CHAPTER 4

ONE-POT DERIVATISATION, EXTRACTION AND ENRICHMENT OF CHLOROPHENOLS INTO A THIN LAYER OF A LOW-DIELECTRIC SOLVENT ASSISTED BY WATER

4.1 BASIS OF THE METHOD

The approach involves taking the sample of suitable size in a required quantity of water to cover the sample completely to such an extent as to find some little excess of water above the level of sample. When MAE is done, MW is instantly absorbed by water as it is a strong dielectric, and the sample gets heated up instantaneously causing the solids to swell and release the analytes from the matrix. The released analytes in the hot condition, rise to the top where it is trapped using a non-polar solvent. Normally, a non-polar solvent like hexane is used to dissolve the acetate derivatives of chlorophenols but the solvent is low dielectric and hence cannot be directly used for MAE. However in this case, water that absorbs MW strongly assists hexane by transferring heat. For the chlorophenolic analytes to be analysed by GC-ECD, acetyl derivatisation is required. Since derivatisation is also the need, the derivatising reagents are also made available in the extraction pot. The advantage with non-polar solvents is that they do not get heated up directly by MW. However when it is used in combination with water, the heat generated in water is

transferred to non-polar solvent by convection. This is a secondary heating and the non-polar solvent will at any time be at less temperature than that of water. The main advantage in this approach is that of solvent minimization achieved by employing water for covering the sample instead of a solvent. Earlier reports reviewed by Eskillson and Bjorklund (2000) pointed out that solvents upto 30 ml are optimized by several studies and this quantity is invariably required to cover the sample rather than to extract or dissolve analytes from the samples. At the same time a solid sample has to be covered fully well by a liquid extractant without which an exhaustive extraction and achieving reproducibility is difficult. Extraction using a MAE technique involving immiscible liquid systems is relatively a rare approach and few such earlier, references are the following: the two publications by Daghbouche et al. (1996) and (1997) particularly in the later reference they employed 7 ml of carbon tetra chloride to extract oil residues from 100 ml water in 1min duration using pressurized MW technique; Onuska and Terry (1995) extracted PCBs from water samples using a solvent and in another work (1995) they extracted chlorobenzenes from 500 ml water using a solvent in dynamic condition and they claimed 1000 folds preconcentration of the analytes. Another feature of the present extraction approach is the incorporation of in situ dervatisation and such in situ derivatisation especially acetate derivatisation incorporated extractions for phenols has been reported by Llompart et al. (1997) for extracting phenols and methyl phenols. But they were not successful with methyl phenols. In another work reported by Criado et al. (2004) acetyl derivatisation and extraction of chlorophenols from fly ash samples was achieved. But those works involved pressurized MW technique, unlike the open vessel approach in this present study. Focused microwave (open vessel) has been employed for derivatisation reaction for organatin compounds (ethylation) by Pereiro et al. (1996). In this

study also a low dielectric solvent is used with assistance of water for MAE. Although such attempts were reported earlier they used more amount of solvent and less water as reported by Numata et al. (2003), Budzinski et al. (1999) and Garcia-Ayuso et al.(1998). Many such reports were reviewed by Eskillson and Bjorklund (2000). An other approach was to use a co solvent like acetone with hexane or other low dielectric solvent as reported by Luque-Garcia and Luque de Castro (2003) ; Budzinski et al. (1999); and by host of reports reviewed by Eskillson and Bjorklund (2000). But the problem is that polar solvents contribute to many polar matrix impurities to the extract which complicate chromatographic separations and detections. The third approach to this problem is the usage of a fluoro polymeric special device as reported by Vetter et. al (1999) and Hummert et al.(1996) to absorb MW and transfer the heat to hexane like solvents. Compared to all the approaches described earlier this work is as economic solution and needs no such investment on accessories in addition to those, being much effective solvent minimization approach. However in this work there may be an extra input of MW energy and extraction duration but this method paves way for green laboratory approach. The employment of water causes swelling of most of the matrices unlike solvents, which also helps in the release of analytes. This study employed Focused open vessel MW extractor device which was used for the extraction of chlorophenols from environmental matrices (soils) by Alonso et al. (1998) in which the extraction was done using 50 ml of a mixture of methanol and water (ratio of 4:1). The study involved optimisation of MW conditions like power, duration of extraction and the optimisation of derivatisation of chlorophenols. Sample weight, water phase covering the sample, solvent volume used for trapping were also studied and reported. Finally, there is enrichment step which is inherent in the method (since the extracting solvent used is at minimum level).

4.2 EXPERIMENT

4.2.1 Equipments

MW equipment of same model; GC–ECD of the same make and model as described in the Experimental sections of chapters 2 and 3 were used.

4.2.2 Reagents

The reagents used are the same as mentioned in Chapter 2.

4.2.3 Reference standard solution of a mixture of chlorophenols

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 daily .The working standards for GC were prepared in the range 0.01-1.00 μg/ ml using hexane.

4.2.4 Preparation of soil sample

The soil free from chlorophenols, was air-dried, pulverized, and sieved to a grain size of 2 mm. A total of 25 g of soil was mixed with acetone until the sample was completely soaked to form slurry. The whole 25 g slurried soil was spiked with an appropriate volume of standard solution to achieve 1 μg/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 2 weeks at room temp. The prepared soil sample was stored in a refrigerator at 4^0C until used for analysis.

4.2.5 Other solid sample preparation

Solid samples like leather were prepared as powders of 100 mesh size and 1 g of each sample was taken for the analysis. The extractions of textile and paper samples were done with 1 g of finely cut (*ca* 1 –2 mm^2) pieces. The solid chemical samples were analyzed by taking 1g after breaking down any lumps if present. Spiking required for these samples were done with an appropriate volume of the standard solution added to the weighed sample so as to achieve 1 μg/g and the sample left for drying overnight at room temperature.

4.2.6 Extraction of solids

5 g of soil sample was weighed and transferred into the MW sample vessel. In the case of leather or textiles, samples of 1 g were taken. To the solid sample, 25 ml of water, appropriate quantity of potassium carbonate to ensure 0.1% solution, were added and shaken well to effect dissolution of carbonate 0.1 g of sodium chloride were added. The acetylating agents, acetic anhydride 0.5 ml and triethylamine 0.5 ml were added. Over this, 1 ml of n-octane was allowed to float. MW energy was varied from 10-40 % for 1-15 min duration for the extraction. The experiment was repeated exactly as discussed above except for changing n-octane volume to 2 ml and then to 5 ml.

4.2.7 Alternate extraction method by MAE employing higher volume of the solvent (n-octane)

5 g of the spiked soil sample was taken in the vessel along with 25 ml of water, suitable quantity of potassium carbonate to achieve 0.1%

concentration and 0.1 g of NaCl and acetylating agents, acetic anhydride 0.5 ml and triethylamine 0.5 ml were added. The contents were shaken well to ensure the dissolution of the salts. 10 ml of n-octane was added over this and MW power of 20% was applied for 10 min (decided from the optimized MAE studies). It was left to cool for 30 min. After 30 min, the condenser was dismantled and the solvent and other contents were transferred into a 100 ml-separating funnel (collected through Whatman 41 filter paper). The aqueous portion was drained and the solvent layer dried over anhydrous Na_2SO_4 and made up to 10 ml before GC analysis. Leather and textile samples were extracted by a similar approach but with 1g quantities.

4.2.8 Soxhlet Extraction

The conventional Soxhlet continuous solvent extractions were employed for solid samples using acetone as the solvent. 150 ml 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; while 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 with additional 10 ml of hexane and the final volume adjusted to 1 ml with hexane. The extract was dried over anhydrous sodium sulphate.

4.2.9 Preparation of liquid samples by MAE

1 ml chlorophenols at 1 μg/ml were spiked into 25, 50,100 ml volumes of Milli-Q water individually and shaken gently to ensure thorough mixing. Water sample of 25-100 ml was taken in a 250 ml MW extraction vessel, appropriate quantity of potassium carbonate to achieve carbonate of 0.1% solution and 0.5 ml each of triethylamine and acetic anhydride for 25-50 ml water volumes or 1 ml each of triethylamine and acetic anhydride for 75-100 ml water volumes were added. Over this 1 ml of n-octane was allowed to float. MWP was rotated from 10-40 % for 1-5 min duration for extraction. The experiment was repeated by varying only n-octane to 2 ml and then 5 ml.

Note: In all the MAE, after completion of extraction of a sample, nearly 30 min was left, only then the condenser was dismantled and the extraction vessel drawn out. The condenser was not rinsed as that would contribute to some more solvent usage and the experiment was tried for finding the analytes only in the solvent trap. The solvent was drawn out from the top with a long slender pipette and then dried with anhydrous sodium sulphate before GC analysis.

4.2.10 Liquid-Liquid Extraction

LLE technique was followed as a referral technique for the aqueous samples and the approach was common for all analytes under the study. Extraction was carried out with a 100 ml volume of sample and 25 ml dichloromethane, made acidic by adding 10% sulphuric acid in a 250 ml separatory funnel and the process was repeated twice with fresh solvent of same

quantity. The extracts were combined and, after the addition of 1 ml of isooctane, evaporated to approx. 1 ml using a rotary evaporator. The extracts were filtered, dried over Na_2SO_4 and the solvent changed to n-hexane. With the residue dissolved in 5 ml hexane and the acetyl derivatisation was carried out following the procedure given in Soxhlet extraction. The hexane solvent fraction was again concentrated in a rotary evaporator, followed by a florisil column cleanup. The extracts were then concentrated by a rotary evaporator and subsequently under a gentle stream of nitrogen to near dryness. The residue was made up to 1 ml with n-octane.

4.3 RESULTS AND DISCUSSION

4.3.1 Selection of n-octane for this extraction:

Chlorophenols are usually acetyl derivatized and extracted with hydrocarbons, mainly with hexane. The hydrocarbons have good solubility for acetates of chlorophenols; they are immiscible with water and more importantly lighter than water. Many hydrocarbons qualify from these features, but n-octane and higher hydrocarbons have higher b.pt. and hence they remain intact compared to n-hexane and other lower hydrocarbons since, the water medium reaches 100°C during MAE using higher power or longer duration resulting in solvent loss. Hence, n-octane and higher alkanes only suit but higher solvents are viscous and comparatively poorer in solvency. Increasing viscosity complicates the easy handling of the solvents by micro syringe and such micro analytical devices. n-Octane compared to other solvents has good solubility for several chloro organics.

4.3.2 Optimisation of MAE of chlorophenols from solid samples

Chlorophenols include highly polar 2-MCP to non-polar PCP. MCPs are volatile than PCP and hence MAE conditions would likely to differ for these analytes. The MAE in this study includes the extraction and the acetyl derivatisation and hence the recovery is meant as combined output. MAE depended on the nature of analyte, its matrix, the volume of aqueous phase and the amount of solid sample present. Hence, just one MAE condition is not possible to adopt for all the analytes or the matrices. MWP and the duration of extraction that enabled the recoveries exceeding 90% alone are reported. The influences of weight of sample, trapping solvent volume and water phase volume are discussed separately. In this study water volume of 25 ml (with carbonate and acetylating agents as described in sample preparation earlier) was used for solid samples of 5 g of soil sample or 2g of leather or textile (cotton). In all the extractions, 1 ml n-octane was used as solvent trap. The experiments were planned to optimize the MAE condition to achieve the best recovery for the analytes for which MWP from 10-50% and duration of 1-30 min were varied. Initially 10% MWP, the lowest possible was used to study the solid samples spiked with a mixture of selected chlorophenols. Of the samples tried, soil samples were found to be relatively tougher matrices although they were successfully extracted at the same MWP condition like other matrices, they demanded an additional 1 to 3 min to attain the recoveries on comparable basis. Soil samples (2 weeks aged) after extraction for 15 min at 10% MWP gave a recovery of 94.2% for PCP. 10% MWP was also successful for MCPs and DCPs with recoveries around 94-96% and 94-97% respectively in 8 min, as MCPs are volatile it is not safer to do prolonged (10 min) heating than what is sufficient. For TCPs it was 94-97% in 10 min recoveries duration of extraction

and for TeCPs at the same 10% MWP, the recoveries were in the range 95-98% in 12 min. The recovery trends of MCPs and PCP (spiked in both cases) are shown in Figures 4.1 and 4.2. MCP the most volatile species is represented by 2-MCP for the extraction from a soil in Figure 4.1 and PCP extraction from a soil sample, which represents the difficult to extract of these analytes, as shown in Figure 4.2. In all these studies the behavior of MCPs and DCPs are almost the same. For leather and textile samples which do not differ much in their MAE conditions for recoveries, 10% MWP produced recoveries in the range 93-95% in 5 min duration of extraction for MCPs, DCPs and for TCPs the recoveries were 95-97% in 5 min while for TeCPs the recoveries were in the range 95-97% in 8 min duration and for PCP the recovery was 95.9% in 10 min. The recovery trend found for cotton textile matrix for the extraction of TCPs is shown in Figure 4.3. Figure 4.4 shows the dependency of recovery of PCP from leather on MW heating duration. When 20% MWP was applied for soil samples, PCP extraction was found to be 94.6% in 10 min duration. For MCPs and DCPs, the recovery values were 93-95% and 94-95% respectively for the duration of 5 min. For TCPs, the recovery was found in the range 93-96% in 5 min and for TeCPs the recovery was 96-97 % in 8 min. For leather and textile samples, at 20% MWP, the recoveries were 94-96% for MCPs and DCPs in 3 min and 96-97% for TCPs in 5 min .The recoveries for TeCPs were 95-99% and for PCP it was 98.7% in 8 min. With 30% MWP, the recoveries for MCPs and DCPs were found exceeding 90% only upto 3 min extraction and with 5 min extractions the recoveries reduced to 80%. TCPs behaved in the same pattern and when the extraction duration extended beyond 8 min there had been a drop (60 -70%) in the recoveries of MCPs, DCPs and TCPs. This results from the failure of n-octane to trap analytes as at higher power, the faster diffusion of analytes occurs. When the condenser was given a washing (it was

not preferred normally in order to curtail solvent usage and also the recoveries were found to exceed 90% in all cases), for TeCPs the recoveries were 86-91% even in 10 min duration from soil samples and the maximum was in 5 min heating with figures of 94-96%. For PCP, the best figure for the recovery (94%) was noticed in 5 min duration and it was close to 90% upto 10 min extraction there after started falling below 70%. For leather and textiles, 30% MWP was good only for 3 min for MCPs, DCPs and TCPs with recoveries in the range of 92-95%, and continued to be acceptable upto 5 min with recoveries around 80% which then fell below 70% beyond 5 min for PCP it was observed as 95-96% with 3 min extraction and 80-90 % range upto 10 min which decreased to 70% for longer duration. The same trend was observed for TeCPs for these matrices. At 40% MWP, MCPs and DCPs, fell below 50% recoveries even in 1 min MWP usage as it was too harsh for these analytes for soil, textile and leather matrices. But for TCPs, it was acceptable upto 3 min with 89-92% recoveries from soil sample while it was around 78-80% for leather and textiles. For TCPs and PCP from soil samples, it was observed as quick analysis in 3 min with recoveries of 90-92%. For TeCPs recoveries were around 92% and for PCP recovery was in the range 81-84%. The extraction beyond 5 min produced values lower than 50%. For leather and textiles it was 90% in 3 min for PCP and 80% upto 5 min and not safe for recoveries beyond that duration for TeCPs. The recoveries of spiked chlorophenols at different concentration levels in different solid samples (matrix effect) and their corresponding recoveries and reproducibilities are given in Table 4.1. The recoveries of these analytes from all the matrices came out well with good reproducibilities as shown in Table 4.1. Since 40% MWP itself carried a loss of analyte, when compared to lower MWP options 50% MWP was not tried.

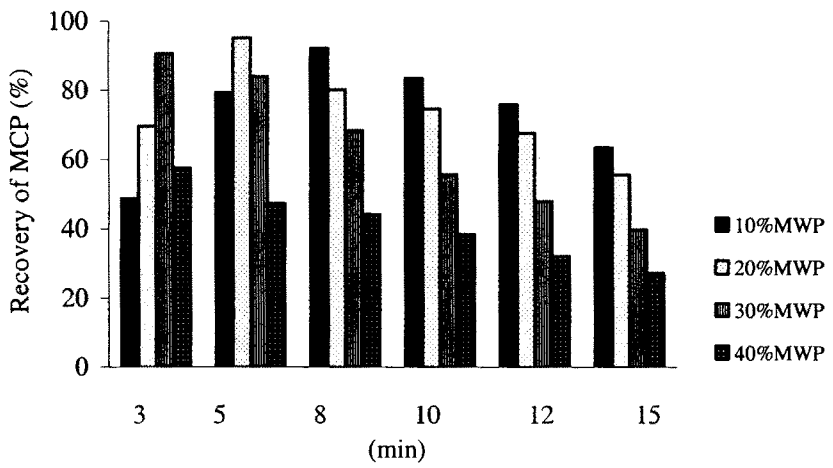

Figure 4.1 Dependency of recovery of 2-MCP from soil sample on MW heating duration

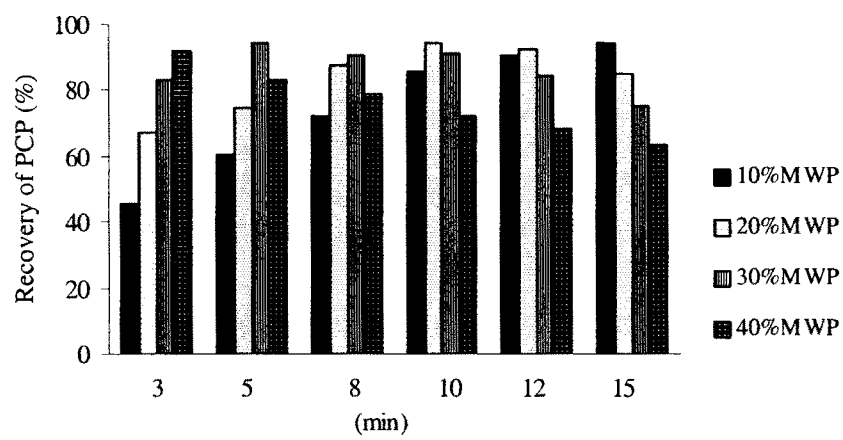

Figure 4.2 Dependency of PCP recovery from soil sample on MW heating duration

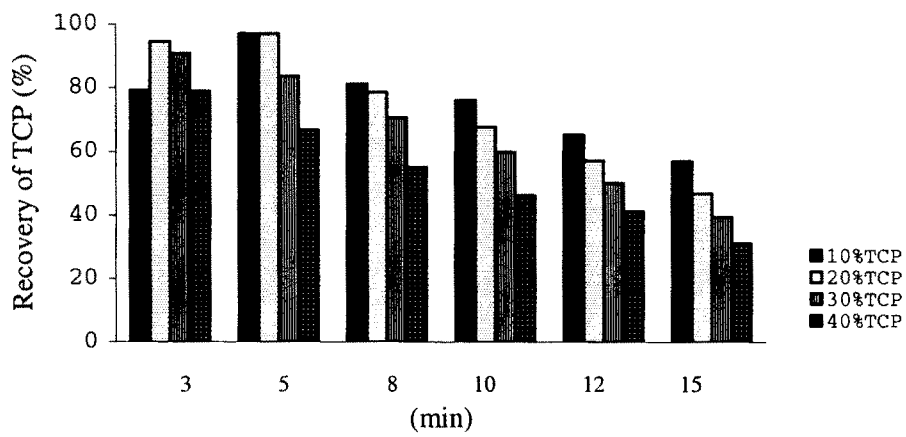

Figure 4.3 Dependency of recovery of 2,4,6-TCP in Textile on MW heating duration

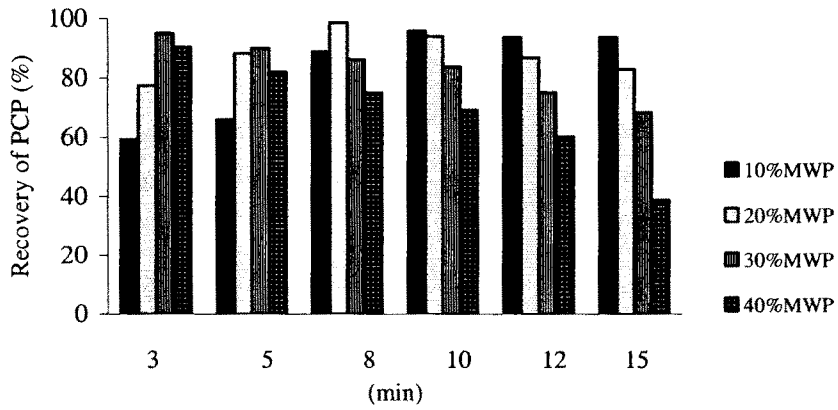

Figure 4.4 Dependency of recovery of PCP from leather on MW heating duration

Table 4.1 LOD and % Recoveries (ng/g) for spiked analytes from solid samples

Nature of solid	Analyte	Method of LOD (ng/g)	Spiked Levels (ng/g)	Detected (ng/g)	% RSD
TEXTILE	2,4,6 TCP	28	25	25.3	4.0
			50	50.7	3.8
SOIL	2-MCP	90	25	24.6	4.7
			50	49.8	3.9
	2,4-DCP	50	25	24.8	3.8
			50	50.1	2.9
LEATHER	2,3,5,6-TeCP	20	25	25.5	3.7
			50	50.8	4.1
	PCP	6	25	25.4	3.2
			50	50.5	2.9

The optimized conditions for extractions of solid samples can be summarized as follows:

For the extraction of soil samples, 10% MWP for 8 min is suitable for MCPs, DCPs; it is for 10 min duration for TCPs; for TeCPs it is 12 min and 15 min for PCP. For leather and textiles 10% MWP for 5 min is required for MCPs, DCPs; while it is 8 min for TCPs, TeCPs and for PCP it is 10 min.

4.3.3 Optimisation of MAE of chlorophenols from aqueous samples

A separate optimisation was required for aqueous samples for the reason that solid samples demand more intensive conditions compared to only milder conditions required for aqueous samples. If higher energy conditions are

prolonged that lead to the loss of more volatile low chlorinated phenols like MCPs, DCPs and a few TCPs. Hence, the optimal duration of the extraction was to be followed. The study started with the application of the lowest 10% MWP and continued upto maximum available 50% MWP. The duration of extraction was done from 1 min-15 min. The study involved the extraction with in situ derivatisation of chlorophenolic species from aqueous sample volume of 25-100 ml using 1 ml of n-octane. The recovery depended on the volume of aqueous sample, as it became poorer with increasing volume of aqueous phase. In general, 10-20% MWP was found optimal for even volumes upto 100 ml of sample but the extraction duration extended with increased volume. Upto 5 min was observed successful for the extraction of volatiles while 8 min was found necessary for semi-volatiles. Figure 4.5 represents the dependency of recovery of DCP on sample volume in water sample in correlation to MW heating duration and Figure 4.6 shows the dependency of recovery of PCP on sample volume in water sample in correlation to MW heating duration.

For 25 ml volume of water sample at 10% MWP, MCPs, DCPs and TCPs produced recoveries in the range 85-89% in 2 min and exceeded 93-95% in 3 min and then at 5 min duration the recoveries declined to 82-84%. Beyond 5 min the recoveries dropped drastically to less than 60%. Hence, for higher volatile low chlorinated phenolic species, 3 min was the ideal duration for the extraction while relatively semivolatiles like TeCPs and PCP produced good recoveries of 92-95% and 93.9 % respectively in 3 min duration at 10% MWP and also for these species recoveries were in the range 83-86% with 2 min extraction. At 20% MWP, the recoveries were best achieved in 1 min with recoveries of 92-94% for MCPs and 99.7% for PCP. The species, MCPs and DCPs were stable upto 2 min duration with recoveries of 84-87% while the

recoveries were in the range 90-93% for TeCPs and PCP. At 30% MWP even 1 min was not useful as only 60% recoveries were found for MCPs, DCPs and TCPs. For TeCPs and PCP, 93-95% recoveries were observed with 1 min extraction and it was not beneficial to try beyond 3 min at this power. Hence, further higher MWP of 40 and 50% were not tried.

For 100 ml volume of water, when 10% MWP was used the more volatile species were found to achieve the better recoveries in 3-5 min duration with 82-85% recoveries found in 3 min, and 92-98% recoveries in 5 min. After 10 min duration the recoveries fell to 78-83%. On prolonged exposure (exceeding 8 min) even at 10% MWP, the volatiles escape due to faster diffusion from aqueous layer and poorer trapping by 1 ml solvent. In the case of TeCP and PCP good recoveries were observed in 3 min and stable upto 8 min extraction. The recovery values at 5 min duration were the best for the TeCP and PCP which were in the range 93-97%. Beyond 8 min, the recoveries gradually fell down to 88-92%. At 20% MWP 3 min was the recommended range for volatiles like MCPs, DCPs and TCPs with recoveries found in the range 90-93% and for semi-volatiles (TeCPs and PCP) 5 min was recommended when 96-98% recoveries were found. In general 30% and 40% MWP conditions were not good for volatiles and for TeCPs and PCP 30% MWP was found good upto 3 min whereas 40% MWP only for a minute. For MCPs, DCPs and TCPs recovery values in the range 60-70% were found in 1 min extraction and when tried for further duration they started falling down below 50% recoveries while for semivolatiles namely TeCPs and PCP, extraction upto 5 min was useful with best recoveries at 3 min with values of 92-96%. 40% MWP for a min was useful for TeCPs and PCP with 90-94% recoveries. Beyond that MWP, it was disastrous from the point of view of the stability of the analytes. Hence 50% was not included in the discussion.

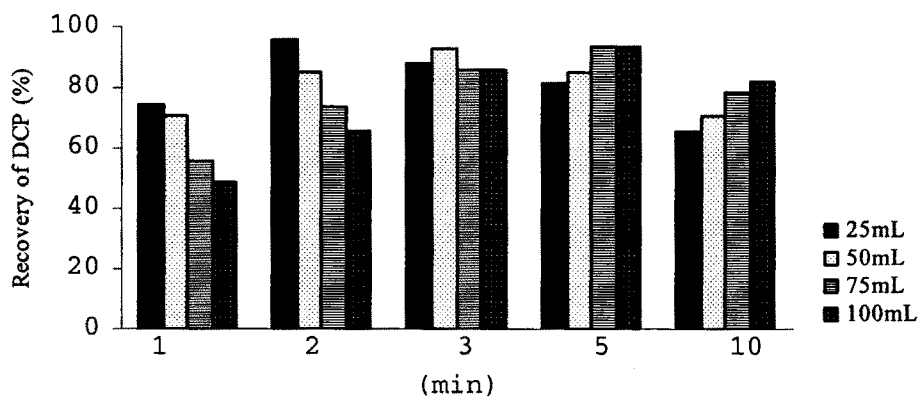

Figure 4.5 Dependency of recovery of DCP on sample volume in water sample in correlation to MW heating duration (MWP 10%)

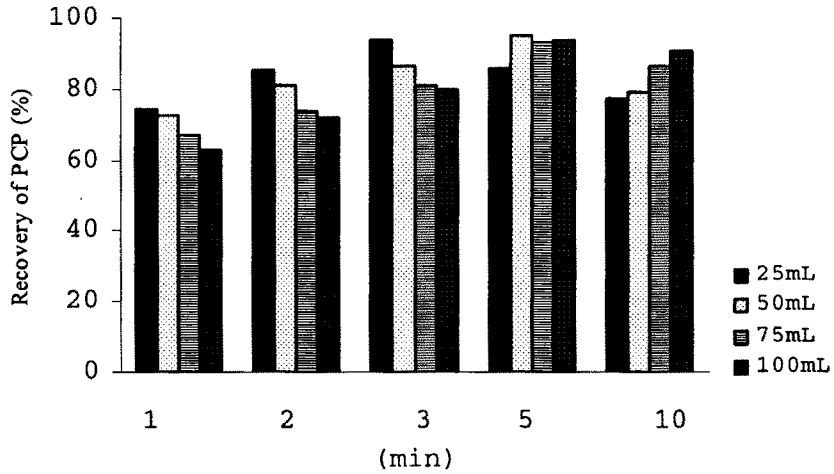

Figure 4.6 Dependency of recovery of PCP on sample volume in water sample in correlation to MW heating duration (MWP 10%)

Table 4.2 LOD and % Recoveries (ng/ ml) for spiked analytes of water samples

Analyte	LOD*	Spikedlevel	Detected	%RSD (n=3)
2-MCP	2.0	5	4.8	4.3
		10	9.8	2.9
2,4-DCP	1.0	5	4.9	5.1
		10	10.1	3.3
2,4,6-TCP	0.5	5	4.9	3.7
		10	10.0	2.8
2,3,5,6-TeCP	0.3	5	5.2	2.5
		10	10.1	2.6
PCP	0.1	5	5.1	2.3
		10	10.2	1.9

* It is applicable for 100 ml water sample extracted into 1 ml n-octane.

For 50 and 75 ml volumes the observation of recoveries showed the trend closer to 100 ml case. Like the recovery of volatile cases MCPs, DCPs and TCPs were observed better in 3 min and good upto 5 min at 10% MWP. The best recoveries were at 3 min and after 6 min the recoveries dropped. At 20% MWP the beneficial conditions were 2-3 min duration with the best at 2 min for volatiles (92-95%) when exceeding 3 min, it produced bad recoveries (less than 70%). The case of semivolatiles namely, TeCPs and PCP at 10% MWP, good recoveries were observed in 3-8 min and the best found at 5 min (93-98%). At 20% MWP good recoveries were found in 2-5 min range with best figures at 3 min (95-101%) and it was not safer to exceed 6 min. At 30% MWP, 50 and 75 ml volumes suffered serious set back in the extraction of volatiles while for semi-volatiles a maximum duration of 3min was acceptable

(91-97%). At 40% MWP just 1 min was acceptable for TeCPs and PCP in the case of 75 ml with recoveries of 91-96% which however did not suit 50 ml. 50% MWP was not tried for any of these aqueous volumes for the main reason that even 1 min was found harsh even for 100 ml. the recoveries for spiked analytes in water samples and their LOD values are given in Table 4.2.

The optimized conditions for aqueous samples are summarized as follows:

For water samples, to extract chlorophenols from 100 ml, 10%MWP for 3 min duration is suitable for MCPs, DCPs and TCPs while it is 5 min for TeCPs and PCPs. For the lowest volume of water tried (25 ml) 10% MWP for 3 min is suitable for MCPs, DCB, TCPs, TeCPs and PCP. For 50 and 75 ml volumes of water 10% MWP for 3 min is suitable for the best recovery of MCPs, DCPs and TCPs whereas for TeCPs and PCP it is 5 min.

4.3.4 Variation of recoveries of isomers in the extractions

The isomers of same chloro member of functionalities are almost with same behavior in the extractions. The dispersal diagrams drawn using their data from the analyses of soil samples (taken after spiking) are given namely Figure 4.7 for the recovery data of MCPs (isomers), similarly Figure 4.8 for DCPs, Figure 4.9 for TCPs, Figure 4.10 for TeCPs and PCP from soil on varying MW heating while Figure 4.11 is shown for the recovery data of TeCP and PCP from leather on varying MW heating. From Figures 4.10 and 4.11 the matrix influence on the recovery of the same analytes is evident. For highly swelling type of matrix like leather the recovery is found higher compared to the lesser swelling matrix like soil. The extractabilities on different MWP conditions at a fixed extraction time (what is observed to be the best for the recovery of the particular type of analytes) are shown.

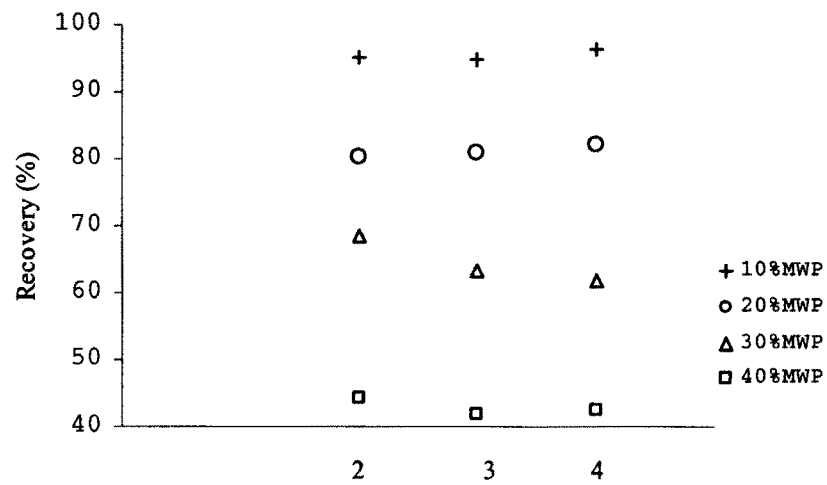

Figure 4.7 Recovery data of MCPs from soil on varying MW heating

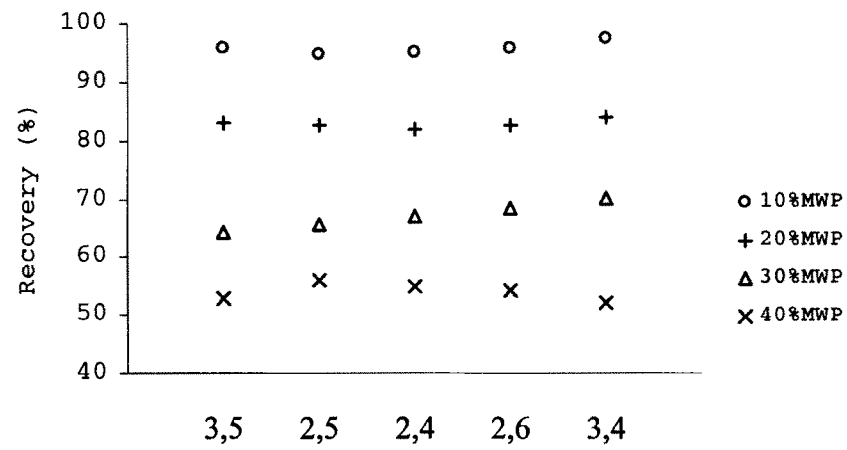

Figure 4.8 Recovery data of DCPs from soil on varying MW heating

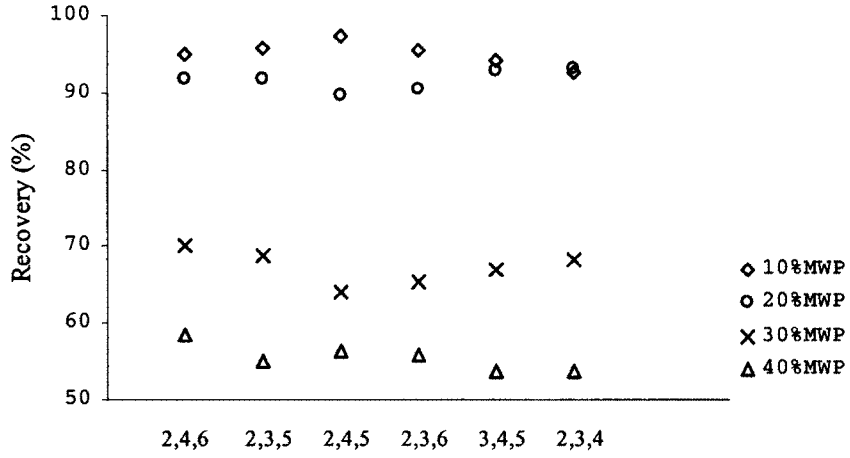

Figure 4.9 Recovery data of TCPs from soil on varying MW heating

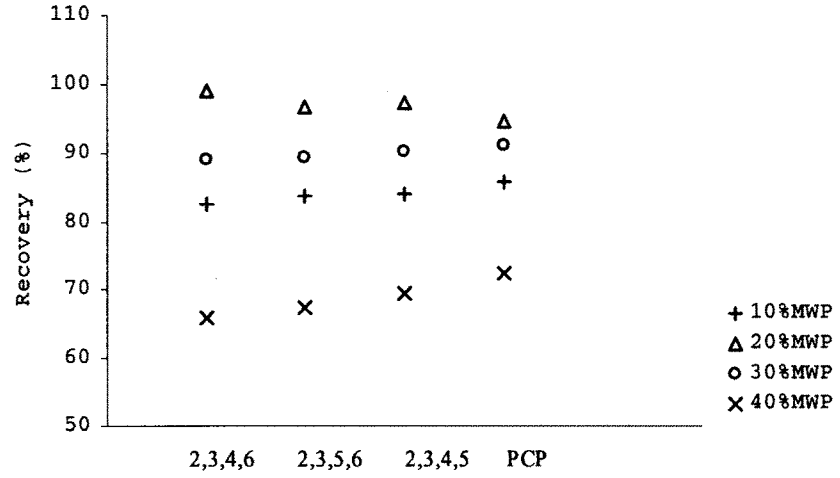

Figure 4.10 Recovery data of TeCPS and PCP from soil on varying MW heating

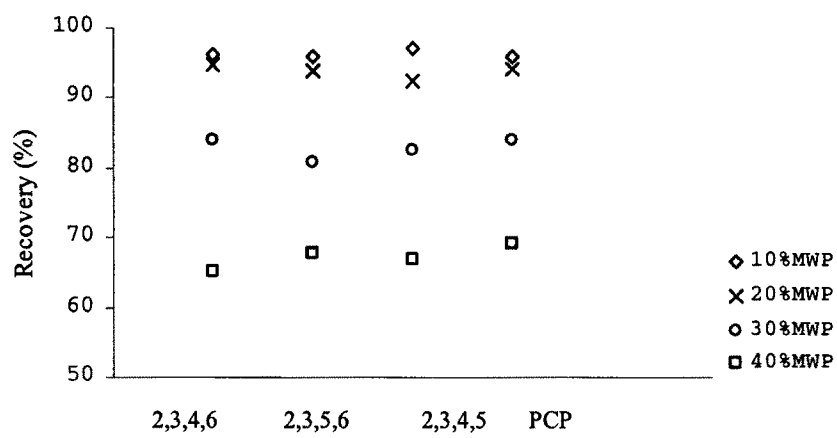

Figure 4.11 Recovery data of TeCPs and PCP from leather on varying MW heating

4.3.5 Duration of derivatisation

The acetylating reaction was carried out with standard chlorophenol mixture, taken in 25 ml of 0.1% carbonate aqueous solution with 0.5 ml each of acetylating agent, acetic anhydride and triethylamine catalyst where the reaction duration was varied from 1 to 5 min at varying MWP from 10-40%. At 20% and 30% MWP even 1min duration was found sufficient for the derivatisation of chlorophenols with 99-101% yields for all the chlorophenolic species. However at 10% MWP, 1.5 min was found necessary for the completion of the reaction. It was tried for varying levels of chlorophenols from 0.2 μg, 0.5 μg, 1.0 μg and 25 μg and in al these cases the completion of the derivatisation was observed in the duration of 1 min at 20% or 30% MWP. Even for 10% MWP, the same trials for 1-5 min duration became valid. At 40% MWP, loss of

chlorophenols like MCPs and DCPs were found that progressively lowered in higher chloro congeners.

4.3.6 Influence of varying concentration of derivatising agents

For an optimal derivatisation, 1.0 ml acetic anhydride and 0.5 ml triethylamine were added to 25 ml of alkaline aqueous extractant used for solid samples. The alkaline solution was 0.1% potassium carbonate. Acetic anhydride concentration range from 0.25-1.0 ml and that of triethylamine 0.25-1.0 ml in the range were tried leading to the observation that there was no any serious change in the yield or the reaction time. The yield of acetyl derivatives of all chlorophenols was uniform and satisfactory. MWP 20% for 1 min was used for the studies.

4.3.7 Influence of variation of volume of the aqueous phase

For solid samples, aqueous phase volumes from 10 ml to 30 ml were tried. 10 ml was not sufficient to wet the samples, since leather and cotton textiles samples are of swelling nature. The idea was to have some water standing in excess above the level of solid samples so that the trapping solvent can be floated on it and also it would be easy to siphon out the solvent enriched by analytes, with a pipette later. 15 ml of water was found to be a minimum requirement to cover the solids, but with this quantity of water the loss of analytes was of higher degree.

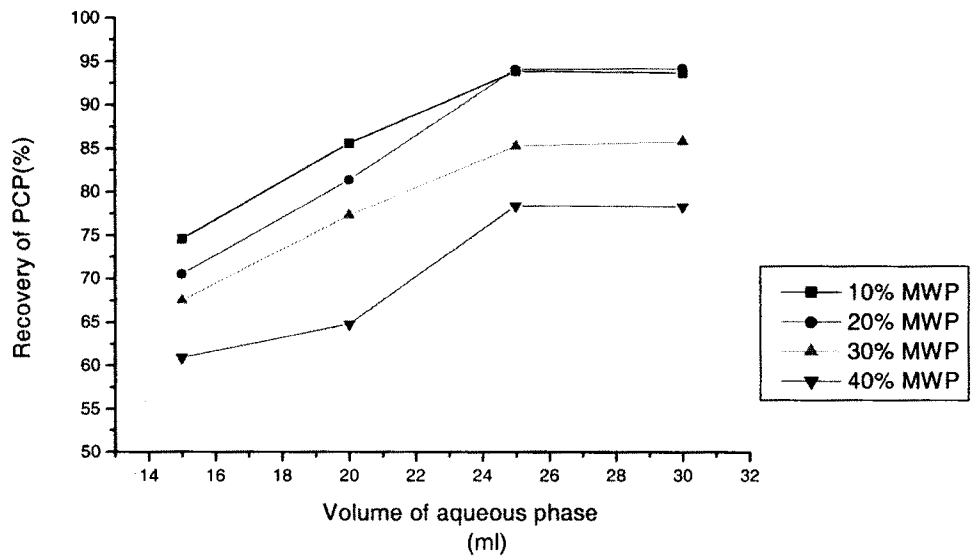

Figure 4.12 The influence of variation of aqueous phase volume on the recovery of spiked PCP using n-octane 1 ml and duration of 3 min

Due to increased temperature by MW heating the faster release of analytes, resulted and the solvent trap was unable to capture them. In the case of 20 ml, the situation was much improved; however 2-MCP and 4-MCP produced poor recoveries (60%) which probably should have been harsher temperature conditions for these species. The next higher volume of 25 ml improved the situation remarkably that all chlorophenols were recovered in the range of 89-99.8% and mainly 2-MCP improved to 89.8%, while DCP improved to 91.6%, TCP improved to 96.5%, TeCP improved to 98.4 and PCP improved to 99.8%.

A similar trend was observed with 30 ml usage. Hence, 25-30 ml were the right volume. However the lowest optimal volume of 25 ml was chosen for

better extractability in difficult matrices like soil as it was also known that the increased volume of the extrantant also affects recovery. The influence of variation of aqueous phase volume is shown in Figure 4.12 from the recovery of spiked PCP using 1 ml n-octane and extraction duration of 3 min.

4.3.8 Influence of varying volume of solvent trap

n-Octane solvent trap was studied by varying from 1 to 5 ml. In the event of 1 ml being successful, the enrichment achieved would be the highest of this trial. Naturally, 5 ml solvent would cause 5 times weaker in response compared to 1 ml. The influence of 1 ml was pronounced high in the case of trace level quantities. In this study, the main observation made was that in most of the cases 1 ml n-octane served well but under circumstances involving volatile analytes like MCPs and DCPs, at high MWP exceeding 30%, 1 ml usage resulted with loss of analytes. That probably could have resulted from the poor trapping of the analytes which were released with higher diffusion rates at higher temperatures. However when 2 ml or 5 ml solvent was (making larger column of the top) used, the trapping of analytes were effectively achieved as shown in Figure 4.13. This was the only noticeable difference on the variation of n-octane.

4.3.9 Influence of the weight of the sample on the recovery of analytes

The quantity of samples required in environmental and toxic residues analyses is often high but there is not much choice left for analysts to lower the quantity of samples because hazardous analytes are in trace quantities (although the lowered weight improves the extractability). However as part of

the study it was carried out. While the variation of sample weight from 1 to 5 g produced marginal influence in the case of volatile analyte, it was serious with the semivolatiles like PCP which also proved to be as relatively difficult to extract analyte. The extraction of chlorophenols from a soil sample which is again a relatively difficult matrix was chosen for this study and the data obtained from these studies are given in Figure 4.14 for which MWP of 10% for 8 min duration was chosen since it also suited for volatiles. 1 ml n-octane was used for trapping and 25 ml aqueous phase was used for covering the sample. From the results lower weights of 1-2 g are suitable for achieving higher recoveries. However for 5 g sample extended duration of extraction can improve the recoveries.

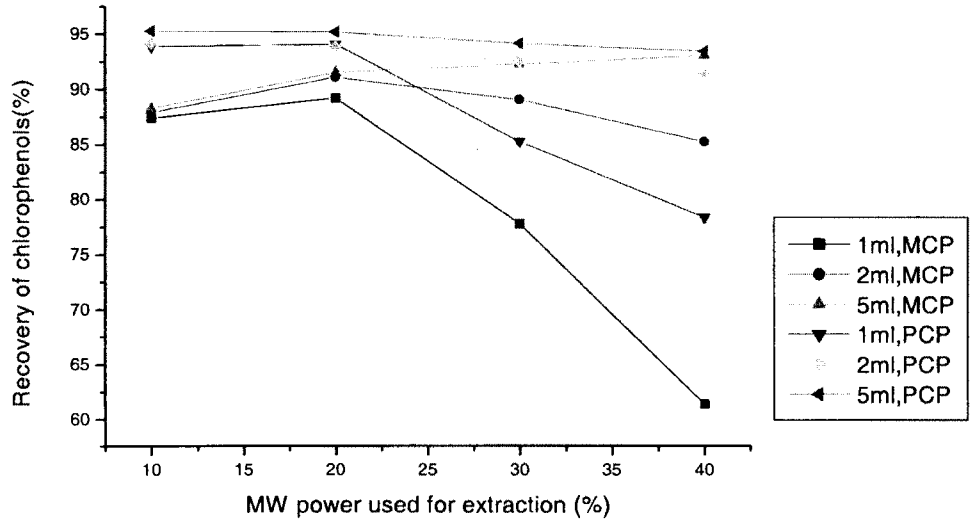

Figure 4.13 Influence of variation of solvent used for trapping in correlation to the variation of MWP from 10-40%. The other conditions: 25 ml aqueous phase and duration of extraction is 3 min

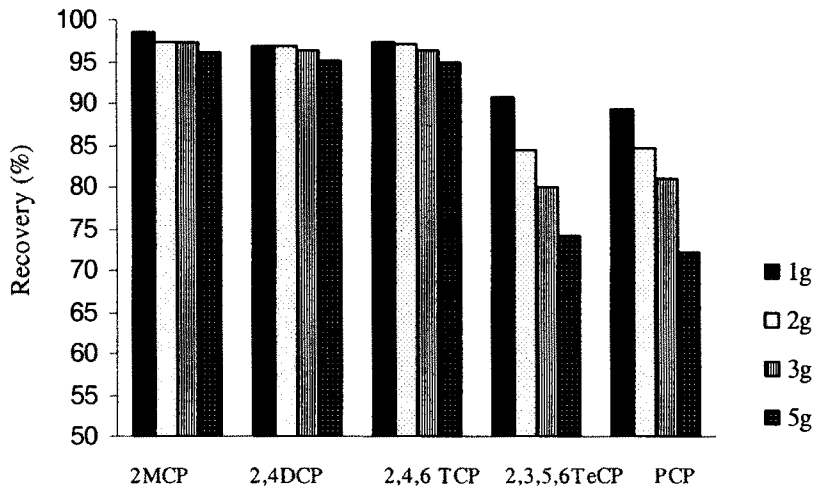

Figure 4.14 **Influence of weight of soil sample on Microwave extraction. Conditions:- 10-40% MWP was varied for a fixed duration of 8min (as that is suitable for volatiles) 25 ml of aqueous phase was used with 1 ml n-octane as trap.**

4.3.10 Analyses of samples (real and spiked)

The recoveries and LOD values obtained for various solid samples used for spiking are given in Table 4.1 and similar data for the spiked analytes in water samples are provided in Table 4.2. Real samples of both solid and water were collected. Many soil and water samples from near by industrial area were collected and other solid real samples were leather and textiles collected as samples from industries. The most probable sample for finding PCP is wood and hence old wood materials were sampled. Figure 4.15 shown represents the PCP detected originally in a leather sample to verify the enrichment, the chromatograms collected for this sample using n-octane 1 ml (A) and 5 ml (B) are shown. The enrichment is clearly observed in this trial. PCP was detected in

a textile sample at its original content and its chromatogram is shown in Figure 4.16. All these samples were analyzed by the proposed procedure and they were also analyzed by Soxhlet extraction in which case external acetyl derivatisation method was followed before GC analysis. Soxhlet extraction is taken as the referral method since it has been in practice for long and is also still part of several official protocols. This aside, MW extraction using higher amount of solvent (10 ml) followed by external derivatisation was also used for comparison. The data for the samples from the proposed procedure and those from parallel procedures are given in Table 4.3.

Table 4.3 Detection (by GC-ECD) of PCP in real samples by different extraction techniques

Nature of Sample	**Soxhlet extraction using acetone**		**MAE derivatised extraction using 1 ml n-octane as solvent trap**		**MAE derivatised extraction using 10 ml n-octane as solvent trap**	
	mg/kg	**%RSD**	**mg/kg**	**%RSD**	**mg/kg**	**%RSD**
Wood	31.5	2.3	32.1	2.9	31.9	1.9
Textile	2.3	1.9	2.8	3.1	2.6	3.1
Leather	29.2	4.4	30.1	2.4	29.9	2.4
Soil	0.2	4.1	0.2	2.9	0.2	3.0
Adhesive tape	19.3	3.1	20.1	2.6	20.2	2.3

4.4 ANALYSIS BY GAS CHROMATOGRAPHY

4.4.1 Instrumental conditions for chlorophenols:

DB-17 capillary column was used which is moderately non-polar in character. The injection port and detector (Electron Capture detector) temperatures were 200°C and 375°C respectively. The initial oven temperature was 80° C held for 2 min; and increased to 220°C at 15°C/min, held for 10 min, then at 15°C/min it was increased to 275°C and held for 5 min. 2 ml solution of chlorophenols prepared in isooctane was injected in splitless mode. Nitrogen gas was used as the carrier gas and as make–up gas for the detector.

The standard mixtures were run and retention times were identified. Then the spiked samples and real samples were run under the same condition.

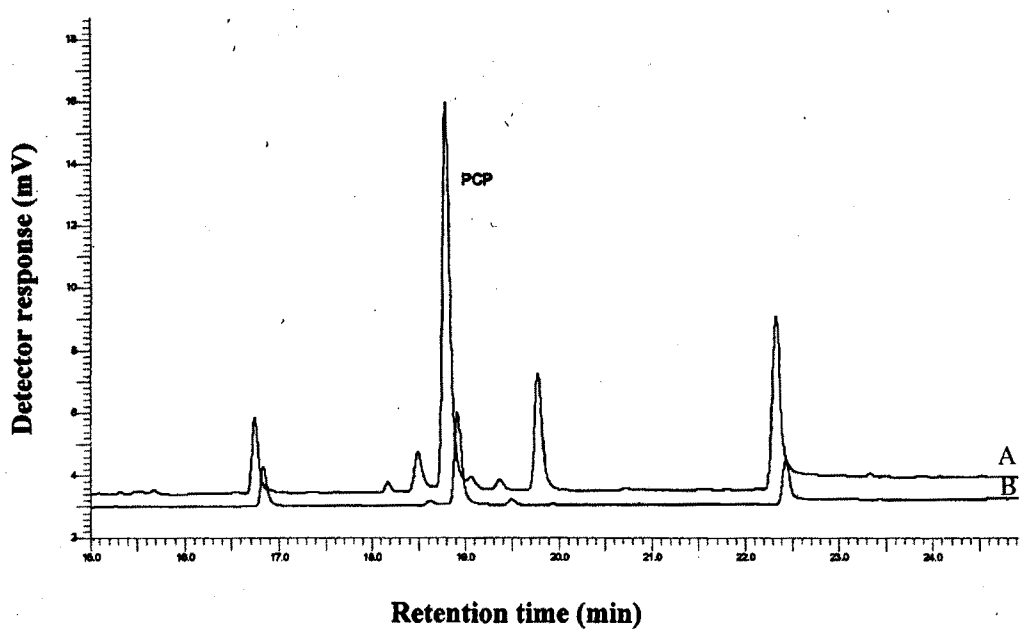

Figure 4.15 **Overlay of chromatograms of PCP extracted (as derivative) from a leather sample (native) A. extracted using 1 ml n-octane; B. extracted using 5 ml n-octane**

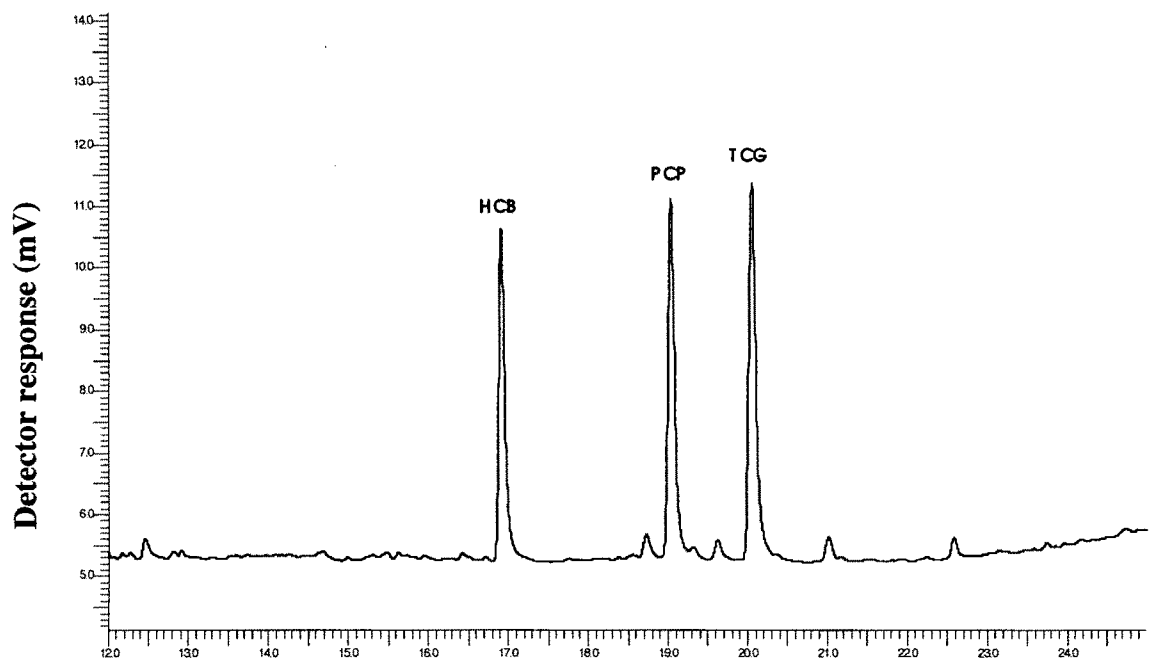

Figure 4.16 **GC chromatogram of a textile sample extracted by in situ derivatisation method using 1 ml of n-octane. PCP originally present in the sample is detected. TCG shown is an internal standard added while HCB is added as an injection marker**