

# Determination of Non-Steroidal Anti-Inflammatory Drugs (Nsaids) in Surface Water at Ho Chi Minh City

Đỗ Vũ Hoàng Anh<sup>1,\*</sup>, Bùi Quang Minh<sup>1</sup>, Nguyễn Duy Linh<sup>1</sup>, Phạm Hồng Nhật<sup>2</sup>

<sup>1</sup>*Institute of Environment Technology, Vietnamese Academy of Science and Technology*

<sup>2</sup>*Vietnam Institute for Tropical Technology and Environmental Protection (VITTEP)*

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**Abstract:** This study aims at setting up an analytical method for determination of 4 Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) including ketoprofen, ibuprofen, diclofenac sodium and mefenamic acid in surface water. The method studied involved solid phase extraction (SPE) using Poly-Sery PSD (Poly styrene divinylbenzene) SPE cartridge and analysis by high-performance liquid chromatography (HPLC) with DAD detector. The recoveries were found in the range of 82% - 98% with relative standard deviations (RSD) of less than 10% and method detection limits (MDL) were from 0.004 to 0.015 $\mu$ g/L. This method was used to determine the selected compounds in fifteen surface water samples collected in Ho Chi Minh City. The analysis results show that their residues currently do exist in surface water in the study area.

**Keywords:** NSAIDs, HPLC-DAD, surface water.

## 1. Introduction

Pharmaceuticals and Personal Care Products (PPCPs) residues present in the environment are now common and recognized as a problem. Thousands of ton of pharmacologically active substances are used every year over the world to fight diseases or to face the stresses of modern life. Studies conducted in Australia, Brazil, Canada, Croatia, England, Germany, Greece, Italy, Spain, Switzerland, the Netherlands and the United States had found the presence of more than 80 compounds including pharmaceuticals and its

metabolites in aquatic environment [1]. Remarkably, Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are increasingly identified in the environment from various sources. These NSAIDs account for many cases of ulcers, or intestinal perforation in chronic users for pain and inflammation. Therefore, PPCPs in general and NSAIDs residues in particular in environment, especially in water environment, should gain better attention from us all. In this context, there is a clear need of an analytical method with proper accuracy and precision to monitor these compounds residues in water environment.

Currently, there are various methods available to identify NSAIDs in the world such

\* Corresponding author. Tel: 84-1219333718.  
E-mail: doanh73@yahoo.com

as: gas chromatography-mass spectrometry (GC/MS) combined with derivatives obtained from previous step; liquid chromatography-mass spectrometry (LC/MS, LC/MS/MS); high performance liquid chromatography with UV and fluorescence detector (HPLC/UV/FL); capillary electrophoresis (CE) methods etc.[2]. Bearing in mind that most of laboratories in Vietnam are not modernly equipped, in this study, we use HPLC/UV to determine the four common NSAIDs which are ketoprofen, ibuprofen, diclofenac sodium and mefenamic acid.

## 2. Materials and methods

### 2.1. Sampling

Samples were collected at the depth of 0.25 to 1.0 meter from the surface of water and stored in 2.5-liter amber glass bottles at 4°C until sample treatment [3].

Fifteen surface water samples at Hồ Chí Minh City were collected on 23 September 2013.

### 2.2. Sample preparation and analysis

#### 2.2.1. Chemicals and reagents

Chemicals and reagents used in the study included acetonitrile (Labscan), methanol (Merck), hexane (Labscan), ethyl acetate (Merck), acetic acid (Merck), ultrapure water (Millipore), ketoprofen, ibuprofen, diclofenac sodium and mefenamic acid standard (IDQC, purity >99.9%).

Stock solutions of 1,000mg/L were prepared in methanol and stored at 4°C. Working solutions were prepared by diluting the stock standard solutions in methanol [4].



Fig. 1. Location of sampling sites.

#### 2.2.2. Instrument

Extraction was performed on solid phase extraction system (Agilent) with vacuum pump and Poly-Sery PSD (Poly styrene divinylbenzene) SPE tubes (250mg, 6mL) of DNW Technologies GmbH.

Chromatographic analysis was performed on a LaChrom Hitachi HPLC instrument. Separations were carried out using an Inspire C18 (250mm x 4.6mm i.d., 5µm) column protected by an Inertsil ODS-3 (10mm x 4.0mm i.d., 5µm) guard column.

#### 2.2.3. Sample preparation

Samples were filtered through a 0.45µm cellulose acetate membrane filter to remove suspended matters. The SPE cartridges were conditioned with 6mL ethyl acetate, 6mL methanol, 6mL ultrapure water. 500mL samples were then transferred to the SPE cartridges. The loaded cartridges were rinsed with 3mL of methanol:water (5:95, v/v) solution and 3mL *n*-hexane. After the enrichment step, the cartridges were vacuum dried. The elution was performed with three 3-mL aliquots of ethyl acetate. Extraction was performed under

vacuum at the flow rate of 3mL/min. The effluent was dried under a stream of nitrogen. The residues were dissolved in 0.5mL methanol and rejected into the HPLC system [5].

#### 2.2.4. HPLC-DAD conditions

Mobile phase (isocratic flow): 40% 1mM Acetic acid– 60% Acetonitrile

Flow-rate: 1.0 mL/min

The UV signal of ketoprofen, ibuprofen, diclofenac sodium, mefenamic acid were measured at 254, 218, 276 and 218nm, respectively.

#### 2.2.5. Calibration curve, method detection limit and method quantitation limit

Calibration standards were prepared in the concentration range of 10 - 2,000 $\mu$ g/L for ketoprofen, diclofenac sodium, mefenamic acid and 20 - 2,000 $\mu$ g/L for ibuprofen.

Method detection limit (MDL) and method quantitation limit (MQL) were determined by analyzing five times of spiked samples which are prepared by adding standard solution so that

their final concentration after post-extracted are 5.0, 10.0, 20.0 $\mu$ g/L, respectively. Minimum concentration ( $C_{\min}$ ) is chosen so that S/N value is ranged from 3 – 10 for each compound.

where:  $V = 0.5\text{mL}$ ;  $V_s = 500\text{mL}$

#### 2.2.6. Recovery

500mL aliquots of sample were spiked five times with the pharmaceutical compounds at three concentration levels (0.5, 2.0, 5.0 $\mu$ g/L). Samples were then extracted as described above. Signals obtained from spiked samples were compared with signals from sample without standard addition.

### 3. Results and discussions

Calibration curves were linear with coefficients of correlation greater than 0.999 for all the pharmaceuticals analyzed. The overall retention time was no more than 18 min.

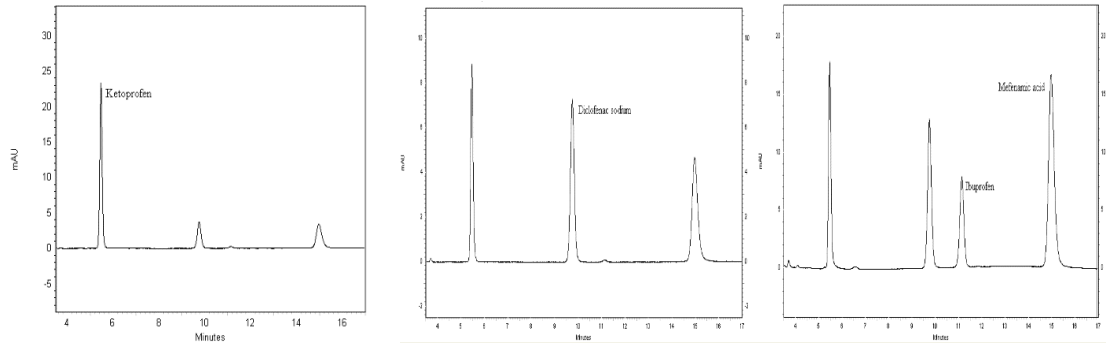


Fig. 2. Chromatogram of standard solution

Table 1. Method detection limit (MDL) and method quantitation limit (MQL) of the pharmaceutical compounds in water

Pharmaceutical compound	MDL ( $\mu$ g/L)	MQL ( $\mu$ g/L)
Ketoprofen	0.004	0.013
Ibuprofen	0.015	0.050
Diclofenac sodium	0.010	0.030
Mefenamic acid	0.004	0.013

The recoveries ranged from 82% to 98% with relative standard deviations (RSD) of all three experiment setup less than 10% (see Table

2). These data indicate that the treatment method acquired are relatively good for the study purpose.

Table 2. Average recoveries of the studied pharmaceutical compounds at different spiking levels (n=5)

Pharmaceutical compound	Spiked concentration					
	0.5 µg/L		2.0 µg/L		5.0 µg/L	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Ketoprofen	90.0	4.52	89.8	2.90	83.5	1.68
Ibuprofen	92.0	7.16	98.0	1.59	86.8	4.39
Diclofenac sodium	91.1	8.64	95.5	4.40	82.0	4.77
Mefenamic acid	84.9	8.41	92.2	5.60	82.6	3.54

Fifteen surface water samples at Hồ Chí Minh City were analyzed by this method. Analytical results are shown in Table 3 and Fig. 3. The results show that residues of the analyzed compounds do exist in the collected water samples. Among them, mefenamic acid concentration is higher than the others and

exists in the surface water at all sampling locations. Particularly, ibuprofen concentration at Lê Văn Sỹ bridge is significantly higher than at other locations. Regarding ketoprofen and diclofenac sodium, they only exist in surface water at a low concentration and at a few sampling locations.

Table 3. Concentration of the analyzed pharmaceutical compounds in surface water samples collected at Hồ Chí Minh City

Sampling site	River/Canal	Ketoprofen (µg/L)	Ibuprofen (µg/L)	Diclofenac sodium (µg/L)	Mefenamic acid (µg/L)
Sài Gòn bridge	Sài Gòn River	<MDL	0.15	<MQL	0.64
Thủ Thiêm bridge		<MDL	<MDL	<MQL	0.39
Bình Triệu bridge		<MDL	0.48	<MQL	0.98
Khánh Hội bridge		0.67	1.29	<MQL	0.91
Chữ Y bridge		<MQL	0.37	0.04	1.67
Chà Và bridge	Bến Nghé – Tàu Hủ – Đôi – Tè	<MQL	1.17	<MDL	2.43
Nhị Thiên Đường bridge		<MDL	<MQL	<MQL	0.88
Nguyễn Tri Phương bridge		<MQL	1.79	<MDL	2.14
Ruột Ngựa small canal		<MQL	1.21	<MDL	3.40
Phú Định ferry		<MDL	<MQL	<MQL	0.56
Ông Buông bridge	Tân Hóa – Lò Gốm	0.80	1.29	<MQL	2.22
Điện Biên Phủ bridge	Nhiều Lộc-Thị Nghè	1.23	0.34	<MQL	0.92
Lê Văn Sỹ bridge		0.74	5.01	<MQL	1.95
Tham Lương bridge	Tham Lương-Bến Cát-Vàm Thuật	1.95	0.95	0.33	2.10
An Lộc bridge		<MQL	<MDL	<MDL	3.48

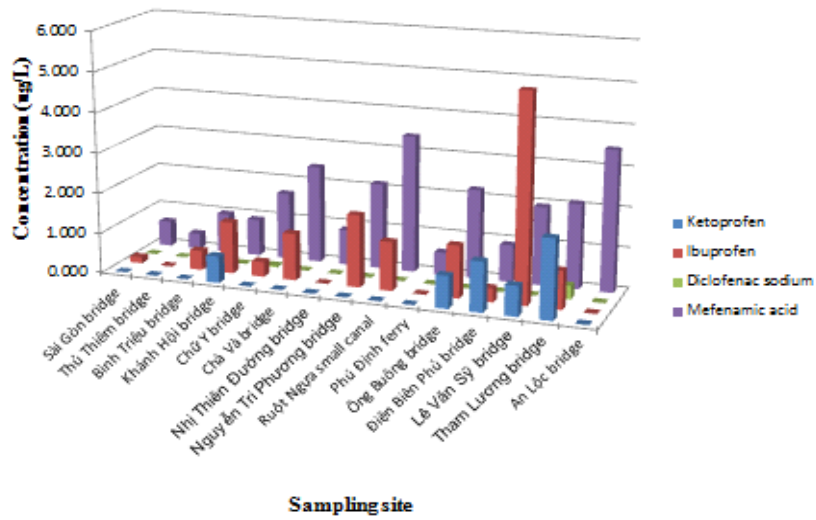


Fig. 3. Concentration of the analyzed pharmaceutical compounds in the water samples.

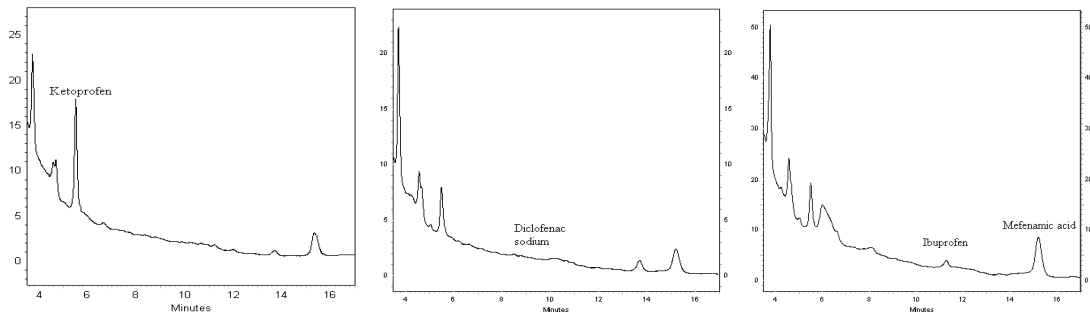


Fig. 4. Chromatogram of the analyzed samples.

#### 4. Conclusions

The HPLC method presented hereby enables the simultaneous analysis of four common NSAIDs including ketoprofen, ibuprofen, diclofenac sodium and mefenamic acid. This method proves to be accurate and effective for the analysis of these compounds.

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## Xác định hàm lượng các hợp chất chống viêm không steroid (nsaids) trong nước mặt thành phố Hồ Chí Minh

Đỗ Vũ Hoàng Anh<sup>1</sup>, Bùi Quang Minh<sup>1</sup>, Nguyễn Duy Linh<sup>1</sup>, Phạm Hồng Nhật<sup>2</sup>

<sup>1</sup>*Viện Công nghệ Môi trường, Viện Hàn lâm Khoa học và Công nghệ Việt Nam*

<sup>2</sup>*Viện Kỹ thuật Nhiệt đới và Bảo vệ Môi trường (VITTEP)*

**Tóm tắt:** Nghiên cứu này trình bày một phương pháp phân tích đồng thời bốn hợp chất chống viêm không steroid (NSAIDs) là ketoprofen, ibuprofen, diclofenac sodium, mefenamic acid trong nước mặt. Phương pháp phân tích bao gồm bước làm giàu và rửa giải bằng chiết pha rắn (SPE) sử dụng cột chiết Poly-Sery PSD (Poly styrene divinylbenzene), sau đó được phân tích với hệ thống sắc ký lỏng hiệu năng cao (HPLC) với đầu dò DAD. Độ thu hồi thu được từ phương pháp này từ 82% tới 98% với độ lệch chuẩn tương đối nhỏ hơn 10%. Giới hạn phát hiện của phương pháp nằm trong khoảng 0,004 – 0,015µg/L. Phương pháp này được ứng dụng để xác định các hợp chất nêu trên trong 15 mẫu nước mặt ở Thành phố Hồ Chí Minh. Kết quả cho thấy tồn tại dư lượng các hợp chất này trong môi trường nước mặt ở khu vực nghiên cứu.

*Từ khóa:* NSAIDs, HPLC-DAD, nước mặt.