Sci & Tech.J.NKRAFA.[2024] 20(2), 36- 44

การหาปริมาณปิโตรเลียมไฮโดรคาร์บอนทั้งหมดในน้ำทะเล ด้วยวิธีสเปกโตรฟลูออโรเมทรีแบบลดสเกล

A reduced scale spectrofluorometric method for determination of total petroleum hydrocarbon in seawater

[1*]นภา ตั้งเตรียมจิตมั่น และ [2#]รัชชานนท์ วงษ์พันธ์
[1,2]ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยบูรพา
[1*]Napa Tangtreamjitmun and [2#]Ruchanon Wongpun
[1,2]Department of Chemistry, Faculty of Science, Burapha University
[*]napa@go.buu.ac.th, [#]jameandjaja_@hotmail.com

Received: June 7,2024
Revised: July 10,2024
Accepted: October 10,2024

บทคัดย่อ

วิธีมาตรฐานในการวิเคราะห์ปิโตรเลียมไฮโดรคาร์บอนทั้งหมด (TPH) ในน้ำทะเลซึ่งจัดทำโดยคณะกรรมาธิการระหว่างรัฐบาลว่าด้วยสมุทรศาสตร์ได้ถูกนำมาปรับปรุงให้สอดคล้องกับแนวคิดเคมีวิเคราะห์สีเขียว โดยทำการลดปริมาตรของตัวอย่างน้ำทะเลและตัวทำละลายอินทรีย์ที่ใช้ในการสกัด จากการลดปริมาตรนี้ทำให้แฟกเตอร์เพิ่มความเข้มข้นลดจาก 800 เป็น 20 ซึ่งเหมาะสมกับสภาพไวในการวิเคราะห์ของเครื่องสเปกโทรฟลูออโรมิเตอร์ที่มีขายในปัจจุบัน กราฟมาตรฐานของไครซีน (ที่ไม่ได้ผ่านการสกัด) มีความเป็นเส้นตรงระหว่าง 10-200 ไมโครกรัมต่อลิตร มีสมการเส้นตรง y=1.567x+2.524 และค่าสัมประสิทธิ์การตัดสินใจ (R^2) 0.9992 ค่าขีดจำกัดการตรวจวัด (3sd/m, n=10) และค่าขีดจำกัดการวิเคราะห์ปริมาณ (10sd/m, n=10) มีค่าเท่ากับ 0.065 และ 0.22 ไมโครกรัมต่อลิตร ตามลำดับ วิธีที่นำเสนอนี้ได้นำไปวิเคราะห์ปริมาณ TPH ในน้ำทะเลชายหาดบางแสน จังหวัดชลบุรี ประเทศไทย วิธีที่นำเสนอนี้ควรได้รับการทดสอบการหาความเที่ยงของวิธีระหว่างห้องปฏิบัติการจากหน่วยงานประกันคุณภาพของวิธีวิเคราะห์เพื่อการยอมรับให้เป็นวิธีมาตรฐานในการวิเคราะห์ TPH ในน้ำทะเล

คำสำคัญ : เคมีวิเคราะห์สีเขียว, น้ำทะเล, สเปกโทรฟลูออโรมิเตอร์, ปิโตรเลียมไฮโดรคาร์บอนทั้งหมด

Abstract

The standard guide for determining total petroleum hydrocarbon (TPH) in seawater, established by the Intergovernmental Oceanographic Commission (IOC), has been adapted to align with principles of green analytical chemistry. This adaptation involves reducing both the sample volume and the amount of extraction solvent used. These modifications have reduced the pre-concentration factor from 800 to 20, making it compatible with the high-sensitivity spectrofluorometers currently available in the market. A calibration curve of chrysene without extraction was linear over a concentration range of 10-200 μg/L. The regression equation was y=1.567x+2.524 with the coefficient of determination (R^2) 0.9992. Limit of detection (3sd/m, n=10) and limit of quantitation (10sd/m, n=10) were 0.065 and 0.22 μg/L, respectively. The method was employed to assess TPH levels in seawater along Bangsean beach in Chonburi province, Thailand. To establish it as a standard method, collaborative trials of this approach should be conducted for validation and acceptance.

Keywords: green analytical chemistry, seawater, spectrofluorometer, total petroleum hydrocarbon

1. Introduction

Marine pollution resulting from petroleum hydrocarbons is a significant concern, given their toxicity to both human and marine ecosystems. Certain Total petroleum hydrocarbons (TPH) compounds can impact the human nervous system, leading to symptoms such as headaches and dizziness. Consequently, it is imperative to take measures to address and mitigate the impact of these pollutants on both our environment and overall health. In recognition of this, the Intergovernmental Oceanographic Commission took a significant step by establishing the standard method for monitoring seawater quality with respect to total petroleum hydrocarbon (TPH) levels back in 1984 [1]. The analysis of TPH is considered semi-quantitative due to the diverse range of hydrocarbons that can contaminate seawater samples. Thus, TPH determination in seawater is quantified in units of "chrysene equivalent" a metric defined by the IOC to account for this variability. Chrysene is a polycyclic aromatic hydrocarbon (PAH) with the molecular formula $C_{18}H_{12}$. There are four fused benzene rings in chrysene structure that benefit for spectrofluorometric determination as depicted in Figure 1. The method was published as a standard guide called "Manual for monitoring oil and dissolved/dispersed petroleum hydrocarbons in marine water and on beach"[1].

This IOC guide suggested extraction procedure, which uses 100 mL of hexane to extract TPH from 3-4 L of the seawater sample using a separatory funnel. The large volumes of sample and hexane used in this extraction process have created operational challenges, making the procedure cumbersome. Furthermore, it generates a substantial amount of waste solution, which is not environmentally friendly. The standard guide employs spectrofluorometric analysis as its technique. A spectrofluorometer features a single light source, two monochromators, and two detectors. One detector measures the initial intensity of the light from the source, while the other detector measures the emitted light from a substance in solution. Despite these challenges, this standard guide remains in use within numerous national and international monitoring programs for TPH determination in seawater [2]. In recent years, technological advancements have accelerated, particularly in scientific instrument technology, resulting in notable enhancements in spectrofluorometer performance, especially regarding sensitivity. With the availability of highly sensitive instruments, smaller sample quantities are now sufficient for precise determination, enabling more efficient and environmentally conscious analytical processes.

To address these concerns, we engaged in the task of aligning the IOC standard guide for TPH determination with the principles of green analytical chemistry. Our approach involved scaling down the analysis process by reducing both the volume of seawater sample and the amount of extracted solvent specified in the IOC standard guide. In accordance with the guide, we utilized hexane and a separatory funnel for extraction. Subsequently, the resulting extracted solution was subjected to measurement using a spectrofluorometer. This transformation aligns with environmentally friendly practices while maintaining the integrity of the analytical process. The modified method was used for determination of TPH in seawater samples in a unit of chrysene equivalent. The analytical figures of chrysene determination using spectrofluorometric technique was evaluated to demonstrate the current performance of spectrofluorometer in use today.

2. Materials and methods

2.1 Apparatus and reagents

A spectrofluorometer model Cary Eclipse (Agilent, USA) was used for the analysis in this work. Deionized water (DW) was obtained from E-Pure Barnstead 3 column model D4632 (Thermo fisher scientific, USA). Chrysene ($C_{18}H_{12}$, analytical standard grade) was purchased from Supelco (USA). Hexane (C_6H_{14}, AR Grade) was purchased from RCI Labscan (Thailand). Sodium sulphate anhydrous, (Na_2SO_4, AR/ACS Grade) was purchased from Loba Chemie (India).

2.2 Procedures

A 100 mL of seawater sample was accurately pipetted into the 125 mL separatory funnel using a volumetric pipet, followed by the addition of 5 mL of hexane using volumetric pipet. After securely sealing the separatory funnel, vigorous shaking for 5 minutes ensued, allowing for proper mixing. Subsequently, the mixture was left undisturbed for phase separation, with the upper organic phase being carefully transferred into a covered beaker. The water phase underwent a second extraction with an additional 5 mL of hexane, and the second organic phase was combined with the first. Anhydrous sodium sulfate was introduced to absorb any remaining water content, and the saturated sodium sulfate was then filtered out using No.1 Whatman filter paper. The extracted solvent was left to evaporate in a fume hood. These residues were dissolved with hexane and transferred into a 5 mL volumetric flask. Finally, the solution was measured using a spectrofluorometer for TPH analysis.

The spectrofluorometer conditions were optimized as following; an excitation wavelength (λ_{ex}) 310 nm and an emission wavelength (λ_{em}) 361 nm, both utilizing a slit width of 10 nm. The average reading time was set at 0.1 s, and a photomultiplier tube (PMT) voltage of 600 volts was applied for precise and sensitive measurements. These settings ensured the accuracy and reliability of the spectrofluorometric analysis.

2.3 Sampling

Seawater samples were collected from a depth of 1 meter below the surface at three distinct locations along the Chonburi coastal region in Thailand as shown in Figure 1. These specific sampling sites were identified as follows: Wonnapa beach (S1 - 13°16'16.3"N 100°55'19.2"E), Bangsaen beach (S2 - 13°17'08.5"N 100°54'44.4"E), and Laemtan beach (S3 - 13°18'11.4"N 100°53'49.3"E). To preserve the samples' integrity, one-liter amber glass bottles were employed for collection, and the bottle caps were sealed with aluminum foil to prevent any external contamination. Subsequently, the samples were stored in a dark and cool environment and analyzed within 3-4 hours after the initial sampling, in strict accordance with the guidelines specified in the IOC standard guide.

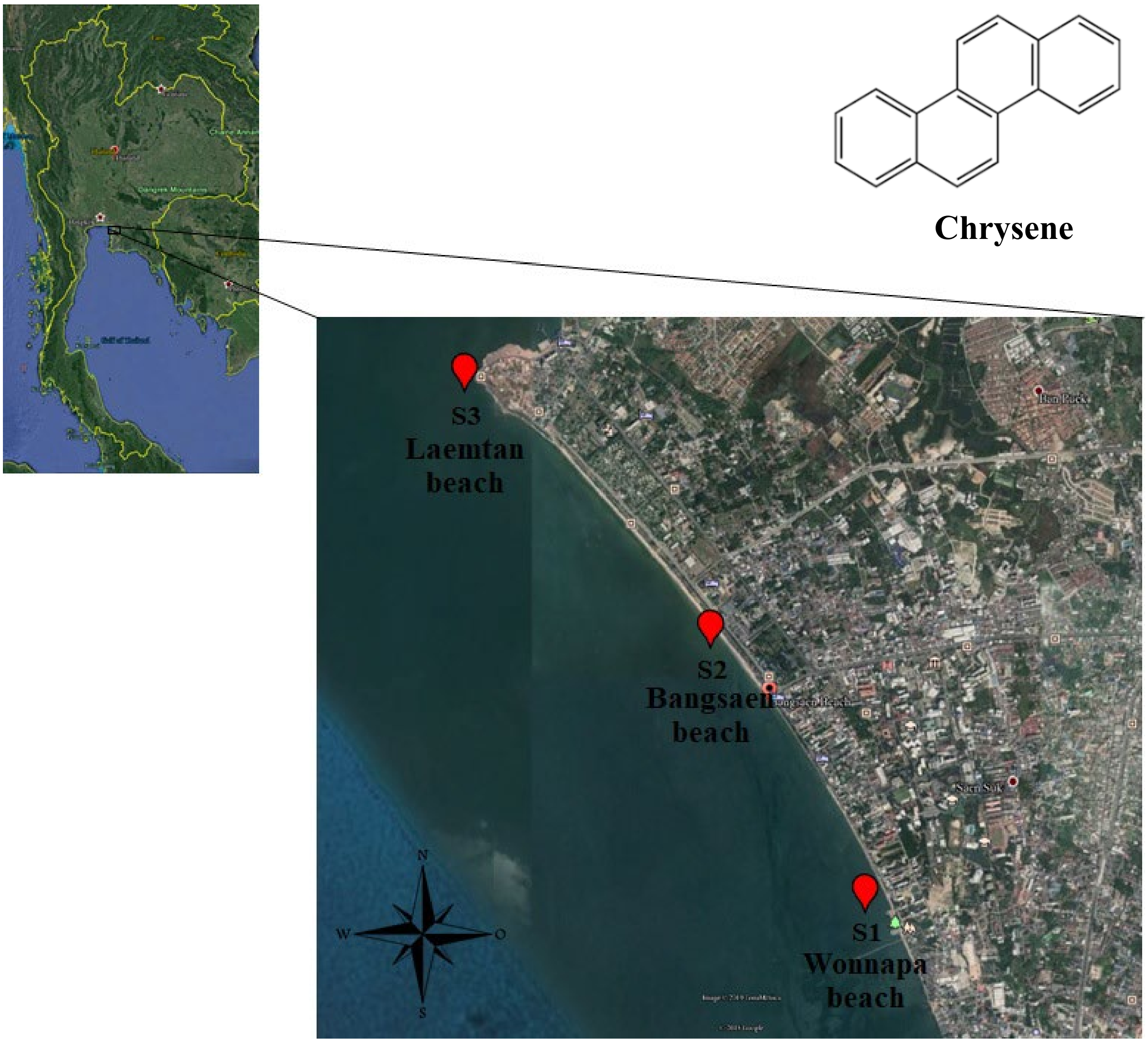

Figure 1. Chrysene standard structure and location of sample site; S1, S2 and S3 along Chonburi coastal in Thailand.

3. Results and discussion

Chrysene was used as a standard for TPH analysis according to IOC standard guide. The analytical figures of merits for chrysene determination by spectrofluorometer were presented as following.

3.1 Calibration curve

The standard solutions of chrysene were prepared in hexane and measured by spectrofluorometer. The calibration curve was linear over a concentration range of 10-200 μg/L as Figure 2. The regression equation was y=1.567x+2.524 with the coefficient of determination (R^2) 0.9992.

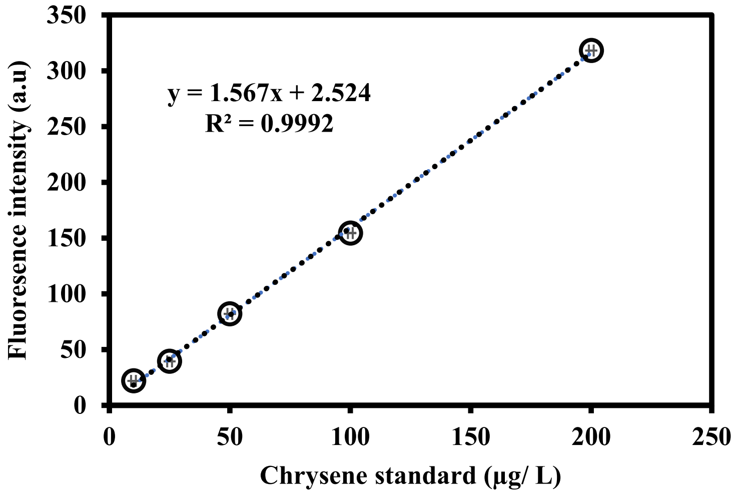

Figure 2. The calibration curve of chrysene standard (n=3).

In Figure 2, the observed chrysene concentration range was notably lower, approximately 10-15 times less, than the recommended calibration curve range stipulated in the IOC standard guide, which was 0.1-3 μg/mL or 100-3000 μg/L [1]. This observation underscores the fact that modern chrysene analysis can be effectively conducted at significantly lower concentration ranges than previously prescribed in the IOC standard guide.

3.2 Limit of detection (LOD) and limit of quantitation (LOQ)

The blank solution, which consisted of deionized water, underwent spectrofluorometric measurement following the procedure outlined in section 2.2. To establish the limit of detection and limit of quantitation, the standard deviations were calculated based on ten replicated measurements of the blank signal. The limit of detection was determined as 0.065 μg/L while the limit of quantitation was found to be 0.22 μg/L, each derived from three and ten times the standard deviation, respectively.

3.3 Precision

Some low molecular weight TPH in seawater samples can be easily lost by volatilization. In order to avoid any lost that would occur during three days storage of samples, the freshly prepared chrysene standard solution was used to study the precision of the proposed method. A 100 mL of 5 μg/L chrysene standard solution was daily prepared and measured through the procedure in section 2.2 for extraction and spectrofluorometric detection. The analysis was performed in duplicate for three consecutive days. The results were shown in Table 1.

Table 1 Chrysene determination using the proposed method over three consecutive days.

Day	Chrysene (μg/L)	
	Duplicate 1	Duplicate 2
1	3.52	3.65
2	3.21	3.36
3	3.79	3.69

One-way ANOVA statistic function was used to determine the mean square between days (MSB) and the mean square within days (MSW) as given in Table 2. The obtained *p*-values 0.0333 is smaller than 0.05, therefore the between day variation is larger than within day variation. Within day variation represents repeatability and between day variation represents the intermediate precision in terms of the within-laboratory reproducibility of the proposed method [3-4].

Table 2 Statistical results of single ANOVA for precision analysis from Excel program.

Source of Variation	SS^{a}	df^{b}	MS^{c}	*F*	*p*-value	*F* crit
Between Groups	0.2140	2	0.1070	13.0	0.0333	9.6
Within Groups	0.0247	3	0.0082			
Total	0.2387	5				

^{a} sum square, ^{b}degree of freedom, ^{c}mean square

The standard deviations for repeatability (s_r) and within-laboratory reproducibility (s_R) were calculated from the MS using the following equation 1 and 2 [5].

For repeatability,

$$S_r = \sqrt{MSW} = \sqrt{0.0082} = 0.091 \ \mu\text{g/L} \quad (1)$$

For within-laboratory reproducibility,

$$s_R = \sqrt{MSW + \frac{(MSB - MSW)}{n}} = \sqrt{0.0082 + \left(\frac{0.1070 - 0.0082}{2}\right)} = 0.24 \ \mu\text{g/L} \quad (2)$$

The repeatability and within laboratory reproducibility in term of relative standard deviations (RSD) would be 2.57% and 6.79%, respectively. Both RSD values fall within the acceptable range as per the guidelines established by the Association of Official Analytical Chemists [6].

The determined analytical figures indicated high sensitivity of spectrofluorometer commonly used in general laboratory. As a consequence, the high sensitivity enables a reduction in the volume of seawater sample required for extraction. In IOC standard guide, 3-4 L of seawater sample was extracted twice using 50 mL of hexane each time. The combined 100 mL of extracted hexane was then concentrated down to 5 mL using a rotary evaporator, resulting in a preconcentration factor of 600-800, necessitated by the wide concentration range of the calibration curve (100-3000 μg/L) mentioned earlier. However, recent research and publications on TPH analysis in seawater using a modified IOC standard guide indicate a trend towards utilizing lower preconcentration factors, as summarized in Table 3.

Table 3 Preconcentration factors for TPH analysis using the modified IOC standard guide in published research.

Seawater (mL)	Hexane volume used in extraction (mL)	Preconcentration factor	References
4000	5	800	[7]
4000	10	400	[8]
2000	5	400	[9]
4000	10	400	[10]
2800	10	280	[11]
1000	5	200	[12]
1000	10	100	[13]
100	5	20	This work

In this study, a sample volume of 100 mL was extracted twice using 5 mL of hexane each time. The resulting 10 mL of combined hexane extract was then reduced to 5 mL before spectrofluorometric analysis. Consequently, the preconcentration factor for this extraction method was 20. The method's quantitation limit was calculated as 0.22/20 = 0.011 μg/L, which is well below 0.07 μg/L detection limit reported by Bicego and colleagues [7] using a preconcentration factor of 800. This indicates that a significantly large preconcentration factor is not essential for TPH analysis.

3.4 Sample analysis

The samples collected from locations S1, S2, and S3 were extracted with hexane and analyzed using a spectrofluorometer, following the procedure described in section 2.2. To determine the TPH concentration in these samples, the calibration curve was employed, with the TPH concentration reported in μg/L being equivalent to that of chrysene, as detailed in Table 4.

Table 4 TPH in seawater samples collected from Chonburi coastal in Thailand (n=3).

Sample site	TPH (μg/L)
Wonnapa beach (S1)	1.05 ± 0.02
Bangsaen beach (S2)	0.90 ± 0.09
Laemtan beach (S3)	0.70 ± 0.08

The TPH limit for seawater in Thailand was classified by Pollution Control Department, Ministry of Natural Resources and Environment into three levels with three standards of seawater quality: 0.5 μg/L for natural resource conservation and aquatic animal breeding, 1.0 μg/L for tourist attraction areas, and 5.0 μg/L for industrial, harbor, and community seawater. The seawater quality from the three consecutive sample sites (S1, S2, and S3) falls into these three distinct groups. S1, located near a small fisherman village with frequent fishing boat discharges, exhibited the highest TPH concentration. S2, a tourist attraction beach with lesser discharges from recreational motor boats, had a lower TPH concentration. S3, a beach with minimal human activity and predominantly oyster farming, showed the least TPH concentration. Notably, the TPH concentration in S3 exceeded the limit for aquatic animal breeding, while the other two sample sites, remained within their respective quality standard limits.

4. Conclusions

This study presents a spectrofluorometric approach for determining TPH levels in seawater. The method involves a modification of the liquid-liquid extraction procedure outlined in the IOC standard method, achieved by reducing the volume of both seawater sample and the solvent used for extraction. This reduction not only lowers the analysis costs due to reduced solvent usage but also minimizes waste production. Moreover, working with smaller seawater sample volumes for extraction and collection proves to be less cumbersome. This reduced scale method is environmentally friendly to conform to green analytical chemistry [14]. Further work on a Collaborative Trials (CT) of this proposed method should be performed involving an accredited quality assurance organization to validate this proposed method and potentially establish it as an accepted standard [5], [15].

5. Acknowledgements

This work was partially supported by Science Innovation Facility, Faculty of Science, Burapha University (SIF-IN-51300014).

6. References

[1] IOC/UNESCO (1984). Manual for monitoring oil and dissolved/dispersed petroleum hydrocarbons in marine waters and on beaches. France. Retrived on March 31, 2024: from https://www.jodc.go.jp/info/ioc_doc/Manual/060618eb.pdf

[2] Wattayakorn, G. (2012). Petroleum pollution in the gulf of Thailand: A historical review. *Coastal Marine Science,.* 35(1): 234-245.

[3] ISO5725-3:1994. Accuracy (trueness and precision) of measurement methods and results –Part 3: Intermediate measures of the precision of a standard measurement method. Geneva, International Organization for Standardization.

[4] ISO3534-2:2006. Statistics – Vocabulary and symbols – Part 2: Applied statistics. Geneva, International Organization for Standardization.

[5] Thompson, M. & Lowthian, P.J. (2011). **Notes on statistics and data quality for analytical chemists**. London: Imperial College Press.

[6] AOAC (2016) Guidelines for standard method performance requirements: Appendix F. (Association of Official Analytical Chemists) Rockville, MD, USA. Retrived on March 31, 2024: from: http://www.eoma.aoac.org/app_f.pdf

[7] Bícego, M.C., Zanardi-Lamardo, E. & Weber, R.R. (2003). Four-year survey of dissolved/dispersed petroleum hydrocarbons on surface waters of Admiralty Bay, King George Island, Antarctica. *Revista Brasileira de Oceanografia.* 51: 33-38.

[8] Wattayakorn, G. & Rungsupa, S. (2012). Petroleum hydrocarbon residues in the marine environment of Koh Sichang-Sriracha, Thailand. *Coastal Marine Science.* 35(1): 122-128.

[9] González, J.J., Viñas, L., Franco, M.A., Fumega, J., Soriano, J.A., Grueiro, G., Muniategui, S., López-Mahía, P., Prada, D., Bayona, J.M., Alzaga, R. & Albaigés, J. (2006). Spatial and temporal distribution of dissolved/dispersed aromatic hydrocarbons in seawater in the area affected by the Prestige oil spill. *Marine Pollution Bulletin.* 53(5): 250-259.

[10] Shriadah, M.M.A. (2000). Levels and distribution of petroleum hydrocarbons in the coastal waters and sediments of the United Arab Emirates in the Arabian Gulf and the Gulf of Oman. *Water, Air, and Soil Pollution.* 119(1): 247-256.

[11] Chouksey, M.K., Kadam, A.N. & Zingde, M.D. (2004). Petroleum hydrocarbon residues in the marine environment of Bassein–Mumbai. *Marine Pollution Bulletin.* 49(7): 637-647.

[12] Nageswar, R.M., Ram, A., Rokade, M.A., Raja, P., Rakesh, P.S., Chemburkar, P. & Gajbhiye, S.N. (2016). Preliminary estimate of total petroleum hydrocarbons in water and some commercially important fish species in the Amba estuary, west coast of India. *Bulletin Environmental Contamination Toxicology,* 97(1), 56-62.

[13] Pikkarainen, A.L. & Lemponen, P. (2005). Petroleum hydrocarbon concentration in Baltic sea surface water. *Boreal Environment Research.* 10: 125-134.

[14] Guardia, M. & Garrigues, S. (2012). **Handbook of green analytical chemistry.** UK: John Wiley & Sons, Ltd.

[15] Miller, J.M. & Miller, J.C. (2010). **Statistics and chemometrics for analytical chemistr**y. 6th ed, Gosport: Ashford Colour Press Ltd.