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Non Steroidal Anti Inflammatory Drug (NSAID) Determination in Environmental Samples by Solid Phase Extraction Coupled to High Performance Liquid Chromatography with Fluorescence Detection

[1] A. Dabwan Ahmed H., [2]K. Satoshi, [2]O. Mika, [2]K. Hideyuki, [3]S. Tohru

[1]*Faculty of Chemical Engineering Technology, TATI University College, Jalan Panchor, Teluk Kalong, 24000 Kemaman, Terengganu, Malaysia.*
[2]*Department of Chemistry for Materials, Graduate School of Engineering, Mie University, Tsu, Mie 514-8507, Japan.*
[3]*Environmental Preservation Center, Mie University, Tsu, Mie 514-8507, Japan.*

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ABSTRACT

A simple and very sensitive pre-concentration method for the determination of Non Steroidal Anti Inflammatory drug (NSAID) mainly naproxen in environmental samples by solid phase extraction method coupled to high performance liquid chromatography with fluorescence detection was developed. Naproxen was preconcentrated on graphite powder as an adsorbent agent, and tetrahydrofuran (THF): methanol (Me); (80:20) mixture was used as a desorbent solvent. The effluent from the column was monitored at two different wavelengths; 272 nm excitation, and 250 nm emission. The Different parameters influencing the enrichment factor such as, flow rate, absorbent amount, pH, THF: Me ratio, and NaCl concentration were investigated. Under the optimal conditions, an enrichment factor of 145 was obtained, the detection limit was found to be 9 pg/mL, and the relative standard deviation was 15% at 10 ppb (n=8). The developed method was successfully applied for the determination of naproxen from environmental samples (river water) with satisfactory results.

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INTRODUCTION

Naproxen is one of the pharmaceuticals consumed in large quantities in modern society. It is mainly used as non-steroidal anti-inflammatory drug (NSAID) (Cuerda-Correa, E.M., Domínguez-Vargas, J.R., Olivares-Marín, F.J., de Heredia, J.B., 2010). Naproxen also considered one of the Pharmaceuticals and Personal Care Products (PPCPs) (García, S.O., Pinto, G.P., Encina, P.G., Mata, R.I., 2013). Significant amounts of these drugs and their metabolites have been reported in wastewater treatment plant (WWTP) effluents and natural waters (Yu, T., Lin, A.Y., Lateef, S.K., Lin, C., Yang, P., 2009). Moreover, the stability of specifically naproxen in the raw materials or in the final products could be changed under abnormal conditions such as temperature, light, humidity, and pH that could produce more toxic products such as 1-(6-methoxy-2-naphthyl) ethanol (MNE) and 2-methoxy-6-ethyl naphthalane (MEN). As a result of this alteration strict, international regulation is being implemented to detect these materials in the raw materials. Although NSAID & PPCPs are usually present at parts-per-trillion levels, studies have shown that long-term exposure to wastewater effluents may cause adverse impacts to aquatic species (e.g., feminization) due to the endocrine disruption activities of some of the mentioned products (Onesios, K.M., Bouwer, E.J., 2012).

Degradation process of naproxen has been covered in different effluents such as wastewater treatment plants (WWTPs) and rivers, and it was found that direct phototransformation and biodegradation considered to be as possible elimination processes (Cuerda-Correa, E.M., et.al., 2010; Onesios, K.M., Bouwer, E.J., 2012; Méndez-Arriaga, F., Esplugas, S., Giménez, J., 2008).

Several liquid chromatography methods were reported for individual and simultaneous determination of naproxen and other anti-inflammatory drugs in human serum and urine and in pharmaceutical preparations. Both recommended methods, United State Pharmacopeia and the British Pharmacopeia, describe the analysis of naproxen in formulations and raw materials for related compounds using thin layer chromatography. However, these methods are time consuming in addition to the complexity of their separation systems when using solvents and other chemicals (Monser, L., Darghouth, F., 2003).

Corresponding Author: A. Dabwan Ahmed H., Faculty of Chemical Engineering Technology, TATI University College, Jalan Panchor, Teluk Kalong, 24000 Kemaman, Terengganu, Malaysia.
E-mail:ahmedmie2000@gmail.com

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To avoid time consuming, complexity of the separation system and chemical use, the preconcentration technique represents an essential step for very low concentration before chromatographic analysis.

Solid Phase Extraction (SPE) coupled with HPLC fluorescence detection could obtain lower detection limit compared with other expensive and time consuming methods in the environmental samples (Katsumata, H., Mizuno, H., Kaneco, S., Ohta, K., Suzuki, T., 2009). Thus in this work SPE was implemented, at the same time THF was used as a solvent. The main factors affecting SPE efficiency were investigated; finally the proposed method was applied for the determination of naproxen in environmental samples from two different rivers with satisfactory results.

Experimental:

Chemicals and reagents:

Naproxen, HPLC grade methanol, Tetrahyrofuran (THF) were purchased from Wako Pure Chemical Industries (Osaka, Japan) and used without further purification. Graphite carbon powder (GCP; 57-106 µm, 15.7 m^2/g) was provided by (Nacalai Tesque Kyoto, Japan).

Preconcentration procedure:

Investigated values of GCP; 50mg to 200mg were packed in a glass SPE cartridge (50 mm, 10 mm i.d.) as an adsorbent agent. Naproxen working standards were freshly prepared from the stock solution by dilution with the appropriate volume of the mobile phase and stored at 4^{o}C. The selected concentrations for naproxen were varied from 5.0 ppb to 20 ppb. Selected initial concentration of naproxen was varied and sample solution (200 ml) was spiked with the desired concentrations of naproxen. After adjusting pH values from 2 to 11, samples were allowed to pass through the SPE cartridge glass at different constant flow rates (1 to 2.5 mL/min). The retained naproxen was eluted from GCP with isocratic mixture of 80/20 (v/v) THF/Me with 1ml/min flow rate. Dissolved naproxen in the eluted sample was measured directly with the HPLC system. Blank samples without naproxen but using the same solvent were set up in the same way and passed through SPE for the samples tested. Blanks and control experiments were carried out in the same manner to make sure that, the quantitative analysis is very accurate. Optimal experimental conditions were obtained when sample flow rate; 1.5mL/min, absorbent amount; 150mg, pH;3, desorption solvent; (THF:Me) 8:2, NaCl concentration; 1%. Under the mentioned experimental conditions, enrichment factor was calculated according to the following equation:

Enrichment factor = C / C_o

Where;

C: Concentration of naproxen in the sample after preconcentration.

C_o: Concentration of naproxen in the sample before preconcentration

Instrumentation and chromatographic conditions:

The HPLC system composed of Model GL-7410 pump (GL Science, Tokyo,, Japan); UV Spectrophotometer model GL-7450 (GL Science, Tokyo,, Japan). Separation was carried out by column (ODS-2), 150 mm L x 4.5 mm i.d., (GL Science, Tokyo,, Japan). HPLC was equipped with a fluorescence detector. Injected samples were 20µL. The mobile phase was consisted of an isocratic mixture of phosphate buffer (may be Na_2HPO_4 with H_3PO_4 at pH 6.4) / EtOH 30/70 (v/v). The flow rate was adjusted at 1.5 ml/min. Stable chromatographic conditions were obtained when the detector wavelengths were set at 272 nm excitation, and 250 nm emission as shown in Fig.1.

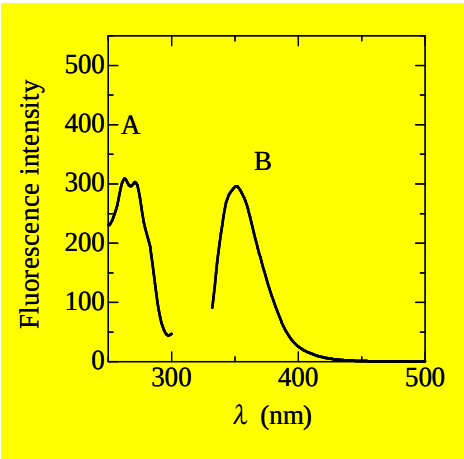

Fig. 1: Naproxen excitation (A) and emission (B) spectra.
Naproxen concentration : 1 µg/mL.

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Preconcentration procedure:

Tested amount of the packing graphite powder was packed in SPE glass cartridge (50mm x 10 mm i.d.). Sample solution (200mL) spiked with 10 ng mL^{-1} of naproxen through the SPE cartridge as a constant flow rate (sucking pump was used to control the flow). pH of the tested samples were adjusted (2-10) with H_2SO_4 or NaOH. The adsorbed amount of naproxen was eluted from graphite powder with 1 ml THF/Methanol (8/2). The analytes in the eluted samples were determined by HPLC.

For quantitative analysis, standards were prepared for comparison and calibration curve was established.

RESULTS AND DISCUSSION

Effect of flow rate:

Flow rate effect was investigated at different flow rate from 1 to 2.5 mL/min as shown in Fig. 2. Under the experimental conditions, as expected increasing flow rate causes decreasing in the enrichment factor. Optimum flow rate was obtained at 1.5 mL/min. therefore this value was taken for further study.

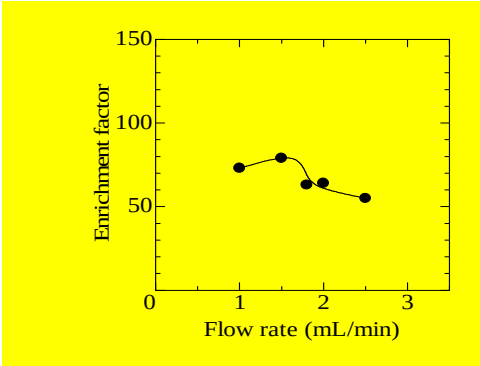

Fig. 2: Effect of sample flow rate on the enrichment factor.
Sample : Naproxen 10 ng/mL, 200mL
pH of sample : 5.6
Adsorbent: graphite powder 200 mg
Desorption: THF:Methanol (8:2) 1 mL

Effect of absorbent amount:

GCP has been used in many applications as a suitable absorbent agent (references). Amount tested was in the range from 50 to 200 g. Fig.3 shows the effect of absorbent amount on the enrichment factor which increases with the absorbent amount till 150g then decreased presumably due to the saturation; therefore 150g was selected for further study.

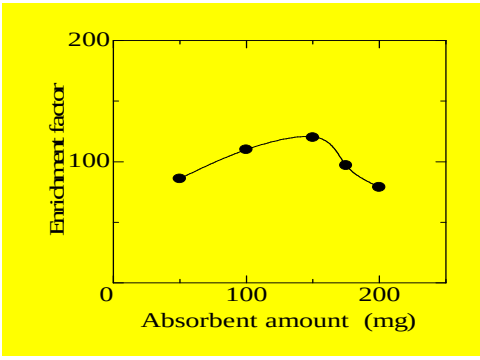

Fig. 3: Effect of absorbent amount on the enrichment factor.
Sample : Naproxen 10 ng/mL, 200mL
pH of sample : 5.6
Flow rate : 1.5 mL/min
Desorption: THF:Methanol (8:2) 1 mL

Effect of initial pH:

The effect of the pH on the enrichment factor was checked with a solution containing 10 ppb of naproxen. Different pH values were checked from 1 to 11. Adjustment was carried out by adding the appropriate drops of

hydrochloric acid or sodium hydroxide solutions to the naproxen samples. The maximum recovery was obtained at low pH range (1-2) as shown in Figure 4. For further study, pH 3 was selected to avoid using more solvent and to protect column damage.

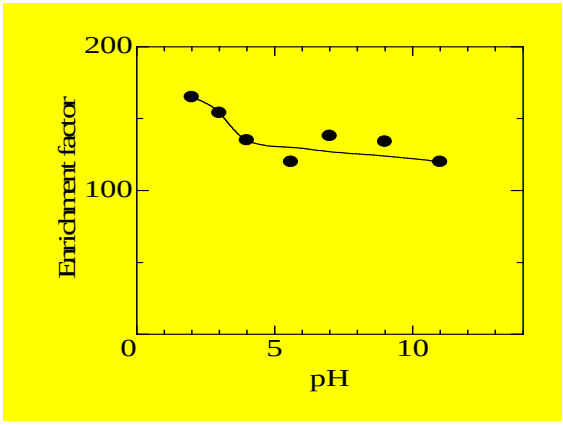

Fig. 4: Effect of initial pH of sample on the enrichment factor.
Sample : Naproxen 10 ng/mL, 200mL
Flow rate : 1.5 mL/min
Adsorbent: graphite powder 150 mg
Desorption: THF:Methanol (8:2) 1 mL

Effect of desorbent solvent:

Suitable desorbent solvent is an essential criterion in solid phase extraction methodology.

In order to examine the effect of the desorbent solvent volume, solution containing different volume percentages of THF/(THF+Me) 10, 50, 60, 70, 80, 90, 100 were tested. Fig. 5 shows the results, where maximum enrichment factor was obtained at THF/ (THF+Me) with 90%, however unstable baseline was noticed, therefore 80% was selected for further study.

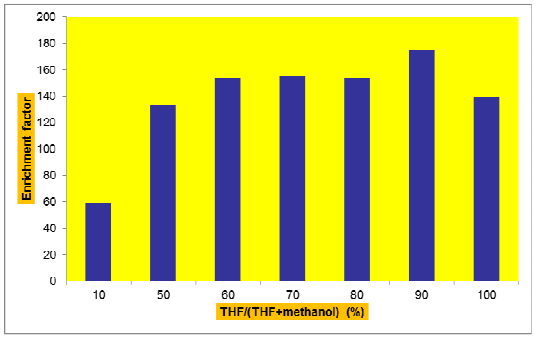

Fig. 5: Effect of desorption solvent on the enrichment factor.
Sample : Naproxen 10 ng/mL, 200mL
Flow rate : 1.5 mL/min
Adsorbent: graphite powder 150 mg
pH : 3

Ionic strength:

It is well known that ionic compounds, when dissolved in water, dissociate into ions. The total electrolyte concentration in solution will affect its important properties such as; the dissociation or the solubility of different salts. In order to evaluate the ionic strength influence different amounts of sodium chloride were added into the tested samples within the range from 0-2% NaCl concentration. Extraction improved for the entire samples treated with NaCl till 1%. Signals also were remained constant in the mentioned range. Sudden decrease was noticed at concentration higher than 1% NaCl. Based on the obtained result 1% salt was selected as the optimal value in order to prevent any negative influence of the original ionic strength of the samples.

Limit of detection and quantification:

For the purpose of quantitative analysis, a calibration curve for naproxen with concentrations ranging from 5 to 20 ppb spiking standards directly into distilled water and extracted under the same optimal conditions

mentioned earlier. As shown in Fig. 6, linearity was observed over the range of 5 to 20 ng/l with a correlation coefficient (R^2) of 0.9999. The detection limit based on a signal-to-noise ratio (S/N) of 3 was 9 pg/mL. The precision of this method was determined by analyzing standard solution at 10ng/L of naproxen for 8 times continuously and the relative standard deviation (RSD) was 15%. The enrichment factor was 145 for 200 ml sample solution.

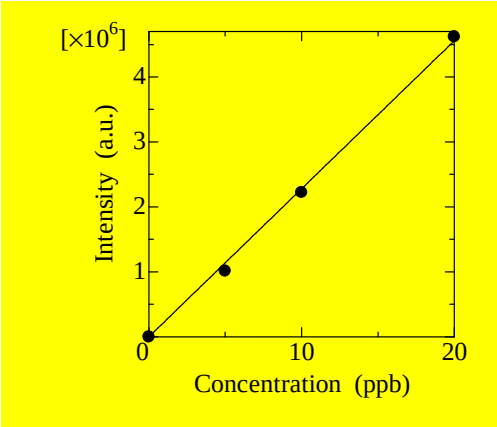

Fig. 6: Relationship between concentration & intensity.
Sample : Naproxen 10 ng/mL, 200mL
pH of sample : 3.0
Flow rate : 1.5 mL/min
Adsorbent: graphite powder 150 mg
Desorption: THF:Methanol (8:2) 1 mL

Determination of naproxen in environmental samples:

The proposed method was applied to the determination of naproxen in environmental samples. Two different rivers were selected for this purpose. The accuracy of this method was assessed through comparing virgin samples with spiked samples. Optimum conditions were used to conduct the assessment (naproxen 10 ng/mL, 200mL; GP 150 mg, flow rate; 1.5 mL/min, THF/Me; 80/20, desorption flow rate 1 mL/min). Results are tabulated in Table 1. River samples were spiked with 10ng/L in order to evaluate the recovery. The recoveries of the spiked samples were in the range from 89% for river A & 94% for river B, with similar relative standard deviations of ±1.7 for both rivers.

Table1: Determination of Naproxen in environmental samples by the proposed method.

Sample	Concentration of Naproxen (ng/L)		Recovery (%)
	Added	Founded	
River A	0	n.d	-
	10	8.9±1.7	89
River B	0	n.d.	-
	10	9.4±1.7	94
Number of analysis; >3, n.d.; not detected			

Conclusion:

A new sensitive and accurate method for determination naproxen in environmental samples was successfully developed by solid phase extraction coupled to high-performance liquid chromatography with fluorescence detection. Enrichment factor reaches to 145 & the detection limit was 9 pg/ml without using excess of solvents and other chemicals. This method could be used to detect other NSAID and/or PPCPs compounds that characterized with very small concentrations in aquatic environmental samples.

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