International Journal of the Physical Sciences Vol. 5(5), pp. 576-581, May 2010
Available online at http://www.academicjournals.org/IJPS
ISSN 1992 - 1950 © 2010 Academic Journals

Full Length Research Paper

Development of a solid-phase extraction method followed by HPLC-UV detection for the determination of phenols in water

B. O. Opeolu*, O. S. Fatoki and J. Odendaal

Department of Chemistry, Faculty of Applied Sciences, Cape Peninsula University of Technology, Cape Town, South Africa.

Accepted 2 December, 2009

A gradient HPLC-UV method for the determination of phenol and 10 of its derivatives was developed. Optimization studies were performed on parameters such as pH, solvents ratio, column temperature, sample volume, run time and flow rate. Calibration experiments were tested for linearity and reproducibility. Milli-Q water (HPLC water) was spiked with phenols' standard solutions for recovery studies using solid-phase extraction (SPE) for analytes enrichment. The total run time was 20 min; the coefficient of determination, R^2 was > 0.99 for all analytes; mean percentage recovery ranged between 67.9±7.28 and 99.6±4.26. Detection limits ranged from 0.51 to 13.79 µg/ml while reproducibility expressed by the coefficient of variation of triplicate extractions was less than 12% for the 11 analytes. Two of the analytes, 2-chlorophenol and 2, 4-dichlorophenol were detected in some drinking water samples analysed.

Key words: Chlorophenols, HPLC-UV, nitrophenols, phenol, solid-phase extraction, waters.

INTRODUCTION

Phenol and its derivatives are common water pollutants due to their widespread usage (Angelino and Gennaro, 1997). They are used in large quantities in the production of plastics, plasticizers, drugs, dyestuffs, explosives, pesticides, detergents, stabilizers and antioxidants (Schmidt-Baumler et al., 1999; Gabriel et al., 2007). They occur in domestic and industrial wastewaters, potable water resources and in nature as building blocks of plants (Baltussen et al., 1999). The different uses of these compounds often results in wastewater and groundwater contamination; consequently, drinking water supplies (Ribeiro et al., 2002). Phenols are introduced into surface waters from industrial effluents such as those from coal tar, gasoline, plastic, rubber proofing, disinfectant, pharmaceutical, agricultural runoffs, chemical spills, steel industries, domestic wastewaters, wood preserving plants, brake and clutching industries and biocides application. (Gupta et al., 1998; Chaliha et al., 2008; Sulisti et al., 1996; Amiri et al., 2004; Huang et al., 2007; Hartung et al., 2007).

*Corresponding author. E-mail: opeolubt@yahoo.com.

The presence of phenol and phenolic compounds in water has been of great public concern due to their high toxicity and persistence (Gupta et al., 1998; Liao et al., 2006 Habibi-Yangjeh and Esmailian, 2008; Schmidt-Baumler et al., 1999; Kartal et al., 2001; Suliman et al., 2006). They are one of the most frequent contaminants in food, water and hazardous waste sites. Phenols can cause bad taste and odour in drinking water even at low concentrations. Apart from anthropogenic emissions, phenolic residues can be formed naturally during decomposition of wood and leaves (Schmidt-Baumler et al., 1999). These compounds which are generally traceable to industrial effluents and landfills display a low taste threshold in potable waters and also may have a detrimental effect on human health at fairly high levels (Toniolo et al., 2007). Some phenolic compounds have been found to accelerate tumour formation Gupta et al. 1998; Suliman et al., 2006). Associated adverse environmental and health effects of phenols have necessitated legislations in many jurisdictions worldwide on set limits for the maximum content of phenols in potable waters and wastewaters (Toniolo et al., 2007; Hartung et al, 2007). Due to their toxicity and ubiquitous occurrence in the environment, the US Environmental

Protection Agency (USEPA) and European Community (EC) have included these phenols in their lists of priority pollutants (Bagheri et al., 2004; Heberer and Stan, 1997; Huang et al., 2009). Determination of these pollutants is of great importance in environmental analysis (Heberer and Stan, 1997; Schmidt-Baumler et al., 1999). The need for inter-comparison studies to validate analytical methods being used therefore cannot be over-emphasized.

Several workers have carried out studies on the analysis of phenols in water samples (Arditsoglou and Voutsa, 2008; Sato and Matsumura, 2003; Santana et al., 2002; Monde et al., 1996; Ozkaya, 2005; Schmidt-Baumler et al., 1999).

Information is however limited on statistical evaluation and validation of methods used. Method development involves the assessment of performance criteria which include precision, accuracy, detection limit, linearity, amongst others. The objective of this study was therefore to develop and validate a high performance liquid chromatographic (HPLC) method for the determination of 11 phenols in water samples.

EXPERIMENTAL

Reagents and Materials

Phenol (P), 4-nitrophenol (4NP), 2-nitrophenol (2NP), 2-chlorophenol (2CP), 2,4-dinitrophenol (2,4-DNP), 2,4-dimethylphenol (2,4-DMP), 4,6-dinitro-o-cresol (4,6 DNoC), 4-chloro-3-methyl phenol (4C-3MP), 2,4-dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP), pentachlorophenol (PCP) were obtained from Chem Services, West Chester, PA. HPLC grade methanol was supplied by Merck, Germany and MilliQ water was from Millipore, Molsheim, France. Solid phase extraction (SPE) cartridges was a polystyrene divinyl benzene material (Chromabond HR, Macherey-Nagel GmbH and Co. KG, Germany), 0.45 µm Magna nylon filters (Osmonics Inc.).

Apparatus

The HPLC system consisted of a binary pump (Waters 1525); a dual wavelength ultra violet (UV) detector (Waters 2487); stationary-phase column was a Waters symmetry C_8 column (3.9 × 150 mm id, 5.0 particle diameter) supplied by Waters, Milford, USA). The system software was Breeze 3.20 (2000) for data acquisition and processing.

Mobile phases

Mobile phases were prepared by adding required (1%) volumes of analytical grade acetic acid to each of methanol and milliQ water. These were filtered through nylon filters (0.45 µm). Gradient programmes, run time, injection volumes, wavelengths, column temperature and flow rate were optimised for phenols' separation on HPLC.

Sample solutions

Stock solutions of each of the phenols were prepared in methanol; Standard solutions of phenolic compounds were prepared daily by diluting stock solutions in methanol and used to calibrate the HPLC system. Milli-Q water was spiked with standard solutions of phenols and processed through SPE. Each sample was acidified to pH <2 with HCl; SPE cartridges conditioned with 6 ml methanol and equilibrated with 6 ml acidified milliQ water (pH <2). Each of solvent was allowed to run successively through the cartridge under gentle vacuum.

Spiked water samples were then passed through the cartridge under similar conditions of vacuum and flow rate. The cartridge was then dried under gentle vacuum and analytes eluted with 2.4 ml methanol in 0.01% HCl. Each extraction was performed in triplicates (Santana et al., 2002).

HLPC conditions

The run time was 20 min, flow rate optimized as 1 ml/min, column temperature was kept at 45°C, injection volume maintained at 10 µl and UV detection at 280 nm. The gradient programme profile used was 30 to 100% B in 20 min.

Sampling and sample pre-treatment

Tap water samples were collected from the Chemistry laboratory of Cape Peninsula University of Technology (CPUT), Cape Town, South Africa. Bottled water was purchased from a grocery store in Cape Town; domestic water samples were collected from a CPUT students' residence and selected informal settlements (Khayelitsha, Langa, Gugulethu) in Cape Town. All samples were filtered through 0.45 µm membrane filter, acidified with HCl to pH < 2 and refrigerated at 4°C until analysis.

Method development

Method development was achieved by optimizing each of the HPLC parameters including solvent ratio, gradient programme, flow rate, column temperature, wavelength and injection volume. Each of the parameters was changed at a time while others were kept constant. Linearity, precision and accuracy of the method were thereafter evaluated.

Quality control

1. Blank determinations to establish the contribution to the analytical signal by reagents, glassware and other materials
2. Triplicate analysis of each sample and variations recorded to assess the precision of the method.
3. Analysis of control samples to ensure instrument's consistency.
4. Calibration events were carried out by injecting in duplicates, phenol standards at each concentration level randomly. This was to ensure that instrument drifts during calibration run would not affect pure error variance (Campo et al., 2006).
5. Limit of detection (LOD) and limit of quantification calculated as signal -noise ratio of 3:1 and 10:1 respectively (Zakeri-Milani et al., 2005).

RESULTS AND DISCUSSION

Linearity

The range studied for the compounds varied; regression equation, ranges of concentration of calibration curves and R^2 values for each compound is presented in Table 1.

Table 1. Calibration data and linearity of the 11 phenols.

Analyte	Range (µgml^{-1})	Calibration plot	R^2 - value	LOQ	LOD
P	0.1 - 50	4780x + 122100	0.9995	0.03	2.11
4-NP	1 - 100	2x -19.23	0.9982	0.90	13.79
2-NP	1 -10	88681x + 93640	0.9978	0.01	0.99
2-CP	1 - 100	2020x + 32250	0.9997	0.18	0.51
2,4-DNP	0.1 - 100	94553x + 10228	0.9993	0.34	6.86
2,4-DMP	0.1 - 30	47130x + 612581	0.9991	0.08	2.17
4,6 DNoC	1 -30	21800x - 109333	0.9982	0.06	1.98
4C-3MP	1 -50	15375x + 114083	0.9998	0.04	1.49
2,4-DCP	1 -100	9055x + 93089	0.9982	0.22	9.26
2,4,6-TCP	1 - 100	11578x + 66137	0.9998	0.09	2.85
PCP	0.1 -30	315x + 4787	0.9999	0.05	1.51

LOQ, Limit of quantitation; LOD, Limit of detection.

Table 2. Retention time (R_T) and peak area repeatability and reproducibility of the 11 phenols.

Analyte	R_T	Repeatability	Reproducibility
P	6.341	6.23	6.8
4-NP	7.979	5.48	5.4
2-NP	8.525	2.11	2.6
2-CP	8.948	2.41	3.8
2,4-DNP	9.181	2.83	4.7
2,4-DMP	10.825	9.35	11.2
4,6 DNoC	11.401	10.08	10.8
4C-3MP	12.197	2.60	4.5
2,4-DCP	12.989	9.30	9.8
2,4,6-TCP	14.797	1.40	2.6
PCP	18.530	7.21	8.1

The external standard method and concentration-peak area relationships described by regression analysis was used (Zakeri- Milani et al., 2005). Calibration data validates linearity in the concentration range studied for all analytes. The goodness of fit (R^2) for all analytes was > 0.99 demonstrating method suitability for the analysis (Chawla et al., 2006; Campo et al., 2006). Detection limits of the analytes were evaluated using a signal/noise ratio = 3; and values ranged between 0.51 - 13.79 µgml^{-1} (Angelino and Gennaro, 1997).

Results are consistent with those reported by Andrade et al. (2006) who analyzed phenol and other compounds in a concentration range of 2.4 and 30 µgml^{-1}. The UV detector response was linear in the concentration range studied. This present study provides a proposed method with greater sensitivity. The lower limits for the linear dynamic range were 0.1 and 1.0 µgml^{-1} for the compounds studied. Most of the compounds can also be analyzed using this method at concentrations above 30 µgml^{-1}.LOD and LOQ were used to determine the sensitivity of the method. The former is the lowest concentration of the analyte detected by the method and the latter, the minimum quantifiable concentration. There is therefore, the possibility of applying the method to industrial wastewaters with high levels of phenols.

Precision

Precision was measured in terms of repeatability and reproducibility (Leon et al., 2006). Data from precision experiments is presented in Table 2. Repeatability was achieved by analyzing 6 replicates of 10 µgml^{-1} of quality control (QC) samples same day; reproducibility on the other hand was measured by analyzing 6 replicates of the QC samples on different days (Zakeri-Milani et al., 2005). Repeatability results showed good precision with mean RSD values range of 1.4% and 10.1 for the 11 phenols. Reproducibility results also indicated good robustness of the method with mean RSD values of 2.6% and 11.2 (Leon et al., 2006).

Accuracy

Accuracy was assessed by taking readings from triplicate

Table 3. Average percentage recovery of phenols from MilliQ water.

Analyte	% Recovery	% RSD
P	67.88	3.59
4-NP	99.64	1.04
2-NP	91.82	8.10
2-CP	87.29	2.58
2,4-DNP	83.74	3.00
2,4-DMP	95.91	5.88
4,6 DNoC	93.28	3.35
4C-3MP	84.92	9.25
2,4-DCP	89.10	1.99
2,4,6-TCP	93.21	4.94
PCP	91.06	0.12

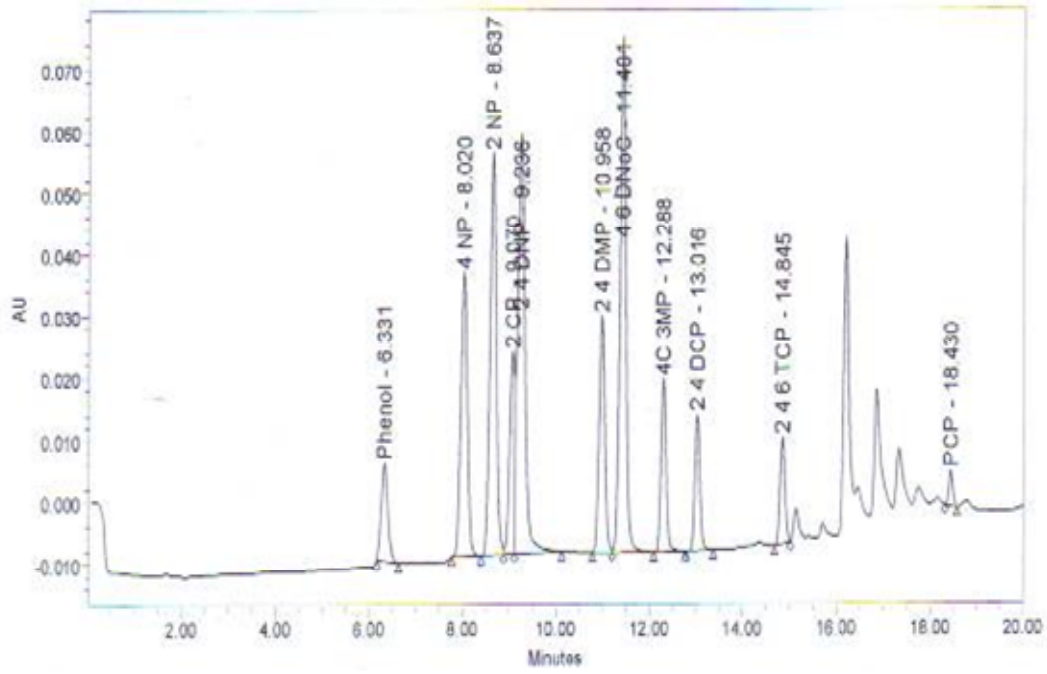

Figure 1. Chromatogram of 11 priority pollutants phenol in methanol (Flow rate = 1 ml/min; Injection volume = 10 µl; wavelength = 280 nm).

measurements. Milli-Q water was spiked with phenols' standards, processed through SPE (for pre-concentration of analytes and removal of interferences) and analyzed using the proposed method. Apparent recovery, calculated as the ratio of measured concentration (given by the calibration curve) to the spiked concentration and expressed as percentage (Andrade et al., 2006; Polo et al., 2006; Xing et al., 2006) for the 11 phenols is presented in Table 3. The recoveries were relatively high for almost all analytes with values ranging between 67.9 and 99.6. Percent RSD values were < 10 for all analytes investigated. These values are consistent with those previously reported (11 - 112%) in literature (Angelino and Gennaro, 1997; Bolz et al., 2000; Chawla et al., 2006; Huang et al., 2009; Patsias and Papadopoulou-Mourkidou, 2000; Polo et al., 2006; Schellin and Popp, 2005). Chromatogram of the phenols mixture is presented in Figure 1.

The potential applicability of the proposed SPE-HPLC/UV method was tested by analyzing domestic water samples from 5 locations. Samples were collected in triplicates thrice from each location and analyzed using this method. Results are presented in Table 4. Most of the analytes were not detected in the water samples; however, 2-chrolorophenol and 2,4-dichlorophenol were found in almost all the samples. These compounds have been reported to be used either as raw materials or intermediate products in the agro-chemical industry, wood preservation and paper production (Ozkaya, 2005; Ribeiro et al., 2002; Santana et al., 2002). Chlorophenols are also formed during dechlorination of natural humic substances in drinking water purification units (Heberer and Stan, 1997); implying possible sources of contamination in the water samples.

Table 4. Analytical results of drinking water samples from different locations.

Analyte	Lab tap	Langa	Khayelitsha	Gugulethu	Bottled	Milli-Q
P	ND	ND	ND	ND	ND	ND
4-NP	ND	ND	ND	ND	ND	ND
2-NP	ND	ND	ND	ND	ND	ND
2-CP	0.831± 0.016	ND	1.135 ± 0.004	1.218 ± 0.008	0.975± 0.003	ND
2,4-DNP	ND	ND	ND	ND	ND	ND
2,4-DMP	ND	ND	ND	ND	ND	ND
4,6 DNoC	ND	ND	ND	ND	ND	ND
4C-3MP	ND	ND	ND	ND	ND	ND
2,4-DCP	0.428 ± 0.034	0.512 ± 0.017	0.509 ± 0.020	0.392 ± 0.019	0.487± 0.024	ND
2,4,6-TCP	ND	ND	ND	ND	ND	ND
PCP	ND	ND	ND	ND	ND	ND

ND = Not detected.

CONCLUSION

A method of simultaneous determination of 11 priority pollutant phenols was developed using SPE- HPLC- UV detection. Calibration data validates linearity in the concentration range studied for each compound with R^2 values > 0.99 for all analytes. Repeatability and reproducibility data showed good precision of the method. Percent recoveries for the compounds were satisfactory when compared with those from previous studies. The method was successfully used to quantify the compounds from some domestic water samples. The proposed method is relatively easy and cheap for quantification of these important pollutants since derivatization is not required and less use of solvents and other chemicals.

REFERENCES

Amiri F, Rahman MM, Bornick H, Worch E (2004). Sorption behaviour of phenols on natural sandy aquifer material during flow through column experiments: The effect of pH. Acta Hydrochim Hydrobiol. 32(3): 214-224.

Andrade LS, Laurindo EA, de Oliviera RV, Rocha-Filho RC, Cass QB (2006). Development of a HPLC method to follow the degradation of phenol by electrochemical treatment. J. Braz. Chem. Soc. 17(2): 369-373.

Angelino S, Gennaro MC (1997). An ion-interaction RP-HPLC method for the determination of the eleven EPA priority pollutant phenols. Anal. Chim. Acta. 346: 61-71.

Arditsoglou A, Voutsa D (2008). Determination of phenolic and steroid endocrine disrupting compounds in environmental matrices. Env. Sci. Pollut. Res. 15(3): 228-236.

Bagheri H, Saber A, Mousavi SR (2004). Immersed solvent microextraction of phenol and chlorophenols from water samples followed by gas chromatograghy- mass spectrometry. T. Chromatogr. A 1046: 27-33.

Baltussen E, David F, Sandra P, Janssen H, Cramers C (1999). Automated sorptive extraction thermal desorption-gas chromatography Mass spectrometry analysis: Determination of phenols in water samples. J. Microcolumn Separations, 11(6): 471-474.

Bolz U, Korner W, Hagenmaier H (2000). Development and validation of a GC/MS method for determination of phenolic xenoestrogens in aquatic samples. Chemosphere 40: 929-935.

Campo P, Sorial GA, Suidan MT, Venosa AD (2006). Statistical evaluation of an analytical GC/MS method for the determination of long chain fatty acids. Talanta.68: 888-894.

Chaliha S, Bhattaryya KG, Paul P (2008). Catalytic destruction of 4-chlorophenol in water. Clean 36(5-6): 488-497.

Chawla S, Ghosh S, Sihorkar V, Nellore R, Shanta Kumar TR, Srinivas NR (2006). High performance liquid chromatography method development and validation for simultaneous determination of five model compounds, antipyrine, metoprolol, ketoprofen, furosemide and phenol red, as a tool for the standardization of rat in situ intestinal permeability studies using timed wavelength detection. Biomed. Chromatogr. 20: 349-357.

Gabriel LPF, Cyris M, Giger W, Kohler HE (2007). ipso-Substitution : A general biochemical and biodegradation mechanism to cleave α-Quartenary alkyl phenols and bisphenol A. Chem. Biodiversity 4:2123-2137.

Gupta YK, Sharma S, Yadav IS, Mohan D (1998). Utilization of bagasse fly ash generated in the sugar industry for the removal and recovery of phenol and p-Nitrophenol from wastewater. J. Chem. Technol. Biotecnol. 71: 180-186.

Habibi- Yangjeh A, Esmailian M (2008). Application of PC-AAN to acidity constant prediction of various phenols and benzoic acid in water. Chin. J. Chem. 26: 875-885.

Hartung R, Lenoir D, Henkelmann B, Schulte-Hostede S, Schramm K (2007). Reductive degradation of poly chlorinated phenols by Pd/cd formate: An eco-efficient remediation method for aqueous chlorinated phenols. Clean. 35(3): 235-238.

Heberer T, Stan H (1997). Detection of more than 50 substituted phenols as their t-butyldimethylsilyl derivatives using gas chromatography-mass spectrometry. Analytica Chimica Acta 341: 21-34.

Huang J, Wang X, Jin Q, Liu Y, Wang Y (2007). Removal of phenol from aqueous solution by adsorption onto OTMAC-modified attapulgite. J. Environ. Mangt. 84: 229-236.

Huang X, Qiu N, Yuan D (2009). Development and validation of stir bar sorptive extraction of polar phenols in water followed by HPLC separation in poly (vinylpyrrolididone-divinylbenzene) monolith. J. Sep. Sci. 32: 1407-1414.

Kartal OE, Erol M, Oguz H (2001). Photocatalytic destruction of phenol by TiO_2 powders. Chem. Eng. Technol. 24(6): 645-649.

Leon VM, Llorca-Porcel J, Alvarez B, Cobollo MA, Munoz S, Valor I (2006). Analysis of 35 priority semivolatile compounds in water by stir bar sorptive extraction-thermal desorption-gas chromatography-mass spectrometry Part II: Method validation. Anal. Chim. Acta 558: 261-266.

Liao C, Chen C, Wang M, Chiang P, Pai C (2006). Sorption of chlorophenoxypropionic acids by organoclay complexes. Environ. Toxicol. DOI10.1002/tox.

Monde T, Kamiusuki T, Kuroda T, Mikumo K, Ohkawa T, Fukube H (1996). High- performance liquid chromatographic separation of phenols on a fluorocarbon-bonded silica gel column. J. Chromatogr.

A. 722: 273-280.
Ozkaya B (2005). Chlorophenols in leachates originating from different landfills and aerobic composting plants. J.Hazard. Mater.B124: 107-112.
Patsias J, Papadopoulou-Mourkidou E (2000). Development of an automated on-line solid-phase extraction-high-performance liquid chromatographic method for the analysis of aniline, phenol, caffeine and various selected substituted aniline and phenol compounds in aqueous matrices. J. Chromatogr. A 904: 171-188.
Polo M, Llompart M, Garcia-Jares C, Gomez-Noya G, Bollain M, Cela R (2006). Development of a solid phase microextraction method for the analysis of phenolic flame retardants in water samples. J. Chromatogr. A 1124: 11-21.
Ribeiro A, Neves MH, Almeida MF, Alves A, Santos L (2002). Direct determination of chlorophenols in landfill leachates by solid-phase micro-extraction gas chromatography-mass spectrometry. J. Chromatogr. A975: 267-274.
Santana CM, Ferrera ZS, Rodriguez JJS (2002). Use of non-ionic surfactant solutions for the extraction and pre-concentration of phenolic compounds in water prior to their HPLC-UV detection. Analyst 127: 1031-1037.
Sato S, Matsumura A (2003). Extraction of phenol in water phase using liquefied dimethyl ether. J. Jpn. Petrol. Inst., 46(6): 375-378.
Schellin M, Popp P (2005). Membrane assisted solvent extraction of seven phenols combined with large volume injection- gas chromatography-mass spectrometric detection. J. Chromatogr A. 1072: 37-43.
Schmidt-Baumler K, Heberer T, Stan HJ (1999). Occurrence and distribution of organic contaminants in the aquatic system in Berlin part II: Substituted phenols in Berlin surface water. Acta hydrochim. Hydrobiol.27(3): 143-149.
Suliman FEO, Al-Kindy SM, Al-Lawati HAJ (2006). Analysis of phenols in water by High Performance Liquid Chromatography using coumarin-6-sulfonyl chloride as a fluorogenic precolumn label. J. Chromatogr.A 1101: 179-184.
Sulisti IA, Watson-Craik EA, Senior E (1996). Studies on the co-disposal of o-cresol with municipal refuse. J. Chem. Technol. Biotechnol. 65: 72-80.
Toniolo R, Pizzariello A, Susmel S, Dossi N, Doherty AP Bontempelli G (2007). An Anionic-Liquid based probe for sequential preconcentration from headspace and direct voltammetric detection of phenols in wastewaters. Electroanalysis 19(19-20): 2141-2148.
Xing A, Zhang M, He Z, Zhang J (2006). Development and optimization of a method for analysis of the products from the transesterification of dimethyl carbonate and phenol. Anal. Bioanal. Chem. 384: 551-554.
Zakeri-Milani P, Barzegar-Jalali M, Tajerzadeh H, Azarmi Y, Valizadeh H (2005). Simultaneous determination of naproxen, ketoprofen and phenol red in samples from rat intestinal permeability studies: HPLC method development and validation. J. Pharm. Biomed. Anal. 39: 624-630.