Chromatographic Determination of Chlorophenols

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(Received 4th January, 2003, revised 19th January, 2006)

Introduction

Because of their extensive use in industrial activities, phenolic compounds are abundant in many wastewaters. They are present in oil refining, petrochemical, plastics, pesticide, carbon liquefaction and food processing industrial wastewaters [1-2]. Because of their importance in pesticides manufacturing, chlorophenols are one of the most studied types of phenols [1]. Chlorophenols are introduced into the environment as industrial effluents, herbicide breakdown products, and through direct use of fungicides [3]. Chlorinated phenols being widely used constitute a major class of organic pollutants that contaminate the ecosystem and accumulate in the food chain [2]. Various industrial effluents contain up to 18 mg/l of chlorophenols, while the municipal wastewater contains from 1 to 21 mg/ml of these compounds [2]. There are 19 different congeners among them. Several chlorinated phenols such as 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, and pentachlorophenol have been classified as the priority pollutants [2, 4]. Monitoring and detection of chlorophenols are of particular importance in environmental control and food analysis for the investigation of human and animal exposure [2]

A continuous flow liquid membrane extraction (CFLME)–C_{18} precolumn-liquid chromatography system was developed for preconcentration and determination of chlorinated phenols (CPs). After preconcentration by CFLME, which is based on the combination of continuous flow liquid–liquid extraction and supported liquid membrane, CPs were enriched in 960 µl of 0.5 mol/l NaOH used as acceptor. This acceptor was on-line neutralized and transported onto the C_{18} precolumn where analytes were absorbed and focused. Then the focused analytes were injected onto the C_{18} analytical column for separation and detected at 215 nm with a diode array detector. CFLME related parameters such as flow rates, pH of donor and acceptor concentration were optimized. The proposed method presents detection limits of 0.02–0.09 µg/l (S/N=3) when 100 ml samples were enriched. The proposed method was successfully applied to determine CPs in tap water and river water samples with spiked recoveries in the range of 70–121% [5]. A procedure for the determination of three chloroanisoles (2,4,6-trichloro, 2,3,4,6-tetrachloro and pentachloroanisol), as well as their precursor chlorophenols (2,4,6-trichloro, 2,3,4,6-tetrachloro and pentachlorophenol), involved in the presence of cork taint in red wine has been developed. Samples, up to 1 l, were concentrated using a 200 mg Oasis HLB solid-phase extraction (SPE) cartridge. Chlorophenols were quantitatively eluted from this sorbent with 3 ml of methanol. Chloroanisoles were mainly recovered in a second fraction of *n*-hexane (2 ml). Both fractions were combined and mixed with an aqueous solution of sodium bicarbonate and 50 µl of acetic anhydride. Chlorophenols were acetylated in the aqueous-methanolic phase and extracted to *n*-hexane. Chloroanisoles remained unaffected in the *n*-hexane layer. Both groups of compounds were determined by gas chromatography–tandem mass spectrometry in the same chromatographic analysis. Using a temperature programmable vaporization injector detection limits from 0.2 to 2.4 ng/l, below their sensorial threshold level in red wine, were obtained for all compounds. Average recoveries higher than 80% and acceptable precision were achieved using red wine samples spiked with the analytes at different concentration levels [6]. Table-1 lists conditions for chromatographic determination of chlorophenols.

A new method, stir bar sorptive extraction (SBSE) with in situ derivatization and thermal desorption (TD)–gas chromatography–mass spectrometry (GC–MS), which is used for the determination of trace amounts of chlorophenols, such as 2,4-dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6-TrCP), 2,3,4,6-tetrachloro-phenol (2,3,4,6-TeCP) and pentachlorophenol (PCP), in tap water,

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Table-1: Conditions for chromatographic determination of chlorophenols

Sr. No.	Technique/Detector	Column/Mobile phase	Analysis conditions	LOD	Reference
1	(CFLME)-C_{18} precolumn LC			0.02-0.09 µg/L	[5]
2	GC-MS			0.2-2.4 ng/L	[6]
3	TD-GC-MS			1-2, 10-20 pg/mL	[7]
4	RP-HPLC	Lichrospher 100 RP-18 column using water-acetonitrile gradient			[8]
5	HPLC	ODS column, 43.7 mM acetic acid with 40 % acetonitrile	pH 2.5	10-60 µg/L	[9]
6	HPLC/Fluorescence detector	ODS column	60°C	0.024-0.08 µM	[10]
7	GC/ECD detector	———	———	0.02 µg/L	[12]
8	Micellar electrokinetic chromatography	——— ———	pH 9, 18°C	0.08-0.46 µg/mL	[13]
9	Micellar electrokinetic chromatography with electrochemical detector	———	pH 6.1	0.1-10 µg/L	[15]
10	HPLC/Fluorescence detector	ODS column/ acetonitrile + triethylammonium phosphate buffer as mobile phase	pH 3 35°C	1 pico mol.	[16]
11	Reversed-phase HPLC/UV detector	ODS minicolumn	———	0.03 µg/L	[17]
12	Reversed- phase HPLC/UV detector	ODS column/ acetonitrile + H_3PO_4	pH 5	0.012-0.38 µg/L	[18]
13	HPLC	ODS column/aqueous acetonitrile	———	0.08-0.16 pico mol.	[19]
14	HPLC/UV detector	Methanol as mobile phase	pH 3	0.2-1.0 ppb	[22]
15	GC/ECD	Argon-methane as mobile phase	315°C	0.72-1.50 µg/L	[24]
16	HPLC/ electro-chemical detector	C_{18} column	pH 7.2	1.0 ppb	[26]
17	Photometric method	———	pH 3.5-4.2	0.08-8 µg/mL	[27]
18	Chloroacetylation method	12 m OV-1 fused silica capillary column	———	0.1-1.0 µg/L	[28]
19	HPLC/ electro-chemical detector	———	———	0.2-2.0 ppb	[29]
20	GC/ECD	12-m OV-1fused silica capillary column	pH 2	0.1 ppb/L	[30]
21	Photometric method	———	pH 10-10.5	0.004 µg/L	[31]
22	Reversed-phase HPLC/UV detector	Hamilton PRP-1 column / CH_3CN+phosphate buffer as mobile phase	pH 7.2-12	in ng range	[32]
23	HPLC/UV detector	ODS column/methanol +0.02M KH_2PO_4	pH 4	10-100 ppb	[33]
24	GC-LC	Methanol + acetonitrile + phosphate buffer as mobile phase	pH 4	In ppb range	[34]
25	HPLC	Methanol+petroleum spirit mixture	———	0.1-1.0 ppm	[35]
26	HPLC/UV detector	C_{18} column/ acetonitrile+acetic acid as mobile phase	40°C	50 ng/mL sample	[36]
27	GC-MS	———	125°C	in ppb range	[38]
28	GC-MS	———	125°C	0.2-1.0 µg/L	[39]
29	HPLC/UV detector	C_{18} column/ methanol+ acetonitrile+water as mobile phase	pH 3.5-4	in ppb range	[40]
30	GC/FID detector	DB-1 capillary column	———	———	[41]
31	GC/ECD	30-m DB-5 capillary column	———	0.5 µg/L	[42]

river water and human urine samples, is described. The derivatization conditions with acetic acid anhydride and the SBSE conditions such as extraction time are investigated. Then, the stir bar is subjected to TD followed by GC–MS. The detection limits of the chlorophenols in tap water, river water and human urine samples are 1–2, 1–2, and 10–20 pg m/l (ppt), respectively. The calibration curves for the chlorophenols are linear and have correlation coefficients higher than 0.99. The average recoveries of the chlorophenols in all the samples are higher than 95% (R.S.D. < 10%) with correction using added surrogate standards, 2,4-dichlorophenol-d_5, 2,4,6-trichlorophenol-$^{13}C_6$,2,3,4,6-tetrachlorophenol-$^{13}C_6$ and pentachlorophenol-$^{13}C_6$. This simple, accurate, sensitive and selective analytical method may be applicable to the determination of trace amounts of chlorophenols in liquid samples [7].

Brominated phenols 2- and 4-bromophenol (2-BP and 4-BP); 2,4- and 2,6-dibromophenol (2,4-DBP and 2,6-DBP) and 2,4,6-tribromophenol (2,4,6-TBP) have been identified as key flavor compounds found in seafoods. Depending on their concentrations, they were responsible for marine or ocean flavor (shrimp/ crab/fish/sea salt-like) or for phenolic/ iodine/ iodoform-like off-flavor. In this work a new analytical methodology was developed to determine, simultaneously, such bromophenols in fish meats, based on reversed-phased high-performance liquid chromatographic separation (RP-HPLC). The separation of bromophenols was made onto a Lichrospher 100 RP-18 column using water: acetonitrile gradient at a flow rate of 1.0 mL/min, using absorbance detection at 286 nm, were the 2-BP, 4-BP, 2,4- and 2,6-DBP show significant absorbtivity values and at 297 nm for 2,4,6-TBP. They were separated in 20 min with a good chromatographic resolution (Rs) for the isomeric compounds: 2- and 4-BP, Rs = 1.23; 2,4- and 2,6-DBP, Rs = 1.63. The calibration curves were linear in the bromophenols concentration range of 200.0–1000 ng/mL. Under optimized conditions, the detection limit of the HPLC method was 127 ng mL^{-1} for 2-BP; 179 ng/mL for 4-BP; 89.0 ng/mL for 2,4-DBP; 269 ng/mL for 2,6-DBP and 232 ng/mL for 2,4,6-TBP. This method has been applied in determination of bromophenols, isolated by combined steam distillation-solvent extraction with 2 mL of pentane/diethyl ether (6:4), from Brazilian fishes samples, collected on the Atlantic coast of Bahia (13°01'S and 38°31'W), Brazil. The concentration range determined were 0.20 ng/g (2-BP) to 299 ng/g (2,4,6-TBP). The method proposed here is rapid and suitable for simultaneous quantification of simple bromophenols in fish meat. As long as we know, it is the first analytical methodology, using RP-HPLC/UV, which was developed to determine simple bromophenols in fish meat [8].

HPLC procedure for simultaneous determination of chlorophenoxy acid herbicides and their radiolytic degradation products in waters is described with the use of octadecylsilica column and spectrophotometric detection at 280 nm. The satisfactory separation was achieved with a mobile phase of pH 2.5 consisting of 43.7 mM acetic acid with 40% (v/v) acetonitrile. Limit of detection values for herbicides and phenol derivatives were in the range of 19–41 μg/l and 10–60 μg/l, respectively. The developed method was applied for monitoring the effectiveness of radiolytic degradation of herbicides. Studies of products of γ-radiolysis of 2,4-dichlorophenol have shown that the efficiency of this process is affected by the presence of naturally occurring scavengers of γ-radiation such as carbonates or nitrates [9].

A sensitive high performance liquid chromatographic method for the determination of phenols and chlorophenols was developed. The fluorescence labeling reaction of phenol with 4-(4,5-diphenyl-1*H*-imidazol-2-yl)benzoyl chloride was completed in 30 min at 60°C. The separation of 4-(4,5-diphenyl-1*H*-imidazol-2-yl)benzoyl derivatives of five representative phenols i.e. phenol, o-, and p-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol was achieved within 35 min with an ODS column using isocratic elution. The detection limit of these 4-(4,5-diphenyl-1*H*-imidazol-2-yl)benzoyl derivatives were in the range of 0.024 to 0.08 μM. Twelve kinds of 4-(4,5-diphenyl-1*H*-imidazol-2-yl)benzoyl derivatives with phenols containing mono-, di-, tri-, tetra-, and pentachlorophenols were also well separated within 208 min by changing the elution conditions. The derivatives were stable for at least 24 hours when they were placed at room temperature in the dark. The proposed method was applied to the assay of human urine samples. The relative standard deviation of the proposed method with in and between day assay were less than 7% and less than 14.2% respectively. The average concentration of free and total phenol found in urine was 4.3±2.5 and 29.5±14μM respectively [10].

A rapid analytical method for the determination of chlorophenols in water was developed. The method is based on direct acetylation of chlorophenols and solid-phase micro extraction and final analysis of the acetates by gas chromatography-mass spectrometry using 4-bromo-phenol as an internal standard. Calibration curves are linear in the range from 0.5 to 100 μg/L. The detection limit is about 0.1 μg/L for most of the chlorophenols. The method does not require organic solvents to be used [11]. A simple but sensitive method for the determination of tri-, tetra-, and pentachlorophenol was proposed. Urine samples, spiked with internal standard, were treated by acid hydrolysis. After a steam bath distillation, the distillates were extracted using solid phase extraction. Derivatization of the chlorophenols was not carried out. Gas chromatography/electron capture detector system was used for detection. Detection limits of the chlorophenols were found in the range of 0.02μg/L

urine (detection limits of the electron capture detector: 0.52 to 2.76 μg/L) [12].

A new method was developed and validated for the determination of chlorophenols in human urine by using micellar electrokinetic chromatography. Separation is accomplished by using a selective buffer consisting of 15 mM borate, 25 mM phosphate and 100 mM SDS at pH 9 in addition to a positive power supply of 25KV at 18°C. The proposed electrophoresis method allows the separation of eleven chlorphenols within 17 min with reproducibility as relative standard deviation between 2.6% and 7.2% and limit of detection between 0.08 and 0.46 μg/mL for all chlorophenols. Urine samples were previously hydrolyzed with 37% HCl at 80°C for 60 min and then clean on a C_{18} minicolumn. Recoveries ranged from 58% to 103%. The overall analysis time is 20 min per sample [13]. An extraction gas chromatographic method to determine chlorophenols in potable water is described. This method is characterized by a low detection limit, high selectivity and rapidity. The range of measurable concentration is 0.05-10 μg/L. The relative standard deviation is 0.05-0.2% and analysis time is 40 minutes [14].

A method was developed for the determination of chlorophenols by micellar electrokinetic chromatography coupled with electrochemical detection. A mathematically model was used to predict optimal separation conditions: seventeen of the twenty compounds of interest were base lined separated in a 50 mmol/L ACES buffer at pH 6.1 with 22 mmol/L sodium dodecylsulfates. Detection was performed with a graphite-epoxy working electrode at a potential of 800 mV vs Ag/AgCl. A Palladium metal union was used to decouple the separation field from the electrochemical cell. Detection limits were in the order of 10 μg/L, one to three orders of magnitude lower than with UV detection. The repeatability was typically ± 0.9% for the mobility and ± 4% for the peak area. Combined with off-line solid-phase extraction on a column with a polystyrene divinylbenzene copolymer packing. The method proved suitable for the analysis of river water samples. When using an internal standard, the detection limit < 0.1 μg/L in 100 mL river water sample [15].

The use of 2-chloro-6,7-dimethoxy-3-quinoline carboxaldehyde as a fluorogenic-labeling reagent in precolumn derivatization for the high performance liquid chromatographic separation of chlorophenols has been investigated. The compounds react (50 min. at 110°C) with 2-, and 4-chlorophenol to give fluorescent ethers that can be separated by reversed phase high performance liquid chromatography and detected at λex =360 nm and λem =500 nm. The experimental conditions for derivatization and chromatographic separation were discussed [16]. A method for the simultaneous determination of chlorophenols and chlorophenoxy acids in drinking water is described. Solid phase extraction on octadecyl silica minicolumns was used for sample preconcentration and the extracts were analyzed by a reversed phase high performance liquid chromatography with UV detection (225 nm). Recoveries of both chlorophenols and chlorophenoxy acids were higher than 94% with standard deviation less than ± 5.6. The detection limits are 0.03μg/L and 0.015μg/L were determined for chlorophenols and chlorophenoxy acids, respectively [17].

A method for the determination of seven chlorophenols including those in the US Environmental Protection Agency, list of priority pollutants in drinking water is described. Solid phase extraction on various alkyl silica bonded phase such as phenyl-, acetyl- and octadecyl silica was used for sample preconcentration. The dependence of recovery on the eluent volume, sample pH and sample volume were investigated for all the sorbents in order to find the optimum sorbent. The sample pH was adjusted to 5 and after concentration the analyte was eluted with 1.2mL of acetonitrile and phosphoric acid. The extract was submitted to reversed phase high performance liquid chromatography with acetonitrile and phosphoric acid as the eluent and analyzed by UV-detector (225 nm). Recoveries after 28 days storage in a refrigerator at 3°C was also studied, the recoveries of chlorophenols were higher then 88%. Extracting a one-litre water sample, the detection limit is 0.12-0.38 μg/L [18].

A simple, rapid and sensitive method for the determination of trace amounts of phenol and chlorophenols by high performance liquid chromatography is reported. The phenols were derivatized by 4-(2-phthalimidyl)benzyl chloride to produce the corresponding fluorescence esters which can be separated on ODS column with aqueous acetonitrile as eluent. The eluates were quantified by fluorescence with peak height method. The detection

limits for the chlorophenols were 0.08-0.16 picomol. [19]. Study of chlorinated water containing 100µg/L phenol has shown that 2-chlorophenol, 4-chlorophenol, 2,4-dichloro-phenol, 2,6-dichloro-phenol and 2,4,6-trichlorophenol were present in varying quantities according to the chlorination level. This paper describes an extraction and concentration technique, which can be used in various analytical methods such as gas chromatography-mass spectrometry, high performance liquid chromatography. The extraction concentration rates of this technique are: 72% for 2-chlorophenol, 70% for 4-chlorophenol, 81% for 2,4-dichlorophenol, 82% for 2,6-dichlorophenol and 92% for 2,4,6-trichlorophenol. The trace analytical methods have the same detection limit about 0.25 µg/L [20].

A sensitive, specific and analytical reliable method for the determination of mono-, di-, tri-, and tetrachlorophenol in human urine was elaborated. After acid hydrolysis and a simultaneous steam distillation of the urine samples, spiked with an internal standard, derivatized with pentafluorobenzoyl chloride and analyzed by capillary gas chromatography-mass spectrometry. The detection limits for the chlorophenols ranged from 0.2 to 2.5 µg/L. Using this method, 4-chlorophenol, 2,4-, 2,5-dichlorophenols, 2,4,6-trichlorophenol, 2,4,5-trichlorophenol and 2,3,4,6-, 2,3,5,6-tetrachlorophenols were detected in urine samples of a group of 258 men and women which had no known occupational contact to hazardous chemical substances [21]. A rapid high performance liquid chromatography method for the determination of chlorophenols in the exhaust and thermal degradation emission gas was studied. Chlorophenols in the sample gas were collected by passing the gas through an impinger containing 10 mL of 1% NaOH solution at a flow rate of one litre/min, the pH was adjusted to 3 and eluted with 2 mL of methanol and the eluate was evaporated to dryness under reduced pressure. The residue was again dissolved in 2mL of methanol/water (1:1). 20 µL portion of the filtrates was analyzed by high performance liquid chromatography with UV-detector (280nm). The recovery was almost 100% and the detection limit was 0.2-1ppb [22].

A gas chromatographic method for the determination of chlorophenols in waste is based on the conversion of chlorophenols into acetyl derivatives (by acetylation with acetic anhydride) followed by extraction with hexane. The detection limits are 0.004, 0.007 and 0.0008 mg/mL for 2,4-dichlorophenol, 4-chlorophenol and 2,3,5-trichlorophenol and 2,3,4,6-tetrachlorophenol respectively. The method was effectively tested in analysis of paper mill wastewater, which revealed the presence of dichlorophenols and trichlorophenols at concentration of 0.026 and 0.0032 mg/L respectively [23]. A method for the determination of chlorophenols in water consists of direct acetylation of chlorophenols in water solid-phase extraction of chlorophenol acetates and gas chromatographic analysis of the eluate. The advantages of this method are as: non-inorganic acetates may be isolated from water easily than high polar free chlorophenols; derivatization in aqueous solution is very fast and results in the formation of products suitable for direct gas chromatographic analysis; and in comparison with repeated liquid-liquid extraction, manipulation with large volumes of solvents are avoided [24].

This article describes the separation and determination of phenolic compounds in wastewater by high performance liquid chromatography. The phenolic compounds in wastewater are saturated with NaCl and condensed by Sep-Pak C_{18} cartridges and then separated and detected by high performance liquid chromatography. The mobile phase was methanol:water (40:60). Recovery of phenols was 81% compared to 19% in the absence of NaCl. Recoveries of o-, m-, and p-cresol and o-, m-, and p-chlorophenol were 96 to 98% and 91 to 98% respectively [25]. A method for the determination of phenols and chlorophenols in water uses electrochemical detection and high performance liquid chromatographic analysis. The selectivity of the electrochemical detection together with the coulometeric efficiency of the electrochemical cell allows the determination of phenols without preconcen-tration. A conventional C_{18} column and CH_3CN + CH_3OH + 0.02M Na_2HPO_4/NaH_2PO_4 buffer solution pH 7.2, eluent are employed. The procedure is rapid and may be adopted for the monitoring of phenols at one ppb level in water [26].

An extraction photometric method is described for the determination of chlorophenols in aqueous solution. The method is based on the formation of complex reaction products in the chlorophenol-$NaNO_2$-Co^{2+} system, which then is easily extracted with an organic solvent from the aqueous phase. The optimal reaction conditions, pH

range, $NaNO_2$ concentration, reaction time were determined for 4-chlorophenol (3.5-4.2; 0.35M; 1 hour heating) and 2,4-dichlorophenol (3.1-3.6; 0.05M; 2.5 min. heating). The complex of 4-chlorophenol was extracted with $CHCl_3$, the other with tributyl amine in $CHCl_3$. The detection limit for 3-, 4-chlorophenol and 2,4-dichlorophenol were 0.08, 0.28 and 8 μg/mL respectively. The method is recommended for chlorophenols determination in tap, natural and wastewater [27]. A quantitative, rapid, sensitive and isomer specific method for the determination of chlorophenols in natural water by in situ chloroacetylation is presented. Without pre-extraction, phenols in water are chloroacetylated by chloroacetic anhydride in the presence of K_2CO_3. If analysis of other classes of neutral aromatic compounds also required, a more elaborated procedure involving solvent extraction of organic compounds and back extraction of phenols into K_2CO_3 solution followed by chloroacetylation is proposed. When a 12m OV-1 fused SiO_2 capillary column was used, twenty-two phenols chloroacetates were easily resolved in a single run. Using distillation and natural water samples, this method was validated and shown to be applicable over a concentration range of 0.1-100 μg/L of the phenols studied [28].

A high performance liquid chromatographic method was developed for the trace determination of chlorophenols in the 0.2-2.0 ppb ranges from spike water samples. Simple liquid-liquid extraction followed by on-line preconcentration of total mono-, and dichlorophenol was performed using a divinylbenzene styrene copolymer as packing material for the precolumn. The chlorophenols were eluted from the pre column on an analytical column and the detection is done with an electrochemical detector. The detection limits for the chlorophenols by means of electrochemical method is in the lower pico gram range, the recoveries of chlorophenols having initial concentration of 2 ppb are usually 70-90% [29]. A sensitive isomer-specific method is described for the simultaneous and quantitative analysis of twenty-one phenols in natural water. The sample was acidified to pH $\leq$ 2, extracted with CH_2Cl_2, evaporated and dissolved in acetone. The phenol extract was then reacted with pentafluorobenzyl bromide to give pentfluorobenzyl ether derivatives. After silica gel column clean up, the ether derivatives were chromatographed on a 12 m OV-1 fused silica capillary column attached to an electron capture detector. The detection limit was 0.1 ppb/L. Recovery of phenols from pH 2 water sample fortified at 10,1 and 0.1 ppb were $\geq$ 80% in most cases except for phenols which was only 30-35%. However phenol recovery was quantitative, when the sample volume was reduced to 100 mL. This method is most suitable for simultaneous recovery of non-chlorinated and monochlorinated phenols as well as other higher chlorophenols at trace level [30].

A simple and rapid extraction photometric method allows determination of phenols, chlorophenols and naphthols in drinking water at a level of maximum permissible concentrations. In determining phenols and chlorophenols, extraction is carried out using n-butyl acetate with the addition of Na_2SO_4 and tributyl phosphate. Determination of 2-chlorophenol and 4-chlorophenol in chlorinated water is based on different extractability or these compounds by n-amyl acetate at pH 10-10.5. Determination of 2-Naphthol includes sorption by a cation exchanger, extraction with a 2.5% solution of HCl at pH 9-10, re-extraction with diazotized sulfonalic acid and photometric determination. The time period is 2-3 hours. Detection limit is 0.004 μg/L [31]. A method for the separation of chlorophenols by reversed phase high performance liquid chromatography at several pH values is given. High performance liquid chromatographic retention time for nineteen chlorophenols isomers relative to pantachlorophenols are given at pH 7.4 to 12. Mobile phase is CH_3CN+Na_2HPO_4+NaH_2PO_4. A Hamilton PRP-1 reversed phase column and UV detector at 254 nm were used the effect of pH on the elution order was studied [32].

The separation of mono-, di-, tri-, and tetra chlorinated phenols and pentachlorophenol was investigated by high performance liquid chromatography using three different systems. Adsorption chromatography on SiO_2 and reversed-phase chromatography on polar bonded phase (amino alkyl) and non-polar bonded phase (octadecyl). A satisfactory separation could not be obtained by adsorption chromatography on SiO_2. A reasonable separation of isomers was obtained with amino columns, but the group separation was not satisfactory. The octadecyl columns were superior with reference both to separation of groups and of isomers with in these groups as well as to stability and speed of analysis. A mixture of nineteen different phenols was resolved on a C_{18} column with in a 30 min., linear gradient of 56-80% methanol and 44-20% of 0.02M KH_2PO_4 (pH=4) [33].

A method is described for the confirmation of chlorophenols in human urine sample. A hydrolyzed urine sample is analyzed by gas chromatography and liquid chromatography with electrochemical detector. The method is sensitive to chlorophenols at low ppb range as they are encountered in most general population samples. A description of these results including factors affecting separation, detection and confirmation of chlorophenols in human urine is present [34]. A method is given for the determination and separation of eight chlorophenols by high performance liquid chromatography. The chlorophenols after extraction from liquid solution by diethyl ether are taken up in 1% CH_3OH-petroleum spirit mixture and injected into the column. Separation of a mixture of all eight chlorophenols can be achieved in 25 min and a linear relation exists for each chlorophenol in the range of 0.1-1.0 ppm [35].

2-Chlorophenol was determined in serum by high performance liquid chromatography after pre-extraction into benzene and then into an alkyl aqueous mixture followed by acidification and re-extraction into chloroform. The detection limit was approximately 50 ng/mL. Reproducibility was good, for example, for one mL serum sample containing 210 ng of 2-chlorophenol was 87%. Recovery was achieved with a relative standard deviation of 4% [36]. In this article, the determination of chlorophenols by thin layer chromatography by using p-(5-fluoro-2,4-dinitro-1-phenyl azo)-N,N-dimethylaniline is described. p-(5-fluoro-2,4-dinitro-1-phenyl azo)-N,N-dimethylaniline was synthesized by coupling of diazotized 2,4-dinitro-5-fluoroaniline with N,N-dimethylaniline and used for thin layer chromatographic determination of phenolic compounds. p-(5-fluoro-2,4-dinitro-1-phenyl azo)-N,N-dimethylaniline was reacted with phenyl and colored reaction products were separated by thin layer chromatography. The Rf values for the reaction products of phenols, p-cresol, p-chlorophenol and p-nitrophenol with p-(5-fluoro-2,4-dinitro-1-1phenyl azo)-N,N-dimethylaniline on silica gel plates were 0.40, 0.35, 0.30 and 0.20 respectively by using 1:2 cyclohexane-benzene as the developing solvent. For determining trace amounts of phenols, the sample solution was boiled with p-(5-fluoro-2,4-dinitro-1-phenyl azo)-N,N-dimethylaniline in DMF for 5 min at pH 8. The DMF extraction was then evaporated to one mL and applied to the plates. Phenols 10^{-5}g in 100 mL could be detected [37].

A method for the determination of chlorophenols in soil samples using accelerated solvent extraction with water as the solvent combined with solid-phase microextraction and gas chromatography-mass spectrometry was developed. Importance of accelerated solvent extraction parameters such as extraction temperature and time were optimized using a spiked wetland soil. The effect of small amount of organic modifiers on the extraction yield was also studied. An extraction temperature of 125°C and 10 min extraction performed three times proved optimal. Two accelerated solvent extraction and solid phase microextraction procedure with and without organic modifiers (5% acetonitrile) were evaluated with respect to precision. Detection limits were in the low ppb range for all chlorophenols [38].

The simple pretreatment gas chromatography-mass spectrometric method for chrolophenol derivatives was developed using the homogenous liquid-liquid extraction in water/pyridine/ethyl chloraecetate ternary component system. The proposed method was performed all process of treatment within 15 min. The determination limits of each chlorophenol derivative were in the range of 0.2-2.1 µg/L. Ten kinds of chlorophenol derivatives were completely separated and were able to determine except 2,5-dichlorophenol and 2,6-dichlorophenol [39]. A technique has been developed for the extraction of a clean concentrate of chlorophenols from cardboard food containers and adhesives of the type used in their manufacture. A simple reversed-phase, isocratic high performance liquid chromatographic system employing an optimized mobile phase permitted the separation of nineteen different phenols. The use of carefully selected internal standard permitted the estimation of ppm levels of pentachlorophenol, 2,3,4,6-tetrachlorophenol and 2,4,6-trichlorophenol in several samples of adhesives and various sections cardboard food containers. Pentachlorophenol (50ng) and 2,4,6-trichlorophenol (20ng) gave significant high performance liquid chromatographic peaks, readily permitting their detection in ppb [40].

Styrene divinylbenzene extraction disks were used for the isolation and trace analysis of phenols, chlorophenols, cresols, xylenols from soils and sediment samples. The extracts were divided into two fractions for free and acetylated compounds. Extraction fraction was then analyzed by gas chromatography/flame ionization detector with DB-1 capillary column. Recoveries of all selected phelnolic compounds from soils and segments were increased after acetylation. Recoveries of tri-, tetra-, and

pentachlorophenol were strongly increased after acetylation from 84.5 – 99.2 to 95.3 – 112 % [41]. A method for the determination of thirty-one chlorinated phenols is based on in situ acetylation procedures. These are guaiacols, catechol, syringol and vanillins in paper and pulp wastewater. Except for 4-chlorocatechol, this procedure provided satisfactory recovery for all phenols at three levels of purification, namely 400, 40 and 4µg/L. The acetyl derivatives were analyzed by gas chromatography using a 30-m DB-5 capillary column interfaced to an electron capture detector. Using a 5 mL effluent sample, the detection limit was 0.5 µg/L for all except the monochlorinated compounds, which have detection, limit one µg/L [42].

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