As. J. Energy Env. **2009**, 10(03), 176-184

Asian Journal on Energy and Environment

ISSN 1513-4121

Available online at www.asian-energy-journal.info

Research Article

Determination of highly toxic phenolic derivative, 2,3-dimethyl phenol in Buriganga River of Bangladesh by gas chromatography - mass spectrometry

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Abstract

A simple, sensitive and rapid gas chromatography-mass spectrometry (GC-MS) method is proposed for the analysis of some environmentally important highly toxic phenols in water. The concentration level of one phenolic derivative, 2,3-dimethyl phenol was determined in water at the sampling stations of Postagalla, Sadarghat and Sowarighat of the Buriganga River, Bangladesh. Water samples were collected from different depths at the sampling stations. The phenolic compounds were extracted with dichloromethane, which was further pre-concentrated by evaporation. Different concentrations of toxic 2,3-dimethyl phenol were obtained in the river water at the various sampling stations. The concentration of highly toxic 2,3-dimethyl phenol was found in the range of 0.222-0.964 $\mu g/L^{-1}$. This method could permit the analysis of water for phenol as well as phenolic derivatives with a detection limit as low as 100 $ng//L^{-1}$.

Keywords: Organic pollutants**,** Toxic Phenolic compounds; 2,3-Dimethyl phenol, Liquid-phase extraction; Pre-concentration, GC-MS.

Introduction

Phenolic derivatives are among the most important contaminants present in the environment. These compounds are used in several industrial processes to manufacture chemicals such as pesticides, explosives, drugs and dyes. They also are used in the bleaching process of paper manufacturing. Apart from these sources, phenolic compounds have substantial applications in agriculture as herbicides, insecticides and fungicides. However, phenolic compounds are not only generated by human activity, but they are also formed naturally, e.g., during the decomposition of leaves or wood. As a result of these applications, they are found in soils and sediments and this often leads to

wastewater and groundwater contamination. Owing to their high toxicity and persistence in the environment, both, the US Environmental Protection Agency (EPA) and the European Union have included some of them in their lists of priority pollutants. They are of great environmental concern owing to their high toxicity [1].

Many analytical techniques have been used for the trace determination of phenols in aquatic environments. High-performance liquid chromatography (HPLC), capillary electrophoresis (CE) and gas chromatography (GC) have been commonly used for the determination of phenolic compounds [2–6]. Although HPLC methods are frequently applied for the analysis of phenols, GC is often preferred, due to its inherent advantage of high resolution, rapid separation, low cost and easy linkage with sensitive and selective detectors.

Recently, Bagheri *et al.* [7] investigated the determination of phenol and some chlorophenols in water samples by SDME–GC–MS analysis. They derivatized the compounds prior to extraction using acetic anhydride in basic media. A drop of butyl acetate was used for extraction of acetylated phenols from water. In this paper, we describe the extraction and quantification of 2,3-dimethyl phenol at different depths of water at various stations along the Buriganga River in Bangladesh.

Experimental

Chemicals and standard solutions

The phenolic compound, 2,3-dimethyl phenol was obtained from Merck (Darmstadt, Germany). Solvent used for chromatographic separation was dichloromethane (GC grade) and water (GC grade), also obtained from Merck (Darmstadt, Germany). All other chemicals used were analytical or GC grade. Anhydrous sodium sulphate (Merck, Germany) was cleaned by heating at 200^{o}C before use. The pH of water samples was adjusted with o-phosphoric acid (Merck). Other reagents were purchased from Merck.

GC–MS analysis

The GC-MS analysis of the crude dichloromethane water extract of the samples was performed using a Varian GC-MS (Model Varian CP 3800, USA) equipped with a VF-5 fused silica capillary column (30m x 0.25 i. d., film thickness 0.25 µm, Varian, USA). An electron ionization system with the ionization energy of 70 eV was used for the detection of GC-MS. Helium was used as carrier gas with constant flow rate of 1 ml/min. Injector and mass transfer line temperature were set at 250 and 300^{o}C, respectively. A 20% split injection mode was selected with a solvent delay time of 3 min. with injection volume 1 µl. The initial column temperature was started at 40°C for 1 min, programmed at 8°C min^{-1} to 200°C and heated until 280^{o}C at 10^{o}C min^{-1}. Injection port was set at 250°C. Helium was used as carrier gas with a flow-rate of 1.0 ml min^{-1}. Molecular ions were monitored for identification. Mass range: 40-500 m/z. Identification of the crude dichloromethane extract was based on GC retention time on VF-5 capillary column, computerized matching of mass spectra with standards (Mainlab, Replib and Tutorial data of GC-MS systems). The reference compound, 2,3-dimethyl phenol was used as marker. The marker was accurately weighed and dissolved in dichloromethane to produce

a series of concentrations. Standard calibration curves were established by plotting the peak areas against different concentrations of the reference compound (varying from 0.5 to 5µg/ml). The external standard method was used for quantification of the marker in the Buriganga River water extract.

River water samples

18 water samples were collected in cleaned amber-coloured glass bottles from three different stations of the Buriganga River on 9 April 2009. The stations were Postagulla, Sadarghat and Sowarighat. Sampling stations were at least 1 km apart. The locations of the sampling points of the river are shown in Fig. 1. Six samples were collected from each of the sampling stations and two samples from each sampling points at surface and 30 cm depth of water. Each sample was collected in 1.1-1 capacity volume, well washed amber-coloured glass bottles. At first, the bottle was lowered slowly into the water and its cork was opened by hand, then marked accordingly in cm at the desired depth. When the bottle was filled with water, it was closed and drawn up carefully. Then 100 ml of water was discharged from the glass bottle. At the same time 10% $CuSO_4$ was added as a stabilizing agent into the water samples, shaken vigorously by hand and closed by the cork.

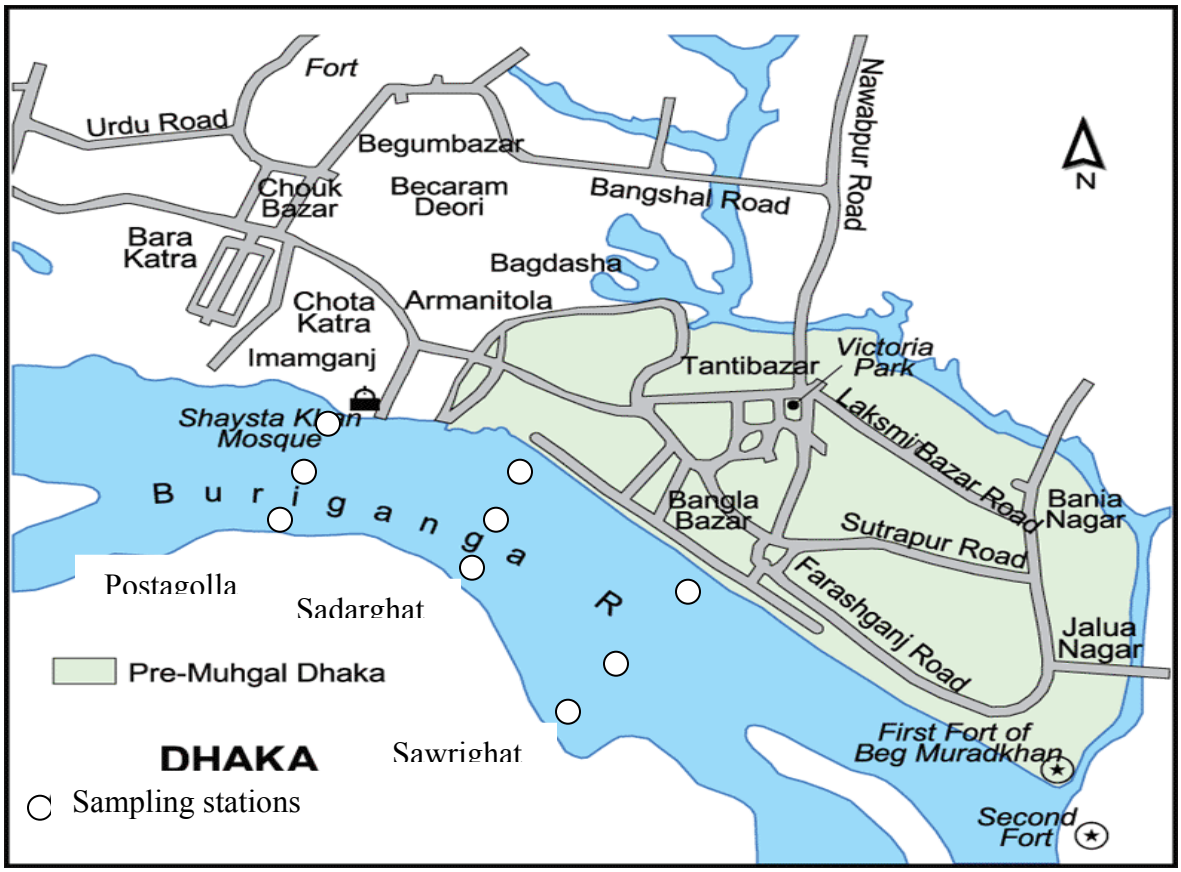

Figure 1. Map of the Buriganga River showing the location of sampling stations and collection points of water samples.

Extraction

The extraction was carried out for 72 hrs., following collection of the samples by solvent extraction method. The method required two 50-50 capacity conical flasks with Teflon stop corks. Each water sample with a volume of 20 ml was poured into the conical flask where 20 ml of dichloromethane was added and the mixture was then shaken vigorously for 1 hr by Lab Tech shaker (Manufacture of Lab. Ind. and Vac. Instrument). Following this, the water-solvent was transferred to the separating funnel and then allowed to stand in a rack for 10 min. The aqueous layer was drained into a jar by means of a Teflon stop cork, leaving the dichloromethane layer (extract) in the separating funnel. The extract was then transferred into a volumetric flask. The aqueous layer was extracted again with 10 ml of dichloromethane and the extract was collected and stored. Both extracts were combined into a volumetric flask and kept in a cool atmosphere. All samples (18) were extracted using the same method.

Removal of residual water from sample extract

In order to remove the residual water from the extract, the extract was treated with anhydrous sodium sulphate. Sodium sulphate (50 gm) was placed in a funnel and slightly moistened to make a solid layer that would not mix with the extract. The extract was then passed through the funnel and collected in a pre-cleaned volumetric flask. The treated water was restored. The operation was done quickly to avoid possible losses of any volatile compounds in the extract. A column (60 cm long x 1 cm i.d.) was used for this operation as well. Fifteen centimeters of the column were packed slowly with silica gel/solid silver nitrate mixture. Before packing the column, the silica gel was activated at 120^{o}C for 10 hr. and deactivated with 3% distilled water by weight. The 10 ml of dichloromethane was introduced into the column to rinse the gel; here 5 ml of dichloromethane was discarded and the remaining 5 ml was retained in the column. Under this condition, the sample extract was passed slowly and carefully through the column. Finally the extract was collected in a suitable container for analysis. All the samples were treated in the same way.

Pre-concentration and analysis of the extract

The extracts were reduced to a volume of 2 ml by evaporation using Kuderna-Danish techniques. By this technique, dichloromethane was slowly evaporated and special attention was given to avoid extra evaporation and the volume of extract (30 ml) was reduced to 2 ml solution. The concentrated solution was preserved in a refrigerator for further analysis. The pre-concentrated solutions were injected into the GC-MS instrument and different peaks of phenolic derivatives were obtained in the chromatogram. The phenolic derivative, 2,3-dimethylphenol was identified and quantified by comparing its retention time and peak area with that of known concentration of standard solution which was also injected into the GC-MS system under the same conditions. The concentration of phenolic derivatives was calculated by using the equation:

Conc. of 2,3-dimethyl phenol = ($_{s}$/A_{std}) x (I_{std}/I_{s})x (C_{std}) x2(Conc. Factor)x1000ppb

Here A_s and A_{std} represent the peak area of component of sample and standard solutions, I_s and I_{std} indicate the injected volume of sample and standards and C_{std} is the concentration of standard solution.

Results and Discussion

Phenols, and their derivatives, are toxic and potentially carcinogenic and they can affect the taste and odour of drinking water with concentrations as low as a few $\mu g/L^{-1}$. As a consequence, both the US Environmental Protection Agency (EPA) and the European Union (EU) have included some phenols, mainly chlorophenols and nitrophenols, in their lists of priority pollutants. EU Directive 2455/2001/EC sets a maximum concentration of $0.5\mu g/L^{-1}$ in water and their individual concentration should not exceed $0.1\mu g/L^{-1}$.

The concentrated water samples and standard solutions were introduced into the GC-MS system. Initially no separation of phenolic derivatives was observed when the GC-MS was operated without the control of column and oven temperature. For separation of the phenolic derivatives of the complex mixtures, it was decided to use column temperature programming resulting in good separation of individual components present in the river water. The optimum conditions for the separation of phenolic derivatives were: injector temperature 250^{o}C, initial column temperature was started at 40°C for 1 min, programmed at 8°C min^{-1} to 200°C and heated until 280^{o}C at 10^{o}C min^{-1}. Helium gas was with flow rate 1ml min^{-1}.

Measurement of phenolic derivative, 2,3-dimethyl phenol in river water samples

Identification of 2,3-dimethyl phenol was carried out by GC-MS analysis, and/or by comparison and combination of their retention times, and mass spectra of the peaks with those of authentic samples. Quantitative data were calculated by GC-MS peak areas compared with those of external standard calibration curves (Fig. 2). The evaluation was performed using three point linear standard calibration curves (r^2 >0.996) calculated by GC-MS.

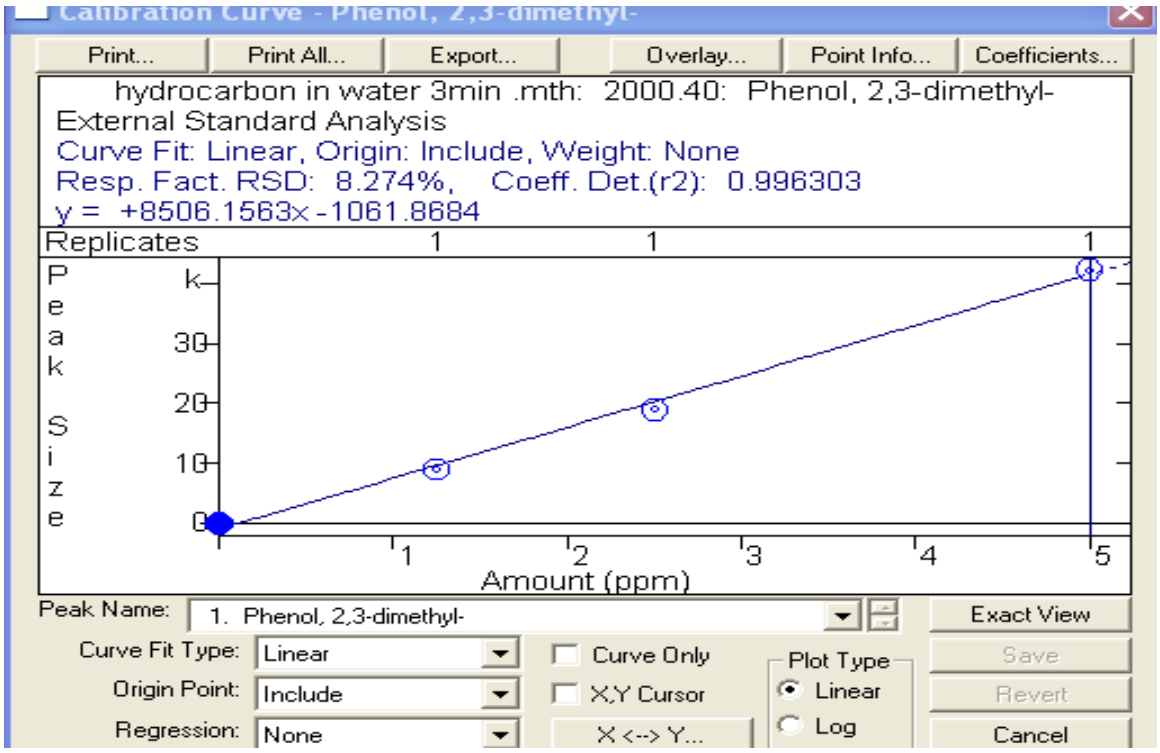

Figure 2. Standard Calibration Curve of 2,3-dimethylphenol.

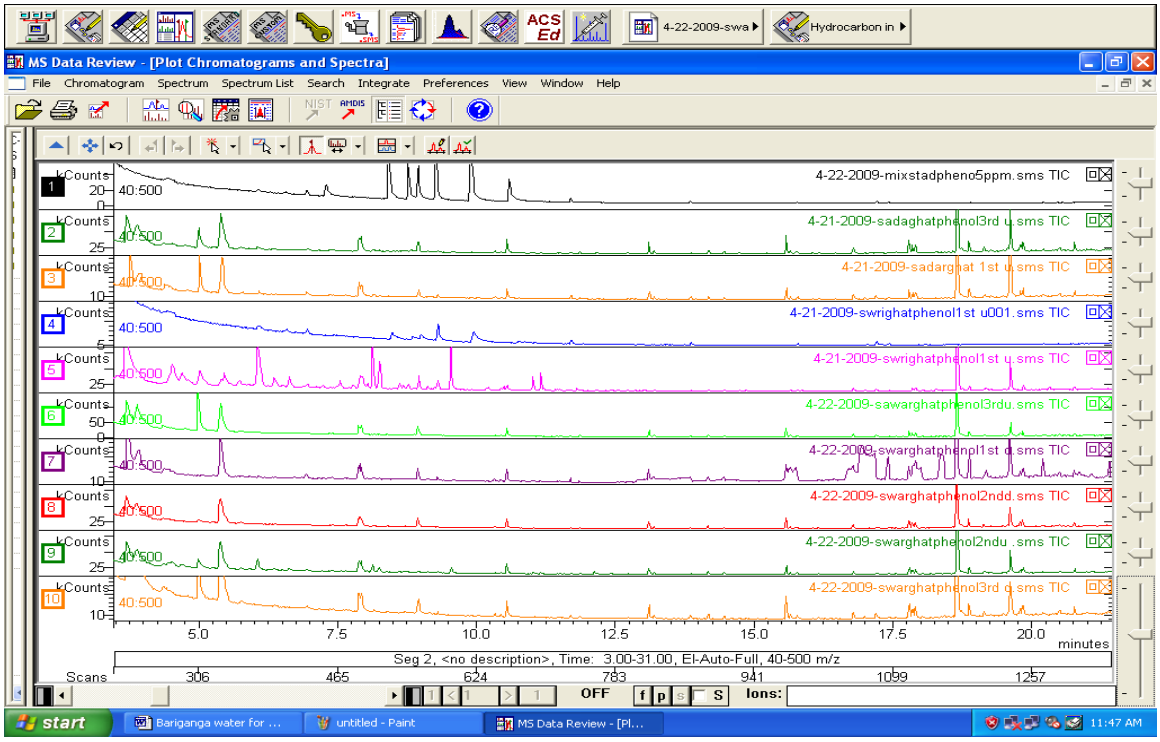

Figure 3. Comparison of chromatograms of retention time of standard and water sample extracts, collected from different sampling stations.

Column: VF-5 (l. 30 m, i.d. 0.25, film thickness 0.25 μm); delay: 3min; Temperature Program: 40°C(1)—›200°C (8°C)—›300°C (10°C); Injector Temperature: 250°C; Split: 20%; Carrier gas: He; Flow rate: 1ml/min.

In order to determine the concentration of phenolic derivatives, pre-concentrated 2,3-dimethylphenol from the surface and 30 cm depth at Postagolla, Sadarghat and Sowarighat stations were injected into the GC-MS instrument. A comparison of mass spectra for the standard solutions of phenolic derivatives with the sample solutions, collected from surface water and sample solutions collected from 30 cm depth at Postagolla station, is shown in Fig. 3.

This shows an excellent similarity of the retention time (9.214 for standard of 2,3-dimethyl phenol) of the separated peak of phenolic derivatives between standard and sample solutions. But other phenolic derivatives were not found. This is probably due to the presence of very low concentrations in water samples, which is well below the detection limit of GC-MS, or may not be present in water samples at the selected locations. Very similar types of chromatograms, not shown in the figure, were obtained for surface and 30 cm water samples collected from the other stations, Sowarighat and Sadarghat.

Dhaka is a mega city and it is situated on the banks of the Buriganga. About 180 million people live in this mega city. There is a lot of small and big industries such as dyeing, painting etc. linked to the river. It is possible these industries are using phenolic derivatives as a raw material to produce finished products, after which they are discharging their effluent directly into the Buriganga River. High concentrations of 2,3-dimethyl phenol were only obtained from the samples collected from surface and 30 cm depth at Sowarighat and Postagolla stations at the northern part of the Buriganga River. The other sampling points did not show 2,3-dimethyl phenol. This is probably due to the presence of very low concentrations in water samples because it is diluted or may not be present in water samples at the selected locations. The absence or lower concentration of other derivatives of phenols is probably due to their volatility, dissolution, biological degradation, photo oxidation and rapid photolysis [8]. It can also be seen that the concentrations of 2,3-dimethyl phenol in surface water are greater than those taken from 30 cm depth. The probable reason is that the higher concentration of 2,3-dimethyl phenol arises from the discharge of effluent from the industry. The concentration of 2,3-dimethyl phenol for surface and 30 cm deep water collected from the various stations of the Buriganga River is presented in Table 1.

Table 1. Concentration of 2,3-dimethyl phenol in surface and 30 cm deep water at different locations of Buriganga River.

Concentration of 2,3-dimethyl phenol, ppm									
2,3-dimethyl phenol	**Sowarighat**			Sadarghat			Postagolla		
	Northern side	Middle	Southern Side	Northern side	Middle	Southern Side	Northern side	Middle	Southern Side
Surface	0.964	nd	0.05	nd	nd	nd	0.418	nd	nd
30 cm depth	0.125	nd	nd	nd	nd	nd	0.222	nd	nd

*nd= not detectable

Table 1 summarizes the concentration of 2,3-dimethyl phenol and shows that this differs at different sampling stations. The concentration at Sowarighat is relatively higher than the other two sampling stations. The highest concentration for toxic 2,3-dimethyl phenol was found 0.964 ppm in surface water at the northern side of Sowarighat and the lowest value was recorded as 0.125 ppm in 30 cm deep water of the same station. On the other hand, almost similar results were obtained from the sampling station at Postagolla.

Conclusions

Phenolic compounds are important priority pollutants in most countries in the world and many related analytical techniques have been developed for detection of phenols. Present work has been done by our previously established method for phenols determination without any derivative process. In conclusion, the concentration of toxic 2,3-dimethyl phenol in water samples at Sowarighat, Sadarghat and Postagolla stations of the Buriganga River were determined. Samples were collected from surface and 30 cm depth of water from each of the sampling stations. The collected samples were extracted, pre-concentrated and analyzed by GC-MS. The highest and lowest concentrations were obtained in the river water samples at Sowarighat station from surface and 30 cm depth. Almost similar results were obtained from Postagolla. The experimental results demonstrated that this GC-MS method offers excellent recoveries and could be employed for environmental sample analysis. In view of the rapidity, sensitivity, simplicity, environment-friendly nature, the proposed method will be an excellent alternative detection technology for phenol analysis and can be widely employed in environmental and other related fields.

Acknowledgements

We are grateful to Engr. Rezaul Bari, Director, Atomic Energy Centre, Ramna, Dhaka for his continuous encouragement and logistic support during the work. We also thank Mr. Zahidul Islam and Mr. Ayub Ali for their help in collection and preparation of the water samples.

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