CHAPTER 2

SOLVENT- FREE AQUEOUS EXTRACTION OF CHLOROPHENOLS FROM SOME SOLID MATRICES

2.1 BASIS OF THE APPROACH

The present study involved MAE of chlorophenols using a simple aqueous carbonate solution followed by detection using HPLC-UV and GC-ECD. The aqueous alkaline extraction is ideal for several cellulosic matrices like wood, vegetables, textile (cotton) and for protein based matrices like fish, animal tissue and leather products. These matrices have the tendency to swell in the aqueous extractant, by which the extraction efficiency improves. The application of aqueous carbonate extractant eliminates exclusive solvent solubles, which are more likely to compete in hydrophobic chromatographic separations affecting quality of detection.

Aqueous extractant was found to serve well and the optimized conditions for extracting chlorophenols from various solid matrices with best recoveries. Luque-Garcia and Luque de Castro (2001) published a clean sample preparation approach by FMW water assisted Soxhlet extraction. In that study they used only water to extract acid herbicides from different types of soils. It has been a pioneering effort for greener laboratory practices. The aqueous carbonate solution was tried for the extraction of chlorophenols from matrices like wood, leather and textiles. Reverse phase SPE cleaning was found

necessary for samples whose analytes were analyzed as such by HPLC-UV and as their acetyl derivatives by GC-ECD.

This particular study is with a goal to develop solvent-free procedure for the extraction of analytes. Open vessel FMW device offers a scope for this. The open vessel MW technique serves also with a possibility to employ higher amounts of sample and extracting solvent. Because the extraction is carried out at the boiling point of the solvent, the unnecessary over heating, and unwarranted reactions can be avoided. Even for MW extraction it has been a common practice to use organic solvents for the extraction of analytes from different matrices; although the consumption is only 10-20% as compared to Soxhlet or Liquid-liquid extraction.

MW extractions were reported for chlorophenols by Lopez-Avila et al. (1995) and Lopez-Avila et al. (1994) but these attempts involved organic solvents. Clean sample preparation involving only water for extraction of organic pollutants like acid herbicides from soils was reported by Luque-García and Luque de Castro (2001), which should be the first completely water based extraction for an organic analyte using FMW. In some studies water replaced some portion of the solvent as reported by Alonso et al. (1998) on the MW extraction of chlorophenols by employing FMW using a mixture of methanol and water (4:1).

2.2 EXPERIMENT

2.2.1 Reagents

Chemicals like Ammonium carbonate, sodium carbonate, potassium carbonate, acetone, acetic anhydride and triethylamine used in this study were

of analytical grade; HPLC grade methanol, ethyl acetate and hexane were procured from Merck (India) ltd., Certified reference materials of individual chlorophenols comprising 2-MCP, 3- MCP, 4-MCP (MCP-monochlorophenol); 3,5-DCP, 2,4-DCP, 2,6-DCP, 3,4-DCP, 2,5-DCP (DCP-dichlorophenol); 2,4,6-TCP, 2,3,5-TCP, 2,4,5-TCP, 2,3,6-TCP, 3,4,5-TCP, 2,3,4-TCP (TCP-trichlorophenol); 2,3,4,6-TeCP, 2,3,5,6- TeCP, 2,3,4,5- TeCP (TeCP-tetra chlorophenol); and pentachlorophenol (PCP), and ENVI-18 RP (reverse phase) solid phase extraction (SPE) material were procured from Supelco, Bellefonte, USA. Water of HPLC grade purity was prepared using Milli-Q, model of Millipore, USA.

Stock solution of a mixture of chlorophenols at 1000 mg/l of individual concentration was prepared using methanol and from this working standard solutions were prepared freshly before analysis. The working standards for HPLC were prepared in the range 0.10-10.00 μg/ml using methanol; and for GC in the range 0.01-1.00 μg/ml using hexane.

2.2.2 Equipments

The microwave system used for this study is a FMW system, model Soxwave 100 along with a programmer for operation procured from Prolabo, Fontenay-sous-Bois, France. The programmer is useful to rotate and fix the experimental conditions of MW power in percentage and time duration in min. This model works at atmospheric pressure and has 300 watts capacity with an available power range 30W-150 W. The frequency of MW radiation was 2450 MHz. Borosilicate glass extraction open vessel was fitted with a solvent collector and an overhead Graham's water condenser. Julabo cooling water

circulator of model FE1800, from Seelbach, Germany was used to support water condenser.

HPLC of Alliance 2695 separations module with quaternary valves, auto injector, photodiode array detector (DAD) of model 996 and Millinnium 32 software version 3.0 from Waters Instruments Corporation, Milford, USA, was used. A column of 250 x 2 mm with 5-micron particles packed, Purosphere STAR (C_{18}) was procured from Merck, Darmstadt, Germany was used. Gas Chromatography, Auto system XL equipped with Electron Capture Detector (ECD) and operated with Turbo Chrome Navigator workstation was procured from Perkin-Elmer (Norwalk, USA). DB-17, a mid polar capillary column of 30 m x 0.32 mm I.D., and 0.25-μm film thickness, procured from J&W Scientific, Folsom, CA, USA was used for GC separation.

Analyses were carried out on a Beckman P/ACE 5510 capillary electrophoresis system (Beckman Instruments, Fullerton, CA, USA) equipped with a diode array detector (DAD). Data were collected and interpreted using P/ACE system 5510 series software. Uncoated fused silica capillaries, 50 cmx 75 μm I.D., of e-cap trademark of Beckman were used. Samples were injected hydrodynamically for 5 seconds (pressure 0.5psi=35mbar). A separation voltage of +15 kV was applied to the capillary (normal polarity). The diode array detector was set to monitor the samples at 214nm and all the analyses were carried out at 25 °C.

2.2.3 Preparation of soil sample

The soil free from chlorophenols, was air-dried, pulverized and sieved to a grain size of 2mm. A total of 100g of soil was mixed with acetone

until the sample was completely soaked to form a slurry. 25 g slurried soil was spiked with an appropriate volume of standard solution to achieve 1μg/g level of analyte. The contents were mixed well for over 3 hours. The bulk of the solvent was evaporated at room temperature by thorough manual shaking. The sample was left for 48 hours in a fume-hood to dry out completely and aged for 1 week at room temp. The prepared soil sample was stored in a refrigerator at 4^0C until it was taken up for analysis.

2.2.4 Other solid sample preparation

Wood and leather samples were prepared as powders of 100 meshes and 1 g of each sample was taken for the analysis. The extractions of textile and paper samples were done with 1g of finely cut (*ca* 1 –2 mm^2) pieces. The chemical samples like dyestuff, were analyzed with 1 g after breaking down any lumps if present. In the case of spiking leather, textile or other solids, an appropriate quantity of standard solution was added to achieve 1 μg/g directly to the weighed sample, left air dried overnight and then taken for analysis the next day.

2.2.5 Soxhlet Extraction

The conventional Soxhlet continuous solvent extractions were employed for solid samples using acetone as the solvent. 150 ml of acetone was allowed for 60 recycles to ensure complete recovery of chlorophenols from solids. After the extraction, the solvent was rotary evaporated to near dryness. The residue was dissolved and made up to 5 ml with methanol. This solution was directly used for the HPLC analysis; whereas 1 ml of this solution was

derivatised for GC analysis. The derivatisation was achieved by adding 0.5 ml acetic anhydride, 1 ml triethylamine and 10 ml hexane to 1 ml of sample solution taken along with 25 ml of 1% carbonate aqueous solution in a separating funnel (50 ml) and then by shaking the contents vigorously in a mechanical shaker for 30 min. The hexane layer was collected separately in a 25 ml volumetric flask. This, LLE was repeated in a similar way with additional 10 ml of hexane and the final volume adjusted to 25 ml with hexane. The extract was dried over anhydrous sodium sulphate.

2.2.6 MAE with aqueous carbonate

The solid samples found positive of chlorophenolic residues (by Soxhlet extraction) were chosen for MAE with 25 ml of ammonium carbonate extractant. Before extracting solid samples, the optimum extraction concentration of aqueous ammonium carbonate of 0.01% to 10% was studied. Carbonate solution strength, which gave the best recovery, was chosen for all further extractions. The optimisation of experimental conditions was studied, by varying MWP from 10-40% of 300 watts at constant time. Then, the influence of time on the extraction was studied by varying the time from 5-30 min at a constant power.

After the extraction, MAE vessel was allowed to cool to room temperature before the condenser was removed; then the aqueous extract was filtered through glass micro fiber filter, made up to 50 ml with carbonate solution and taken directly for HPLC analysis. For analysis by GC-ECD, 10 ml portion of the extract was acetyl derivatised as described under Soxhlet extraction method.

2.3 RESULTS AND DISCUSSION

2.3.1 Influence of Carbonate strength

In order to study the influence of carbonate on the recovery of chlorophenols from solid samples, aqueous solutions of ammonium carbonate of various strengths from 0.001 to 10.0 % were tried. This study was done using samples, which were found to contain PCP originally (by Soxhlet). When only pure water was employed for the extraction under the same experimental conditions, the recovery was 65% for PCP. 0.001% concentration of carbonate was not effective as it was as weak as water in the extraction and yielded a recovery of 67.5%. The recovery was maximum with 0.01% carbonate which then decreased with higher concentrations of carbonate and found to be the lowest with 10% carbonate. Figure 2.1 is shown for the influence of carbonate strength on the recovery of PCP extracted from leather. Because there is no change in the trend in Figure 2.1, concentration upto 5% of carbonate is shown and the decreasing trend is continuous upto 10%. The recovery trend was found to be the same for all the solid samples chosen. Carbonate solutions also serve to keep chlorophenols in polar forms in which their volatility is low compared to their neutral forms and thus the loss of analytes at higher temperatures is prevented. The reason for the falling recovery in extractions of solid samples with increasing concentration of carbonate should be due to the increased level of ions that hinder MW propagation. Although carbonates of sodium, potassium, or ammonium suit, the latter (being volatile) extraction helps to take the sample directly to techniques like electro spray LC-MS, CE-MS and for CE as reported by Jeevan et al. (2002).

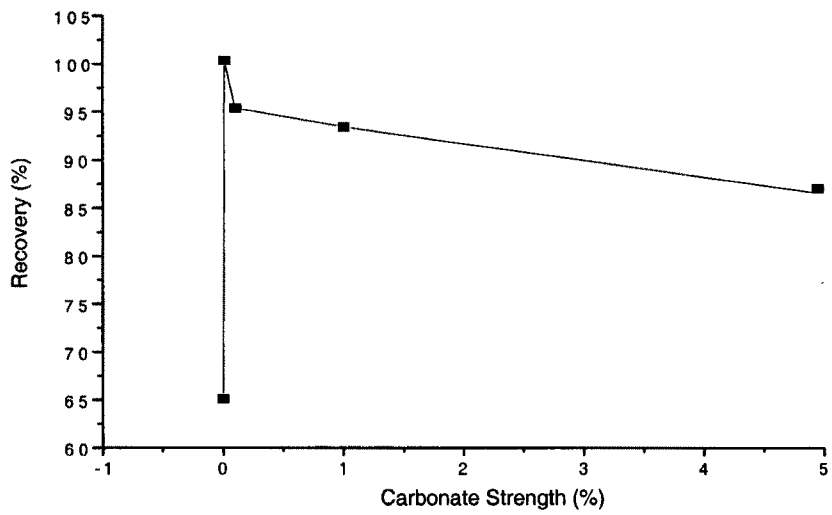

Figure 2.1 Influence of varying carbonate strength from 0.001-5.0% (shown) on the recovery of PCP extracted from leather

2.3.2 Optimisation of MAE

The weight of the solids and the extractant ratio are important factors in the recovery. The main issue in deciding the sample size is the concentration of analytes in a sample. In this aspect environmental samples are likely to carry toxic residues in low concentrations (need for higher sample size for soil) where as in other solids this is not a serious issue. Also, in the cases of leather and textile, samples undergo more swelling compared to soil. Samples with higher swelling nature also consume more of aqueous phase resulting in shrunken volume of the extractant and hence, higher sample size causes inconvenience in dealing. For the optimisation of the volume of extractant and the sample size, mainly for soil and leather (which is a swelling matrix) were chosen. Soil sample was spiked with PCP standard at 0.5 μg/g levels to samples of different weights of 1, 2 and 5g while the leather sample that was found to contain PCP originally at 1.3 μg/g (by GC-ECD) were chosen at different weights like 1 g,

2 g and 5 g. These samples were tried for studying the extraction efficiencies by varying the volumes (from15 ml-50ml) of 0.01% carbonate extractant. The results from this study are given in Table 2.1. It was observed that the recoveries with lower extractant volume of 15 ml were less due to excessive heat generated leading to loss of analytes whereas in higher volumes of more than 40 ml also the extraction was less effective. The recovery observed was better with 25 ml in the 20-30 ml volume range and hence 25 ml was chosen. For 25 ml volume of extractant, it was possible to use upto 5 g of soil and a maximum of 3 g each of leather, textiles, paper or wood. To use 5 g leather samples, 40 ml extractant is required.

MWP was varied from 10 to 40% of the maximum allowed 50% limit. Initially increasing the power resulted in increased recoveries. This trend was observed up to 30% but when the power was opted for 40%, the recovery decreased which resulted from excessive heat generated. Accordingly, 20-30% MWP was chosen. Optimisation of time was done by varying extraction time from 5 to 30 min at constant power and determining the corresponding recoveries. The dependence of the recovery of PCP from a leather sample (2 g) on MWP and duration of extraction is shown in Figure 2.2. It is inferred from the figure that MWP 30% for 5 min or 20% for 10 min duration should be satisfactory (as they produce recoveries exceeding 95%) for the solid samples investigated. 20% for 10 min has been chosen for MAE of the samples in this study. The extraction trend is similar for all solid samples chosen for the study. The other chlorophenols were not present in solid samples and hence a mixture of these species taken from the respective standard solutions and was spiked to achieve 1 μg/g of each of the isomers and the extraction was done with 0.01% carbonate acetyl derivatised and detected by GC-ECD. Recoveries obtained for the spiked chlorophenols from wood and leather samples are provided in Table 2.2.

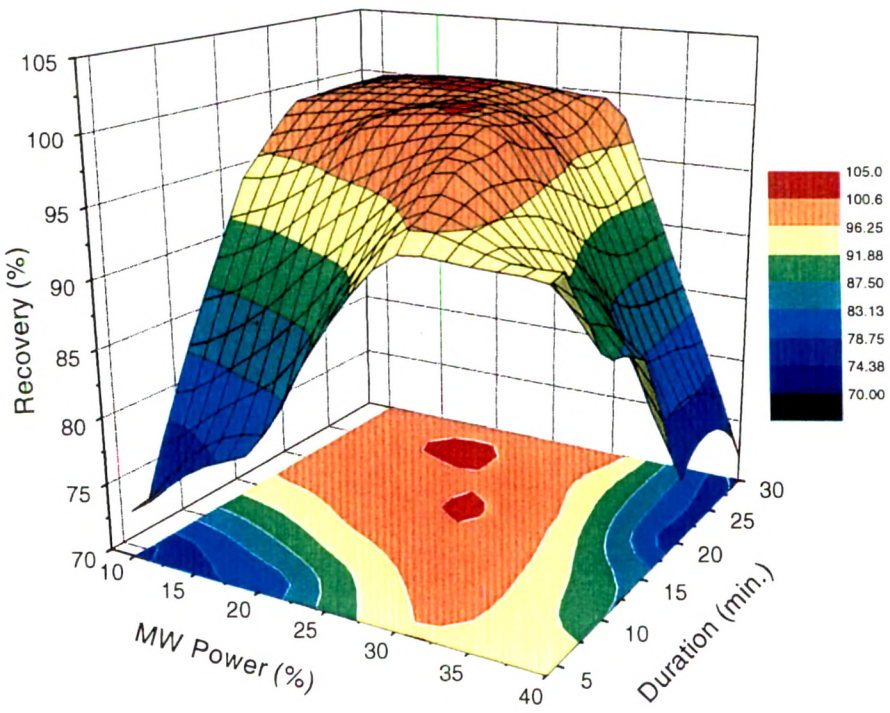

Figure 2.2 **The correlation of % MW power, duration of extraction and the recovery of PCP present originally in a solid sample (leather of 2 g was used)**

Table 2.1 Optimisation of extractant volume with respect to weight of solid sample to achieve the best recovery. Recoveries of PCP for soil sample spiked with standards, at 0.5 μg/g and leather sample (originally having PCP at 1.3 μg/g). Detection by GC-ECD. (MAE conditions: 20% MWP for 15 min duration).

Volume of the 0.01% carbonate (ml)	Recovery (%) of PCP from Soil			Recovery (%) of PCP from Leather		
	1g	2g	5g	1g	2g	5g
15	89.7	82.5	81.4	93.1	92.7	90.5
20	95.5	93.1	92.4	94.3	92.8	91.8
25	95.7	92.9	96.1	94.9	95.0	93.2
30	95.1	93.9	95.8	93.6	92.9	93.9
40	91.7	92.4	90.9	89.1	93.2	95.4
50	90.5	89.7	87.5	91.5	90.8	91.3

2.3.3 SPE clean up for HPLC

SPE cleaning and enrichment of chlorophenols have been reported in the following publications by Puig and Barceló (1996); Pocurull et al. (1996); Brouwer and Brinkman (1994); Rodriguez et al. (2000) and Lacorte et al. (1999). On completion of the MW extraction of a solid sample, the whole carbonate extract of about 25 ml along with 2-3 ml of 0.01 % carbonate solution washings, was adjusted to pH 2.5 with 10% sulphuric acid and then passed through 0.5 g of ENVI-18 cartridge previously conditioned with

methanol, at a flow rate of ~2 ml/min. The elution was done with 5x1 ml of ethyl acetate and made up to 5 ml. This was filtered through 0.45 μm PTFE filter and used for HPLC analysis. LOD for various chlorophenols by HPLC-UV detection are given in Table 2.2.

At first, the recovery of the chlorophenols was ascertained for ENVI-18 SPE with a standard solution of mixture of chlorophenols. When break through studies have been for chlorophenols separately by ENVI-P and ENVI-18; ENVI-P had 125 ml while ENVI-18 had 75 ml. These break through volumes are much higher than the volume of extractant (25 ml) in this study. For this, the standard solution of chlorophenols (1 μg of individual concentration of all congeners) was spiked into 25 ml of ammonium carbonate extractant, adjusted to pH 2.5 with H_2SO_4 and passed through ENVI-18 cartridge. SPE was carried out in a similar way explained earlier. The SPE recovery from ENVI-18 was observed from 81.7% for more polar analytes like 2-MCP to 101.5% for PCP, which is relatively a non-polar analyte.

2.3.4 SPE cleaning for GC analysis

For GC analysis, only acetyl derivatives of chlorophenols suit. If the SPE cleanup discussed earlier in sec. 2.2.5 is done, chlorophenols are collected into an organic phase of very low volume which becomes difficult to handle for derivatisation. Hence, it was planned to complete the derivatisation first, followed by SPE using ENVI-18 of 0.5 g. In this case SPE is for acetyl derivatives of chlorophenols. SPE clean up for acetyl derivatives of chlorophenols has been discussed earlier in a review by Rodriguez et al. (2000), using C_{18} RP cartridge. The recovery of acetylated chlorophenols is much higher compared to chlorophenols by a C_{18} SPE.

Initially, the recovery of acetyl derivatised chlorophenols was studied with a standard solution of chlorophenols. That was done by adding 1 ml of standard solution of chlorophenols equivalent to 1 μg of each of the species of chlorophenols to 25 ml of 0.01% potassium carbonate extractant and carrying out acetyl derivatisation as explained in 2.3.3 but omitting hexane. After the derivatisation step the extract was passed through ENVI-18 cartridge at 2 ml/min. The elution was done using 5x1 ml of n-hexane and made up to 5 ml. The extract was used for GC-ECD analysis. The solid sample extracts after MW carbonate extraction and acetyl derivatisation, were cleaned up with ENVI-18 and analysed by GC-ECD. The recovery value obtained for standard chlorophenols from this study showed that the lowest was 100.2% for 2-MCP and the highest was 102.7% for PCP. This study also supports the fact that the SPE done using a C_{18} material, improves recovery of chlorophenols if they are acetyl derivatised and it is more pronounced in highly polar analytes likes 2-MCP and other MCP isomers. LOD values obtained for various chlorophenols by GC-ECD are given in Table 2.2.

2.3.5 Recoveries of chlorophenols by aqueous MAE

Standard solutions were spiked at different levels from 0.2 -1.0 μg/g to each of the chosen solid matrix. The samples were analysed by MAE using 0.01% carbonate and ENVI-18 cleaning method. The spiked studies were carried out only with selected (at random) isomers. 3-MCP and 2,4-DCP only at 0.50 and 1.00 μg/g, as their LOD (by GC-ECD) values are 0.51 and 0.25 μg/g respectively; whereas for 2,4,6 TCP; 2,3,4,6-TeCP and PCP, their LODs are <0.1μg/g, and hence, a lower level of (0.20 μg/g) spiking was feasible. The spiked recoveries (at different levels) from various solid samples are given in Table 2.3. The general trend in the spiked recoveries from solid samples reveals

that the spiking at lower quantity give less recovery compared to higher quantity. HPLC chromatogram of leather sample spiked at 1 μg/g with a standard mixture of chlorophenols (50 μl was injected to enable the detection of all the species) is shown in Figure 2.3. The GC-ECD chromatogram of spiked recovery of reference chlorophenols mixture except PCP (that was present originally) from a wood sample is shown in Figure 2.4.

2.3.6 Analysis of samples (real and spiked)

Paper, soil (collected from an industrial site), adhesive tape, dyes and other solid samples mentioned earlier were all taken for analyzing chlorophenols. PCP was the only chlorophenol found in all the real samples taken for analyses. PCP detected in real samples ranged from 0.2 mg/kg in a soil sample to 114.5 mg/kg in a dyestuff and hence, the proposed method works for a wider range. Both the proposed FMW aqueous extraction and Soxhlet techniques produced very close results for the same samples, confirming the validity of the method. This is revealed from the data given in Table 2.4, obtained for real solid samples which were extracted with 0.01% carbonate by MW, derivatised, cleaned by ENVI-18 and detected by GC-ECD. PCP extracted from real samples gave 101.3-115.4% (all recovery values are relative to Soxhlet solvent extraction) with %RSD1.8-4.2. Both the efficiency of the technique and %RSD values are either better or comparable to Soxhlet extraction with good reproducibility. In the case of real sample analyses, matrix effects were quite dominant and ENVI-18 clean up was found to give cleaner matrices. Figure 2.6 shows the chromatogram of PCP extracted from a real (leather) sample using, 0.01% carbonate and optimized MAE conditions followed by ENVI-18 SPE enrichment of acetyl derivatised analyte (contains 29.8 mg/kg; Table 2.4).

Table 2.2 LOD of chlorophenols found by spiking the standards, MAE with 0.01% carbonate solution and ENVI-18 cleanup (by HPLC-UV); and derivatised before ENVI-18 cleanup for GC-ECD detection. Recovery (%) of chlorophenols found by spiking standards at 1 μg/g (each analyte) to solid samples by aqueous MAE and ENVI-18 follow-up (% RSD 5 replicates)

Nature of Chlorophenol	LOD by GC-ECD μg/g	LOD by HPLC-UV μg/g	Recovery (%) for wood sample %RSD		Recovery (%)for leather sample %RSD	
2-MCP	0.51	0.22	89.2	3.6	85.7	2.7
3-MCP	0.54	0.40	91.2	3.1	85.0	3.9
4-MCP	0.90	0.38	92.5	1.9	93.1	2.6
3,5-DCP	0.38	1.43*	95.5	2.3	96.3	3.2
2,5-DCP	0.17	0.40	94.3	1.7	91.7	2.9
2,4-DCP	0.25	1.32*	97.1	4.1	101.8	1.9
2,6-DCP	0.35	0.89	95.1	2.8	95.5	2.2
3,4-DCP	0.08	1.25	96.1	3.4	94.2	2.7
2,4,6-TCP	0.13	1.15*	95.1	3.5	96.3	4.2
2,3,5-TCP	0.11	2.33*	97.7	2.6	96.3	1.8
2,4,5-TCP	0.05	1.72*	94.9	2.8	98.8	2.5
2,3,6-TCP	0.11	0.24	96.2	3.3	95.4	2.7
3,4,5-TCP	0.13	2.45*	97.9	1.9	99.1	3.4
2,3,4-TCP	0.80	3.02*	98.1	2.7	96.8	2.3
2,3,5,6-TeCP	0.09	1.05*	100.3	1.6	99.3	3.1
2,3,4,6-TeCP	0.10	1.02*	100.1	1.1	99.1	2.4
2,3,4,5-TeCP	0.09	3.52*	99.7	3.1	100.5	1.9
PCP	0.04	3.54*	101.3	4.2	102.0	3.9

* 50 μl (10 μl in other cases) injection done to achieve detectability.

Table 2.3 Recovery values of chlorophenols of different spike levels, from solid samples extracted with 0.01% aqueous carbonate by MW, acetyl derivatised followed by ENVI-18 cleanup. (Detection by GC-ECD % RSD from 3 replicates)

Nature of Chlorophenol	Spike level µg/g	Soil	%RSD	Leather	%RSD	Wood	%RSD
3-MCP	0.5	92.5	3.7	91.6	4.1	85.5	4.9
	1.0	89.2	3.4	85.0	3.9	91.2	3.1
2,4-DCP	0.5	81.7	5.3	94.3	3.5	92.9	2.9
	1.0	90.1	4.7	101.8	1.9	97.1	4.1
2,4,6-TCP	0.2	85.2	5.7	89.3	5.1	80.9	5.9
	0.5	91.8	3.5	97.2	3.0	93.6	4.5
	1.0	93.2	4.1	96.3	4.2	95.1	3.5
2,3,4,6-TeCP	0.2	80.5	5.4	84.4	5.7	86.3	4.8
	0.5	90.7	5.2	89.7	4.5	90.1	3.9
	1.0	91.9	3.1	99.1	2.4	100.1	1.1
PCP	0.2	86.5	4.5	93.2	2.9	95.0	4.3
	0.5	90.9	5.2	95.3	4.8	91.7	5.2
	1.0	96.3	4.6	102.0	3.9	101.3	4.2

Table 2.4 Detection (by GC-ECD) of PCP in real samples by different extraction techniques

Nature of Sample	Soxhlet extraction using acetone		MAE using aqueous carbonate. (SPE after acetyl derivatisation)	
	mg/kg	%RSD	mg/kg	%RSD
Wood	31.5	2.3	31.9	4.2
Textile	2.3	1.9	2.7	2.5
Leather	29.2	4.4	29.8	3.9
Paper	0.6	2.3	0.7	2.9
Dyestuff	114.5	1.4	116.9	1.8
Soil	0.2	4.1	0.2	3.5
Adhesive tape	19.3	3.1	19.8	1.9

2.4 ANALYTICAL TECHNIQUES FOLLOWED

2.4.1 HPLC analytical conditions

RP-HPLC with UV detection for these analytes was reported earlier by Puig and Barceló (1996); Pocurull et al. (1996) and Brouwer and Brinkman (1994). In this study, a gradient elution programme was followed for mobile phase consisting of methanol and water containing 0.3% formic acid, added to adjust the pH to 2.5. Methanol was at 50% initially and then increased to 90% in 40 min on a linear gradient programme and held at the same composition for a further period of 10 min. The flow rate was 0.20 ml/min initially, and then it was raised to 0.35 ml/min on linear gradient in 40 min. From there it was

increased to 0.40 ml/min in 10 min. 10 μl sample was used for injection (50 μl was used for some poorly responding analytes as referred in Table 2.2) and the detection was done at 280 nm. The separation of chlorophenols was completed in 40 min duration as shown in Figure 2.3.

2.4.2 GC-ECD analytical conditions

A temperature gradient programme was employed. The oven temperature was initially at 80^0 C for 2 min, then raised to 220^0 C at the rate of 15^0C/min and held at 220^0 C for 10 min. Finally it was raised to 275^0 C at 15^0 C/min. The electron capture detector (ECD) and injection temperatures were maintained at 375^0 C and 250^0 C respectively while the carrier gas was set at a constant pressure mode of 8 psi. The separation was done using DB-17, a mid polar capillary column. Nitrogen gas of high purity was used as carrier gas and also as detector make-up gas. 2 μl of sample was injected by split-less mode. The GC separation was completed in 30 min. The GC separation of chlorophenols is shown in Figure 2.4. The GC analysis of a real sample (Leather) in which PCP was detected is shown in Figure 2.5.

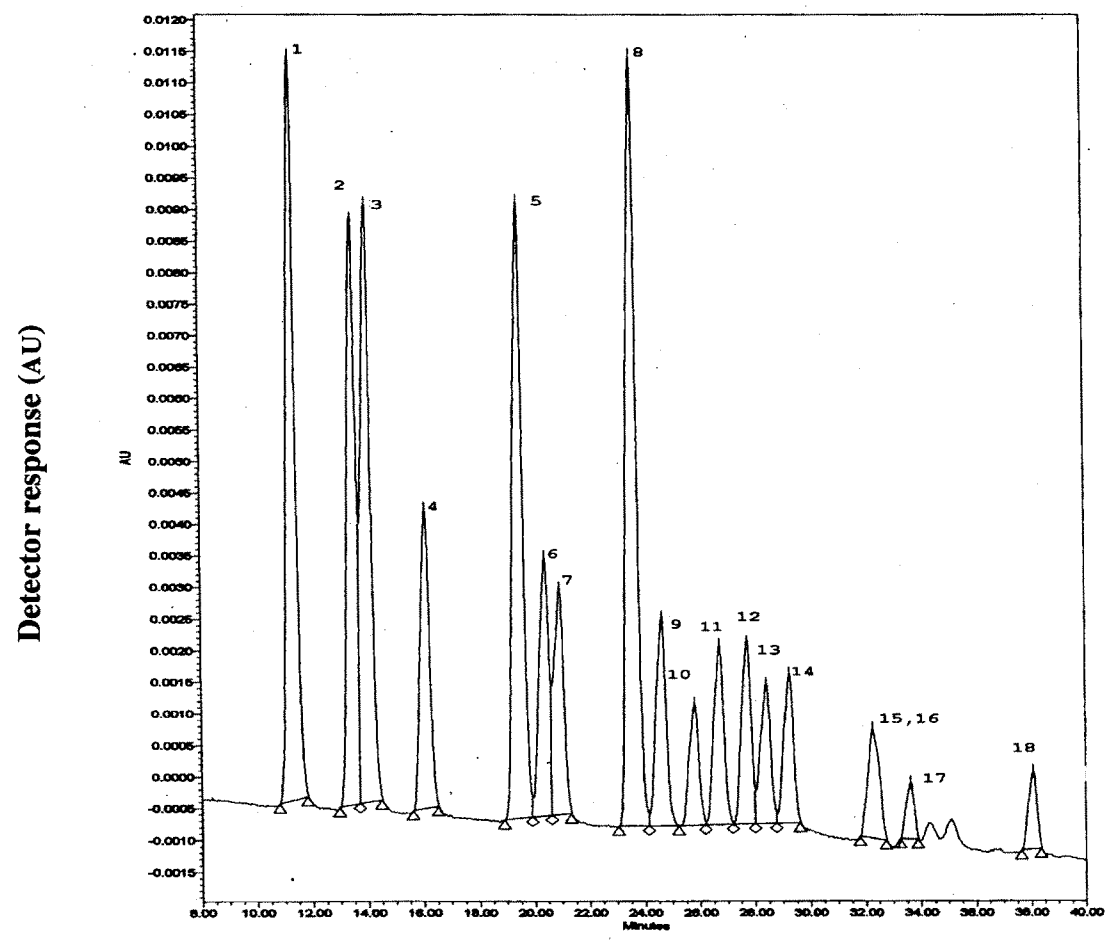

Figure 2.3 **HPLC-UV chromatogram of a leather sample spiked (1 μg/g of each of the species) with standard mixture of chlorophenols except PCP that is originally present (50 μl was injected; Detection at 280 nm):(1). 2-MCP, (2) 4-MCP, (3) 3-MCP (4) 2,6-DCP (5) 2,5-DCP, (6) 2,4-DCP, (7) 3,4-DCP, (8) 2,3,6-TCP, (9) 3,5-DCP, (10) 2,4,6-TCP, (11) 2,3,4-TCP, (12) 2,4,5-TCP, (13) 2,3,5-TCP, (14) 3,4,5-TCP, (15) 2,3,5,6-TeCP, (16) 2,3,4,6-TeCP, (17) 2,3,4,5-TeCP, (18) PCP**

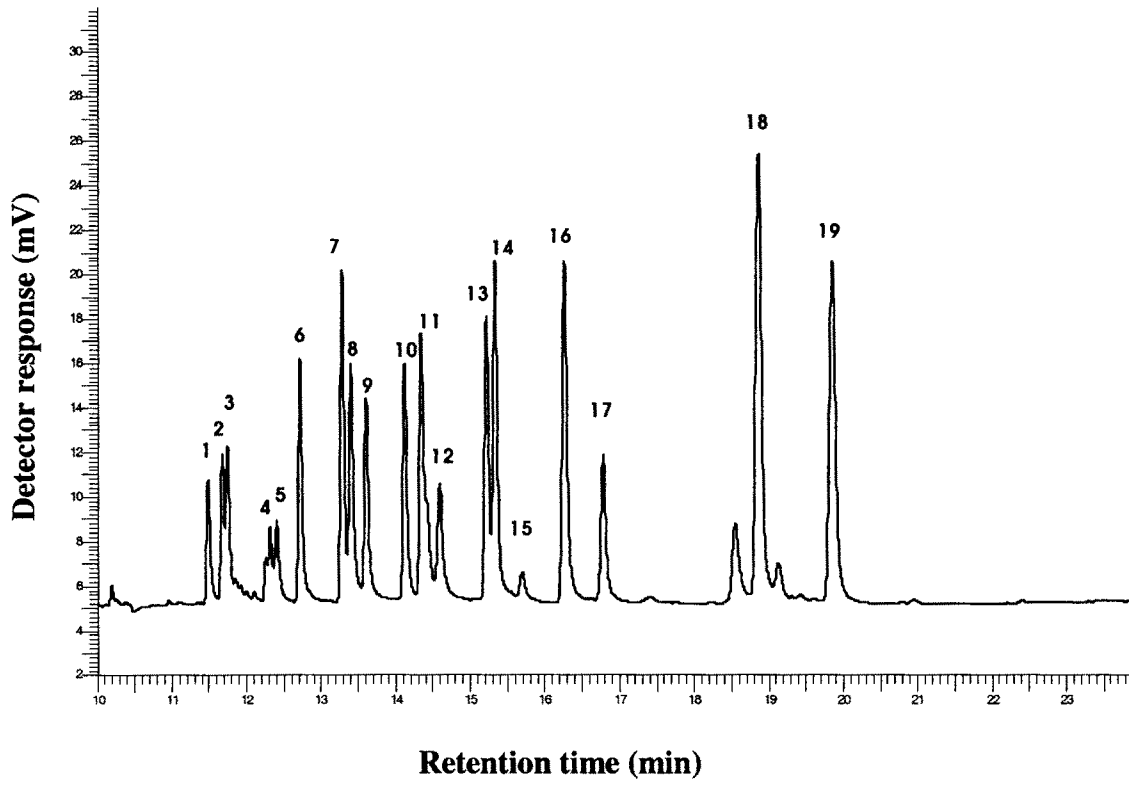

Figure 2.4 GC-ECD chromatogram of a wood sample spiked (1 μg/g of each of the species) with standard mixture of chlorophenols except PCP that is originally present :(1). 2-MCP, (2) 3-MCP, (3) 4-MCP (4) 3,5-DCP, (5) 2,5-DCP, (6) 2,4-DCP, (7) 2,6-DCP (8) 3,4-DCP, (9) 2,4,6-TCP, (10) 2,3,5-TCP, (11) 2,4,5-TCP, (12) 2,3,6-TCP, (13) 3,4,5-TCP, (14) 2,3,4-TCP, (15) 2,3,4,6-TeCP, (16) 2,3,5,6- TeCP, (17) 2,3,4,5-TeCP (18) PCP (19) Tetrachloroguiacol (Internal standard)

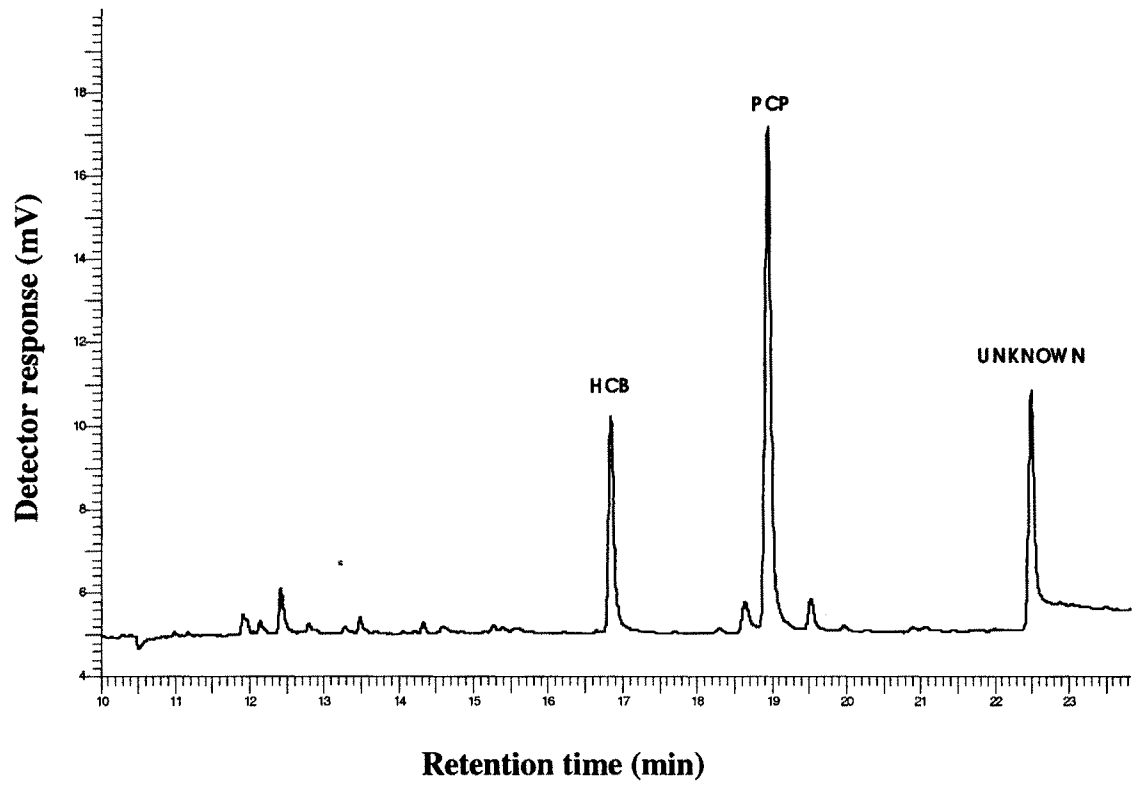

Figure 2.5 **GC-ECD chromatogram of PCP extracted from a real (leather) sample using 0.01% carbonate and optimized MAE conditions, followed by ENVI-18 SPE enrichment of acetyl derivatised analyte**

2.4.3 Capillary electrophoresis

2.4.3.1 Cyclodextrin electrokinetic chromatography

Analyzing the samples prepared directly in carbonate is not possible by Gas chromatography and High performance liquid chromatography. Hence, CE technique was resorted, and carbonate concentration in the range of 10 mM to 100 mM. Cyclodextrins have been in increasing by used in capillary electrophoresis techniques named as Cyclodextrin electrokinetic chromatography (CD-EKC) first reported by Terabe et al. (1985) in which the charged β-cyclodextrin (CD) acts as a pseudo stationary phase as discussed in the two publications of Chankvetadze (1997).The same technique is employed for separating several neutral compounds such as pesticides by Schmitt et al. (1997), polychlorinated biphenyls by Garcia-Ruiz et al. (2001) and chlorophenols by Araki et al. (2000), Jauregui et al. (2000) and Jeevan et al. (2001). In the present study, β-cyclodextrin is found to improve the resolution with good gain in the analysis time. Hence β-cyclodextrin assisted capillary electrokinetic chromatography has been opted. Capillary electrophoretic analyses with carbonate as electrophoretic run buffer could bring good compatibility with sample preparation based on carbonate. Samples can be analyzed without further additional steps by this procedure.

For the entire preparation of electrophoresis buffer and sample dilutions, HPLC grade water of purity 18.0 Ω cm was used. The electrophoresis buffer used for analysis consisted of 100 mM ammonium carbonate, and 50 mM β-cyclodextrin (Merck, Mumbai, India), at pH 8.5. Buffers were prepared freshly on the day of analysis. The preparation of electrophoretic buffer involved dissolving ammonium carbonate first and then

dissolving β-CD with the help of an ultrasonicator. 50 mM concentration was found to be stable only for 10-12 hours. Buffer solutions were filtered prior to use through 0.22 μm Supor filter of Gelman Sciences and degassed. Stock reference solutions were prepared by dissolving chlorophenols in HPLC grade methanol, and then the experimental solutions were prepared by diluting with HPLC grade water to a concentration of 15 μg/ml. Between the runs, the capillary was rinsed for 2 min with electrophoresis buffers. After every run rinsing was done for 2 min with 0.1N NaOH, 2 min with HPLC grade water and 2 min with the electrophoresis buffer. To avoid decomposition of the buffer, fresh buffer was placed after every run. Optimisation of experimental parameters led to the choice, 15 kV for separation that offered selectivity of not less than 1.00 in a reasonable analysis time of less than 15 min. The observation led to the choice of electrophoretic buffer of carbonate 100 mM with β-CD 50 mM concentration at pH 8.5. When β-cyclodextrin had been spared and only carbonate was been employed the separation was not achieved and retention of the species was long since the species are anionic. Cyclodextrins α,β,γ were tried for this study and only β-cyclodextrin caused interesting development in the separation of these species. It is in harmony with the earlier studies using cyclodextrin for the separation of chlorophenols as reported elsewhere by Araki et al. (2000). The hydrogen-bonding formation has been responsible for retentions by the charged pseudo β-CD stationary phase. Although γ-CD is better capable of hydrogen bonding, the fit between CD and the analyte species is also a point to consider and in the case of chlorinated phenols β-CD offers better fit.

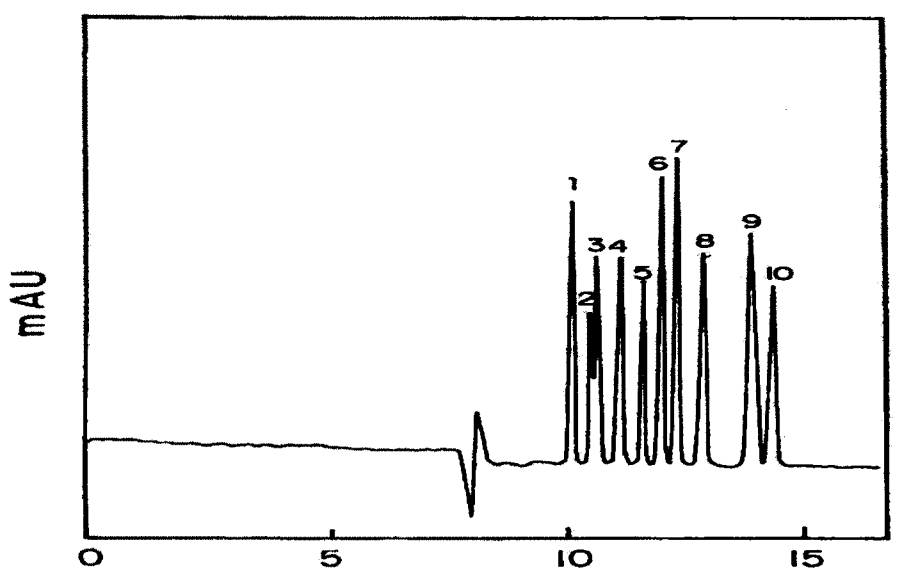

Figure 2.6 **Electrophoregram of chlorophenols detected at 214 nm Peaks: (1) 2,3,4-TCP, (2) 2,3,4,6-TeCP, (3) 2,4,5-TCP, (4) 3,4,5-TCP, (5) 2,3,4,5-TeCP, (6) 2,4,6-TCP, (7) PCP, (8) 2,3,6-TCP, (9) 2,3,5,6-TeCP, (10) 2,3,5-TCP. Conditions: 100mM Ammonium Carbonate, 50mM β-CD, pH 8.5, voltage +15kV, current 143.6 μA.**

The complete separation of chlorophenols employing cyclodextrin electro kinetic chromatography is achieved using aqueous carbonate sample preparation, which is shown in Figure 2.6.

The CD-EKC separation method has worked effectively for the separation of chlorophenols, but the limit of quantitation for chlorophenols has to be improved to apply for the real samples. The observed quantitation limit for chlorophenols was in the range of 15-20 μg/g (calculated as five times the base line noise).