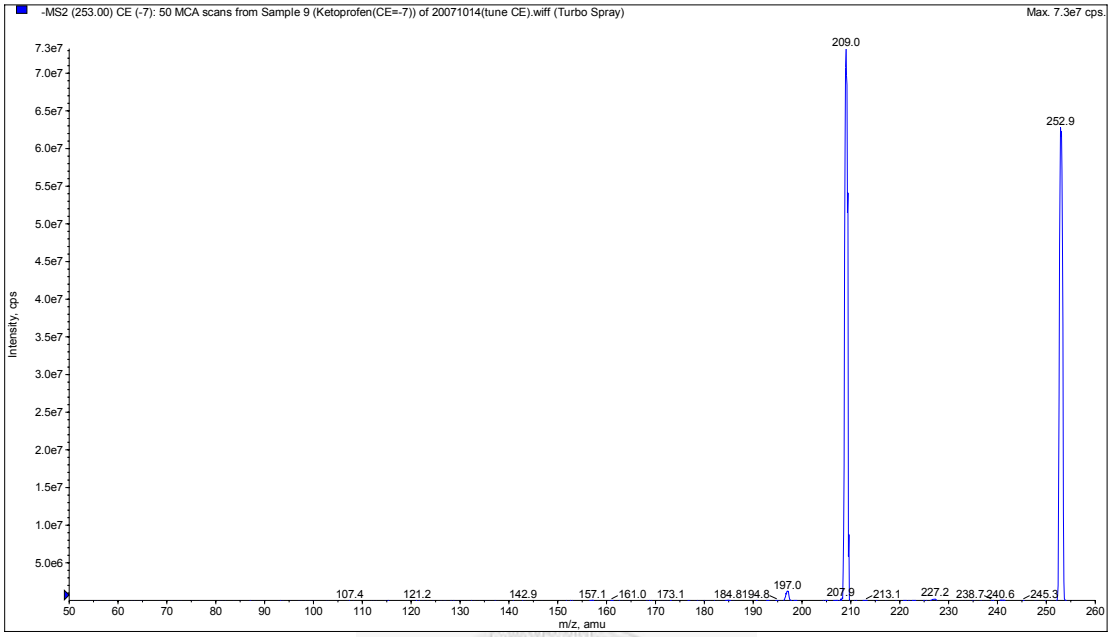


Figure 4.13 The mass spectrogram of Ketoprefen and its product ion on ESI(-) mode

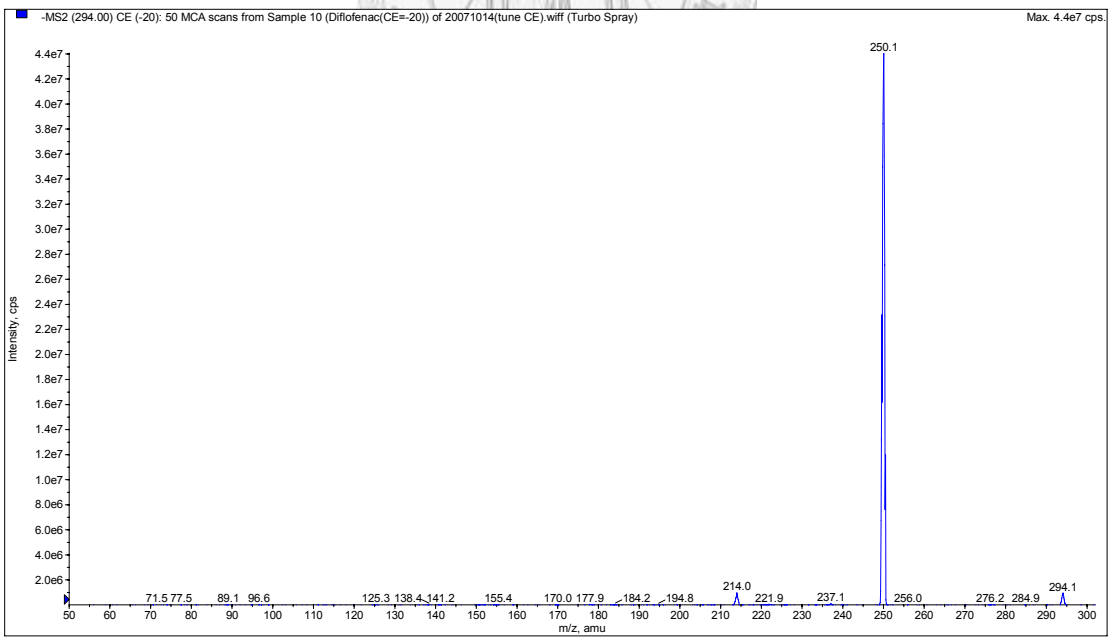


Figure 4.14 The mass spectrogram of Diclofenac and its product ion on ESI(-) mode

4.3 Method recovery, Method detection limits, Quality control and Quality assurance

In this study, high rates of recovery were obtained by varying several parameters. First, we tested the effect of pH values (pH 4.0, 6.0, 7.0, and no adjusting) before experimenting, and the results indicated that the recovery of compounds was higher at pH 7.0. The result is similar with M. Jose Gomez et al. who found the highest recovery at pH 7.0. (Gomez, Petrovic et al. 2006). There were two kinds of cartridges, 50 mg and 500 mg, and we had compared it to see which one renders the better performance. The result showed 500mg cartridge was more effective. The standards' concentration of level 50 and 500 ng/L were spiked into D.I. water and blank sample at pH 7.0 to determine the recoveries of the spiked target compounds. The recoveries (mean of three replicate samples) and standard deviation of the target compounds are presented in **table 4.1**. In D.I. water sample, the recovery ranged from 66.1 to 126.3 % when the concentration of standard was 50 ng/L and 67 to 115 % when the concentration of standard was 500 ng/L; in blank sample, the recovery ranged from 67.1 to 139.7 % when the concentration of standard was 50 ng/L and 68.3 to 110.3 % when the concentration of standard was 500 ng/L; We could conclude it to a result that better recoveries could be acquired when high concentrations of standards were applied.

Table 4.1 The recoveries of target compounds

Compound	**Recovery (%) (n=3) in D.I. sample**		**Recovery (%) (n=3) In Blank water sample**	
	50 ng/L	**500 ng/L**	**50 ng/L**	**500 ng/L**
Positive mode	**Mean±SD (%)**		**Mean±SD (%)**	
Acetaminophen	98.8 ± 2.8	100.9 ± 2.1	101.2 ± 2.4	100.3 ± 0.6
17β-Estradiol	113.3 ± 3.1	103.7 ± 3.5	118.3 ± 14.0	101.4 ± 2.9
Propranolol	73.0 ± 1.9	78.6 ± 2.6	94.0 ± 2.7	94.6 ± 0.8
Estrone	69.4 ± 4.1	72.0 ± 3.5	71.5 ± 2.2	70.7 ± 1.1
17α-Ethynylestradiol	112.7 ± 9.5	100 ± 2.2	102.3 ± 9.4	97.3 ± 0.9
Negative mode				
Ibuprofen	94.1 ± 6.1	84.7 ± 5.1	139.7 ± 6.7	116.3 ± 5.7
Naproxen	99.5 ± 4.9	86.0 ± 3.7	97.6 ± 3.0	80.9 ± 0.2
Gemfibrozil	66.1 ± 0.5	67.0 ± 1.3	67.1 ± 4.9	68.3 ± 7.1
Ketoprofen	113.7 ± 3.5	104.0 ± 3.0	121.7 ± 7.2	99.1 ± 1.7
Diclofenac sodium	126.3 ± 2.5	115.0 ± 2.6	136.0 ± 2.0	110.3 ± 8.3

The accuracy and precision are lower than 20 %. The accuracy and precision were determined by the replicate samples (n=6) of blank sample extracts spiked at 50 and 500 ng/L over a period of 3 days. The parameters are showed in **table 4.2**. The accuracy range at concentration levels of 50 and 500 ng/L were -13.3 to 18.4 % and -8.0 to 17.8 %, respectively. The precision ranged from 1.0 to 13.9 % for 50 ng/L and 0.5 to 6.8 % for 500 ng/L. These values are all well within the acceptable range.

The linearity of calibration curves was established using blank sample spiked with analyses and fitted a linear mode, least-squares linear regression analysis (y=a+bx) in the concentration range studied. For all target compounds, nine points (0.5, 1, 5, 10, 50, 100, 500, 1000, 2000 ng/L) calibration curves were constructed. The method detection limit (MDL) was determined from the minimum detectable concentration of analyses in the linear range with a signal-noise ratio of 3. The method validation parameters are presented in **table 4.3**. The method detection limits of acetaminophen, propranolol and gemfibrozil were 0.2 ng/L; of naproxen was 2 ng/L; of ketoprofen and diclofenac were 5 ng/L; of estrone, 17α-ethylnylestradiol, 17β-estradiol, and ibuprofen were 10 ng/L. The calibration curves were linear with correlation coefficients of all target compounds greater than 0.99.

Table 4.2 The mean concentrations, accuracies and precisions of target compounds

Compounds		Day 1			Day2			Day3		
	Spike Conc. (ng/L)	Mean Conc. (ng/L)	Accuracy (%)	Precision (%)	Mean Conc. (ng/L)	Accuracy (%)	Precision (%)	Mean Conc. (ng/L)	Accuracy (%)	Precision (%)
Acetaminophen	50	58.6	17.1	2.7	52.2	4.4	1	54.2	8.4	3
	500	577	15.4	2.9	534	6.8	2.7	535	7	3
17β-Estradiol	50	56	11.9	13.9	53.6	7.2	8.7	52.7	5.4	4.2
	500	557	11.4	2.2	594	18.7	4.6	560	12	4.8
Propranolol	50	59.2	18.4	2.6	58.1	16.2	3.9	57.4	14.8	6
	500	581	16.2	0.5	571	14.2	3.3	576	15.3	3.2
Estrone	50	54.2	8.3	11	55.3	10.6	5.2	51.6	3.2	3.7
	500	508	1.6	3.4	570	14.1	3.1	543	8.7	5.3
17α-Ethynylestradiol	50	47.2	-5.6	6.3	56.3	12.6	4	56.3	12.6	1.9
	500	502	0.5	2.9	593	18.6	5.1	559	11.7	3.5
Ibuprofen	50	48.4	-3.3	1.8	54.5	9	6.1	54.9	9.8	3.8
	500	509	1.9	3.5	532	6.4	6.5	589	17.8	6.8
Naproxen	50	43.4	-13.3	4.2	47.7	-4.6	3.7	53.7	7.4	2.5
	500	460	-8	3.1	490	-2	4.3	540	8	2.7
Gemfibrozil	50	47.7	-4.7	5.1	50.3	0.6	7.1	55.6	11.3	9.3
	500	477	-4.6	4	494	-1.2	4.8	559	11.8	5.4
Ketoprofen	50	47.5	-4.9	3.7	53.3	6.6	4.8	52.5	5	6.7
	500	471	-5.9	3.6	570	14.1	4.9	548	9.6	4.4
Diclofenac	50	53.7	7.4	6.2	55.3	10.6	5.9	54.2	8.3	6.9
	500	537	7.4	0.9	535	6.9	5.9	550	10	3.7

Table 4.3 The MDLs and linear range

Compounds	MDL(ng/L)	Linear (ng/L)	r
Acetaminophen	0.2	0.2~2000	0.9998
Ibuprofen	10	10~2000	0.9993
Naproxen	2	2~2000	0.9922
Ketoprofen	5	5~2000	0.9997
Diclofenac	5	5~2000	0.9991
Estrone	10	10~2000	0.9985
17α-Ethynylestradiol	10	10~2000	0.9998
17β-Estradiol	10	10~2000	0.9999
Propranolol	0.2	0.2~2000	0.9999
Gemfibrozil	0.2	0.2~2000	0.9987

4.4 Occurrences of selected compounds

For the ease of discussion, target compounds were grouped into three categories based on their therapeutic classes; the first one is NSAID group which includes acetaminophen, ibuprofen, naproxen, ketoprofen and diclofenac; the second group is the estrogen group which includes estrone, 17α-ethylnylestradiol and 17β-estradiol, and others are grouped into the third group which includes propranolol and gemfibrozil. **Table 4.4** summarizes the concentrations of ten target compounds at six sampling locations measured day and night for three consecutive weeks alone Sin-Dian River. The minimum, maximum, and median for n =36 data points were calculated and reported.

Occurrence of NSAIDs: NSAIDs were mostly detected with high concentrations with acetaminophen ranging from 8.3 ng/L to 9170 ng/L and ibuprofen ranging from n.d. to 4350 ng/L, The concentrations of acetaminophen measured didn't agree well with other researches; Boyd et al. only found trace amount of acetaminophen (maximum 0.2 ng/L (Boyd, Reemtsma et al. 2003) and Nakada et al. found acetaminophen up to 52 ng/L(Nakada, Komori et al. 2007). Apparently, the concentrations of acetaminophen detected in Taiwan were much higher than that of other countries. The concentrations of ibuprofen detected by Thomas and Hilton ranging from n.d. to 928 ng/L, and they were similar to my data since the median concentration of ibuprofen is 231.5 ng/L (Thomas and Hilton 2004). Naproxen and diclofenac are the compounds with the lower concentrations by tens of ng/L in this group; however, the median concentration of naproxen (65.5 ng/L) is approximately 6 times bigger than of diclofenac (12.9 ng/L). Ternes had detected naproxen and diclofenac with median concentrations of 70 ng/L and 150 ng/L, respectively in 1998, and the result of naproxen was similar to mine,

however, the result of diclofenac didn't match that of mine (Ternes 1998). In the case of ketoprofen which almost absent in Sin-Dian River, was found to have a similar results in Ternes, 1998 and Nakada et al., 2007 with median concentrations of ketoprofen as n.d. by Ternes and 24 ng/L (mainstream) and n.d. (tributary) by Nakada et al.(Ternes 1998; Nakada, Komori et al. 2007).

Occurrence of Estrogens: Estrogens include estrone, 17α-ethylnylestradiol and 17β-estradiol. In my research, it was similar to other researchers in the world that hormone compounds stayed in relatively low concentrations. It could be seen from **table 4.8** that most of the times, they were absent in the water samples. The median concentrations of estrone, 17α-ethylnylestradiol and 17β-estradiol in my research were 13.05 ng/L, n.d., and 15.6 ng/L, respectively. Kim et al., 2007 had detected the three compounds with mean concentrations of estrone, 17α-ethylnylestradiol and 17β-estradiol in Korean surface water as 3.6 ng/L, n.d. and n.d., respectively(Kim, Cho et al. 2007), and Ternes et al., 1999 had not detected the three compounds with the median concentrations in 15 German rivers and the results were the same with Boyd et al., 2003 who detected no such compounds in surface waters in Louisiana, either(Boyd, Reemtsma et al. 2003). Although the median concentrations of estrone and 17β-estradiol are detected in Sin-Dian River, but after checking **table 4.5 ~ 4.7**, there were many no detection in a lot of samples. It matched others' reports that most estrogens were detected consistently under the detection limits (Ternes, Stumpf et al. 1999; Boyd, Reemtsma et al. 2003; Kim, Cho et al. 2007).

Propranolol and Gemfibrozil: Propranolol (anti-hypertensive) and gemfibrozil (lipid regulator), despite being often detected in the rivers, their aqueous concentrations were general low. The median concentrations of gemfibrozil and propranolol in this study were 115.15 ng/L and 13.05 ng/L. Kim et al., 2007 had detected the mean concentration of gemfibrozil as 6.6 ng/L (Kim, Cho et al. 2007) and Bound and Voulvoulis, 2006 had detected no propranolol in UK rivers(Bound and Voulvoulis 2006). Compared to their results, the concentrations of the two compounds were relatively high.

Variation among weeks and between day/night: According to **table 4.4,** I would like to discuss the difference of occurrences of the target compounds measured during day and night. In general, the concentration of each compound during the three weeks did not show a big day-night variation except for that of acetaminophen and ibuprofen in certain week. For example, the median concentrations of acetaminophen and 17β-Estradiol in the second week were much different from day to night; in the same way, the concentrations of ibuprofen in the third week had a big difference in the whole day. We can observe that naproxen and gemfibrozil had the similar situation in the third week and with little difference in the first and second weeks. I supposed that the regional or hospital wastewater discharge quantity was much bigger in the night than in the day in the third week. However, estrone is quite an unstable compound with a big variety of concentration trends in the day time and night time of the three weeks. In addition, the median concentrations of ketoprofen and 17α-Ethilnylestradiol were all not detected in the three weeks no matter the day or night.

According to **table 4.10**, we can find out an interesting phenomenon that after gathering all the data of the three weeks and derived the median concentration of every compound in each sampling location by day and night, than checked the result, it's

apparent that the concentration of every compound would be higher in night time than in day time. However, we can not conclude it to the result that the photodegradation is useful because if we checked the graphs of every single compound in a specific week (**Appendix 1**), there were no definite correlation between day and night. We could only summarize a result from **table 4.10** that most of the time, the concentration of every compound would be higher in the night time. I supposed that there might be two reasons. One is the discharge of regional and hospital in night time is more than in day time; the other is related to the habit of taking medicine in our country. We are used to take medicine after having dinner, and it would match the time when I went sampling.

Impact from nearby wastestreams: According to **table 4.9** and **figure 4.15~4.24**, the occurrence of every compound in each sampling point was showed. First of all, it could be found that the median concentrations of acetaminophen, ibuprofen, gemfibrozil and naproxen were the highest four compounds in every point of the river, and others' were under 40 ng/L in common. I would like to pick the four compounds to discuss in order to verify the difference of occurrence in every point. Acetaminophen and naproxen had the same trends that their concentrations descended from A1 to A4 and than increased to A6; nevertheless, ibuprofen was totally opposite to the former group that its trend was raised from point 1 to point 4 and than decreased to point 6. The trend of concentrations of gemfibrozil was straightforward which increased from A1 to A6. Why I only picked four compounds is that they had higher concentrations and might result in some risks in the environment. Moreover, the median concentrations of acetaminophen were apparently higher than other compounds in every point by nearly or more than thousands of ng/L. However, it was strange that some detected concentrations of points A4, A5 and A6 (**table 4.5 ~ 4.7**) in the second and third weeks appeared relatively low

concentrations by 8.3 ng/L to 32,4 ng/L in five samples. I supposed that there were two reasons; first one may be the timetable and quantity of regional discharge was uncertain, and the other may be the streamway was getting larger since A3. Based on the reason that there were no hospitals near points A4 to A6, I thought the biggest source affecting this area might be the regional discharge. Based on **figure 3.1,** I conclude that A1, A2, A5, and A6 were most contaminated since more regional discharge and hospital wastewater discharge points were near these points. Actually, the distances of each point are not very long so I supposed that the nearby points wouldn't have big differences.

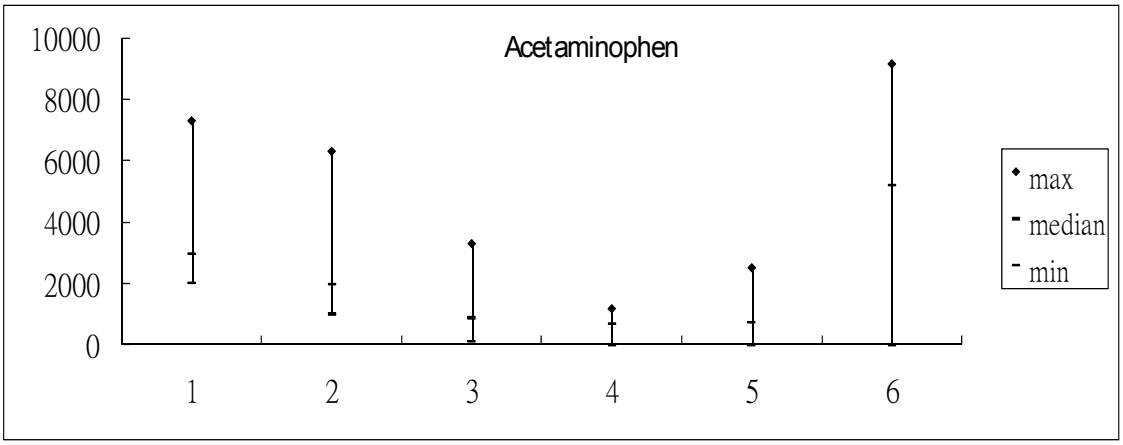


Figure 4.15 The max, min, and median concentrations of Acetaminophen along downstream Sin-Dian River

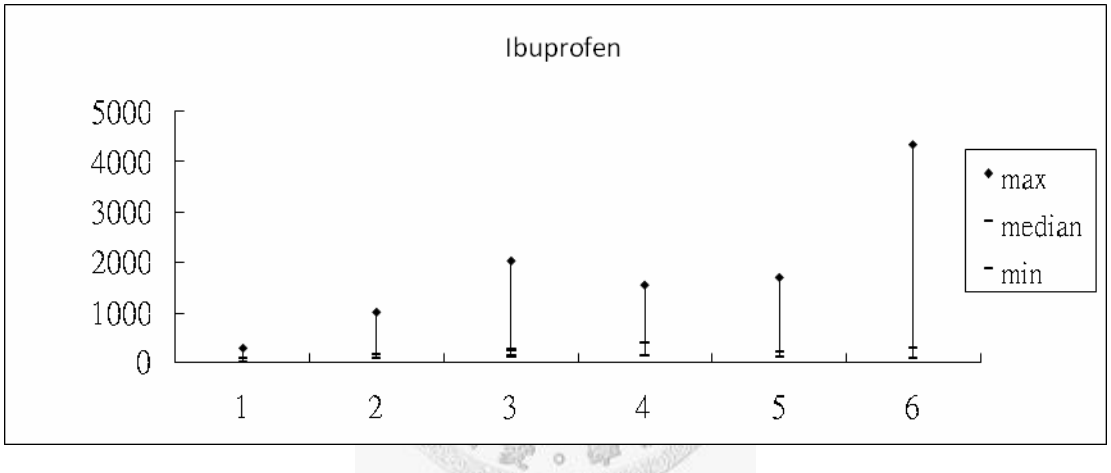


Figure 4.16 The max, min, and median concentrations of Ibuprofen along downstream Sin-Dian River

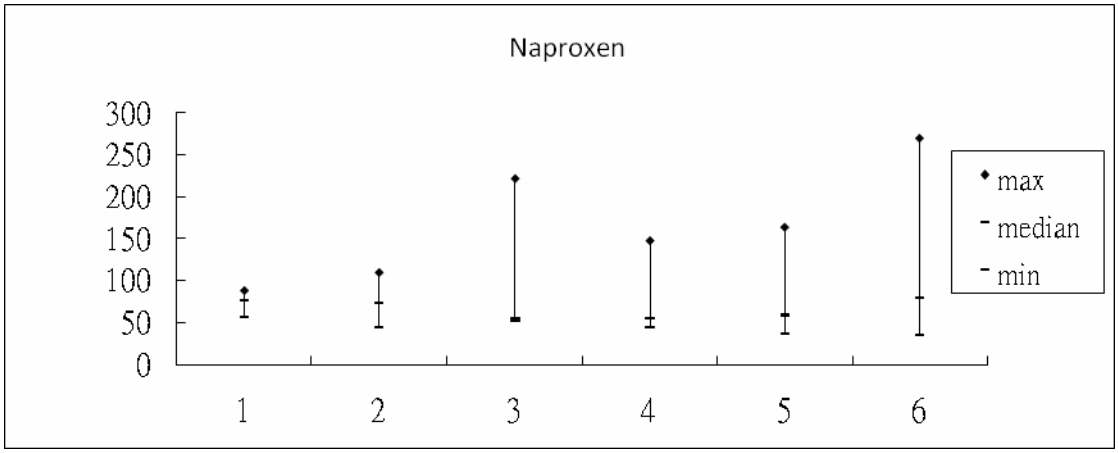


Figure 4.17 The max, min, and median concentrations of Naproxen along downstream Sin-Dian River

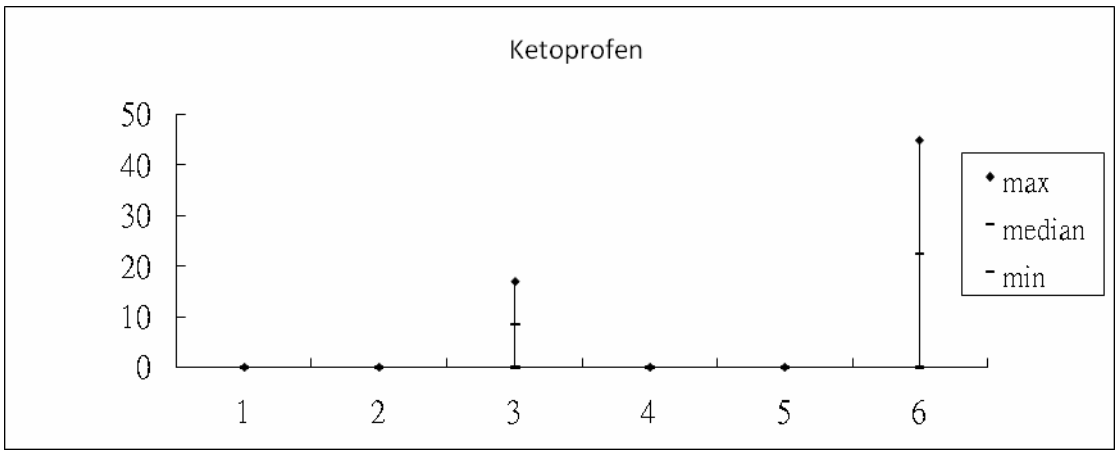


Figure 4.18 The max, min, and median concentrations of Ketoprofen along downstream Sin-Dian River

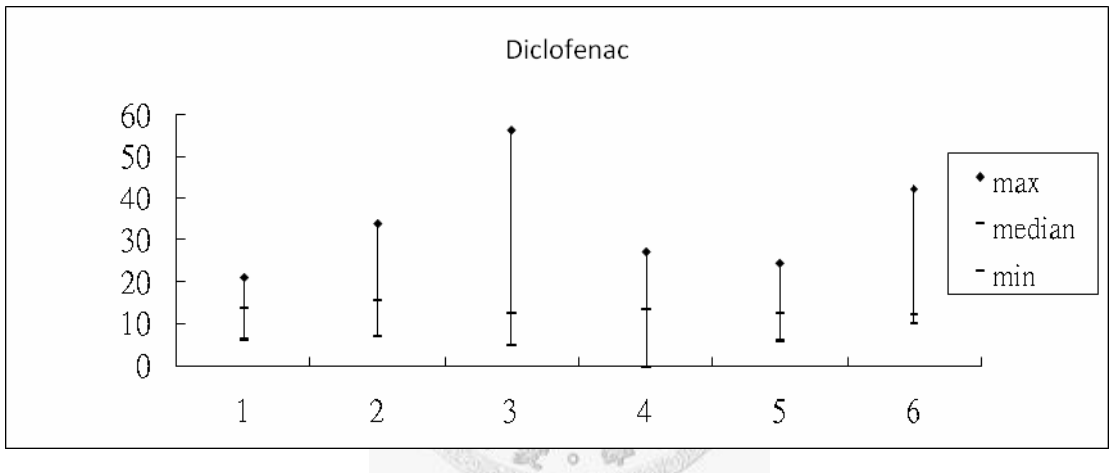


Figure 4.19 The max, min, and median concentrations of Diclofenac along downstream Sin-Dian River

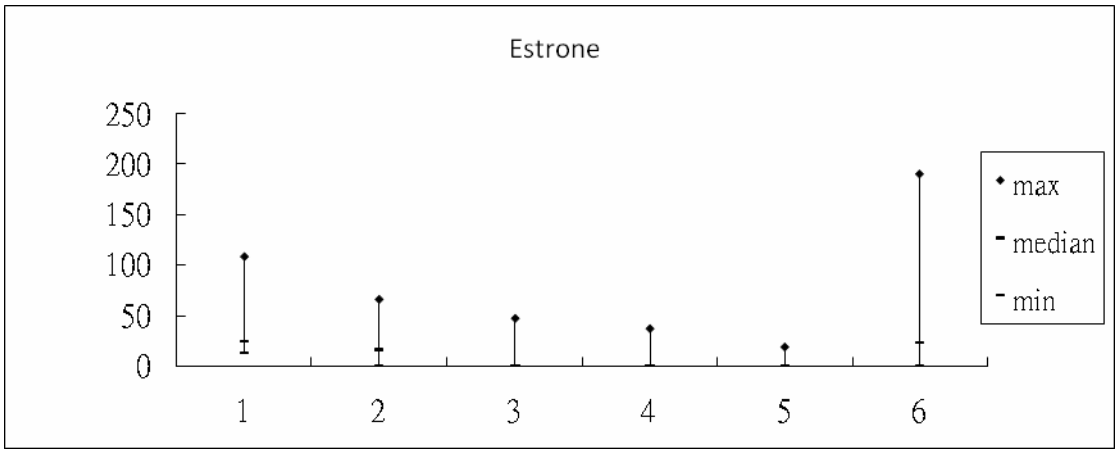


Figure 4.20 The max, min, and median concentrations of Estrone along downstream Sin-Dian River

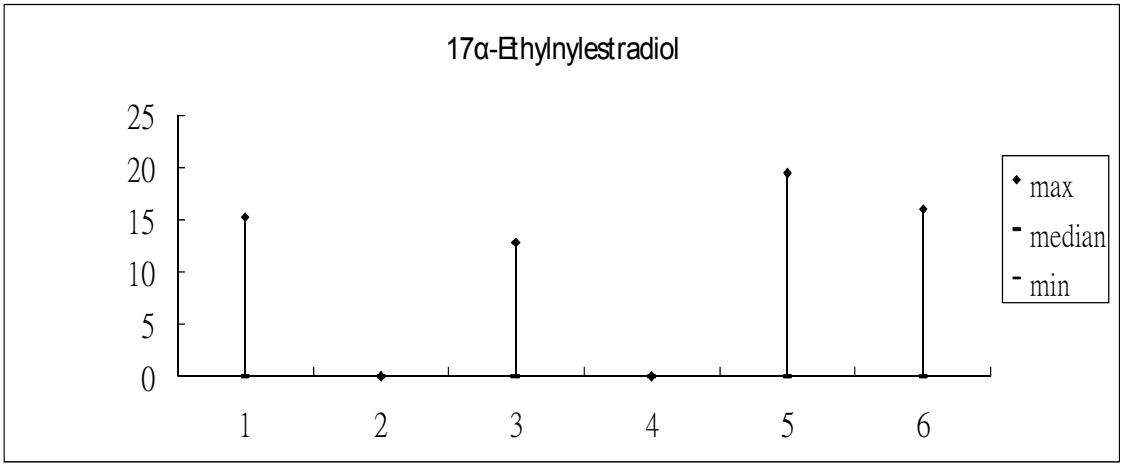


Figure 4.21 The max, min, and median concentrations of 17α-ethilnylestradiol along downstream Sin-Dian River

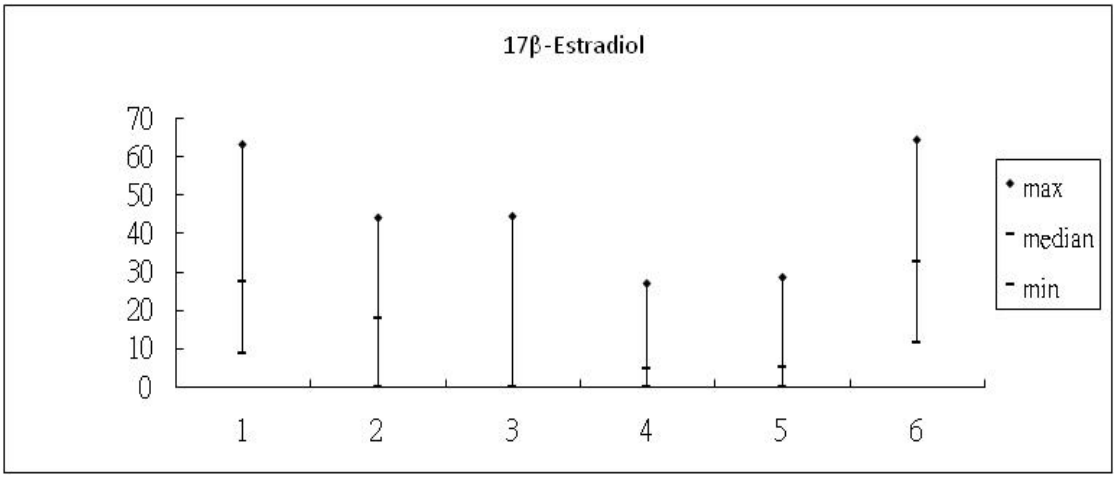


Figure 4.22 The max, min, and median concentrations of 17β-Estradiol along downstream Sin-Dian River

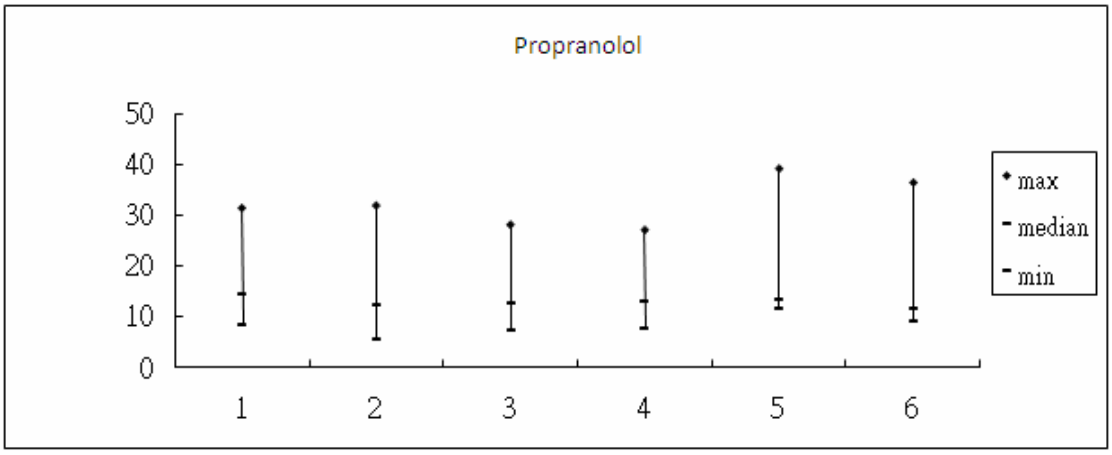


Figure 4.23 The max, min, and median concentrations of Propranolol along downstream Sin-Dian River

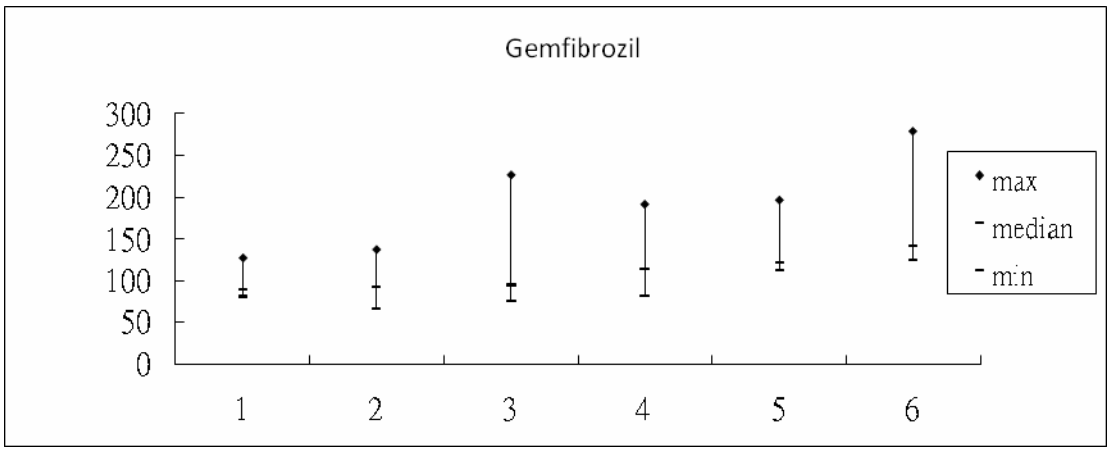


Figure 4.24 The max, min, and median concentrations of Gemfibrozil along downstream Sin-Dian River

Table 4.4 Median concentration (ng/L) of each compound in the three weeks

	Day/night	First week	Second week	Third week
Acetaminophen	Day	1540.0	512.2	1730.0
	Night	2370.0	4800.0	1930.0
Ibuprofen	Day	92.7	303.0	365.5
	Night	125.0	237.0	1635.0
Naproxen	Day	50.4	63.2	65.7
	Night	49.4	79.9	156.0
Ketoprofen	Day	n.d.	n.d.	n.d.
	Night	n.d.	n.d.	n.d.
Diclofenac	Day	8.1	10.4	14.9
	Night	7.3	23.3	24.3
Estrone	Day	12.3	n.d.	n.d.
	Night	n.d.	56.5	29.6
17α-Ethilnylestradiol	Day	n.d.	n.d.	n.d.
	Night	n.d.	n.d.	n.d.
17β-Estradiol	Day	7.9	5.3	24.4
	Night	5.0	44.5	24.6
Propranolol	Day	13.9	8.4	10.6
	Night	13.6	31.8	12.2
Gemfibrozil	Day	109.8	95.7	91.5
	Night	107.8	126.5	194.5

*n.d. is not detected

Table 4.5 Concentrations (ng/L) of selected compounds in the first week

	MDL	Day/ Night	A1	A2	A3	A4	A5	A6
Acetaminophen	0.2	Day	3680.0	1630.0	203.0	361.0	1450.0	3910.0
		Night	3100.0	2240.0	132.0	188.0	2500.0	3490.0
Ibuprofen	10	Day	53.5	80.0	109.0	120.0	105.5	79.5
		Night	83.3	76.0	107.5	152.0	169.0	142.5
Naproxen	2	Day	83.5	58.0	55.5	45.2	36.0	35.5
		Night	71.3	74.0	54.5	44.3	42.9	35.2
Ketoprofen	5	Day	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		Night	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Diclofenac	5	Day	6.5	10.1	9.6	n.d.	6.2	10.3
		Night	13.4	8.4	5.1	5.5	6.2	12.1
Estrone	10	Day	13.1	13.1	n.d.	n.d.	19.2	11.4
		Night	13.0	n.d.	n.d.	n.d.	n.d.	13.3
17α-Ethilnylestradiol	10	Day	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		Night	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
17β-Estradiol	10	Day	20.9	15.8	n.d.	n.d.	n.d.	64.7
		Night	27.6	n.d.	n.d.	10.0	n.d.	20.0
Propranolol	0.2	Day	20.9	14.4	12.6	12.9	13.6	14.2
		Night	17.1	13.8	12.9	13.3	14.9	12.4
Gemfibrozil	0.2	Day	109.0	66.9	93.4	110.5	120.5	125.5
		Night	90.7	93.8	99.3	120.0	116.3	128.5

*n.d. is not detected

Table 4.6 Concentrations (ng/L) of selected compounds in the second week

	MDL	Day/ Night	A1	A2	A3	A4	A5	A6
Acetaminophen	0.2	Day	2010.0	1010.0	1580.0	14.3	8.30	11.9
		Night	7310.0	6310.0	3290.0	1160.0	32.40	9170.0
Ibuprofen	10	Day	n.d.	79.5	274.0	555.0	392.00	332.0
		Night	230.0	233.0	223.0	241.0	270.00	309.0
Naproxen	2	Day	69.7	44.0	56.7	53.5	77.50	71.1
		Night	88.5	96.1	71.3	59.9	61.30	123.0
Ketoprofen	5	Day	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		Night	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Diclofenac	5	Day	10.0	7.3	9.5	10.8	21.90	12.4
		Night	21.2	34.0	21.9	19.3	24.60	39.2
Estrone	10	Day	18.7	n.d.	n.d.	n.d.	15.60	n.d.
		Night	109.0	66.5	46.5	37.4	n.d.	191.0
17α-Ethilnylestradiol	10	Day	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		Night	11.3	n.d.	n.d.	n.d.	19.50	16.1
17β-Estradiol	10	Day	12.8	n.d.	n.d.	n.d.	10.50	11.5
		Night	63.5	44.1	44.8	27.3	n.d.	51.6
Propranolol	0.2	Day	8.8	5.8	7.7	8.0	13.20	10.0
		Night	31.5	32.0	28.3	27.3	39.10	36.4
Gemfibrozil	0.2	Day	92.0	76.1	91.3	99.4	141.00	153.0
		Night	128.0	130.0	107.0	120.0	125.00	235.0

*n.d. is not detected

Table 4.7 Concentrations (ng/L) of selected compounds in the third week

Unit:ng/L	MDL	Day/ Night	A1	A2	A3	A4	A5	A6
Acetaminophen	0.2	Day	2820.0	2010.0	160.0	254.0	1450.0	6480.0
		Night	2480.0	1980.0	1880.0	117.0	23.00	9030.0
Ibuprofen	10	Day	98.6	496.0	475.0	524.0	119.0	256.0
		Night	279.0	1020.0	2040.0	1560.0	1710.0	4350.0
Naproxen	2	Day	81.5	72.5	52.8	58.8	57.6	86.6
		Night	57.0	110.0	222.0	148.0	164.0	270.0
Ketoprofen	5	Day	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		Night	n.d.	n.d.	17.0	n.d.	n.d.	45.0
Diclofenac	5	Day	14.2	25.7	15.6	16.2	8.8	12.4
		Night	16.6	21.2	56.5	27.3	16.5	42.5
Estrone	10	Day	32.8	18.4	n.d.	n.d.	n.d.	39.9
		Night	29.2	29.9	47.6	n.d.	n.d.	33.2
17α-Ethilnylestradiol	10	Day	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		Night	15.3	n.d.	12.9	n.d.	n.d.	n.d.
17β-Estradiol	10	Day	43.1	20.0	n.d.	n.d.	28.7	39.8
		Night	27.3	24.9	13.1	15.4	24.3	25.4
Propranolol	0.2	Day	12.0	10.0	10.0	9.3	11.9	11.2
		Night	10.3	11.1	20.5	14.2	13.3	9.4
Gemfibrozil	0.2	Day	88.1	94.9	75.4	81.9	114.0	130.0
		Night	80.9	138.0	227.0	192.0	197.0	279.0

*n.d. is not detected

Table 4.8 The max, min, and median concentrations (ng/L) regardless of different points

(n=36)

	No. of n> MDLs	Min	Median	Max
Acetaminophen	36	8.3	1755.0	9170.0
Ibuprofen	35	n.d.	231.5	4350.0
Naproxen	36	35.2	65.5	270.0
Ketoprofen	2	n.d.	n.d.	45.0
Diclofenac	35	n.d.	12.9	56.5
Estrone	12	n.d.	13.1	191.0
17α-Ethylnylestradiol	5	n.d.	n.d.	19.5
17β-Estradiol	26	n.d.	15.6	64.7
Propranolol	36	5.8	13.1	39.1
Gemfibrozil	36	66.9	115.2	279.0

*n.d. is not detected

Table 4.9 The max, min, and median concentrations of target compounds focusing on each sampling point

Acetamonophen	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**
max	7310.0	6310.0	3290.0	1160.0	2500.0	9170.0
min	2010.0	1010.0	132.0	14.3	8.3	11.9
median	2960.0	1995.0	891.5	707.0	741.2	5195.0
Ibuprofen	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**
max	279	1020	2040	1560	1710	4350
min	0	76	107.5	120	105.5	79.5
median	91.1	156.5	248.5	382.5	219.5	282.5
Naproxen	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**
max	88.5	110	222	148	164	270
min	57	44	52.8	44.3	36	35.2
median	76.4	73.3	56.1	56.2	59.5	78.9
Ketoprofen	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**
max	n.d.	n.d.	17	n.d.	n.d.	45
min	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
median	n.d.	n.d.	8.5	n.d.	n.d.	22.5
Diclofenac	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**
max	21.2	34	56.5	27.3	24.6	42.5
min	6.5	7.3	5.1	n.d.	6.2	10.3
median	13.8	15.7	12.6	13.5	12.7	12.4
Estrone	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**
max	109.0	66.5	47.6	37.4	19.2	191.0
min	13.0	n.d.	n.d.	n.d.	n.d.	n.d.
median	24.0	15.6	n.d.	n.d.	n.d.	23.3
17α-Ethylnylestradiol	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**
max	15.3	n.d.	12.9	n.d.	19.5	16.1
min	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
median	0.00	0.00	0.00	0.00	0.00	0.00

17β-Estradiol	A1	A2	A3	A4	A5	A6
max	63.5	44.1	44.8	27.3	28.7	64.7
min	8.8	n.d.	n.d.	n.d.	n.d.	11.5
medium	27.5	17.9	n.d.	5.0	5.3	32.6
Propranolol	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**
max	31.5	32.0	28.3	27.3	39.1	36.4
min	8.8	5.8	7.7	8.0	11.9	9.4
median	14.6	12.5	12.8	13.1	13.5	11.8
Gemfibrozil	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**
max	128	138	227	192	197	279
min	80.9	66.9	75.4	81.9	114	125.5
median	91.4	94.4	96.4	115.3	122.8	141.5

*n.d. is not detected

Table 4.10 The difference between day and night of 10 target compounds

	Day/night	Median concentrations (ng/L)
Acetaminophen	**Day**	1450.0
	Night	2360.0
Ibuprofen	**Day**	119.5
	Night	237.0
Naproxen	**Day**	57.8
	Night	72.65
Ketoprofen	**Day**	n.d.
	Night	n.d.
Diclofenac	**Day**	10.2
	Night	20.3
Estrone	**Day**	5.7
	Night	21.3
17α-Ethylnylestradiol	**Day**	n.d.
	Night	n.d.
17β-Estradiol	**Day**	11.0
	Night	24.6
Propranolol	**Day**	11.6
	Night	14.6
Gemfibrozil	**Day**	97.2
	Night	126.5

*n.d. is not detected

4.5 Environmental risk assessments

According to Castiglioni et al. and Bound et al.(Castiglioni, Fanelli et al. 2004; Bound, Kitsou et al. 2006), risk assessment using predicted environmental concentrations (PEC) guidelines are debatable because the validity of PECs base on many factors, such as the credibility of any information adopted to the model. I would like to verify these uncertainties and try to find out a PEC prediction that is most suitable to Taiwan.

PECs were compared with measured environmental concentrations (MECs) in this study. Results are shown in **Table 4.11** In general, PECs fall in the range of detected river concentrations except for that of estrogens. The median MECs for diclofenac, ibuprofen, gemfibrozil and propranolol (12.9, 231.5, 115.2 and 13.1ng/L) are very close to what we predicted with PECs (21.1, 313.2, 173.5 and 8.4 ng/L). For naproxen and acetaminphen, PECs are slightly lower than that of median MECs. This might due to the underestimation of them because they could be sold without prescription. Ketoprofen was almost not detected in every sample, and according to the report of Lin et al.(Lin and Reinhard 2005), I was not sure about how to judge the removal rate with 100% or 99%, however, after considering about there's not always big sunlight and I did detect two samples with slight concentrations in the river in the third sampling week (table 4.6). Then I finally decided the removal rate of ketoprofen was 99%. PECs for estrogens (estrone, 17α-ethylnylestradiol and 17β-estradiol) were severl orders lower than that of the MECs even at the minimum MECs. PECs are 0.0002, 0.0001 and 0.0222 ng/L for estrone, 17α-ethylnylestradiol and 17β-estradiol while median MECs measured are 13.1, 0 and 15.6 ng/L. Apparently, only 17α-ethylnylestradiol has the similar result and the others get much higher by median MECs. This maybe due to the underestimation of

usage quantity or I neglected the process of removal in hospital wastewater.

There are many factors influencing the validity of PECs calculated. The biggest variable is the removal rate estimation. Many researchers had incooperated the WWTPs removal efficiency into the model. For example, ketoprofen is found to have the removal rates by STPs from 51% to 100% (Lindqvist, Tuhkanen et al. 2005); the range of ibuprofen was investigated from 14% to 99% (Bound, Kitsou et al. 2006). I didn't consider WWTP removal efficiency in my calculation because there were not any wastewater treatment plants along the river. Consequently, I took only the metabolism and natural attenuation mechanisms into account. However, the variation of the two removal mechanisms which I took into consideration would be affected by the weather and geological conditions depending on the different regions. In many cases, the metabolites of pharmaceuticals are thought to be unharmful to the environment, but acetaminophen was surveyed to be more toxic in metabolite than parent compound in previous researches (Bedner and Maccrehan 2006). Bound and Voulvoulis had questioned the inaccuracy of ignoring the improper disposal of unused medicines. It would get into the environment by the original state rather than metabolite (Bound, Kitsou et al. 2006). After considering all these factors it, I separated the source into two groups. One is discharged after digesting by human beings, and the other is dumped directly to the environment. Their proportions are respectively set to be 0.8 and 0.2. The results demonstrated that the PECs estimated are similar to the measured environmental concentrations (MEC) except for estrogens (estrone, 17α-ethylnylestradiol and 17β-estradiol). However, in order to make this module more accurate, I think the process of hospital wastewater treatments are needed to know to correct the removal rate, but it's hard because the men who worked in hospitals wouldn't let me know.

The predicted no effect concentration (PNEC) of each compound is a varying parameter due to spotty and limited ecotoxicity data for each compound.

PNEC= $\frac{ecotoxicity\ data}{assessment\ factor}$. PNEC is used to predict the concentration which wouldn't be harmful to the environment. Ecotoxicity data are recommended to use the chronic toxicity of NOEC (no-observed-effect concentration) rather than acute toxicity because most compounds in the environment are long existence and low concentration. However, acute toxicity data was more comfortable to be gotten, so EC50 (50% of effective concentration), and LC50 (50% of lethal concentration) were adopted by many researchers. EC50 represents the concentration of each compound would affect 50% organisms in a specific condition; LC50 is the concentration of each compound would kill 50% organisms in a specific condition. Assessment factor is decided by which ecotoxity is chosen; for example, if EC50 is used, than the assessment factor would be 1000. It's proper to have a consistent system like using the same organism (i.e., algae, daphnids, or fish) to derive the ecotoxicity data (NOEC, EC50 or LC50). However, in this study, I used the different ecotoxicity data because there aren't the consistent data in the recent study. Ferrari et al. had indicated some problems of this procedure. For example, chronic toxicity was proved to be more appropriate to represent environmental hazardous pharmaceuticals. Nevertheless, it's not practical to wait for such experimental works because of time and financial problem. They also brought up some improvement such as increasing the assessment factor when only acute toxicity is available.(Ferrari, icirc et al. 2004)

The risk quotients (RQs) were calculated with PECs, and mininum, median and maximum values of MECs respectively. Results were indicated in **table 4.11**. The risk quotients (MEC/PNEC) showed that propranolol and three estrogens pose

environmental and health risk; their RQs are 3.64, 2.3875, 650 and 1.625 respectively using maximum MECs data. However, if median MECs data were used, only propranolol poses risk. When PECs were used to evaluate RQs, all RQs are lower than one. This result demonstrates that PECs could sometimes underestimate the environmental health risk of these pharmaceuticals, especially in the case of estrogens. For example, some researchers had questioned the mechanism of performing single pharmaceutical risk assessment; if taking the same therapeutic pharmaceuticals as a group, the PEC/PNEC value would be much higher than single pharmaceutical (Halling-Sorensen, Nors Nielsen et al. 1998; Jones, Voulvoulis et al. 2002)

Table 4.11 PEC,MEC, PNEC (ng/L)and RQ values of target compounds

Compounds	PEC	MEC(min~max)	MEC(median)	PNEC(toxicity data, AF)	RQ(PEC/PNEC)	RQ (min)	RQ (max)	RQ (median)
Acetaminophen	848.1	8.3~9170	1755.0	9200(EC50,1000) [a]	0.0922	0.0009	0.9967	0.1908
Ibuprofen	313.2	0~4350	231.5	7100(EC50,1000) [a]	0.0441	0	0.6127	0.0326
Naproxen	27.3	35.2~270	65.5	37000(EC50,1000) [a]	0.0007	0.0010	0.0073	0.0018
Ketoprofen	1.7	0~45.0	n.d.	15600(NA) [b]	0.0001	0	0.0029	0
Diclofenac	21.1	0~56.5	12.9	10000(NOEC,10) [a]	0.0021	0	0.0057	0.0013
Estrone	0.0002	0~191	13.1	80(LOEC,100) [c]	0.000003	0	2.3875	0.1638
17α-Ethynylestradiol	0.0001	0~19.5	n.d.	0.3(LOEC,100) [c]	0.0003	0	65	0
17β-Estradiol	0.0222	0~64.7	15.6	40(LOEC,100) [c]	0.0006	0	1.6175	0.39
Propranolol	8.4	5.8~39.1	13.1	10(NOEC,50) [d]	0.8400	0.5800	3.9100	1.3100
Gemfibrozil	173.5	66.9~279.0	115.2	440(EC50,1000,) [e]	0.3943	0.1520	0.6341	0.2618

Ref. a.(Carlsson, Johansson et al. 2006); b.(Santos, Aparicio et al. 2007); c. (Press-Kristensen, Ledin et al. 2007); d. (Ferrari, icirc et al. 2004); e. (Isidori, Nardelli et al. 2007)

Chapter 5 Conclusion

1. A multi-component LC-MS/MS method was developed for analyzing ten pharmaceuticals in the aqueous matrix. Low method detection limits were achieved: 0.2 ng/L for acetaminophen, propranolol and gemfibrozil; 2 ng/L for naproxen; 5 ng/L for ketoprofen and diclofenacand; 10 ng/L for estrone, 17α-ethylnylestradiol, 17β-estradiol and ibuprofen.

2. Occurrence of pharmaceuticals in Sin-Dian River was monitored and results showed that all the target compounds were detected in at least two water samples. Among them, acetaminophen, ibuprofen, naproxen, diclofenac, propranolol and gemfibrozil were most often detected; they present in all the water samples. Acetaminophen and ibuprofen have the highest measured concentrations (9170 and 4350 ng/L respectively) along the Sin-Dian River.

3. The concentrations detected varied (up to two order of magnitude difference) between day and night and among weeks for several compounds, such as 17α-ethynylestradiol, acetaminophen and ibuprofen. When there were potential sources of contamination nearby, the detected concentrations were higher. This indicated that hospitals and regional discharges nearby greatly influenced the water quality along Sin-Dian River.

4. Our occurrence data agree closely with what was reported worldwide except for that of acetaminophen. Acetaminophen was measured in much higher concentrations in Sin-Dian River than in other reported studies. This may due to the high medicine usage

in Taiwan. In fact, acetaminophen is the number one prescribed medicine in National Health Insurance Plane for year of 2004 and 2005. The different results between Ternes (Ternes 1998) and I might due to the reason that there are no waste water treatment plants along the Sin-Dian River, and water were directly discharged into the river without being treated. According to Ternes's investigations, acetaminophen can be easily removed by STPs; therefore, without the treatment procedure, the concentration of acetaminophen stays high as demonstrated in this study..

5. PECs were calculated and then compared with the median MECs. Results showed that they were comparable except for that of estrone and 17β-Estradiol; the PEC values of these two compounds were much lower than the detected concentrations.

6. Risk quotients showed that propranolol and three estrogens (estrone, 17α-ethylnylestradiol, 17β-estradiol) posed environmental and health risk; their RQs are 3.6, 2.4, 650 and 1.6 respectively calculated with maximum MECs data. However, if median MECs data were used, only propranolol poses risk. PECs data could sometimes underestimate the environmental/human health risk, especially in the case of estrogens since PECs of estrone and 17β-estradiol were much lower than MECs. On the other hand, the higher PECs and MECs compounds such as acetaminophen, ibuprofen and gemfibrozil were not thought to be harmful to the environment according to the risk assessment calculation.

Engineering Significance: The multi-compounds analytical method, based on SPE procedure followed by HPLC-MS/MS analysis, could monitor selected ten pharmaceuticals in ng/L level. The occurrence data obtained and risk assessment

procedures/results demonstrated in this study could provide significant information to future development of standard analytical procedures and regulations in Taiwan's aqueous environment.

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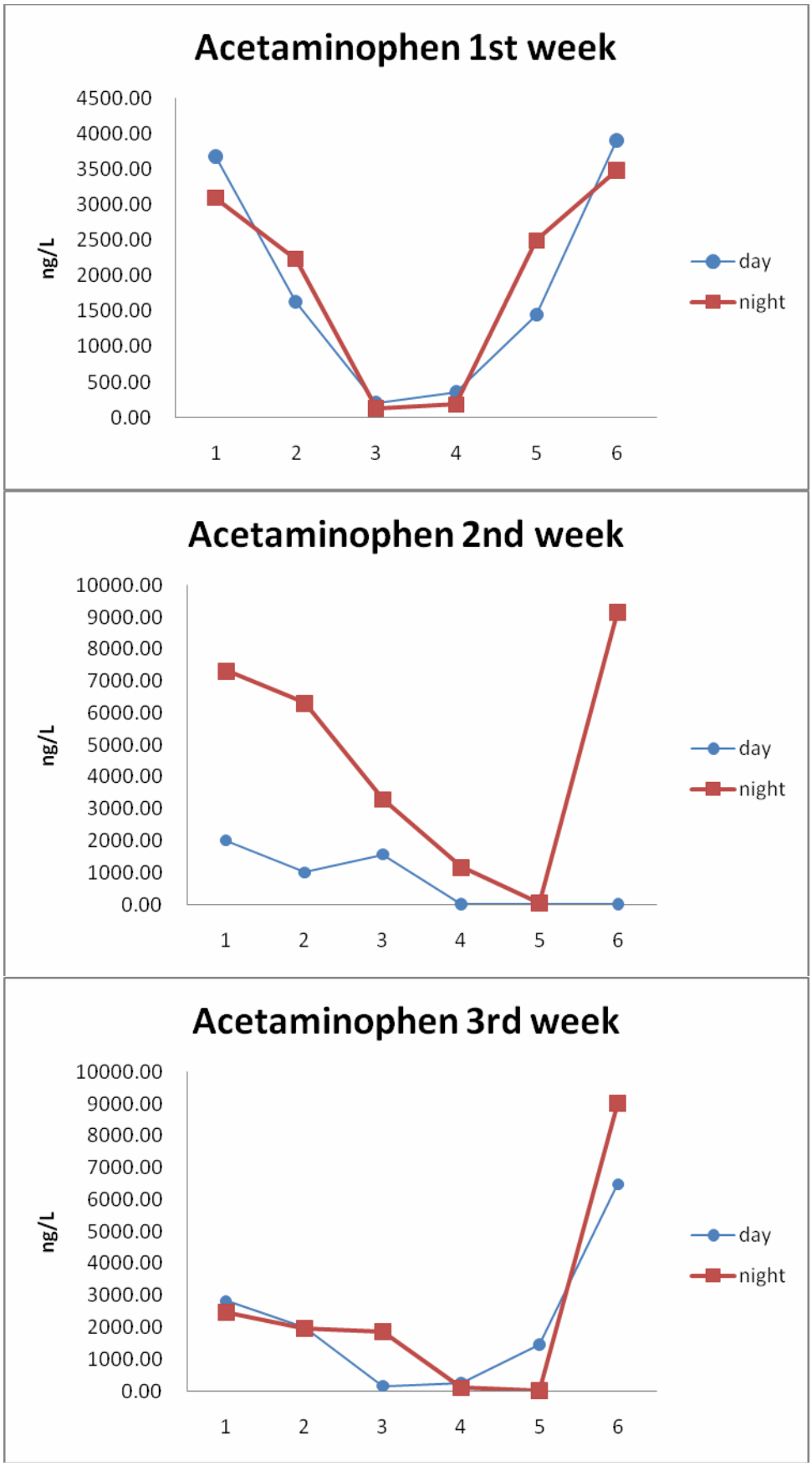
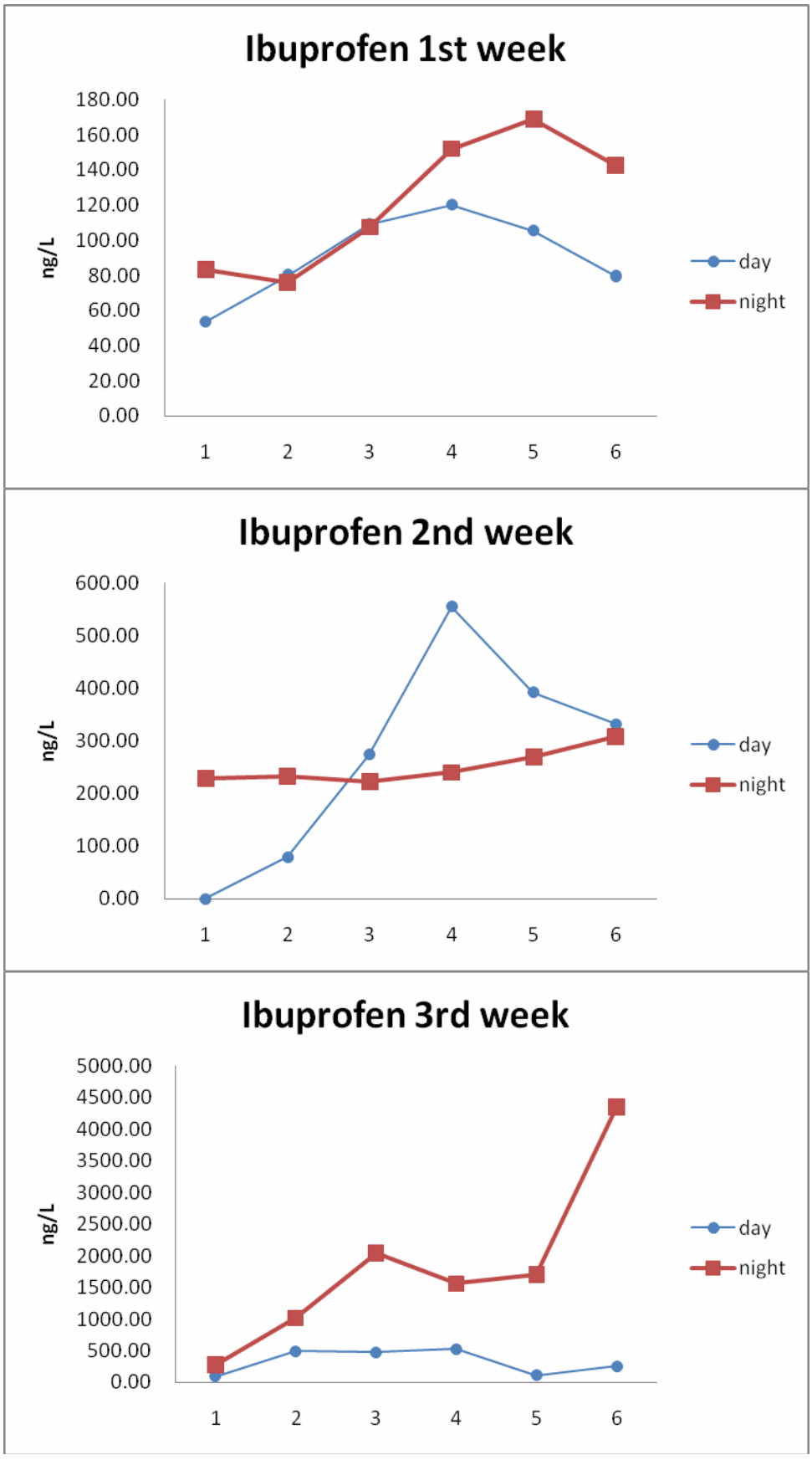
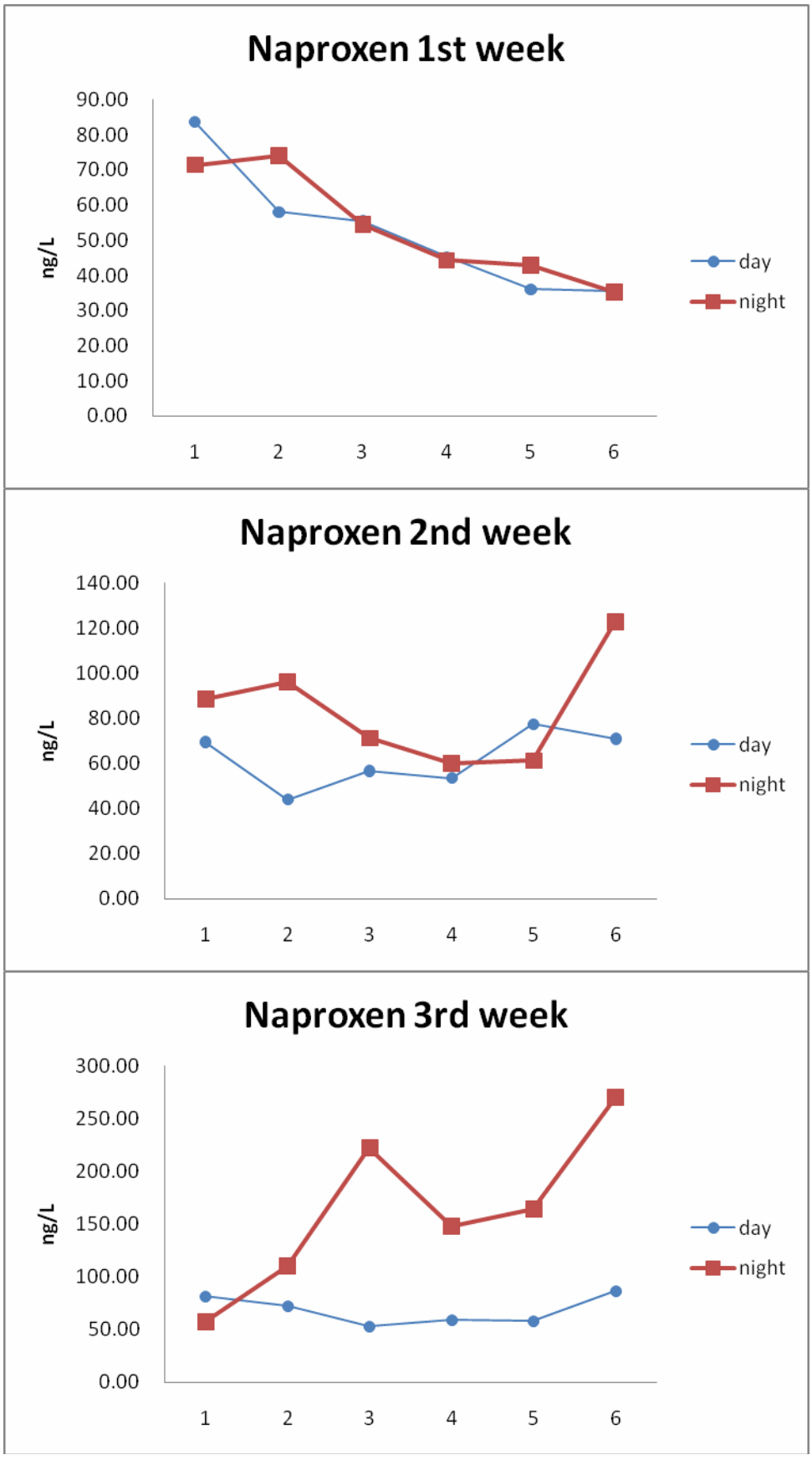
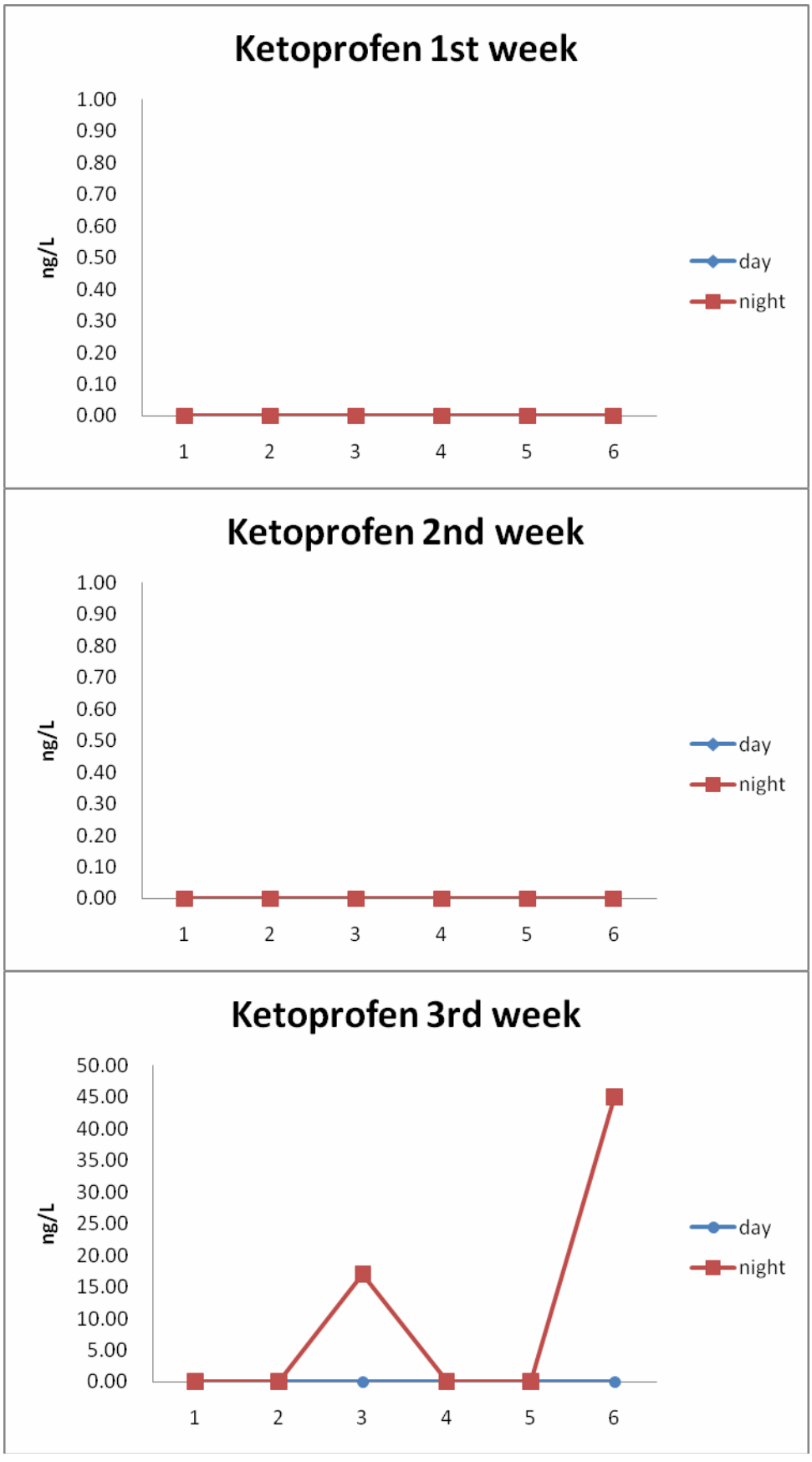
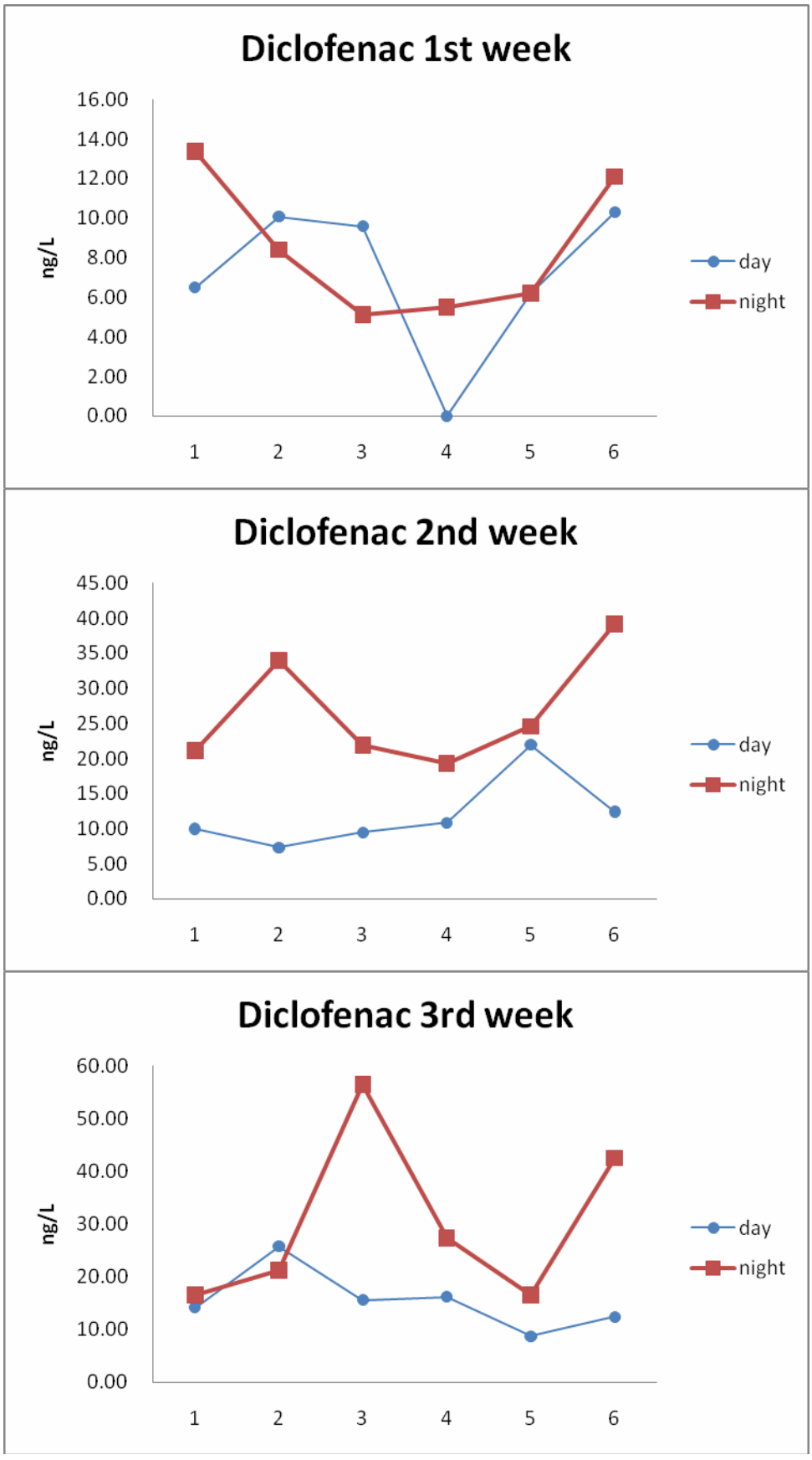
Appendix The trend of every compound in different points by day and night of each week

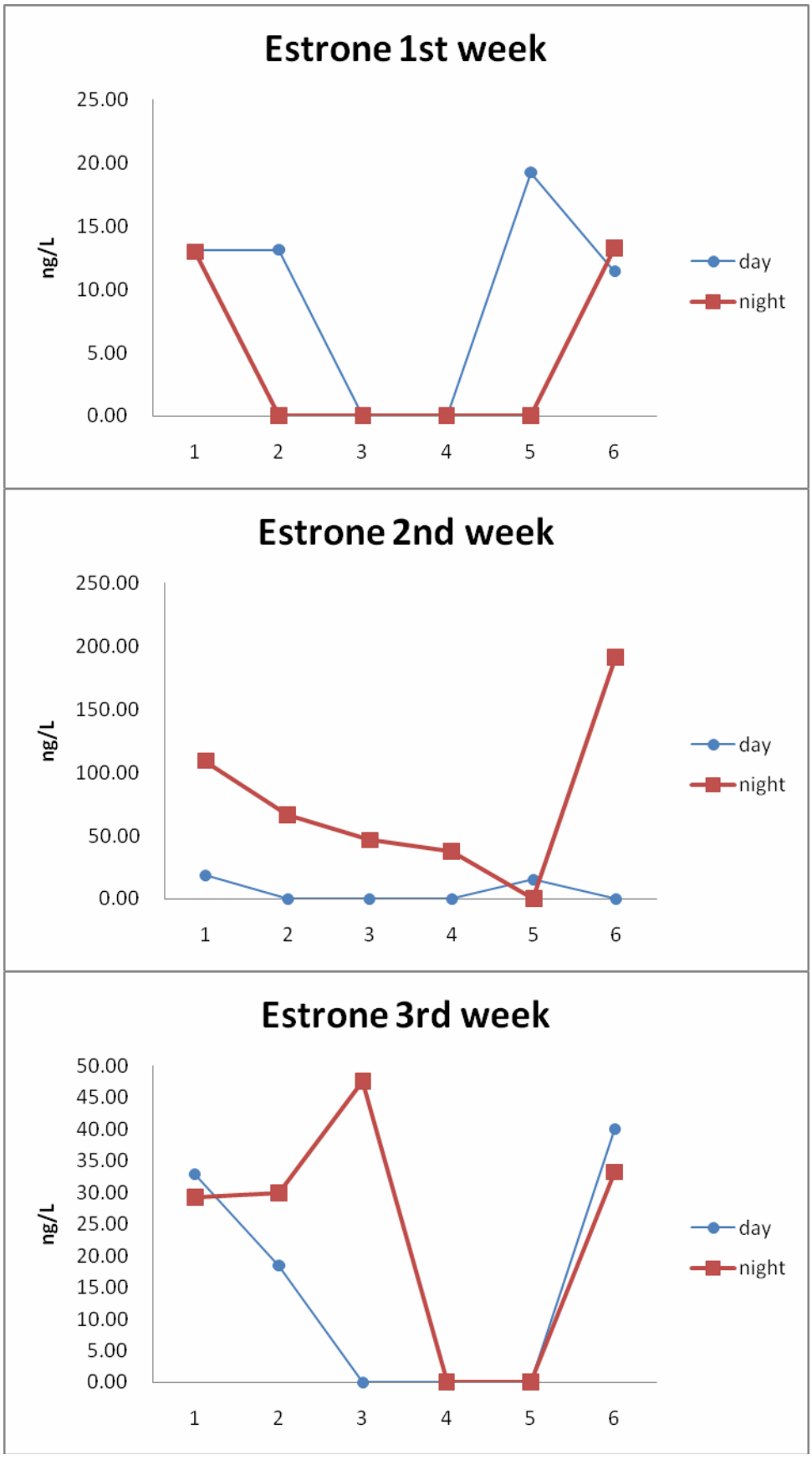


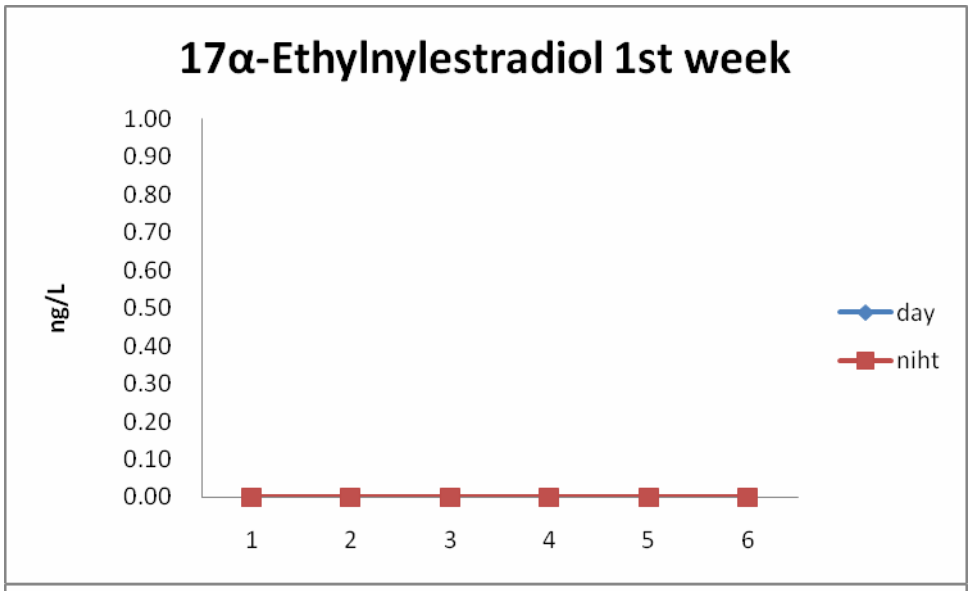

Ibuprofen 1st week
180.00
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
ng/L
1
2
3
4
5
6
day
night
Ibuprofen 2nd week
600.00
500.00
400.00
300.00
200.00
100.00
0.00
ng/L
1
2
3
4
5
6
day
night
Ibuprofen 3rd week
5000.00
4500.00
4000.00
3500.00
3000.00
2500.00
2000.00
1500.00
1000.00
500.00
0.00
ng/L
1
2
3
4
5
6
day
night

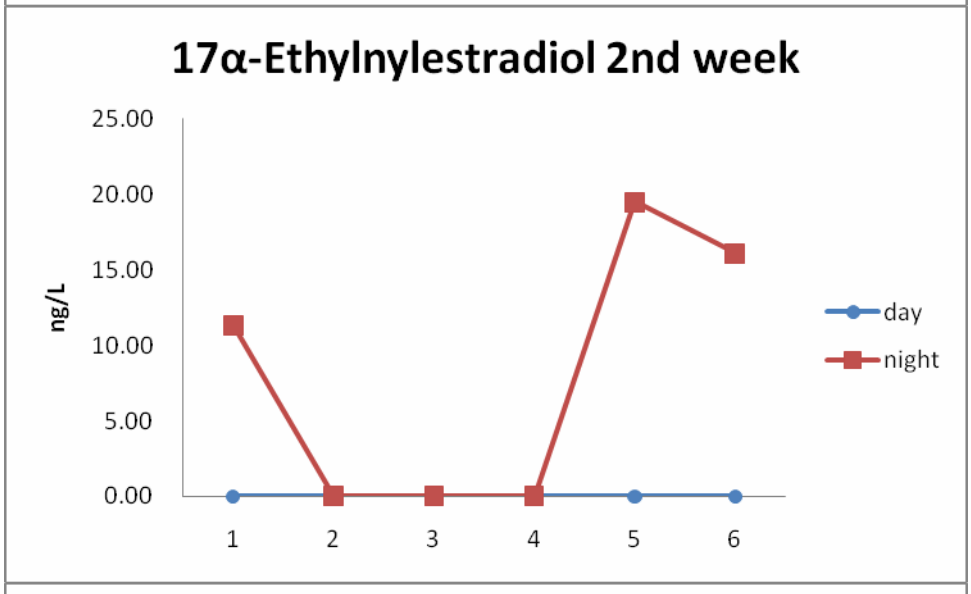

Naproxen 1st week
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
ng/L
1
2
3
4
5
6
day
night
Naproxen 2nd week
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
ng/L
1
2
3
4
5
6
day
night
Naproxen 3rd week
300.00
250.00
200.00
150.00
100.00
50.00
0.00
ng/L
1
2
3
4
5
6
day
night

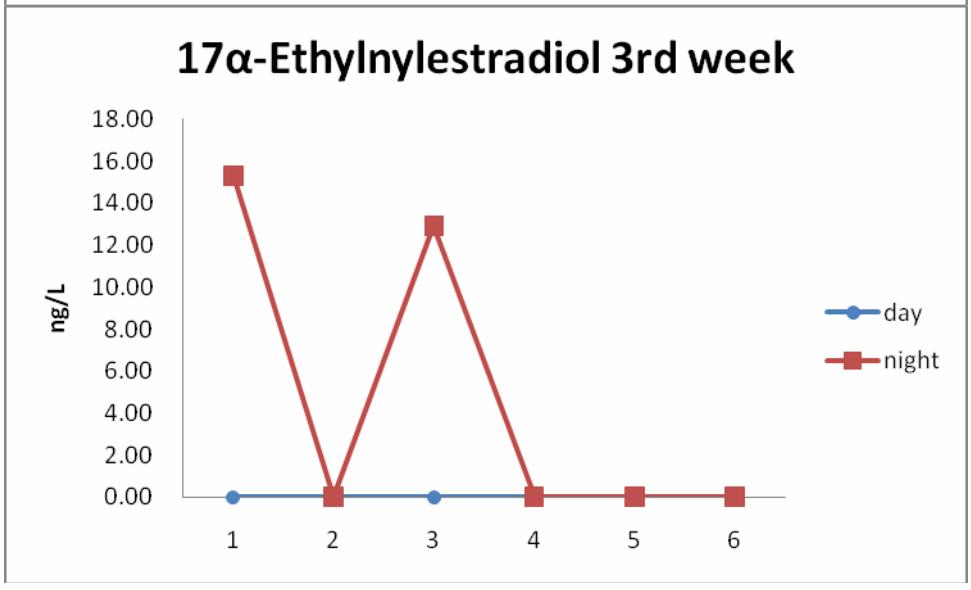

Ketoprofen 1st week
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
ng/L
1
2
3
4
5
6
day
night
Ketoprofen 2nd week
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
ng/L
1
2
3
4
5
6
day
night
Ketoprofen 3rd week
50.00
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
ng/L
1
2
3
4
5
6
day
night

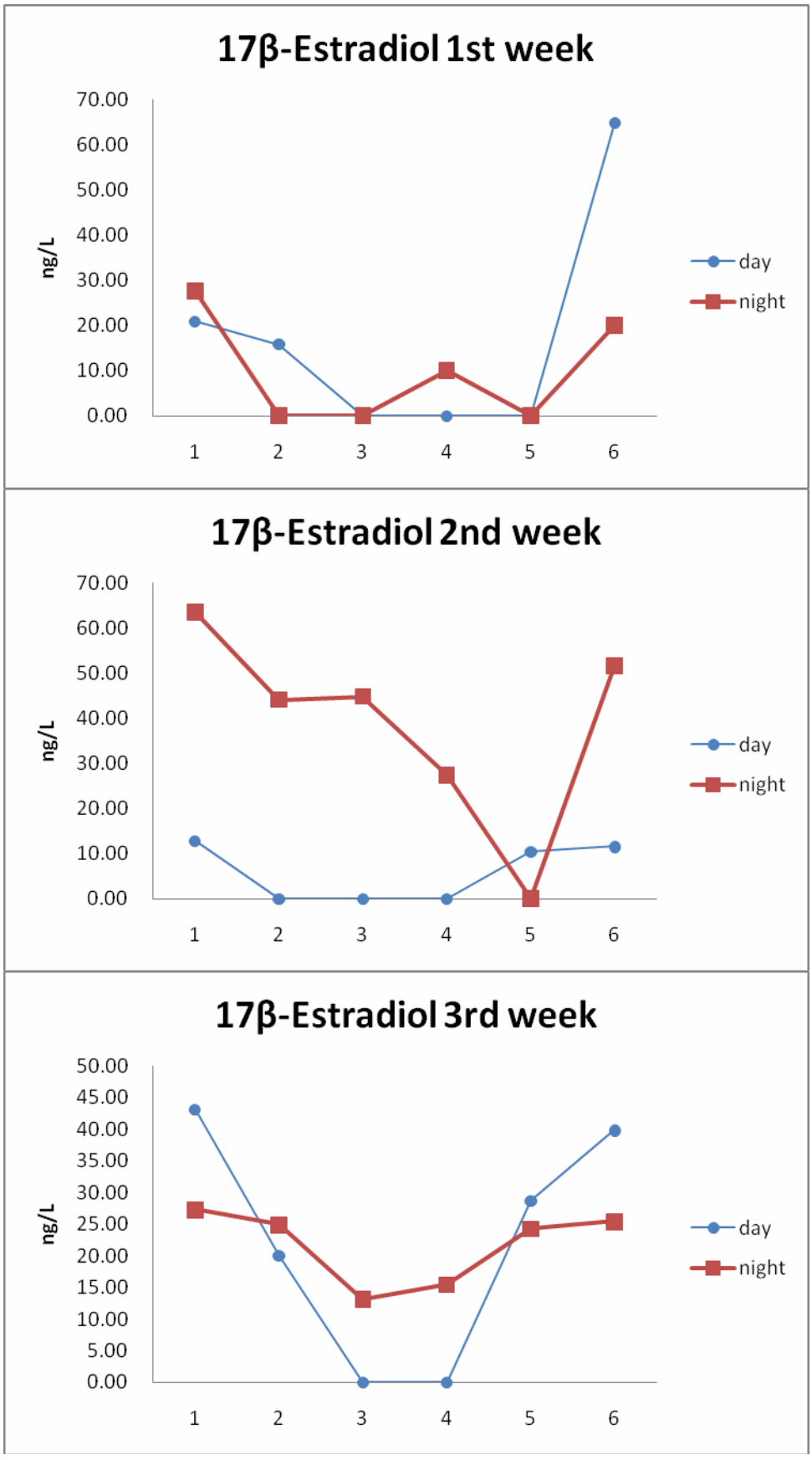

Diclofenac 1st week
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
ng/L
1
2
3
4
5
6
day
night
Diclofenac 2nd week
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
ng/L
1
2
3
4
5
6
day
night
Diclofenac 3rd week
60.00
50.00
40.00
30.00
20.00
10.00
0.00
ng/L
1
2
3
4
5
6
day
night

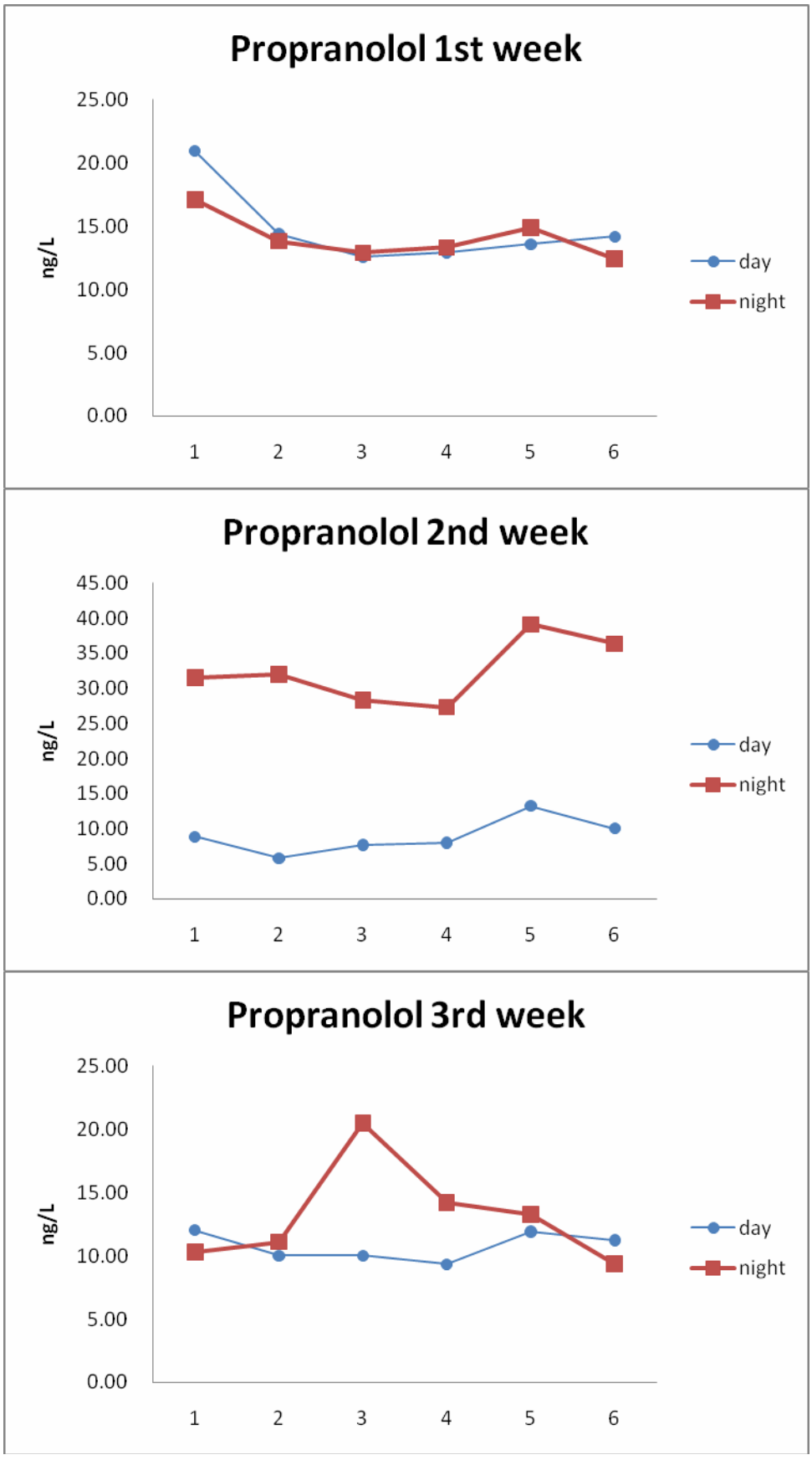

Estrone 1st week
25.00
20.00
15.00
10.00
5.00
0.00
ng/L
1
2
3
4
5
6
day
night
Estrone 2nd week
250.00
200.00
150.00
100.00
50.00
0.00
ng/L
1
2
3
4
5
6
day
night
Estrone 3rd week
50.00
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
ng/L
1
2
3
4
5
6
day
night

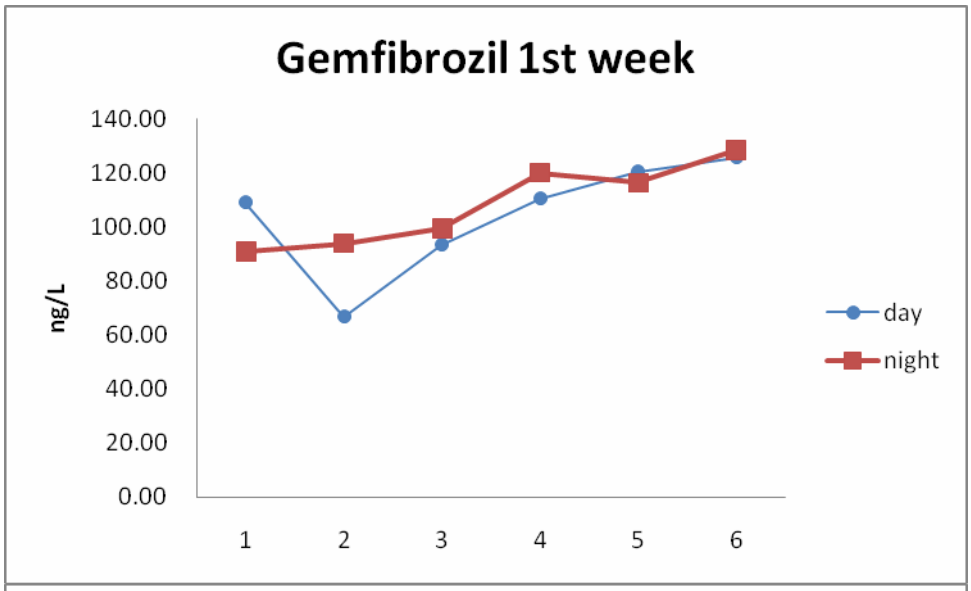

17α-Ethylnylestradiol 1st week
ng/L
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
1
2
3
4
5
6
day
niht

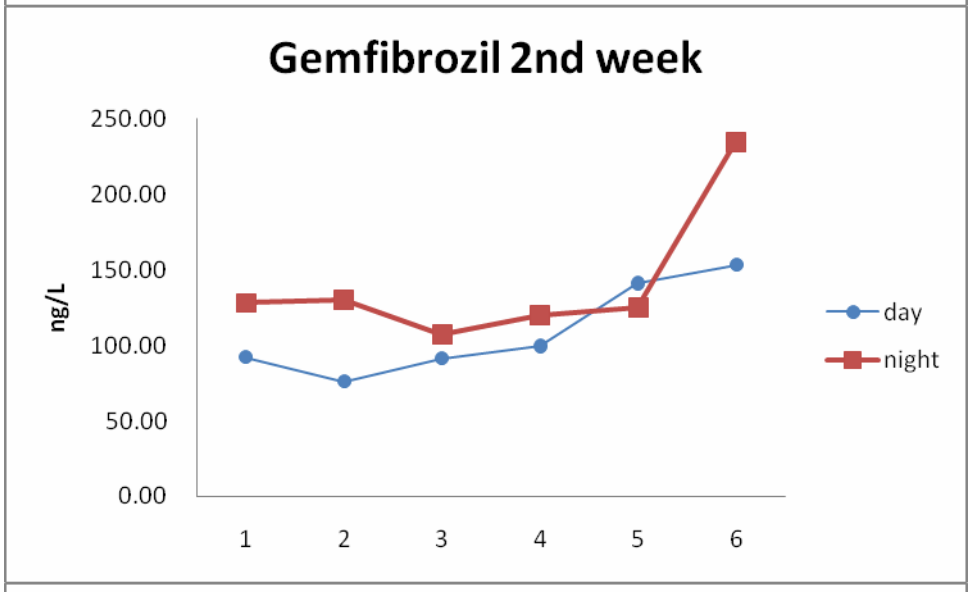

17α-Ethylnylestradiol 2nd week
ng/L
25.00
20.00
15.00
10.00
5.00
0.00
1
2
3
4
5
6
day
night

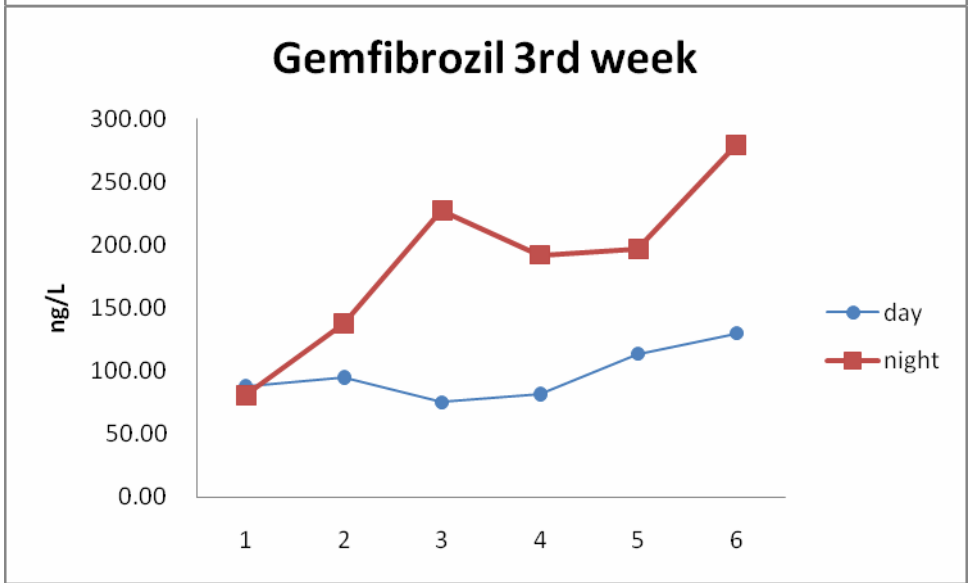

17α-Ethylnylestradiol 3rd week
ng/L
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
1
2
3
4
5
6
day
night


17β-Estradiol 1st week
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
ng/L
1
2
3
4
5
6
day
night
17β-Estradiol 2nd week
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
ng/L
1
2
3
4
5
6
day
night
17β-Estradiol 3rd week
50.00
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
ng/L
1
2
3
4
5
6
day
night


Propranolol 1st week
25.00
20.00
15.00
10.00
5.00
0.00
ng/L
1
2
3
4
5
6
day
night
Propranolol 2nd week
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
ng/L
1
2
3
4
5
6
day
night
Propranolol 3rd week
25.00
20.00
15.00
10.00
5.00
0.00
ng/L
1
2
3
4
5
6
day
night


Gemfibrozil 1st week
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
ng/L
1
2
3
4
5
6
day
night


Gemfibrozil 2nd week
250.00
200.00
150.00
100.00
50.00
0.00
ng/L
1
2
3
4
5
6
day
night


Gemfibrozil 3rd week
300.00
250.00
200.00
150.00
100.00
50.00
0.00
ng/L
1
2
3
4
5
6
day
night