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ABSTRACT

This study is based on the SPE process, and the method employed was validated on 11 compounds of phenol using phenol calibration mix. It focuses on the determination of phenols in water by high performance liquid chromatography with a UV-detector

The background section deals with phenols, its subsequent group, the analytical methods and laboratory equipments used in determining these compounds in water. The experimental section provides information on the validation and optimization of analytical method according to the ISO standard or EPA method concentrates on specific group of phenols.

High performance liquid chromatography with a UV-detector is the main analytical equipment used in determination of phenols in water. The water samples to be analyzed were taken from five different locations in Poznan, Poland. The experiments were done in the water and soil testing laboratory of regional sanitary and epidemiological station in Poznan, Poland. The objective of the developed method was to achieve a concentration of 0.1 mg/l of each phenol from water samples and excellent recovery between 80-110% based on the column employed

In conclusion, with the method developed, it can be prove that phenols were not present in water within the working range of 0.003-0.250 mg/l

INTRODUCTION

The human race is influenced by the quality of water resources in the natural environment In order to guarantee a secure and healthy life, the water consumed by everyone must be free from harmful chemical substances. The water released into the environment by water purification plants and private organizations should be regularly accessed and examined to maintain complete yielding to water framework act. Thus, treated waters should be safe for .consumption and free from harmful compounds to guarantee a healthy life

However, there are environmental agencies for water which have given standards to ensure that water is free from contaminants, thus making safe water consumption possible. Water treatment requires adequate analysis and monitoring which includes determination of low level contaminants. The maximum level of contaminants present in drinking water should be so small that the effect is harmless to human life. Thus analytical methods are important to quantify the contaminants

Phenol and nitro-phenols are natural harmful substances found in water. They are used in many industrial activities for the production of pesticides, insecticides, herbicides, and synthetic products. Phenols which contain certain amount of pesticides and wood preservatives can leads to specific health damages even at the lowest concentration levels Nevertheless, it is important to be aware of phenols and substituted phenols in environmental and biological samples. Substituted phenols can be named using suitable prefixes which are the ortho- (1, 2), meta- (1, 3) and para- (1, 4) system depending on the placement of the substituent from the hydroxyl group. Liquid chromatography with a UV detector is one analytical approach used due to its high selectivity for phenols. However methods have been developed for the determination of phenol by gas chromatography with a flame ionization detector, which is quite effective but this research will concern a different method for determining the eleven possible phenols as listed in the U.S.EPA pollutant guideline

Solid phase extraction is a known method used for sample preparation techniques. It is the most recognized technique for aqueous sample extraction, sample cleanup and concentration. This method is applied to my research. Method detection limits of liquid chromatography techniques for direct sample injection in determining low level concentration in natural waters are not easily quantified, and thus it is necessary to carry out sample pre-concentration before analysis using solid phase extraction. Method detection limits can be used to create an environment which enhances a specific compound retention on the chosen solid phase extraction column

This research study concerns the validation and optimization of an analytical method for phenol determination in water. The aims of this thesis is to determine phenols present in water by high performance liquid chromatography with an ultraviolet detector and secondly, to test different columns for their retention property of analytes as well as other matrixes

The objective of this study is to develop a method with high recovery rate under various conditions and to estimate the performance characteristics of an analytical procedure including limit of detection and quantification, accuracy, recovery, precision, trueness of an analytical method and uncertainty. This research work is limited to phenols and .phenolic compounds in water, method development and verification

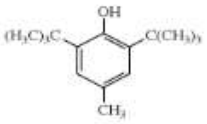
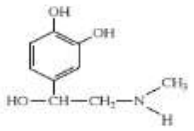
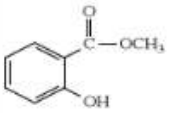
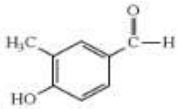
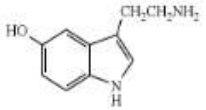
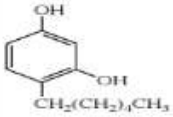
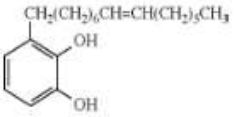
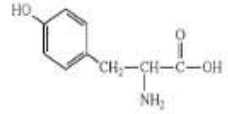
The research questions concerning this study are the following: Can the developed method ?be applied and is it a trusted method in determining phenols in water? Is it fit for purpose Does the developed method pass the requirement set by the EPA guidelines? The purpose of the developed method is to achieve a concentration of 0.1 mg/l of each phenol from water samples and excellent recovery base on the column employed

The water samples used for the research work are from five different locations in Poznan

THEORETICAL FRAMWORK

Phenols are classified as organic compounds similar to alcohols, but they form stronger hydrogen bonds. They are characterized by the hydroxyl (-OH) group which is attached to a carbon atom and is part of an aromatic ring. Phenols which have a structural formula (C6H5OH), that is the formula of phenol only, as a simplest member of phenols, and ,subsequent phenols with structure R- C6H4OH, where R represents some groups like CH3 and NO2. However, phenols are more soluble in water than alcohols and possess a higher .boiling point. Phenols are highly toxic colorless liquids or white solid at room temperature (.Wade 1999)

TABLE 1. The structural formula of some groups of phenol compounds (Wade 1999)

 phenol	 2,6-di-t-butyl-4-methyphenol	 epinephrine(adrenalin)
 methylsalicylate	 vanillin	 serotonin
 4-hexylresorcinol	 urushiol	 tyrosine

However, some commonly used names of certain phenolic compounds are vanillin salicylic acid, pyrocatechol, resorcinol, cresol, hydroquinone, and eugenol. Hence, phenol

having one substituent can be classified with the right number or the ortho (1,2), meta (1,3)

and para (1,4) pattern (Wade 1999)

TABLE 2.Structural formula of substituent phenols (Wade 1999)

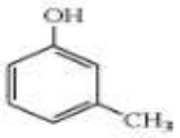
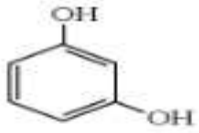
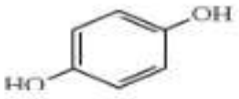
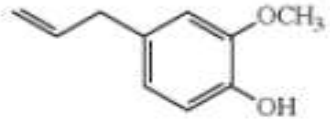
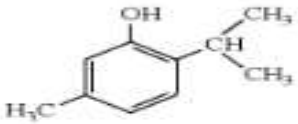
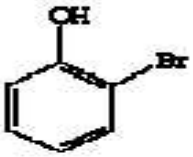
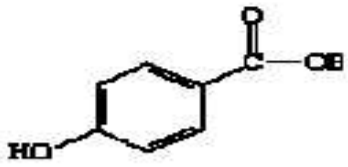
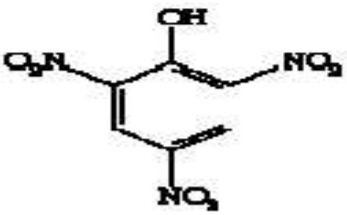
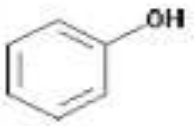
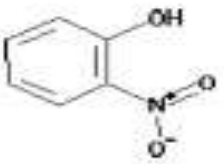
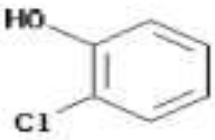
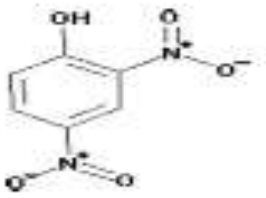
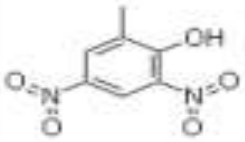
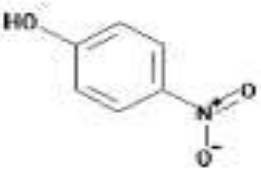
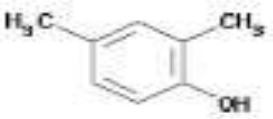
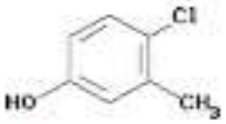
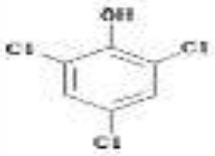
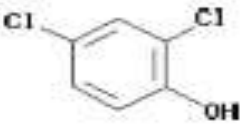
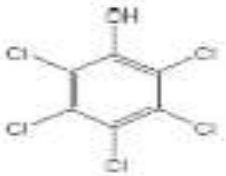
 meta-cresol	 resorcinol	 hydroquinone
 eugenol	 thymol	 2-bromophenol (ortho-comopund)
 4-hydroxybenzoic acid (para-compound)	 2,4,6-trinitrophenol	

TABLE 3. Phenolic compounds, their parent compounds and examples of environmental sources (BCER COTC Fact sheet on phenols 2007

	Chemical name	Abbreviation	Parent compound if applicable	Additives, commercial & personal product exposure sources
1	Bisphenol A			Polycarbonate containers and coatings (cans, cups), dental sealant
2	Benzophenone-3(2-hydroxy-4-methoxy-benzophenone), (oxybenzone)	BP3		Sunscreen agent, photostabilizer for synthetic resins
3,4,5	2,4-dichlorophenol and trichlorophenols (chlorinated phenols)	24DCP, 245TCP, 246TCP	Phenoxy- and other derivatives(245,246 TCP are metabolites of hexachlorobenzene and hexachlorocyclohexane)	Herbicides (organochlorine pesticides)
6	2,5-dichlorophenol	25DCP	4-dichlorobenzene (metabolite of p-DCB)	Mothballs
7	ortho-phenylphenol	o-PP		Fungicide
8	4-tert-octylphenol	4-t-OP		Detergent surfactant
9	Triclosan[5-chloro-2-(2,4-dichlorophenoxy)phenol]	TRCS		Microbicide in home cleaning and personal care products

TABLE 4. Structures of the eleven phenols specified in the U.S.EPA priority pollutants list (Dionex Corporation 2008)

 Phenol	 2-nitrophenol	 2-chlorophenol	 2,4-dinitrophenol
 2-methyl-4,6 dinitrophenol	 4-nitrophenol	 2,4-dimethylphenol	 4-chloro-3-methylphenol
 2,4,6-trichlorophenol	 2,4-dichlorophenol	 Pentachlorophenol	

Sources of phenols

Phenols mainly occur in nature as a product of coal tar or crude petroleum. However phenols are formed as natural decay of organic compounds. Phenols present in nature include tyrosine found in proteins which belong to the class of amino acid. Epinephrine is an adrenaline that produces hormone. Serotonin is a neurotransmitter found in the brain and urushiol, which causes irritation generated by poison ivy to prevent animals from doing certain things. Some phenol can be gotten from plant like thymol, separated from thyme and eugenol, extracted from cloves. (Wade 2009)

Uses of phenols

Phenol is used as raw material in the manufacture of a wide range of important chemicals including phenolic resins, bisphenol-A, caprolactam, alkylphenols and adipic acid. Phenol is widely used for the treatment of injuries. It is suitable for making aspirin drug also .antiseptics and local anaesthetics (Wade 2009)

Phenol is used in the manufacture of paints and varnish removers, lacquers, rubber, ink and illuminating gases, tanning dyes, perfumes, toys and soaps (Wade 2009). Phenol is used as an industrial chemical in the manufacturing of certain products such as “resins plastics, fibers, adhesives, iron, steel, aluminum, leather, and rubber”, also phenol is present in disinfectants, cigarette smoke, and emissions from vehicles (EHC 161, 1994)

Phenols have a wide range of applications in household products and industrial synthesis They are used as disinfectants in household cleaners, lotions, salves, ointments and in mouth wash. However, compounds of phenols are used in dye industries to make colored azo dyes and used as components in making wood preservatives such as creosote Industrial applications of phenols are used in making plastics, explosives like picric acid and drugs such as aspirin. (Wade 1999)

Physical properties of phenols

Phenols which are identical to alcohols having a hydroxyl group attach to an aromatic ring which enables them to undergo intermolecular hydrogen bonding. They have the ability to form stronger hydrogen bond than alcohols. Nevertheless, the presence of hydrogen bonds in phenols makes them to be more soluble in water. Hence, the occurrence of hydrogen bonds in phenols results in higher melting and boiling points. (Wade 2009)

Table 5 summarizes the physical and chemical data of eleven phenols specified by certainenvironmental agencies in their priority pollutants lists. The eleven groups of phenols aredetermined in water to ensure that the concentration does not exceed the limit provided bythe environmental agencies

TABLE 5. Physical and chemical data of Phenols (Merck chemicals 2010

	Formula	Molar mass (g/mol)	Melting point (°C)	Boiling point (°C)	Density (g/cm^3)
Phenol	C_6H_5OH	94.11	40.8	181.8	1.06 (20 °C)
2-nitrophenol	$2\text{-}(NO_2)C_6H_4OH$	139.11	43 - 45	215 - 216	1.26 (20 °C)
2-chlorophenol	$2\text{-}(Cl)C_6H_4OH$	128.55	7	174	1.26 g (20 °C)
2,4-dinitrophenol	2,4-DNP	184.11	114 - 115		1.68 (20 °C)
2-methyl-4,6-dinitrophenol	$C_7H_6N_2O_5$	198.14	82 - 85	312	
4-nitrophenol	$O_2NC_6H_4OH$	139.11	110 - 115	279	1.48 (20 °C)
2,4-dimethylphenol	$2,4\text{-}(CH_3)_2C_6H_3OH$	122.16	25	211	1.016 (25 °C)
4-chloro-3-methylphenol	$4\text{-}(Cl)\text{-}3\text{-}(CH_3)C_6H_3OH$	142.58	63 - 65	235 - 239	1.37 (20 °C)
2,4,6-trichlorophenol	$C_6H_3Cl_3O$	197.44	65 - 68	244 - 246	1.675 (25 °C)
2,4-dichlorophenol	$2,4\text{-}(Cl)_2C_6H_3OH$	163	40 - 43	209 - 211	
Pentachlorophenol	C_6HCl_5O	266.34	190-191	309-310	1.978 (22 °C)

METHODS FOR DETERMINING OF PHENOLS IN WATER

There are methods which have been developed over the years for the determination of phenolic compounds in water and waste water. Some of these methods are spectrophotometry, electrochemical methods, capillary electrophoresis, the gas chromatographic (GC) method using liquid-liquid extraction and either using flame ionization detection (FID) or derivatization and electron capture detection (ECD) to analyze different phenols at a low concentration. In determination of phenol at high concentration, the gas chromatography/mass spectrometric (GC/MS) method with liquidliquid liquid extraction is employed

In water treatment plant, chlorine applications have resulted in producing chlorophenol The method which can be applied in analyzing phenol, ortho- and meta- substituted phenols is known as 4-aminoantipyrine colorimetric method. However, para-substituted phenol with sub group known as carboxyl, halogen, methoxyl or sulfonic acid group cannot be determine using 4-aminoantipyrine method under certain pH ranges. Thus, 4aminoantipyrine

method is suitable for water samples with high sensitivity. The

disadvantage of this method is that any color produced by the reaction of any phenolic compounds is proved to be phenol

Chemiluminescence (CL) is an analytical detection method suitable for very low detection limit, fast and large linear working range that can be obtained using simple instrumentation. Chemiluminescence method is applied for the determination of phenol including luminal CL system and acidic KMnO4CL system. However, due to lack of selectivity for phenol, chemiluminescence systems cannot determine phenol in water samples directly. Phenol can be determined only when the CL system is combined with some separation precession like per-distillation, liquid chromatography and capillary Liquid-liquid extraction gas chromatographic method is applied in analyzing phenols and some substituted phenols in water either in municipal or industrial released. During

confirmation of an unknown compound, the use of derivatization, cleanup and electron capture detector gas chromatography (ECD/GC) are used to determine results obtained by flame ionization detector gas chromatographic

gives a wide number of options for the determination of phenols in water and soil samples. Phenols are separated from water at pH < 2 with methylene chloride using iquid-liquid or continuous liquid extraction. Phenols is analyzed by FID using one column or double column procedure after solvent evaporation and replacing the solvent to 2propanol

hus, sensitivity may not be suitable for the underivatized phenols. Phenols can be derivatized with diazomethane to produce methyl ester of phenol and can be determined by FID. A suitable approach for sensitivity and selectivity can be achieved by derivatizing the analyte extracts with pentafluorobenzylbromide (PFBBr) and detecting the derivatized phenols using electron capture detector (ECD)

Hence, three phenols: 2, 4-dinitrophenol 2_methyl-4, 6-dinitrophenol and dinoseb are not derivatized by PFBBr. During cleanup process, a silica gel is used after the derivatization

Another method for derivatization is the use of acetic anhydride (C4H6O3). The sample is adjusted to pH≈7 using sodium hydroxide or phosphoric acid and adding potassium carbonate and acetic anhydride to the sample. After mixing, hexane is used to extract the derivatives then the extract is injected into gas chromatography column. This method is not .suitable for nitrophenols because of the poor effectiveness of the derivatization reaction

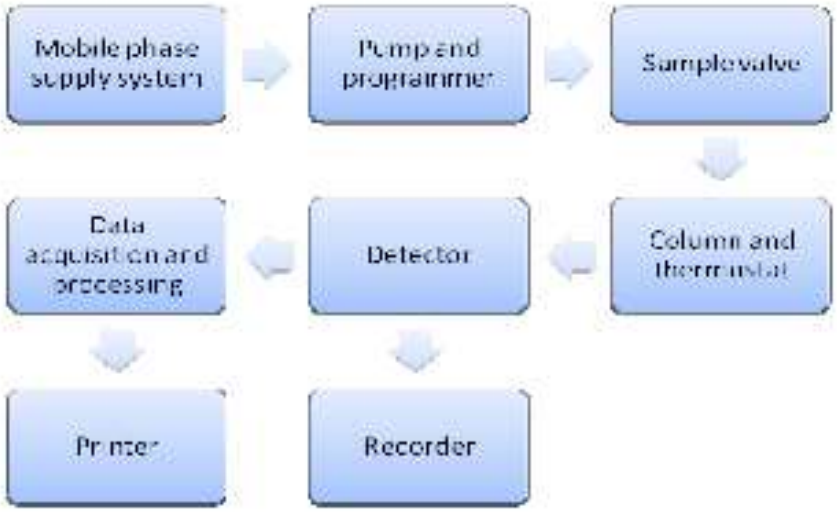
The characteristics of high performance liquid chromatography (HPLC) are proved to be an effective method for the separation of phenols. However, improvements have been made in HPLC analysis of compounds. In recent years, devices for detection and identification coupled to HPLC have been developed to make separation, qualification and quantification of compounds possible. HPLC methods for analysis of compound avoid the difficulties and time-consuming separation of compounds for the subsequent individual identification of each compound. Thus, in this research, information of the analysis of phenols by HPLC is provided

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

In our present time, there is a rising interest in applying high performance liquid chromatography (HPLC) which is not subject to temperature dependence to the determination of not just volatile organic compounds like aliphatic and polyaromatic hydrocarbons, saturated and unsaturated aliphatic halogen compounds, haloforms and some esters, phenols unlike the gas chromatography, but for all organic and inorganic matter present in water samples. Hence, in liquid chromatography, a liquid passes through a porous solid stationary phase and the elute flows through a detector. In HPLC, the mobile phase is pumped at high pressure

he essential parts of liquid chromatography include the mobile phase of solvent, high ,pressure and low pressure gradient programmers, pumps (piston and diaphragm pumps syringe and rapid refill pumps), valves and oven column. The detectors used in high performance liquid chromatography include UV detector which can be fixed or variable wavelength, the fluorescence detector and the refractive index detector. An HPLC stationary phase includes irregular and spherical silica gel

Graph 1 gives a clear illustration of the essential parts of liquid chromatography from one component part to another

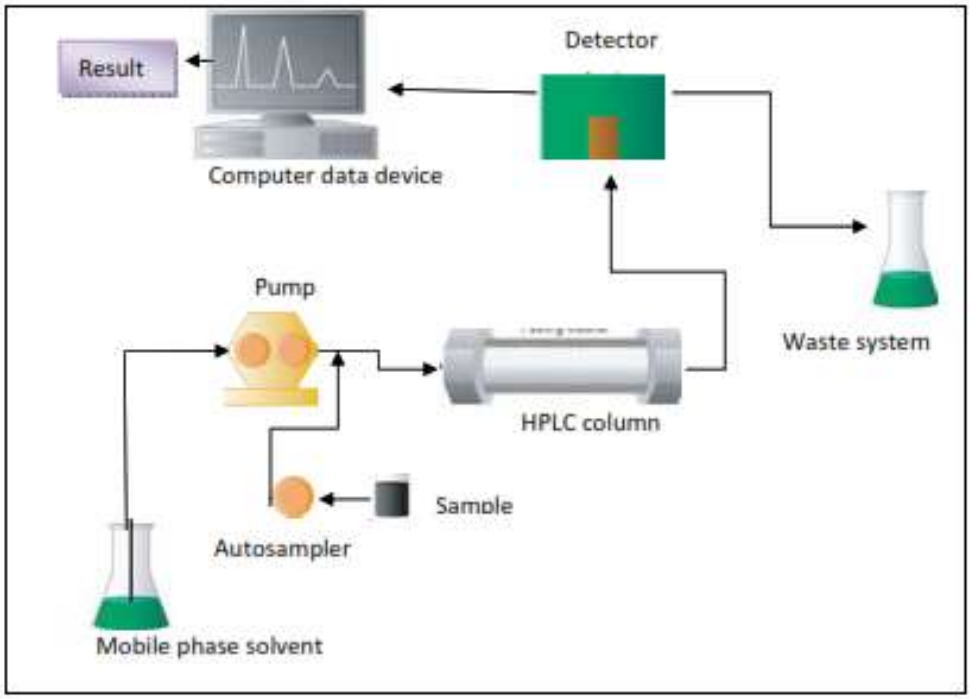


GRAPH 1. The essential parts of liquid chromatography copyright by Scott 2008

The mobile phase which is a solvent is contained in a bottle. A high pressure pump is needed to deliver the mobile phase at a certain flow rate apparently in milliliters per minute. Also, an injector known as auto-sampler passes samples into a constant moving mobile phase stream that moves the sample into the HPLC column. The stationary phase is a chromatographic material packed in a column and it is needed to effectively carry out the sample separation. Furthermore, a detector helps to visualize the separated sample bands eluting the HPLC column and the mobile phase leaves the detector which is collected by waste system. However, the detector is connected to a data collection system that stores electrical signal that produces the chromatogram on its screen. The end results are seen as chromatogram appearing as peaks of various heights depending on the concentration of the

.sample constituents

Graph 2 gives a concise picture on the operation of high performance liquid chromatography (HPLC) system



GRAPH 2. Operation of HPLC system copyright by Waters Corporation 2010

Types of chromatography column

High performance liquid chromatography (HPLC) have been created so as it can perform to a very high level by combining selective stationary phases of different material sizes with it, also with adequate columns with big amount of plates per liter. There are different types of chromatography column used in high performance liquid chromatography which are reversed-phase chromatography, reversed-phase ion-pairing chromatography, ionsuspension

chromatography and ion-exclusion chromatography. Reversed phase

chromatography (RPC) column are mostly used in all HPLC applications

LIQUID CHROMATOGRAPHY DETECTOR

In choosing the right kind of detector in liquid chromatography, it is highly important to consider the selectivity, response, sound, specific values and linearity (Scott 2008). The different detectors that can be used in analytical procedures include some refractive index equipments, detectors associated with an ultraviolet absorption, also fluorescence detection. These detectors are widely used in LC applications because of its sensitivities and linearity (Scott 2008

EXPERIMENTAL SECTION FOR DETERMING OF PHENOLS

This chapter gives an overview of all analytical equipments, materials, chemicals used in this project. The experiments were performed in Water and Soil Testing Laboratory in Poznan, Poland. This method is suitable for determination of phenol, 4-nitrophenol, 2, ,4dinitrophenol

chlorophenol, 2-nitrophenol, 2,4-dimethylphenol, 4-chloro-3methylphenol-2

methyl-4,6-dinitrophenol, 2,4,6-trichlorophenol, 2,4-dichlorophenol and-2

pentachlorophenol in tap water using high performance liquid chromatography (HPLC) with UV-detector after solid phase extraction

Principle: Phenols which are present in water are extracted to an appropriate stationary phase. The elution process of analytes is done using some organic solvent and the purity of the solvent is suitable for HPLC analysis. Phenols are separated on HPLC column using gradient, identification of analytes and quantification is done with UV-detector with .optimization of maximum absorption of each analytes

Interferences: All compound present in water samples having the same maximum absorption and the same retention time have the possibility of interfering with the analytes

Reagent: All chemicals of analytical purity should be used to prevent additional contamination. Thus, the glass purity and the purity of chemicals need to be checked by

analyzing blank sample

,Compounds used for extraction and HPLC analysis: Acetonitrile (CH3CN), distilled water methanol (CH3OH), sodium thiosulfate pentahydrate (Na2S2O3˙5H2O) to inactive the free chloride present in water sample, nitric acid (suprapur: a stage of purity). Standard stock solution with a concentration of 2000 mg/l of each phenol must be kept in dark at low temperature and should be protected from evaporation, also to avoid contact with skin

TABLE 6. Chemical and solvent details

Name	Producer	Details
604 phenols calibration mix	Restek	2000 µg/ml each in methanol
Acetonitrile	Merck KGaA	2.5 l
Methanol	Merck KGaA	2.5 l
Distilled water	Millipore GmbH	Milli-Q Plus
Acetic acid	Merck KGaA	2.5 l (100%-vol)
Nitirc acid	Merck KGaA	1000 ml (100%-vol)
Tap water	Aquanet	

Apparatus: The usual laboratory apparatus and in particular; SPE columns filled with appropriate stationary phase

TABLE 7. Description of all analytical equipments

Name	Producer	Details
SPE vacuum unit	Mallinckrodt Baker	SPE-12 G vacuum with tap
SPE column	J. T. Baker	
Glass vial		5 ml, 50 ml
Microlitre syringes (Chromatography syringes)	Hamilton thermoscientific	50 µl
Glass material	Schott duran	500 ml light brown sample bottles
Graduated pipette	Eppendorf	100-1000 µl
Measuring cylinder	Simax Czech republic	500 ml
Beakers	Simax Czech republic	50-250 ml
Transferpipette	Eppendorf	0.5-50 µl
Filter paper		Nylon 0.45 µm

Standard for calibration curve: Standard for calibrations were prepared by using standard stock solution to achieve 5 calibration solutions distributed as evenly as possible over an expected working range. However, checking the accuracy of calibration curve, some standard solution from manufacturer is used independently and the curves were checked at .different levels

HPLC systems containing UV-detector with photodiode array detector (DAD) and appropriate software for data collection. In general, it consists of the following: eluent ,reservoir, a degassing unit, HPLC pump with gradient system, sample injection system separation column, photodiode array detector (DAD) capable for UV-VIS measurement .from 200 nm to 800 nm

Quality requirement for the separation column: A typical HPLC column with length up to mm, internal diameter from 2 mm to 4.6 mm packed with reverse phase and a size of 0300 particles from 3 µm to 5 µm

TABLE 8. HPLC operating conditions (Confidential)

HPLC condition: The HPLC system was set up according to the manufacturer's instruction. Eluent was run for few minutes to ensure that the baseline is stable

Calibration checking: Standard solutions of different phenols were measured to lower and

upper working range making sure the calibration curve remain valid before blank and other samples are measured

Detection, confirmation and identification: Peaks for particular phenols are identified by comparing the retention time with those from calibration solutions. Thus, changes of retention time should not exceed ±10% within a batch. The occurrence of peaks in chromatogram shows the probability of presence of analytes. To ensure a peak comes from analytes, UV spectrum of the analyte is compared with UV spectrum of calibration standard. (ISO/IEC.nr 35,1989)

Quantification: The concentration of analytes was calculated taken into account dilution steps and recovery which was calculated during validation process. The concentration can be calculated

SOLID PHASE EXRACTION OF PHENOLS FROM MATRIX

This method is suitable for tap water only but can be tested for fitness for purpose regarding waste water, river water and lake water

Sample pretreatment: A clean dark brown bottle for sampling with volume 500 ml to 1000 ml was filled with water to the brim with no free space to avoid evaporation of analytes Thus, 50 mg of sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$) was added to the bottle to avoid reaction of analytes and some free chlorine that might be present in the water and 1 ml nitric acid (HNO_3) to the sample for preservation with pH < 7. Thus all samples were cooled, kept in dark and the extraction was done within 48hours

TABLE 10. Sample preparation procedure

FIELD	Environmental
SAMPLE	604 Phenols calibration mix; 2000 µl/ml each in methanol
MATRIX	Tap water
EXTRACTION COLUMN	(Confidential)
SAMPLE PREPARATION	25 µl of reference material was added to 500 ml of water sample when checking recovery. 1 ml of HNO_3 was added to the sample for preservation
COLUMN CONDITIONING	(Confidential)
SAMPLE ADDITION	With vacuum off; 500 ml sample was connected to the column through a rubber tube. Attach SPE-12 G reservoir to the column, turn on the valve to allow extraction to occur at a steady flow rate
SAMPLE ELUTION	For HPLC analysis; vacuum dry the column between 5-10 minutes.
ANALYTICAL METHOD	HPLC with UV detector for analytes analysis

RESULTS AND DISCUSSIONS

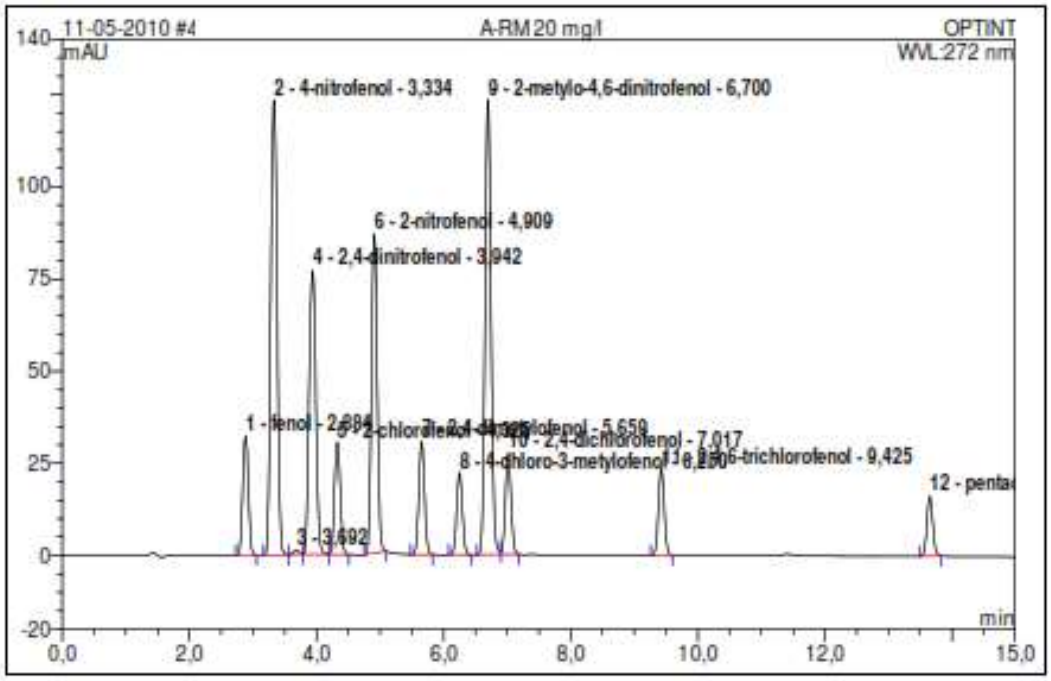
This research is base on SPE process and the method was validated on a particular column to test water samples on 11 compounds of phenols using phenols calibration mix

However, the aim is to determine if there are other available columns that are suitable for the method as well as different matrixes. Before any analysis, the accuracy of calibration curves was checked to ensure they are still valid with a standard solution of 1 mg/l and 20 mg/l. The results from the analysis of phenols from standard solutions to test calibration curves using this validated method can be seen in Table 10

TABLE 11. Results of the 11 phenols obtained from standard solution of 1 mg/l

No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount mg/l	Type
1	2.92	Phenol	1.588	0.166	5.19 0	1.034	BMB
2	3.37	4-nitrophenol	6.042	0.702	21.95	1.025	BMB
3	3.98	2,4-dinitrophenol	3.755	0.469	14.68	1.031	BM
4	4.37	2-chlorophenol	1.491	0.161	5.050	1.035	MB
5	4.94	2-nitrophenol	4.276	0.445	13.93	1.033	BMB
6	5.69	2,4-dimethylphenol	1.495	0.155	4.850	1.004	BMB
7	6.28	4-chloro-3-methylphenol	1.075	0.112	3.520	1.014	BMB
8	6.73	2-methyl-4,6-dinitrophenol	5.925	0.647	20.25	1.020	BMB
9	7.05	2,4-dichlorophenol	1.292	0.131	4.080	0.972	BMB
10	9.46	2,4,6-trichlorophenol	1.154	0.121	3.790	1.017	BMB
11	13.68	Pentachlorophenol	0791	0.087	2.710	1.002	BMB
Total:			28.884	3.197	100.00	11.186	

Table 11 presents the results of the 11 phenols obtained from standard solution of 1 mg/l The calculation of concentration of the compounds are based on the area of peaks (mAU*min). The nominal values of concentrations of each phenol were 1 mg/l to check the accuracy of calibration curve at the lower working range, thus the result gotten from the standard solution shows an excellent accuracy of all calibration curves of all phenols on the lower level



GRAPH 9. Typical chromatogram obtained from a standard solution of 20 mg/l

Graph 9 shows a typical chromatogram obtained from a standard sample containing the 11 phenols from water sample with a standard solution of 20 mg/l. The baseline is smooth due to high concentration of the standard solution

Consequently, using the same validated method for the 11 phenols for water samples and the same solid phase extraction procedures, wastewater sample was also analyzed using the same columns to determine if the method is suitable for wastewater matrix. However series of results was obtained after carrying out the analysis. The results from the analysis of phenols from spiked wastewater samples using this validated method can be seen in Table 13

TABLE 13. Results obtained from spiked wastewater sample

No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount mg/l	Type
1	2.92	Phenol	15.333	1.805	9.20	0.113	BMB
2	3.37	4-nitrophenol	31.962	3.592	18.32	0.052	BMB
3	3.96	2,4-dinitrophenol	20.586	2.446	12.47	0.054	BMB
4	4.36	2-chlorophenol	7.935	0.819	4.18	0.053	BMB
5	4.94	2-nitrophenol	26.765	2.740	13.97	0.064	BMB
6	5.68	2,4-dimethylphenol	8.369	0.909	4.64	0.059	BMB
7	6.28	4-chloro-3-methylphenol	6.197	0.630	3.21	0.057	BMB*
8	6.73	2-methyl-4,6-dinitrophenol	41.106	4.389	22.38	0.090	BMB*
9	7.04	2,4-dichlorophenol	8.463	0.868	4.42	0.065	BMB
10	9.45	2,4,6-trichlorophenol	8.255	0.873	4.45	0.093	BMB
11	13.66	Pentachlorophenol	4.914	0.539	2.75	0.062	BMB
Total:			179.885	19.611	100.00	0.761	

The Table 13 shows the results of the 11 phenols obtained from spiked wastewater sampleswith a particular column. Thus, the nominal value of concentration of each phenols were0.1 mg/l but the results are less than 0.1 mg/l indicating that there are some seriousinterferences between matrix and analytes. Thus, recovery for this analysis needs to becalculated because recovery for this matrix is different. The chromatogram of .wastewatershows that there are no phenols present within the working range

CONCLUSIONS

This study is based on the SPE process, and the method employed was validated for tap water using phenol calibration mix which aims at checking whether there are other columns that could be used for the developed method as well as other matrixes. However for the validated method five different columns were analyzed to determine their properties. The ability of each column to retain analytes and the results achieved show that C18, polar plus, Quart. Amine columns are not suitable for determination of phenols from water samples using HPLC with a UV-detector but C18/SDB columns can be use due to .excellent recoveries of analytes which were between the working ranges of 80-110%

Thus, the validated method was concluded to be suitable for analyzing different matrixes which gave excellent recoveries within the working range. It can also be concluded that phenols are not present in lake water but the validated method is suitable for analyzing different water matrixes. Samples were collected from Uli Lake(52.25.38.17.22.)

Baba Lake 52.25.14.17.21.57)

As regards waste water, the recoveries were very low due to interferences between matrix and analytes. Nevertheless, the results from samples differ greatly making it impossible to calculate the mean value of recoveries. A different approach is needed for determining concentrations. Thus, one approach might be to calculate recovery for each waste water .sample separately

The experimental results show that phenols are not present in water within the working range of concentrations 0.003-0.25 mg/l. The guideline base on drinking water directive for Polish regulation act shows that the concentration of 2, 4, 6-trichlorophenol should be less than 0.2 mg/l. In every tested tap water, phenols were not detected showing that water meets the requirement of Polish act of drinking water quality. In comparison with the result

from the experiments obtained from spiked water samples and that of standard samples, it show that the concentration was 0.093 mg/l for 2,4,6-trichlorophenol which supports the conclusion that the method is suitable for the determination of 2,4,6-trichlorophenol and .the rest of phenols in water using HPLC with a UV-detector

Referring to the research questions of this study, the developed method is trustworthy and suitable for the determination of phenols in water. The method was checked for accuracy .(using internal quality control (checking the calibration curves and recovery testing However, a known volume (25 µl) reference material of concentration 2000 mg/l phenol mix was diluted in order to achieve an end concentration of 0.1 mg/l of each phenol. The reference material was used to create spiked samples to check recoveries of each phenol as well as to determine if the error present was less or greater than 10% after analysis, for the method to be valid. Also, using an external quality control for checking the results of .(phenols obtain from inter-laboratory proficiency testing (LGC standard

In suggestion for further research concerning method validation, more research should be conducted on method for determination of drugs and hormones present in water due to .large consumption of these chemical compounds by humans

This research has ignited in me a further interest and motivation in method development and validation not only in the environmental sector but also in forensic sciences. It has vastly enhanced my knowledge in the field of environmental analysis, research study .which will enable me to overcome future challenges in working life