Microfluidic Precolumn Derivatization of Environmental Phenols with Coumarin-6-Sulfonyl Chloride and HPLC Separation

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Abstract

A simple, fast, sensitive and versatile method for the analysis of phenols in water is proposed using microfluidic precolumn derivatization with the fluorogenic label coumarin-6-sulfonyl chloride (C6SCl) and HPLC separation on monolithic columns. Phenols react with C6SCl within 3.0 min in the microreactor at ambient temperature to produce phenol–coumarin sulphonamides derivatives which were separated within 3.0 min in the microreactor at ambient temperature to produce phenol–coumarin sulphonamides derivatives which were separated within 3.0 min in the microreactor at ambient temperature to produce phenol–coumarin sulphonamides derivatives which were separated within 3.0 min in the microreactor at ambient temperature to produce phenol–coumarin sulphonamides derivatives which were separated within 3.0 min in the microreactor at ambient temperature to produce phenol–coumarin sulphonamides derivatives which were separated within 3.0 min in the microreactor at ambient temperature to produce phenol–coumarin sulphonamides derivatives which were separated within 3.0 min in the microreactor at ambient temperature to produce phenol–coumarin sulphonamides derivatives which were separated within 3.0 min in the microreactor at ambient temperature to produce phenol–coumarin sulphonamides derivatives which were separated within 3.0 min in the microreactor at ambient temperature to produce phenol–coumarin sulphonamides derivatives which were separated within 3.0 min in the microreactor at ambient temperature to produce phenol–coumarin sulphonamides derivatives which were separated within 3.0 min in the microreactor at ambient temperature to produce phenol–coumarin sulphonamides derivatives which were separated within 3.0 min in the microreactor at ambient temperature to produce phenol–coumarin sulphonamides derivatives which were separated within 3.0 min in the microreactor at ambient temperature to produce phenol–coumarin sulphonamides derivatives which were separated within 3.0 min in the microreactor at ambient temperature to produce phenol–coumarin sulphonamides derivatives which were separated within 3.0 min in the microreactor at ambient temperature to produce phenol–coumarin sulphonamides derivatives which were separated within 3.0 min in the microreactor at ambient temperature to produce phenol–coumarin sulphonamides derivatives which were separated within 3.0 min in the microreactor at ambient temperature to produce phenol–coumarin sulphonamides derivatives which were separated within 3.0 min in the microreactor at ambient temperature to produce phenol–coumarin sulphonamides derivatives which were separated within 3.0 min in the microreactor at ambient temperature to produce phenol–coumarin sulphonamides derivatives which were separated within 3.0 min in the microreactor at ambient temperature to produce phenol–coumarin sulphonamides derivatives which were separated within 3.0 min in the microreactor at ambient temperature to produce phenol–coumarin sulphonamides derivatives which were separated within 3.0 min in the microreactor at ambient temperature to produce phenol–coumarin sulphonamides derivatives which were separated within 3.0 min in the microreactor at ambient temperature.
L suction from Milli Q system (Millipore Corporation, Milford, USA) was used for all preparations and HPLC separations.

**Phenols standards**
Phenol (PH) was obtained from BDH Chemicals Ltd (UK, Pool). Standard chlorophenols: 2-chlorophenol (2-CP), 3-chlorophenol (3-CP), 4-chlorophenol (4-CP), 2, 3-dichlorophenol (2,3-DCP), 2, 5-dichlorophenol (2,5-DCP), 2, 6-dichlorophenol (2,6-DCP), 3, 4-dichlorophenol (3,4-DCP), 3, 5-dichlorophenol (3,5-DCP), 2, 4, 6-trichlorophenol (2,4,6-TCP) were purchased from Dr Ehrenstorfer GmbH (Germany, Augsburg). 2, 3, 5-trichlorophenol (2, 3, 5-TCP) 2, 3, 6-trichlorophenol (2, 3, 6-TCP) are supplied by Acros Organics (NJ, USA).

**Preparation of phenols derivatives of C6SCI**
A solution of phenol or chlorophenol (0.01 M) in sodium carbonate pH 9.0 (100 mL) was stirred at room temperature while a solution of C6SCI (0.1 M) in acetone (25 mL) was added dropwise over a period of 20 min. After stirring for further 2 h under subdued light, a solid product was formed. The content was filtered, and the residue was collected, washed with dilute HCl until the washing was neutral and then with water and dried in a vacuum desicator. The crude was then recrystallized from 50% ethanol and toluene mixture to give white crystals with a yield of 40–70%. Melting point, NMR, IR and mass spectrometry were used for identification and the spectroscopic data were confirmed.

**HPLC instrumentation**
HPLC system used for this study consists of Waters 1525 binary HPLC pump, 2489 UV/Visible detector and 2475 multi λ fluorescence detector (Waters, Milford, USA) connected to a personal computer. The two detectors are configured with Breeze chromatography workstation where data communications and control are manipulated. For derivatization and separation systems, 50:50 acetonitrile:water was used as a mobile phase at flow rate of 1 mL min⁻¹ and a UV-detector over a range of 210–254 nm. However, for postcolumn alkaline hydrolysis, the fluorescence detection was carried at λex 360 nm and λem 460 nm. NaOH was introduced via T-junction where the reaction took place in Teflon loop of 1 mm long and 0.49 mm i.d. The mobile phases were passed through a 0.45 μm filter and degassed by sonication for few minutes. The monolithic columns used in this study are Onyx™ Monolithic C18, (50 × 4.6 mm i.d. and 50 × 2.0 mm i.d.) (www.phenomenex.com).

**Microfluidics**
All microreactions were carried out using a borosilicate glass microreactor, FC-R150.332.2, Fluidic Connect 4515 purchased from Micronit Microfluidics (www.micronit.com) with two inlets and one outlet. The channel 150 μm (wide) × 150 μm (deep) × 332 mm (long) with an internal volume of 6 μL and the channel structure in the microreactor is powderblasted. The two inlets are connected with fused silica capillary tubing with protective polyimide coating and the outside (150 μm i.d., 375 μm o.d.) with ‘Nanoport’ ferrules made of PERLAST™, which is chemically resistant to most solvents. ‘Nanoport’ nuts made of PEEK (PolyEtherEtherKetone) with low dead volume (20–90 nL) connections were used. The cartridge around the chip was made of PP (Polypropylene) with outer dimensions (l × w × h) 75 × 25 × 4 mm and maximum working temperature of 80°C. The chip holder was made of stainless steel (80 μ 55 × 9.5 mm). The chip holder (Inverter) frame was made of POM (Polycetal) (100 × 75 × 24) mm with maximum working temperature of 80°C. The chip was connected to an external syringe pump (Bioanalytical Systems Inc, BASI, West Lafayette, USA), which simultaneously deliver two solutions at set flow rates ranging between 0.1 and 100 μL min⁻¹. The product stream was collected from the outlet through same tubing used for the inlet into a small vial immersed in ice bath in order to quench the reaction.

**Procedure**
Standard stock solutions of phenol and chlorophenols (each of 200 mg L⁻¹) were prepared in sodium carbonate solution pH 8.5. A working mixture solution of these six phenols was prepared from the above stock solutions to give 50–30 mg L⁻¹ for each phenol daily. Series of solutions were then prepared from this working mixture ranging from 0.75 to 12.5 mg L⁻¹. A solution of C6SCI (50 mg L⁻¹) in acetonitrile (at a molar ratio to highest phenol concentration of about 4 in the final volume) was introduced to one inlet of the microfluidic chip and a solution of phenols mixture standard solution into the other inlet. The final solution was collected from the outlet in a vial immersed in ice bath and 10 μL injected into the column. Sodium hydroxide (1 M) was added via T-junction after the column through a separate pump with 0.5 mL min⁻¹ flow rate. Triplicate injections for each standard solution were made and the peak area of the analyte was plotted against the corresponding phenol concentration. The effectiveness of using ice bath to quench the reaction outside the microfluidic reactor was examined by placing a mixture of the phenol and the label in an ice bath in a bench-scale and the peak area of the derivatized phenols was monitored using UV-detector. The signal was observed to remain almost constant up to 90 min indicating that the ice bath used efficiently quenches the reaction.

**Sampling and sample treatment**
Tap water samples from the laboratory and fountain water samples were analyzed. The samples were collected in 500 mL clean bottles and passed through a 0.45 μm filters to remove suspended particulates and analyzed immediately to avoid degradation. The pH of all samples was adjusted to 8.5–9.0 using sodium carbonate solution (0.1 M). A known volume of water sample (10 mL) was then spiked with different concentration (0.75–7.5 mg L⁻¹) of phenolic standard mixture. The treated sample was subjected to the derivatization through microfluidic and then directly injected into the HPLC system for separation and postcolumn alkaline hydrolysis processes. Triplicate injections for each sample solution were made and the recoveries were calculated by relating the concentration determined in the water sample to the calibration standards. The HPLC detection system was programmed in a way to skip the label and the hydrolysis signals which eluted earlier before the derivatives.
Results

Derivatization in microfluidic

In order to optimize derivatization conditions in microfluidics, four parameters were considered: reaction time, pH, temperature of the microreactor, and the ratio of the phenols to the label that will lead to a maximum yield of the labeled phenols. The effect of time on the reaction yield was followed by reacting a mixture of phenols, 3-CP and 2, 3-DCP in carbonate buffer, pH 8.5 with C6SCI. Optimum reaction time was studied and compared with bench-scale experiments that are performed using the same solutions of phenols and C6SCI.

Different syringe pump flow rates ranging from 0.4 to 100 μL min⁻¹ were used and the residence times were calculated on the basis of the internal volume of the microchip. Figure 1 shows the increase of the reaction yield of 3-CP and 2, 3-DCP derivatives of C6SCI with time. This is represented by the evolution of the peak area of these compounds with time. Clearly, the formation of both derivatives increases with time for the bench scale and the microreactor systems. In the bench-scale experiment, 2, 3-DCP derivatization reaction reaches equilibrium after 1 h, however, for 3-CP, the yield is continuously increasing even after 1 h has elapsed. In this figure, we compared the results obtained from the micro- and bench-scale experiment at pH 8.5.

The influence of pH of media and the relative concentration of the label on the relative yield of the derivatized phenols were re-optimized in the microreactor. This was investigated by the reaction of a mixture of phenols, 3-CP and 2, 3-DCP prepared in various pH buffers ranging from 7.0 to 10.0 with C6SCI. The flow rate of 2.0 μL min⁻¹ was used in this experiment.

The effect of pH and concentration of the label are similar to those obtained in the batch method. Therefore, a pH of 8.5 and a label to phenol ratio of 4.0 was selected as the optimum value.

On the other hand, a residence time of 3.0 min in microfluidic was selected as a compromise between sensitivity and speed of analysis.

We further investigated the effect of temperature on the reaction yield by heating the microreactor using a thermostat. It was observed that with an increase in temperature, the peak area of the derivatives either decreased slightly or remained constant. Therefore, in this study, the derivatization reaction was conducted at room temperature.

HPLC separation

In an attempt to shorten run times, a monolithic column was used for the separation of phenolic derivatives. Monolithic columns can be used with high flow rate of mobile phases compared with particle-based columns and its high porosity besides its continuous form increases the reliability and reproducibility of the separation.

The optimum composition of the mobile phase was evaluated based on optimum resolution of the phenols derivatives. A series of mobile phases were prepared ranging from 20 to 80% acetonitrile. The phenolic mixture to be tested was prepared as described before. The best separation accompanied by good peak shapes was obtained using solvent composition of 50:50 (acetonitrile:water).

Postcolumn ring opening

After establishing the optimum chromatographic system, the method was used for the analysis of a standard mixture of phenols in different water samples. However, the optimum conditions of derivatization and separation described earlier can only be applied to high concentration of phenols; trace amount of phenols in water sample could not be detected by UV. To improve the sensitivity of the method, we need to use a fluorescence detector.

As has been previously reported, the derivatives are weakly fluorescent under neutral conditions, and require a high pH to exhibit fluorescence (24). Hence ring opening of the coumarin nucleus with NaOH is used where the fluorescence intensity of the derivatives was significantly improved. To achieve ring opening of the phenol–C6SCI derivatives, we mixed NaOH with the column effluent in a reaction coil before the detector.

The effect of the flow rate and the concentration of NaOH on the intensity of the derivatives were studied. An increase in the fluorescence intensity of all ring-opened phenolic derivatives compared with the parent derivatives was observed when 1 M NaOH solution was pumped at a flow rate of 0.5 mL min⁻¹. Using higher flow rates of NaOH results in a drastic decrease of the peak area.

The effect of temperature on the ring-opening reaction was also studied by placing the postcolumn reaction coil in a thermostat at temperatures that range from 25 to 60°C. All the six phenols have almost the same trend; as the temperature increases, the fluorescence intensities of ring-opened derivatives decreases.

Since the main target of this study was to reduce the analysis time with minimum tradeoff in the sensitivity, the effect of the mobile phase flow rate on the ring opening reaction must be carefully scrutinized. Figure 2 shows the relation of the fluorescence intensity of the six phenols while changing the flow rate from 0.2 to 0.5 mL min⁻¹ using the narrower monolithic column (2 mm i.d.). All the six phenols exhibit the same decreases in their fluorescence intensity with increasing the flow rate. It is
observed from Figure 2 that increasing the flow rate (from 0.2 mL min\(^{-1}\) to 0.4 mL min\(^{-1}\)) is accompanied by three times reduction in the peak area of the derivatives.

**Analytical applications**

Using the optimum conditions discussed earlier, we separated and analyzed six environmental phenols by the proposed procedure. A typical chromatogram is shown in Figure 3. The reagent and its hydrolysis product did not interfere with the analysis because they are eluted early before 1.8 min. Excellent separations were obtained for all derivatives using 50:50 mobile (acetonitrile : water) for both columns under 7.0 min at a flow rate of 1.0 mL min\(^{-1}\) (using 50 × 4.6 mm i.d. column). A shorter separation time can be obtained using higher flow rate or narrower columns with minimum losses with regard to the separation.

Ring-opened derivatized phenols were detected at \(\lambda_{\text{exc}}\) 360 nm and \(\lambda_{\text{em}}\) 460 nm. Retention factors (\(k'\)), separation factors (\(\alpha\)) and resolutions (\(R_s\)) were obtained and are summarized in Table I.

Calibration curves in the range 0.75–12.5 mg L\(^{-1}\) for all phenols were obtained. A least square regression method was used to generate the calibration data. The figures of merit for all phenol are summarized in Table I. The \(R^2\) values were >0.99 for all derivatized phenols and demonstrated a direct proportional relationship between the response signals and the corresponding concentration. Furthermore, the developed method was used to analyze drinking water and recycled water samples. The water samples were collected and the proposed method was applied to the determination of phenols in spiked water samples. The recoveries of phenols from tap water and the recycled water samples spiked with 0.75 mg L\(^{-1}\) are shown in Table II. The recoveries obtained are satisfactory for practical use.

**Discussion**

The analysis of phenols in water using C6SCl as a precolumn derivatization agent followed by alkaline hydrolysis and ion pair RP-HPLC has been investigated by us (24). The reaction scheme of phenol or substituted phenols with the label C6SCl is illustrated in Figure 4. This derivatization reaction is followed by alkaline hydrolysis in which the ring of the coumarin–phenol derivative opens to produce two species, cis and trans isomers in the presence of light. Both isomers are highly fluorescent (24). In this method, the precolumn derivatization step is slow, requiring more than an hour to reach completion. On the other hand, the ring-opened anionic phenol derivatives require an ion-pairing reagent in order to separate in a reversed phase column. Additionally, because the ring-opening reaction is reversible, the use of alkaline mobile phase is mandatory. Therefore, in such

<table>
<thead>
<tr>
<th>Compound</th>
<th>(%) Recovery ± SD</th>
<th>Tap water</th>
<th>Fountain water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ph</td>
<td>89.7 ± 3.3</td>
<td>110.6 ± 3.3</td>
<td></td>
</tr>
<tr>
<td>4-CP</td>
<td>110.3 ± 1.9</td>
<td>101.5 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>2,3-DCP</td>
<td>116.8 ± 3.2</td>
<td>111.9 ± 2.9</td>
<td></td>
</tr>
<tr>
<td>3,5-DCP</td>
<td>112.1 ± 3.0</td>
<td>106.1 ± 3.1</td>
<td></td>
</tr>
<tr>
<td>2,4,6-TCP</td>
<td>120.3 ± 3.5</td>
<td>129.7 ± 3.3</td>
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<tr>
<td>2,3,5-TCP</td>
<td>116.4 ± 3.0</td>
<td>121.8 ± 3.2</td>
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</table>

\(n = 7.\)

Table I

<table>
<thead>
<tr>
<th>Compound</th>
<th>(k')</th>
<th>(\alpha)</th>
<th>(R_s) Slope ± SD</th>
<th>Intercept ± SD</th>
<th>(R^2)</th>
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<tbody>
<tr>
<td>Ph</td>
<td>7.8</td>
<td>2.1</td>
<td>(2.2 ± 0.06) (\times 10^5)</td>
<td>(4.1 ± 2.9) (\times 10^4)</td>
<td>0.9967</td>
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<tr>
<td>4-CP</td>
<td>11.4</td>
<td>3.0</td>
<td>(2.8 ± 0.08) (\times 10^5)</td>
<td>(1.3 ± 5.3) (\times 10^5)</td>
<td>0.9970</td>
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<tr>
<td>2,5-DCP</td>
<td>15.0</td>
<td>2.7</td>
<td>(3.2 ± 0.09) (\times 10^5)</td>
<td>(1.5 ± 2.3) (\times 10^5)</td>
<td>0.9996</td>
</tr>
<tr>
<td>3,5-DCP</td>
<td>18.2</td>
<td>1.9</td>
<td>(1.1 ± 0.03) (\times 10^5)</td>
<td>(1.2 ± 5.1) (\times 10^5)</td>
<td>0.9975</td>
</tr>
<tr>
<td>2,4,6-TCP</td>
<td>21.4</td>
<td>2.1</td>
<td>(3.5 ± 0.06) (\times 10^5)</td>
<td>(6.6 ± 2.5) (\times 10^2)</td>
<td>0.9989</td>
</tr>
<tr>
<td>2,3,5-TCP</td>
<td>23.8</td>
<td>1.1</td>
<td>(1.5 ± 0.02) (\times 10^5)</td>
<td>(5.1 ± 1.1) (\times 10^4)</td>
<td>0.9990</td>
</tr>
</tbody>
</table>

\(\lambda_{\text{exc}} = 360, \lambda_{\text{em}} = 460\) (postcolumn ring-open). Elution order: Ph, 2, 4-CP; 2, 3-DCP, 3, 5-DCP, 2, 4, 6-TCP; 2, 3, 5-TCP.

Figure 2. Effect of flow rate on the peak area of the phenols using the monolith column: Onyx Monolithic C18, (50 × 2.0 mm i.d.). Filled diamond, 4-CP; filled star, 2, 3-DCP, filled pentagon, 3, 5-DCP; filled square, 2, 3, 5-TCP; filled circle, 2, 4, 6-TCP. This figure is available in black and white in print and in color at JCS online.

Figure 3. A chromatogram showing the separation of six phenolic components for spiked water sample (2 ppm) at 0.5 mL min\(^{-1}\) (1.0 M NaOH flow rate). HPLC flow rate: 1.0 mL min\(^{-1}\), column: Onyx Monolithic C18, (50 × 4.6 mm i.d.), mobile phase: 50% CH₃CN, detection: fluorescence, \(\lambda_{\text{exc}} = 360, \lambda_{\text{em}} = 460\) (postcolumn ring-open). Elution order: Ph, 2, 4-CP, 2, 3-DCP, 3, 5-DCP, 2, 4, 6-TCP; 2, 3, 5-TCP.

Table II

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Recovery ± SD ((n = 3))</th>
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<td></td>
</tr>
</tbody>
</table>

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cases, we need to use special columns because silica-based columns will dissolve at high pH.

In this work, a fast, simple, versatile, and sensitive method for the analysis of phenols is developed. In order to develop such a method, three routes have been endeavored: reducing the pre-column derivatization time by using microreactor; decreasing the separation time by using monolith column; and enhancement of the sensitivity by postcolumn ring-opening. After setting all the parameters of the reaction and separation condition, the optimum condition is invested in analytical application.

As has been shown in Figure 1, a much higher yield is produced in microfluidic within shorter reaction times compared with that produced in bench-scale experiments. For example, the yield obtained for a residence time of 15 min for 3-CP on-chip derivatization is comparable to that obtained after 55 min for bench-scale derivatization.

For microreactors, heat and mass transfer capacities are greatly enhanced compared with macroscale reactors. Therefore, it is important to realize that these parameters are greatly influenced by the dimensions and architecture of channels which greatly determines the speed of the reaction. Collision of two fluid streams in microchannels apply high shear rates to reactant fluids and therefore shorten mixing time. The Y-shape mixer which has been used in this study is an example of microreactors using this mixing principle (25–28). The enhancement of mixing is further obtained by discontinuities in the fluid flow within microfluidic by the specifically designed serpentine channel to enhance disruption of the flow of a liquid. When a liquid flow passes a sharp bend, the change in flow direction gives rise to a secondary flow field perpendicular to the flow of the liquid. This lateral flow field improves mixing performance in a micromixer where mixing by turbulence is not feasible. On the other hand, the separation of boundary layers leads to the generation of vortices, which results in enhanced mixing performance. Vortices tend to break the stream up into layers and each layer curls in a different manner. These breaking and curling actions reduce the diffusion distance between the molecules of two liquids in a mixing process (25). The reduced dimensions of the microfluidic system increase mass transfer efficiencies. Therefore, narrower channels shorten the diffusion transport distances for the molecules and by increasing the length of the channel more mixing results (25–28). All of these processes play a significant role in reducing the reaction time in microfluidics.

One of the advantages of the use of continuous flow microreactors is that it allows automation of sample preparations at a very low cost. However, the major disadvantage of using microreactors in organic synthesis is associated with the difficulty by which large amounts are produced. This hurdle can be surmounted in chromatographic applications by using highly sensitive detection systems, using narrow micropore columns or by operating several devices in parallel to sustain enough material for less sensitive detection systems.

A postcolumn ring-opening step has contributed a great deal to the sensitivity of the method. However, as shown in Figure 2, using higher flow rates of NaOH results in a drastic decrease of the peak area. It has been reported that base hydrolysis of coumarins is a fast step (29). This step is followed by the slow opening of the pyrone ring and the formation of coumarinic acids which is believed to be the species that exhibit very high quantum yield. The rate is reported to show first-order kinetics with respect to the concentration of OH−.

Ring-opening reactions of coumarin and thiocoumarin were reported to be endothermic; therefore, phenol–C6SCl derivatives were expected to show a similar behavior (29). The
decrease in intensity by increasing the temperature could be due to the decrease in fluorescence quantum yields of the derivatives due to an increase in radiationless transitions of the molecules.

The proposed method was found to be rapid, simple, environmentally friendly and reproducible. This qualifies the method to be an attractive procedure for the analysis of phenols in environmental water. Within 6 min, six-phenols were separated using flow rates as low as 0.3 mL min⁻¹. This results in reduction of consumption of solvents by at least four times compared with when particle-based columns were used. On the other hand, the introduction of postcolumn ring-opening reduced the sensitivity of the method compared with precolumn ring-opening methods. Moreover, the peak width has been increased by about 10%. Ideally, calibration curves are expected to pass through the origin, however, in this work, relatively large intercept values were obtained mainly due to the blank signal which is usually not corrected in chromatography systems.

In the absence of light, the coumarin ring-opened derivatives produce cis-coumarinic acid; however, when exposed to light, these derivatives are known to undergo photochemical isomerization to the trans isomer. The trans-coumarinic acid is characterized by a much higher quantum yield compared with the cis-isomer. The sensitivity of the method can, therefore, be improved greatly using a postcolumn photochemical reactor.

The method performance was further investigated by applying it successfully to the separation and determination of phenols in fountain and tap water. All phenol concentrations were obtained at recoveries 89–129% indicating that this method is suitable for the determination of phenols in environmental samples. All phenol–C₆SCl derivatives were well separated using a monolithic column at all flow rates investigated. Therefore, a tradeoff between the sensitivity and the analysis time can be made. Clearly, if the levels of the phenols in the samples are high enough to be detected, then the analysis time can be reduced at least by 50% by increasing flow rates. On the other hand, to improve the sensitivity for low concentrations of phenols, we can use low flow.

Conclusion
Derivatization reactions within microreactors generated products in high yield within shorter time, compared with the equivalent batch reactions. Consequently, the application of microreaction technology is of great environmental importance as it has the potential to reduce the quantity of raw materials required, while efficiently converting them into the desired product with minimal generation of side products and waste. The optimum derivatization and separation conditions were: 3.0 min reaction time, ambient temperature, pH:8.5, 1:4 (phenol: label) and 50: 50 acetonitrile: water mobile phase. Postcolumn ring opening of the coumarin nucleus has resulted in the generation of highly fluorescent compounds that improved the sensitivity and the detection limit of the method. This in turn allowed using a versatile monolithic column through which the separation time was greatly reduced. A simple, rapid and sensitive method suitable for the analysis of phenols in tap and treated waste water in <10 min per run is developed. The consumption of reagents is significantly reduced compared with the conventional batch method derivatization techniques. In addition, the short separation time results in further reduction in the cost of the method, considering the prices of high-quality solvents are inflating every year.

Acknowledgments
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References
1. Bayer, T., Himmeler, K.; Mixing and organic chemistry; Chemical Engineering Technology (2005); 28: 285.
3. Wegner, J., Ceylan, S., Kirschning, A.; Ten key issues in modern flow chemistry; Chemical Communications, (2011); 47: 5483.
19. Duonga, C.T., Roper, M.G.; A microfluidic device for the automated derivatization of free fatty acids to fatty acid methyl esters; *Analyst*, (2012); 137: 840.
27. Aoki, N., Mac, K.; Effects of channel geometry on mixing performance of micromixers using collision of fluid segments; *Chemical Engineering Journal*, (2006); 118: 189.