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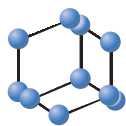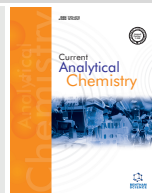
Determination of Monoaromatic Hydrocarbons in Water Samples by Nano-Liquid Chromatography using a Composite Carbon Nanotubes-Lauryl Polymethacrylate Capillary Monolithic Column

Ahmad Aqel[1,*], Soad S. Alzahrani[1,2], Asma'a Al-Rifai[3], Mohammed Alturkey[1], Kareem Yusuf[1], Zeid A. ALOthman[1] and Ahmed-Yacine Badjah-Hadj-Ahmed[1]

[1]*Department of Chemistry, College of Science, King Saud University, Riyadh 11451, KSA;* [2]*Department of Chemistry, College of Science, Al Imam Mohammad Ibn Saud Islamic University, Riyadh, KSA;* [3]*Department of Chemistry, College of Science, Princess Nourah bint Abdulrahman University, Riyadh, KSA*

Abstract: ***Background***: This work reports a green analytical method for the determination of organic environmental pollutants using nano-liquid chromatography with a self-made column for rapid, sensitive, inexpensive and efficient analysis of BTX pollutants in water. The applications of monolithic nano-scale columns for quantitative analysis of environmental real samples are very limited in the literature.

Methods: A capillary column containing a composite of multi-walled carbon nanotubes incorporated into a lauryl methacrylate-*co*-ethylene dimethacrylate porous monolithic polymer was fabricated for the determination of BTX pollutants in real water samples.

Results: Baseline separation was accomplished at 0.4 μL/min flow rate with UV-detection set at 208 nm. Under the optimum conditions, the calibration curves were validated over the range of 1.0-500 μg/L with R^2 more than 0.9992. The detection limits of benzene, toluene, *o*-xylene and *m/p*-xylene were 0.25, 0.05, 0.075 and 0.05 μg/L, respectively. After a simple extraction process with a theoretical preconcentration factor equal to 200, the recovery values in Milli-Q, tap and sea water samples were found to be ranged from 84.85 to 97.84% with %RSD less than 7.5. Furthermore, we reported a comparison between our prepared composite column with a commercial C_{18} silica based column which is the most used in such analytical field. Each column demonstrated its advantages from different analytical aspects.

Conclusion: The application of monolithic columns and nano-scale LC for routine analysis of environmental samples is very promising as the use of monolithic capillary columns offers several advantages over conventional scale particulate packed columns.

1. INTRODUCTION

Monocyclic aromatic hydrocarbons, such as benzene, toluene and xylene isomers, abbreviated as (BTX), are among the Volatile Organic Hydrocarbons (VOHs). They are relatively abundant in petroleum derivatives such as gasoline [1]. BTX compounds are characterized by their physical properties; they have moderate solubility in water, high vapor pressures and they are all liquids at room temperature [2]. The common feature of all these substances is the single aromatic ring. They are important industrial solvents which are extracted from petroleum products, and widely used in synthetic rubber, inks, paints, food, pharmaceuticals, adhesives and as chemical intermediates [3-5]. They are also used in replacement of alkyl-lead compounds, as additives in gasoline to enhance octane rating and to improve the combustion process [6].

Because of their toxicity, volatility, migration abilities and negative impacts on human health and environmental, BTX compounds are listed as priority pollutants [7]. They enter the body through breathing at gas stations or absorption into the skin while swimming in contaminated waters [8]. BTX have major effects on the central nervous system, and may produce symptoms such as damage to bone marrow, liver and kidney disease [9], weakness and memory loss [10], abnormality, vision alteration [11], headache and eye irritation [12], immune system depression, dizziness, drowsiness [13] and even cancer [4, 7, 14]. In addition, BTX contributes to many environmental problems such as odor nuisance, global warming and photochemical ozone formation [1]. Among all of these compounds, benzene is considered

*Address correspondence to this author at the Department of Chemistry, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, KSA; Tel: +966 11 4674198; E-mail: aifseisi@ksu.edu.sa

the most toxic one [12]. According to the US environmental protection agency (EPA), the Maximum Contaminant Levels (MCLs) for BTX compounds in drinking water are 0.005, 1 and 10 mg/L of benzene, toluene and xylenes, respectively [12, 14, 15].

Determination of BTX compounds in environmental matrices is difficult because they are highly volatile and are degraded by microorganisms. In addition, they are present in trace-levels and their partial loss can occur during sample handling and extraction. Therefore, it is very important to develop rapid, sensitive, reliable and accurate analytical methods to detect their presence, in order to minimize their harmful effects on the human and environment [16].

Various analytical procedures have been developed for the determination of mono aromatic hydrocarbons in several environmental samples including waters. Since the concentration of BTX compounds in water is rather low at μg/L trace-levels, samples need some treatments, such as preconcentration and extraction to reach an adequate concentration before analysis [3, 14]. In general and regardless of their advantages or disadvantages, three classes of preconcentration techniques have been used for BTX compounds. The first class makes BTX hydrocarbons concentration in gas phase, such as headspace extraction and purge and trap process [17]. In the second approach BTX are concentrated in a liquid phase using different solvents, such methods include liquid-liquid extraction [18], headspace solvent microextraction [5], ionic liquid based single-drop microextraction [19] and suspended droplet microextraction [14]. Thirdly, concentration on a solid phase has been also widely used for BTX aromatics enrichment, such as solid-phase extraction and microextraction [20-22].

The presence of BTX and other light aromatic hydrocarbons in water is an indicator of the presence of oil pollution. The quantitative determination of BTX compounds in environmental samples is commonly carried out by GC-FID, which is one of the most preferred techniques by various health and environmental organizations and was cited in many reports [13, 15, 21]. However, other detectors have been recommended and used after gas chromatographic separation such as photoionization and mass spectrometry [6, 13, 22, 23]. On the other hand, there are relatively few studies on BTX analysis using an HPLC with UV and even fluorescence detection [7, 24, 25]. In general, the determination of BTX pollutants by HPLC is limited by its relatively low sensitivity and longer analysis time [7]. Furthermore, the commercially available HPLC columns are not able to separate all monocyclic aromatic alkyl-substituted hydrocarbons, since the isomers have usually similar retentions [26].

The use of organic monolithic polymers as stationary phase in chromatographic separation has continued to grow since the first report in 1992 [27]. Because of their high permeability and presence of only large through pores in their structure, the first generation of organic polymer monolithic columns exhibited fast separation of large molecules such as proteins at high mobile phase flow rates [28, 29]. However, the lack of mesopores means a limited surface area and hence does not provide a sufficient number of interaction sites in the monolithic structure, thus making the unmodified monoliths unable to separate small molecules, especially in isocratic mode [28].

Several approaches have been proposed to enhance the separation efficiency of the monolithic columns. Some groups tried to optimize the polymerization conditions including time and temperature as well as monomers and porogenic solvents composition [30, 31]. Other groups attempt to use a single crosslinker [32], introducing a new hypercrosslinking reaction [33], termination of the polymerization reaction at early stage [34] and incorporation of nano or microparticles into the naked porous monolithic structure such as Metal-Organic Framework (MOFs) [35, 36], carbon nanotubes (CNTs) [37-39], C_{60} fullerene [39, 40] and sporopollenin microparticles [41]. Due to the large surface-to-volume ratio of carbon nanotubes [42], the small surface area in addition to the lack of mesopores and sufficient interaction sites in the unmodified monolithic framework could be overcome through the incorporation of these carbon nanoparticles into the polymer monolithic structure.

The miniaturization of the liquid chromatography system including capillary, micro- and nano-LC is one of the present trends in separation science; these techniques have been established as a complementary or competitive to the conventional scale HPLC systems [43]. Miniaturized liquid chromatography techniques use columns with smaller internal diameter (*i.d.*) than in conventional HPLC. Nowadays, it is widely accepted that the definition of nano-LC is a chromatographic separation performed using capillary columns with *i.d.* range from 10 to 100 μm, and mobile flow rate less than 500 nL/min [43, 44]. The main advantage of using smaller *i.d.* columns in HPLC is the increased detection sensitivity that can be obtained as a result of reduced sample dilution during the analytical process [43-45]. This is very important to improve detection limits and allow determination of compounds present at low concentrations in limited volume samples. Other advantages include the ability to analyze rare compounds of interest in lower samples, as well as the reduction of solvent consumption. This addresses both the cost of solvents and chemicals and diminishes the environmental impact of the toxic chemicals.

This work focused on the development of a new analytical method for determination of BTX compounds in water samples. Due to their amazing properties, the combination of carbon nanotubes, monolithic material and a miniaturized liquid chromatography system might provide fast, sensitive, efficient and cost efficient method for routine analysis of BTX and related pollutants in water samples. The analytical method was validated and the prepared composite capillary column was compared with a commercial C_{18} silica based packed column.

2. EXPERIMENTAL

2.1. Materials and Samples

Sodium hydroxide, toluene, hydrochloric acid, 1-propanol and acetic acid were provided from BDH (Lutterworth, UK). Benzene, *o*-, *m*- and *p*-xylenes were acquired from Acros Organics (Geel, Belgium). Ethylene dimethacrylate (EDMA), 3-(trimethoxysilyl)propyl methacrylate, azo-bis-isobutyronitrile (AIBN), lauryl methacrylate (LMA) and 1,4-butanediol were obtained from Aldrich (Steinheim, Germany). HPLC grade solvents acetone, acetonitrile, tetrahydrofuran, ethanol and hexane were purchased from Fisher Scientific (Leices-

tershire, UK). The purified water was prepared using Millipore system (Milli-Q Advantage Elix, Millipore S.A.S. 67120 Molsheim, France).

Empty fused silica capillary with 0.100 mm *i.d.* and 0.365 mm outer diameter (o.d.), coated with polyimide was provided from Restek (Bellefonte, USA). Multi-walled carbon nanotubes with 2-5 nm *i.d.*, less than 8 nm o.d., 10-50 µm length and 95% purity were provided from Chengdu Organic Chemicals Co. Ltd. (Chengdu, China).

Two types of water samples were used in this study for evaluation of the prepared column and proposed method; sea water and tap water. Sea water samples were prepared by collecting various water samples from Al-Dammam city coast (Eastern Province, Saudi Arabia) from various locations at various days in brown amber glass bottles. Tap water sample was taken after flowing for 3 min from various water taps in our school in different days, and then used to generate a composite water sample. All water samples were collected from their resources by filling the water to the top of the bottles, and stored at 5°C after adding small amounts of HCl. All analyses were carried out in less than one week from sampling. Prior to use, all mobile phase solvents were filtered using a vacuum glass filtration system through 0.20 µm nylon membrane filters obtained from Millipore (Bedford, MA, USA). On the other hand, water samples were not filtered to avoid loss of volatile compounds.

2.2. Capillary Columns Preparation

In order to enhance formation of silanol groups (Si-OH) along the inner wall of the capillary tube, the inner surface of the empty fused silica capillary was modified by flushing with acetone, water, 0.20 M sodium hydroxide solution, water, 0.20 M hydrochloric acid and ethanol. The fused silica capillary was then pumped with 3-(trimethoxysilyl)propyl methacrylate in ethanol 10% (v/v) adjusted to pH 5 using acetic acid solution for 4 h; then, the capillary was washed with ethanol and dried with a highly pure nitrogen gas. The interior wall of the activated silica tubing was modified with the 3-(trimethoxysilyl)propyl methacrylate to improve the covalent attachment of the monolith stationary phase to the capillary inner wall surface.

The monomeric mixture was prepared in 40 mL clear glass vial at the following weight percentages: 24% LMA, 16% EDMA, and 35% 1,4-butandiol with 25% 1-propanol as porogenic solvents and 1% (with respect to monomers) AIBN initiator. In order to ensure the homogeneity of nanotubes inside the column after monolith synthesis, the mixture was incorporated with 1.0 mg/mL CNT (0.1 wt% with respect to monomers), vortexed for 1 min, purged with stream of nitrogen for 3 min and subjected to high-power sonication for 10 min at 40°C in water bath. The grayish CNT-monomeric mixture was then filled in the activated capillary column and both ends were plugged with two pieces of rubber. The polymerization was completed in the same water bath at 70°C for 20 h. Then, rubber seals were removed, the capillary column was then cut to 200 mm length, connected to nano-LC system and washed with pure acetonitrile. A control column (LMA-*co*-EDMA) without incorporated nanotubes was prepared with the same monomeric mixture percentages and by an identical procedure.

2.3. Extraction Procedure and Sample Preparation

A stock solution of each BTX with a concentration of 1000 µg/L was prepared using HPLC grade acetonitrile. Working standard solutions, from 1.0 to 500 µg/L, were prepared by appropriate dilutions of the stock solution using HPLC grade acetonitrile. As a control sample, a sample of 1 L of Milli-Q water was spiked with 0.50 µg/L of each pollutant, 5.0 mL HPLC grade hexane was added and the mixture was shaken for half an hour. The phase separation was then carried out using a suitable separatory funnel. The final extract was diluted with HPLC grade acetonitrile and directly injected into the nano-LC system at optimum chromatographic conditions. The same procedure has been repeated for both tap and sea water samples.

2.4. HPLC Analysis

All chromatographic evaluations were carried out using a Dionex Ultimate 3000 RSLC nanosystem (Sunnyvale, CA, USA), equipped with a 3 nL Ultimate 3000 variable wavelength detection cell and an external injector with a fixed 4 nL inner sampling loop (Vici Valco, Houston, USA). A mixture consisting of water/acetonitrile/tetrahydrofuran (60:35:5, v/v) was used as mobile phase in isocratic mode. The flow rate was 0.4 µL/min with a column temperature of 70°C. The detector was set at a 208 nm. Chromeleon 7.2 data package was used to control the nanosystem and to acquire the chromatographic results.

Nucleodur C_{18} Gravity column with 100×4.0 mm dimensions, packed with 3 µm size particles, was purchased from Macherey-Nagel GmbH (Düren, Germany). Shimadzu HPLC system (Kyoto, Japan) including a pump (LC-20AD), a Rheodyne 7125 manual injector equipped with a 10.0 µL sample loop and a UV detector (SPD-M20A) was used for the comparative study. For quantitative experiments, peak areas were used, while each standard or sample was injected five times. Microsoft Office Excel software 2010 package was used for statistical parameter calculations.

2.5. Validation

The proposed method was validated in terms of precision, limit of detection, quantitation and linearity (LOD, LOQ and LOL) and recovery. Standard solutions with concentrations ranging from 1.0 to 500 µg/L of pollutants were prepared. In order to study the method precision and linearity, each solution was injected in five replicates and peak areas were plotted versus the respective concentrations of pollutants. LOD and LOQ corresponded to the pollutant concentrations that lead to signal-to-noise ratio of 3:1 and 10:1, respectively. In order to study the efficiency of the extraction, recovery test was performed by spiking method for the three Milli-Q, sea and tap water samples in five replicates.

3. RESULTS AND DISCUSSION

3.1. Columns Preparation and Characterization

As mentioned in section 2.2, the capillary columns preparation procedure consists of two main steps; capillary tube inner surface activation and *in-situ* polymerization of monomeric mixture in the column. Several works proved that the composition type and percentages, and the polymerization
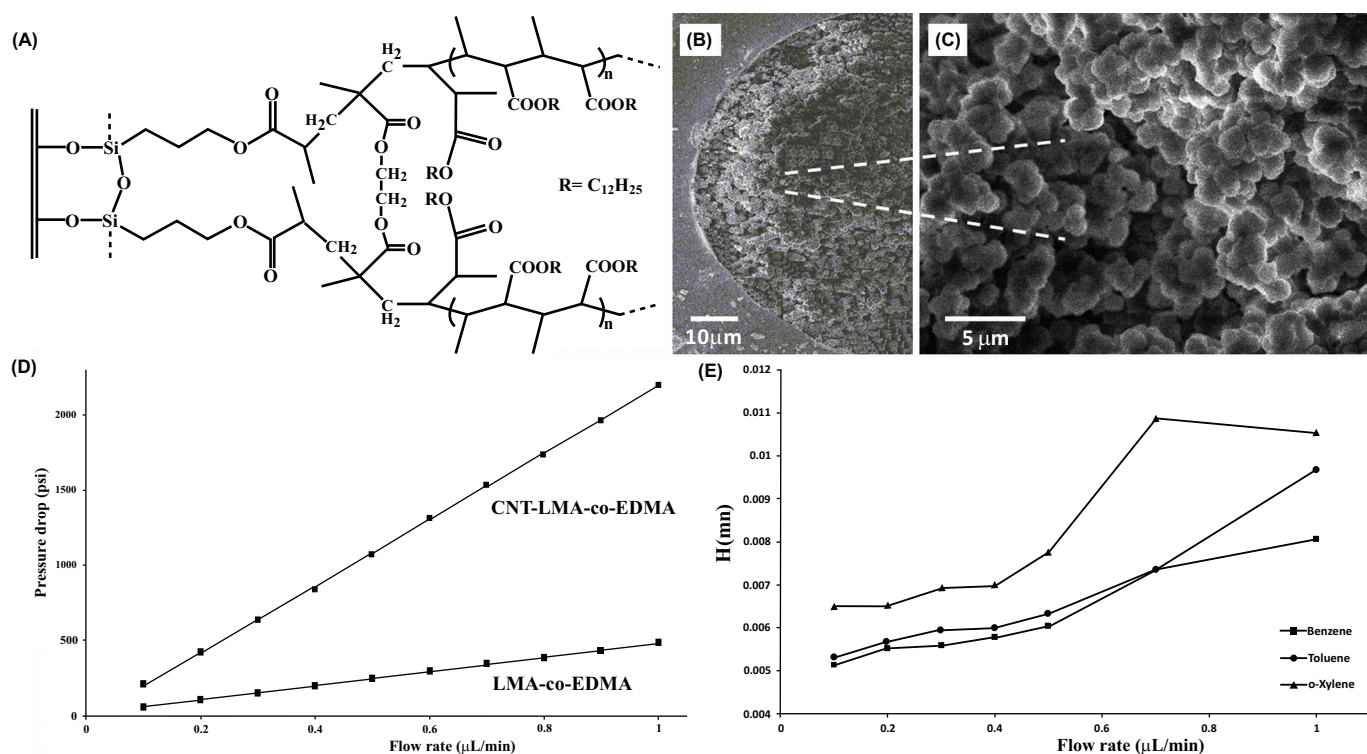
Fig. (1). (**A**) Chemical structure of the synthesized LMA-*co*-EDMA monolith. (**B** and **C**) Cross-sectional and bulk region SEM images of the prepared column. (**D**) Mechanical stability curve of the two columns backpressure as a function of acetonitri*l*e flow rate. (**E**) van Deemter plots for benzene, toluene and *o*-xylene on the CNT-LMA-*co*-EDMA composite column.

conditions strongly affect the final morphology and chromatographic performance of the prepared column [30, 31]. In this work, a composite stationary phase made of MWCNT incorporated to LMA based monolithic polymer was prepared and chemically attached to the capillary inner wall. The structure of the synthesized LMA-*co*-EDMA monolith is illustrated in Fig. (**1A**). As a control column, LMA-*co*-EDMA has been prepared using the same procedure but without incorporated CNTs. Both LMA (with its long C_{12} alkyl chain) and CNT (having a hydrophobic character) are strong candidates for interacting with most non-polar pollutants including BTX compounds. The content of MWCNT in the monomeric mixture composition was set based on the previous reports and preliminary experiments [38].

To characterize the prepared column, the morphology of the polymer monolithic material was evaluated by scanning electron microscopy (SEM). Representative cross-sectional and bulk region SEM images of the prepared column are shown in Fig. (**1B** & **C**). The images demonstrate a permeable monolithic bed, with continuous and homogeneous structure. As shown in the cross-sectional SEM image (Fig. **1B**), the synthesized monolith was well attached to the inner silica surface of the capillary tube. Large macropores and interconnected microglobules were obtained and clearly observed in the bulk monolith material (Fig. **1C**). In order to evaluate the permeability and mechanical stability of our composite material, acetonitrile was used for the measurement of the pressure drop across the column at different flow rates ranging from 0.10 to 1.0 µL/min. Fig. (**1D**) illustrates the effect of the flow rate through the prepared columns on backpressure using acetonitrile as eluent. An excellent linear depend-

ence with regression factors R^2 better than 0.9996 indicates that mechanical stability and permeability of both stationary phases were excellent. The linear dependence of the CNT-LMA-*co*-EDMA composite column backpressure and eluent flow rate confirmed also that there was no bleeding of the CNTs from the prepared column.

Numerical values for the permeability of the two capillary columns were measured at room temperature while pure acetonitrile passed through the columns at 0.5 µL/min volumetric flow rate. The permeability values of the prepared columns were 4.13×10^{-14} and 1.03×10^{-15} m^2 corresponding to a measured pressure drop of 249 and 1070 psi for LMA-*co*-EDMA and CNT-LMA-*co*-EDMA columns, respectively. The total porosity of 0.72 and 0.69 for LMA-*co*-EDMA and CNT-LMA-*co*-EDMA columns, respectively, was calculated by injecting uracil as unretained solute. As expected, addition of small amount of nanotubes to the monomeric mixture induced lower permeability and porosity values and a higher column backpressure. The determined values of porosity and permeability are in good agreement with that published previously for similar types of monolithic capillary columns [37, 38].

The prepared capillary columns were used for separation and analysis of BTX pollutants in water samples. Fig. (**2A** & **B**) demonstrates the separation chromatograms for pollutants at optimum chromatographic conditions. In fact, both columns did not exhibit a complete separation of the five pollutants, since no separation has been obtained for the three xylenes using LMA-*co*-EDMA control column, and between *m*- and *p*-xylene isomers using CNT-LMA-*co*-EDMA col-
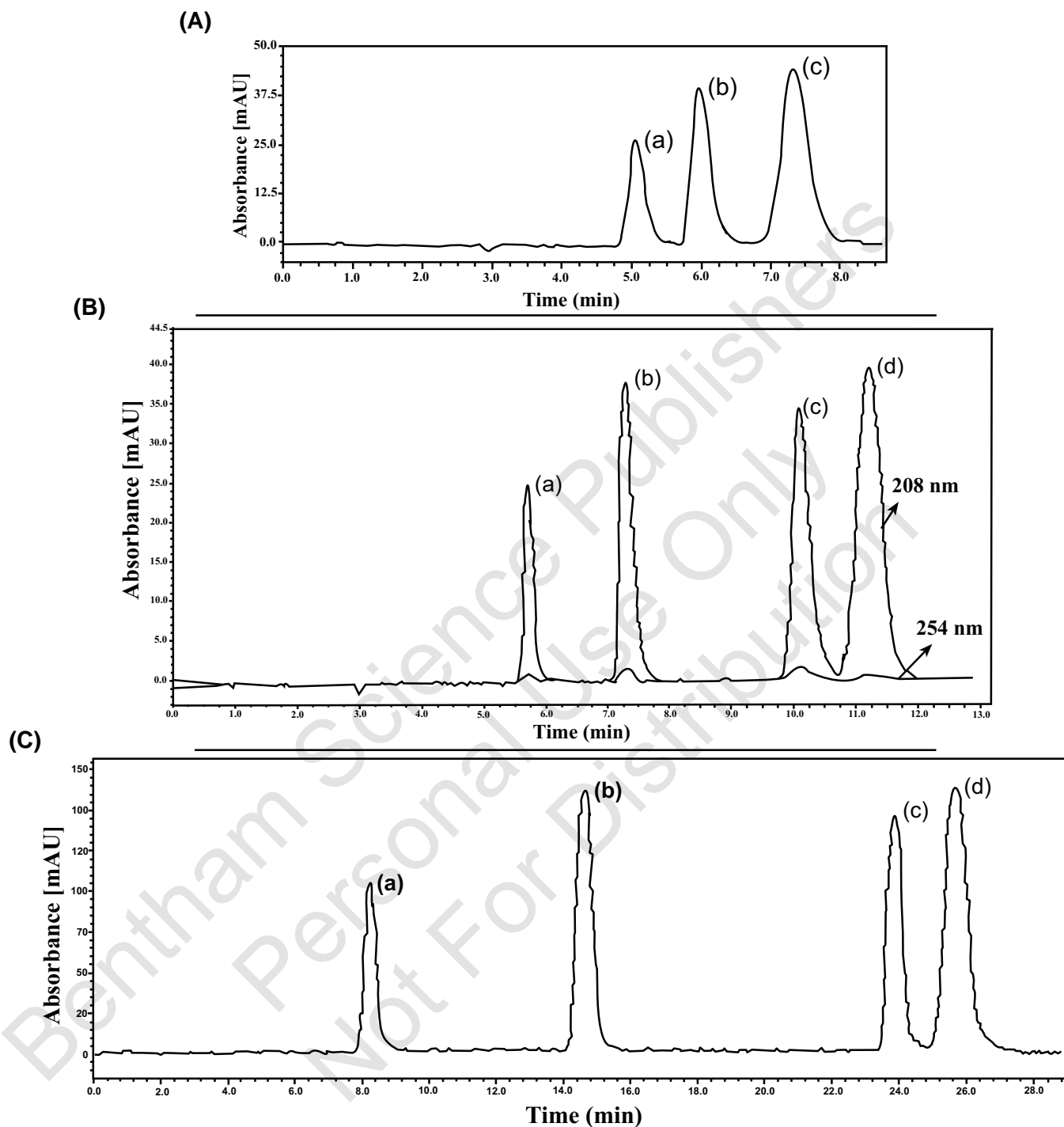
Fig. (2). Chromatograms of 0.25 µg/mL standard BTX pollutants at optimum conditions using (**A**) LMA-*co*-EDMA control column at 208 nm detection wavelength, where: (**a**) benzene, (**b**) toluene, (**c**) total xylenes. (**B**) CNT-LMA-*co*-EDMA composite column at 208 and 254 nm detection wavelength and (**C**) Nucleodur C$_{18}$ gravity packed column at 208 nm detection wavelength, where: (**a**) benzene, (**b**) toluene, (**c**) *o*-xylene and (**d**) *m/p*-xylenes.

umn, while it is usually achieved using C$_{18}$ silica based HPLC columns and even by capillary GC columns [6, 7, 13, 21-25]. However, with the exception of *m*- and *p*-xylenes (which were co-eluted), the other three pollutants were completely separated in about 12.0 min at 0.4 µL/min mobile phase flow rate using CNT-LMA-*co*-EDMA composite column.

Several parameters are important for evaluation of the CNT-LMA-*co*-EDMA composite column efficiency: plate number, height equivalent to a theoretical plate, band broadening and chromatographic resolution. These factors have been calculated for each pollutant at different mobile phase flow rates. The height equivalent to a theoretical plate *H* fluctuates from 0.005 to 0.01 mm on the examined mobile

Table 1. Packed C$_{18}$ conventional column versus CNT-LMA-*co*-EDMA composite capillary column.

-	Nucleodur C$_{18}$ Gravity Conventional Column	CNT-LMA-*co*-EDMA Capillary Column
Column dimension	100×4.0 mm, 3 µm particle size	200×0.10 mm
Flow rate (µL/min)	500	0.40
Mobile phase	water/acetonitrile 50:50, v/v/v	water/acetonitrile/tetrahydrofuran 60:35:5, v/v/v
Injection volume	10.0 µL	4.0 nL
Column temperature (°C)	24	70
Detector cell volume	10.0 µL	3.0 nL
Run time (min)	27.0	12.0
N/m (for benzene)	36,600	35,500
N/m (for toluene)	48,900	33,400
N/m (for *o*-xylene)	83,700	28,600
R$_s$ (benzene - toluene)	8.31	4.72
R$_s$ (*o*-xylene - *m/p*-xylene)	1.78	1.33
LOD (for toluene, µg/L)	0.25	0.05
LOL (for toluene, µg/L)	1000	750
Sampling rate (sample/h)	≈ 2	≈ 5
Solvents consumption (mL/h)	30.0	0.024

phase flow rate range which was 0.1 to 1.0 µL/min. The best efficiency was obtained for benzene at 0.1 µL/min flow rate which corresponded to 39,000 theoretical plates per meter. Efficiency and resolution values are summarized in Table **1** and the van Deemter plots for benzene, toluene and *o*-xylene on the prepared composite column are shown in Fig. (**1E**).

3.2. Analysis Conditions

Various studies have reported a wavelength of 254 nm to be used for the determination of BTX pollutants by HPLC [28]. Fig. (**2B**) clearly shows that a wavelength of 208 nm could be considered as an optimal value to get a significant higher sensitivity for detection of BTX pollutants by UV detector. The results indicated that the sensitivity for benzene, toluene, *o*-xylene and *m/p*-xylenes detection at 208 nm was about 25, 38, 34 and 39 times higher than that measured at 254 nm, respectively. Therefore, a wavelength of 208 nm has been selected for detection of BTX pollutants in this work.

The effect of mobile phase composition and flow rate on the separation of pollutants was investigated to find a good compromise between peaks resolution and separation time. The injection volume was fixed at 4 nL using an external injector with electric actuator. Based on the previous findings [37, 38], the nanotubes cross-through the monolithic bed pores and decrease the polymethacrylate monolith channels radius, inducing an increase of the surface area of the column and improving the chromatographic performance. This effect was accompanied by an increase in column back-

pressure, which, in turn, limits the applicable high flow rates even with only 0.1% of CNTs in the monomeric mixture. This problem has been solved by increasing the column oven temperature that was set at 70°C.

A systematic optimization of the mobile phase has been carried out, and the mixture of water/acetonitrile/tetrahydrofuran (60:35:5, v/v) was selected. Addition of 5% tetrahydrofuran to water and acetonitrile in the mobile phase enhanced the separation efficiency from 28,400 to 35,500 plates/m (1.25 folds) for benzene and from 27,200 to 33,400 plates/m (1.23 folds) for toluene at 0.4 µL/min flow rate. The presence of tetrahydrofuran in the mobile phase leads to swell some of the polymethacrylate bed which could enhance the surface diffusion of the analytes and improves the separation efficiency. Moreover, adding tetrahydrofuran to the mobile phase also improved the peak shape by reducing the tailing of all analytes [31, 37].

By varying the mobile phase flow rate, the plate numbers gradually decreased as the mobile phase flow rate increased from 0.1 to 1.0 µL/min as demonstrated in Fig. (**1E**) by the van Deemter curves for benzene, toluene and *o*-xylene on the prepared column. However, the mobile phase flow rate has been set at 0.4 µL/min to get a compromise between full separation of the four peaks and minimum run time. Under all these chromatographic parameters, the total run time of BTX pollutants was about 12.0 min with highly reproducible retention times for all studied compounds and %RSD less than 3.6 through the whole analysis.
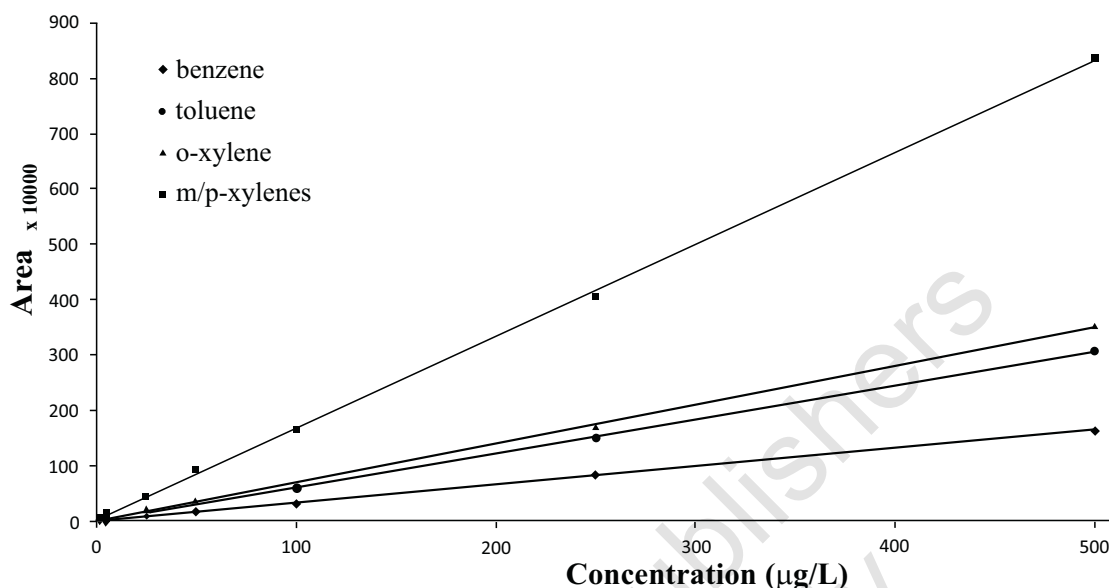
Fig. (3). External calibration curves of benzene, toluene, *o*-xylene and *m/p*-xylenes using a CNT-LMA-*co*-EDMA composite column at optimum conditions.

Table 2. Calibration data and the values of LOD, LOQ and LOL for the BTX pollutants (n=5).

BTX	Equation	R^2	LOD (µg/L)	LOQ (µg/L)	LOL (µg/L)
Benzene	A = 3289 c + 6095	0.9998	0.25	0.8	1000
Toluene	A = 6068 c + 33032	0.9992	0.05	0.2	750
o-xylene	A = 6940 c + 34239	0.9994	0.075	0.25	750
m/p-xylenes	A = 16718 c + 26011	0.9995	0.05	0.2	500

3.3. Validation of the Method

In order to validate the suitability of the prepared composite column for the determination of BTX compounds in water samples, the developed method was optimized in terms of linearity, limits of detection and quantitation, precision and recovery. Seven-point calibration curves have been plotted for each BTX solute, after preparation of standard solutions by dilution of the stock solution in acetonitrile into the appropriate concentrations.

When peak area was used for signals estimation, the method was found to be linear in the dynamic concentration range between 1.0-500 µg/L for the four peaks. The experimental results are shown graphically in Fig. (**3**). A good linearity was obtained for the four calibration curves with regression factor R^2 better than 0.9992. The linear equations of the obtained calibration curves were: benzene: *A = 3289 c + 6095* with R^2 = 0.9998, toluene: *A = 6068 c + 33032* with R^2 = 0.9992, *o*-xylene: *A = 6940 c + 34239* with R^2 = 0.9994 and *m/p*-xylenes: *A = 16718 c + 26011* with R^2 = 0.9995; where *A* is the peak area and *c* the concentration of each compound in µg/L.

LOD and LOQ values of the method were estimated based on the signal-to-noise criteria as mentioned in the ex-

perimental section. The measured values found for LOD were 0.25, 0.05, 0.075 and 0.05 µg/L for benzene, toluene, *o*-xylene and *m/p*-xylenes, while the LOQ values were 0.8, 0.2, 0.25 and 0.2 µg/L for benzene, toluene, *o*-xylene and *m/p*-xylenes, respectively. The precision of the method was also checked by calculating the %RSD of five consecutive injections of different BTX standards concentrations ranging from 1.0 to 500 µg/L. The %RSD value was less than 4.2 for all cases and confirmed that the developed assay has a good precision. Calibration curves data and the values of LOD, LOQ and LOL for the BTX pollutants are summarized in Table **2**.

3.4. Water Samples Analysis and Recovery

Since both real water samples (tap and sea water) were free of any BTX pollutant according to our procedure, as shown in Fig. (**4A**), water samples including highly pure Milli-Q (control water) were spiked with the five pollutants at 0.50 µg/L concentration level. Simple liquid-liquid extraction procedure was performed in five replicates and the extracts were directly analyzed at optimum chromatographic conditions. Unspiked water samples were also analyzed according to the same procedure.

Table 3. Analytical results for water samples (added concentration = 0.50 µg/L, n = 5).

BTX	Control Water	Tap Water		Sea Water	
	%Recovery ± %RSD	Detected	%Recovery ± %RSD	Detected	%Recovery ± %RSD
Benzene	86.83 ± 7.5	ND	84.85 ± 6.6	ND	86.25 ± 6.6
Toluene	94.75 ± 6.1	ND	92.29 ± 7.1	ND	93.58 ± 6.4
o-xylene	96.09 ± 5.5	ND	94.91 ± 6.2	ND	93.66 ± 4.5
m/p-xylenes	97.84 ± 4.1	ND	96.74 ± 5.8	ND	96.46 ± 5.9

The applicability and reliability of the prepared column and developed method to the analysis of BTX pollutants in water samples was demonstrated by means of recovery percentage experiments. 1,000 mL of each aqueous solution containing the added BTX pollutants has been extracted by 5.0 mL HPLC grade hexane, one milliliter of the final extract was then diluted with 1.0 mL HPLC grade acetonitrile in order to minimize the chromatogram baseline variation. Finally, 4.0 nL of the extracted samples were injected into the nano-LC system operated under the optimized conditions. Based on the above simple extraction process, the theoretical enrichment factor is equal to 200 and the targeted concentrations for all pollutants correspond to 100 µg/L.

The recovery percentage values ranged between 84.85-86.83 for benzene, 92.29-94.75 for toluene, 93.66-96.09 for *o*-xylene and 96.46-97.84 for *m/p*-xylenes. With the exception of benzene which is the most volatile hydrocarbon, the recovery percentage values demonstrated good extraction efficiencies. The respective values of %RSD were <7.5% for control water, <7.1% for tap water and <6.6% for sea water performed in five extraction replicates. %RSD results indicate that the precision of the method was satisfactory at this concentration level. The typical chromatograms of the spiked tap water and sea water samples are shown in Fig. (**4B & C**), respectively. Spiked recovery percentages and %RSD values of the BTX compounds in control and real water samples are given in Table **3**. The results indicated that the developed method could be used for extraction of BTX compounds and in the analysis of environmental water samples.

3.5. Comparison of the Prepared Column and C$_{18}$ Packed Column

The aim of this section was to compare the performance of the prepared composite capillary column with a packed C$_{18}$ HPLC column which is the most popular used for separation of BTX compounds. To the best of our knowledge, this type of comparison is very limited in the literature. Both columns and optimized methods were compared in terms of separation efficiency, chromatographic resolution, run time, LOD, LOL, solvents consumption and sampling rate. The typical chromatograms of BTX compounds separation are shown on Fig. (**2B**) using a CNT-LMA-*co*-EDMA composite column and on Fig. (**2C**) using Nucleodur C$_{18}$ Gravity packed column. A summary of the comparison data is given in Table **1**.

In order to enable an honest comparison, the developed methods have been pushed to their optimum chromatograph-

ic conditions. Both columns correspond to the reversed-phase HPLC mode which is the right choice for this type of environmental mixtures. Fig. (**2B & C**) shows that the developed methods could effectively separate BTX analytes (except *m*- and *p*-xylenes) in about 12.0 min at 0.4 µL/min flow rate using CNT-LMA-*co*-EDMA composite column and in 27 min at 0.5 mL/min flow rate using conventional C$_{18}$ packed column. Several experimental parameters were then evaluated and the comparative results are presented in Table **1**.
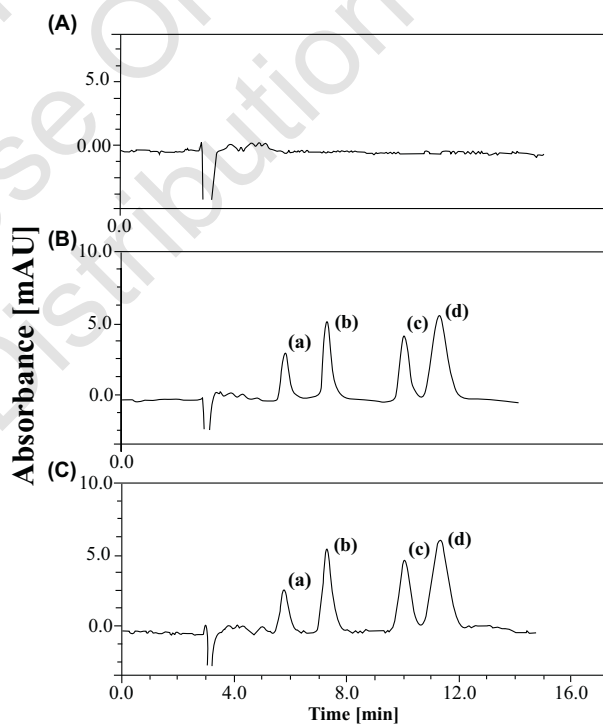

Fig. (4). Chromatograms of BTX pollutants using a CNT-LMA-*co*-EDMA composite column at optimum chromatographic conditions on real water samples using liquid-liquid extraction for (**A**) un-spiked sea water, (**B**) spiked tap water and (**C**) spiked sea water, where: (**a**) benzene, (**b**) toluene, (**c**) *o*-xylene and (**d**) *m/p*-xylenes.

The data show some advantages of the conventional packed C$_{18}$ column (3 µm particle size) compared to the CNT-LMA-*co*-EDMA composite capillary column in terms of separation efficiency and chromatographic resolution. At optimum conditions, both columns provided almost the same number of theoretical plates for the least retained solute benzene: 36,600 plates/m for C$_{18}$ column versus 35,500 plates/m for the CNT-LMA-*co*-EDMA composite capillary column. The difference between the numbers of theoretical plates

increased for the more retained solutes as the retention time becomes higher. The same observations have been noted for the chromatographic resolution values. As it can be seen in Table **1**, C_{18} packed column led to higher resolutions with 1.76 and 1.34 folds higher than those obtained using CNT-LMA-*co*-EDMA composite capillary column for benzene-toluene and *o*-xylene-*m/p*-xylene couples, respectively. Although the conventional C_{18} packed column exhibited better separation efficiency, the obtained chromatographic performance obtained using the CNT-LMA-co-EDMA capillary column was satisfactory and sufficient for complete separation and determination of BTX aromatics.

On the other hand, the obtained data explicit advantages of CNT-LMA-*co*-EDMA stationary phase prepared for nano-scale HPLC, compared to the conventional scale particulate packed C_{18} column (3 µm particle size) for analysis of BTX aromatics. This was very obvious in a shortened analysis time (more than the half time) and a drastically reduced consumption of mobile phase solvents (1,250 fold/h) and injected sample volumes (2,500 fold/injection) which contribute to decrease the time and cost of analysis. In addition, monolithic columns have a significantly higher porosity and permeability compared to the particulate packed ones which also contribute to decrease the column backpressure [27, 28].

Moreover, the amount and cost of a polymeric stationary phase material used for preparation of monolithic capillary columns is significantly less than that of conventional C_{18} silica based columns. In terms of analysis time, comparison of the present work with many previous reports dealing with BTX compounds determination shows that the run time of 12.0 min was shorter or comparable with those of most previous studies carried out using several HPLC and even GC techniques, which were in the range 8.0-27.6 min [6, 7, 13, 21-26]. Furthermore, as a result of the significantly lower consumption of mobile phase solvents, reagents and stationary phase materials, the amount of toxic waste generated and the risk of contamination using capillary columns is negligible relative to conventional scale columns.

From the validation point of view and as expected, the composite capillary column with 0.1 mm internal diameter, exhibited lower LOD and LOL values compared to those of the conventional column with 4.0 mm internal diameter for all studied solutes. These effects are due to the fact that the smaller internal diameter in capillary columns reduces analyte dilution but also limits the column loading capacity. According to all these conclusions, the CNT-LMA-*co*-EDMA composite capillary column has shown to be an acceptable alternative to conventional scale C_{18} packed columns for an accurate and rapid determination of hydrocarbon traces in water.

CONCLUSION

A CNT-LMA-*co*-EDMA composite capillary column was fabricated, characterized and successfully applied for the determination of BTX aromatics in water samples. A quite simple, fast, cost-effective and sensitive method has been implemented for detection of these toxic pollutants in tap and sea waters by nano-LC technique. The results showed good precisions, low detection and quantitation limits, acceptable extraction recoveries and satisfactory linear ranges. Under the optimum conditions of chromatographic and extraction procedures, the limits of detection reached by this method are all lower than the maximum contaminant levels set by the EPA for drinking water. In addition to demonstrating an efficient method for the analysis of BTX compounds, this work also presented a specific comparison between our prepared CNT-LMA-*co*-EDMA composite column and a conventional packed C_{18} column for the analysis of BTX aromatics. In summary, the CNT-LMA-*co*-EDMA capillary column provided lower run time, more sampling rate and much less solvent consumption, while the C_{18} conventional packed columns still remain the best choice for higher separation efficiency and chromatographic resolution.

Because of the high maturity, availability and versatility of the conventional silica based particulate packed columns, limited number of quantitative analysis applications have been reported using capillary monolithic columns in spite of their promising properties and advantages. In this regard, capillary monolithic columns performance and selectivity can be still enhanced and more researches are needed to improve the efficiency and applicability of these promising columns. Nevertheless, the results obtained from the present work make the proposed method applicable to routine analysis of BTX and related other pollutants in water samples.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this study are available within the article.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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