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Tandem supercritical fluid extraction and liquid chromatography system for determination of chlorinated phenols in solid matrices

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ABSTRACT

A tandem supercritical fluid extraction–liquid chromatography system for determination of chlorinated phenols in various solid matrices is described. The system permitted direct introduction of supercritical fluid extracts into the liquid chromatograph, allowing quantitation down to the sub-parts per million (w/w) levels without any sample clean-up. The system performance compared favorably with the traditional methodologies in terms of both the analysis speed and the selectivity.

INTRODUCTION

Supercritical fluids with the low viscosities and relatively high densities are efficient solvents for a number of compounds. These fluids can be made selective solvents through change in density, brought about by relatively simple temperature and pressure manipulations. Due to rapid equilibration periods and variable solvating strength of these fluids, there has been considerable interest in the application of supercritical fluid extraction (SFE) in analytical chemistry. SFE has been used for rapid extraction of a variety of xenobiotics and extraction efficiencies ranging from 70–98% have been obtained for non-polar and moderately polar analytes such as polychlorinated biphenyls, chlorinated pesticides and phenols [1–3]. The extractions can be accomplished in much shorter periods than the liquid solvent-based extraction methods. In addition, SFE extracts are cleaner due to lower concentrations of interfering co-extractants. This last feature permits the direct introduction of extracts into analytical systems such as gas chromatography (GC), supercritical fluid chromatography (SFC), liquid chromatography (LC) and mass spectrometry (MS) [4–10].

The coupling of SFE and LC results in an integrated system which is suitable for a number of moderately polar and polar chemicals and a few applications of such systems have been reported in literature. Nieass *et al.* [11,12] used a coupled SFE–LC system for solubility assessment of organic compounds. Evaluation of a similar system for extraction and determination of valtrate and didrovaltrate from *Radix valerianae* was reported by Unger and Roumeliotis

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[13]. Schneiderman et al. [14] used a SFE–HPLC system with electrochemical detector for the determination of anthraquinone in paper and wood. Direct coupling of SFE with microcolumn LC has recently been described by Cortes et al. [15]. In this system micro-LC was used as a clean up (fractionation) step prior to GC separation and analysis.

Solvation and extraction of chlorinated phenols from solid matrices such as soil, wood and biological tissue have been under investigation in our laboratory. These studies have been facilitated by a tandem SFE–LC system. The system also performed well in the determination of phenols at trace levels, details of this application are presented in this article.

EXPERIMENTAL

A schematic of the SFE–LC system is shown in Fig. 1. The system consisted of a pneumatic amplifier (Model AAD-30; Haskel Engineering, Burbank, CA, USA), a pressure surge tank, extraction vessels and a liquid chromatograph. The pressure surge tank and extraction vessels were placed in a thermostated water bath. Car-

bon dioxide from the cylinder was compressed to desired pressure with the pneumatic amplifier. The compressed CO_2 was introduced into the 660-ml capacity surge tank. The surge tank acted as a reservoir for compressed CO_2 and also served to bring the CO_2 temperature down to the operating level. Samples were loosely packed into a glass wool lined wire mesh sample holder. The sample holders were placed in the extraction vessel and extraction vessel sealed. Extraction vessels were pressured by opening the inlet valve. The contents were allowed to equilibrate for periods ranging from 30 min to 2 h. After a set equilibration period on aliquate of equilibrated CO_2 was transferred to LC by operating the appropriate three-way valve and a vessel selection valve (V-3) and the sampling loop valve (V-2). Extraction vessels with internal volume of 150 ml were used. These vessels were designed to operate at pressures up to 400 atm (1 atm = 101 325 Pa) and were fabricated at the Science Instrument Shop, University of Missouri–Columbia. Three-way stainless-steel valves were purchased from High Pressure Equipment (Erie, PA, USA). The vessel selection valve (V-3) and sampling loop valves (V-1 and V-2) were ob-
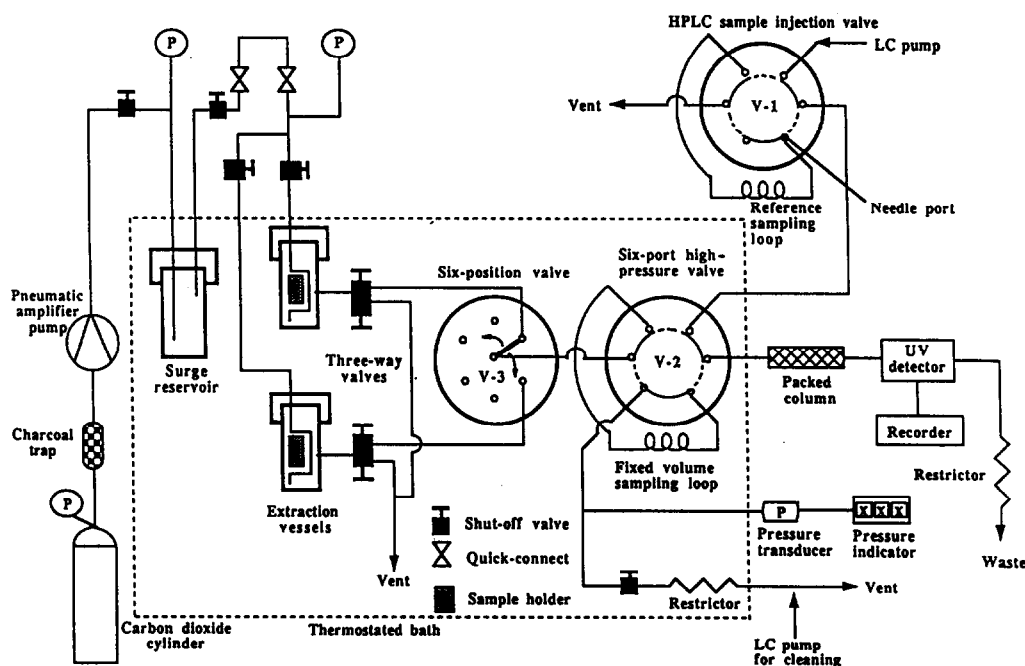

Fig. 1. A schematic of the on-line SFE–HPLC system.

tained from Rheodyne (Cotati, CA, USA). Incorporation of valve V-3 permitted sequential sampling of up to six extraction vessels. The details of sample preparation and SFE apparatus have been presented elsewhere [16].

A 20-μl loop (V-1) was used for introducing samples during the routine LC mode of operation, whereas a 50-μl loop (V-2) was used for introduction of the SFE extract. The LC system consisted of a bonded C_{18} column, a LC pump (Model series 4; Perkin-Elmer, Norwalk, CT, USA) and a UV–Vis detector (Model LC-85, Perkin-Elmer). To prevent formation of CO_2 bubbles, two linear restrictors consisting of 8 cm $\times$ 25 μm I.D. fused-silica tubing were attached at the end of the detector and the vent tube. The restrictor tubing was obtained from Polymicro Technologies (Tucson, AZ, USA). The selection of restrictors was based on the pressure limit of the detector cell, mobile phase flow-rate and composition. A low-volume pressure transducer (void volume *ca.* 10 μl) was installed in the back of sample loop restrictor to ascertain the pressure difference between the extraction vessel and the sampling loop.

The separation of chlorinated phenols was accomplished by reversed-phase chromatography with a 250 $\times$ 4.6 mm I.D. stainless-steel column with 5 μm C_{18} bonded silica packing (Supelcosil; Supelco, Supelcopark, PA, USA). Water–acetonitrile was used as the mobile phase, the composition being changed from 100% A (water–acetonitrile–acetic acid, 94:5:1) to 100% B (acetonitrile–acetic acid, 99:1) in 35 min using a linear solvent gradient. The absorbance of separated components was measured at 275 nm.

The initial evaluation of the SFE–LC system was carried out with a mixture of chlorinated phenols spiked on glass beads. However, optimal partition parameters were established for each matrix separately. This optimization involved selection of equilibration pressure, temperature, modifier, modifier concentration. The minimum detection limit (MDL) and linearity of response were determined by spiking different matrices with the phenol mixture over a concentration range of 1–500 ppm (w/w). For comparative purposes soil and wood shaving samples were also extracted by conventional liquid solvent
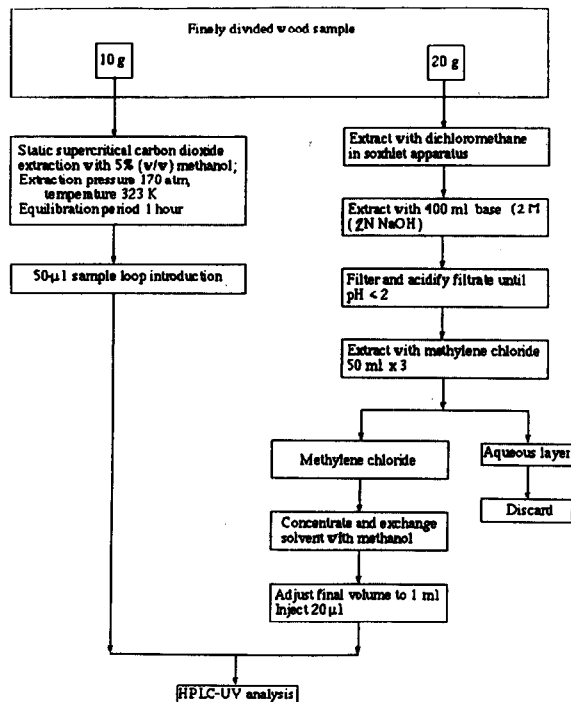

Fig. 2. Flow scheme for the determination of chlorinated phenols in wood shavings.

based methods which entailed Soxhlet extraction followed by partitioning of phenols and other acidic components into a strong base, neutralization of the base and back extraction of the phenols into methylene chloride. The extraction and clean-up schemes are outlined in the flow schemes in Fig. 2.

RESULTS AND DISCUSSION

The optimization of the system involved selection of SFE and LC parameters. These parameters were first selected independently through off-line extraction experiments and collection of extracts in methanol. Extracted phenols were analyzed by introduction of methanol into the liquid chromatograph through valve V-1. The objective of optimization experiments as to establish conditions which permitted highest selectivity, *i.e.*, where recovery of components of interest was highest and amount of coextractants lowest. In earlier studies it was pointed that optimal conditions are dependent not only on

the analytes but also on the matrix. For instance, while the non-polar analytes such as polychlorinated biphenyls (PCBs) and chlorinated biphenyls can be readily removed from biological matrices with carbon dioxide, recoveries of the same analytes is much worse from soil or sediments. These differences arise from the fact that these non-polar analytes are generally associated with non-polar portions of the biological tissues with which carbon dioxide exhibits better wetting properties than polar humic portions of soil and sediments. Distribution coefficients for a number of non-polar and moderately polar organics in different supercritical fluids and matrices have been determined in our laboratory. Most of these studies were conducted with an off-line extraction set-up [17]. These experiments showed that optimal extraction parameters for all matrices were in the near critical region, i.e., the extraction temperature of 40°C and the pressure of approximately 170 atm. Under these conditions a minimum equilibration period of 30 min was required to reach steady state concentration. As a result, in all experimental extractions, an equilibration period of 1 h was employed.

Under the optimized LC parameters separation of all phenols of interest was achieved in approximately 25 min. The first step in the evaluation of tandem SFE–LC was to monitor the integrity of the chromatographic separation. The introduction of pressurized carbon dioxide led to considerable deterioration in the chromatographic performance. The primary cause of this deterioration was bubble formation (entrapment of CO_2) at the exit end of the chromatographic column and the detector cell. High diffusivities of solutes molecules in the CO_2 band also led to peak broadening (Fig. 3). The band broadening problem was addressed through the modification of solvent gradient which entailed a longer initial hold and lowering of acetonitrile concentration in the eluent A from 30% to 5%. These changes allowed CO_2 band to elute away from most solutes of interest. The overall effect of these changes was an elongation of elution time from 25 min to 35 min. The problem of CO_2 bubble formation was eliminated by installing a restrictor at the outlet of the detector.
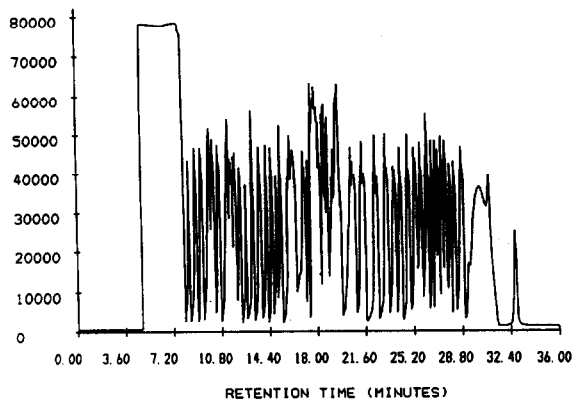

Fig. 3. Output of UV–Vis detector in the integrated system without outlet restrictor. y-Axis is reponse in arbitrary units.

Linear restrictors with 50 μm I.D. were used for the purpose. The back pressure was determined by the length of the restrictor and the flow-rate. In the present study an over pressure of 850 p.s.i. (1 p.s.i. = 6894.76 Pa) was found to be adequate for preventing bubble formation. The chromatographic separation of phenols obtained with the integrated SFE-LC is shown in Fig. 4. This separation was achieved by introducing 250 μg of phenols on 10 g of glass beads. Spiked glass beads were placed in extraction vessels and equilibrated with CO_2 at 170 atm and 40°C for 1 h. A 50-μl aliquot of CO_2 extract was introduced into the LC system through the sampling valve (V-2). The chromatographic performance of the system remained largely unchanged except for peaks co-eluting of CO_2 or immediately after it.
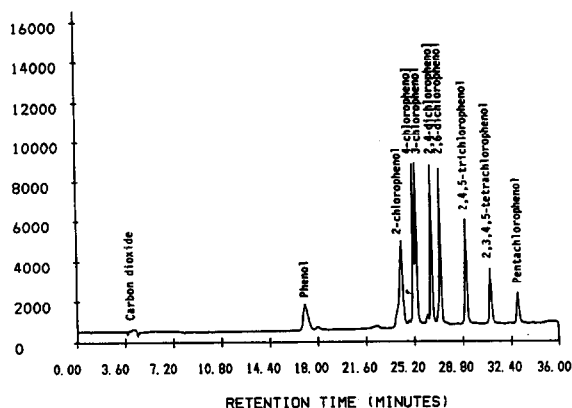

Fig. 4. Chromatogram of chlorinated phenols obtained with SFE–HPLC system under optimized gradient conditions and with outlet restrictors. y-Axis is reponse in arbitrary units.

The performance of the system was evaluated over a concentration range of 1–500 ppm with two other solid matrices: soil and wood shavings. A linear response was obtained over the entire range for each analyte with all three matrices. The calibration curves obtained with glass beads are shown in Fig. 5. A high degree of precision was obtained with the system. The standard deviation for six replicate analyses was less than 2%. It should be pointed out that detection limit and linear range in the system are interrelated and are depended on a number of parameters which include sample size, extraction vessel volume and the sampling loop volume. These parameters are in turn depended on partition ratios of the analyte in the given matrix/supercritical fluid system. The MDL of the system can be calculated through the following expression.

$$\text{MDL (ppm)} = d_1 \cdot \frac{V_{\text{EX}}}{V_{\text{L}}} \cdot \frac{1}{K} \cdot \frac{1}{S_{\text{w}}}$$

where d_1 is the instrument detection limit (μg), V_{L} is the volume of sampling loop (ml), V_{EX} is the volume of the extraction vessel (ml), K is the partition ratio of the analyte and S_{w} is the sample mass (g).

While little difference in chromatographic performance was observed in case of soil samples, the recoveries were generally low, falling in 60–65% range. To achieve better extraction efficiencies ($\geqslant$80%) a polar modifier such as methanol had to be introduced into the extraction vessel, and 5% (w/w) methanol was found to give optimal results. Under these conditions the complete analysis was performed in 1.5 h which compared favorably to the *ca.* 15 h required for traditional analytical methodologies.

System performance was found to be decidedly superior in case of complex matrices which contain high levels of interfering compounds, *e.g.*, wood samples with high pigment content. The analyses of chlorinated phenols in such
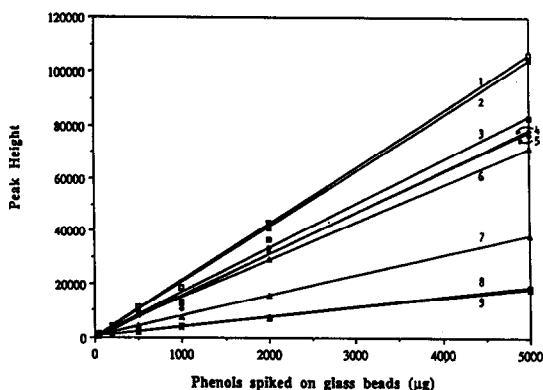
Fig. 5. Calibration curves for chlorinated phenol. Lines: 1 = phenol ($y = 499.62 + 3.4372x$, $R^2 = 0.999$); 2 = 2,4-dichlorophenol ($y = -775.79 + 15.790x$, $R^2 = 0.995$); 3 = 4-chlorophenol ($y = 95.502 + 16.680x$, $R^2 = 0.995$); 4 = 2-chlorophenol ($y = -81.644 + 15.496x$, $R^2 = 0.995$); 5 = 3-chlorophenol ($y = -570.43 + 20.923x$, $R^2 = 0.999$); 6 = 2,4,5-trichlorophenol ($y = -142.98 + 21.288x$, $R^2 = 0.999$); 7 = tetrachlorophenol ($y = 227.97 + 14.250x$, $R^2 = 0.999$); 8 = pentachlorophenol ($y = 253.30 + 75623x$, $R^2 = 1.000$); 9 = 2,6-dichlorophenol ($y = 279.73 + 3.6365x$, $R^2 = 0.999$).
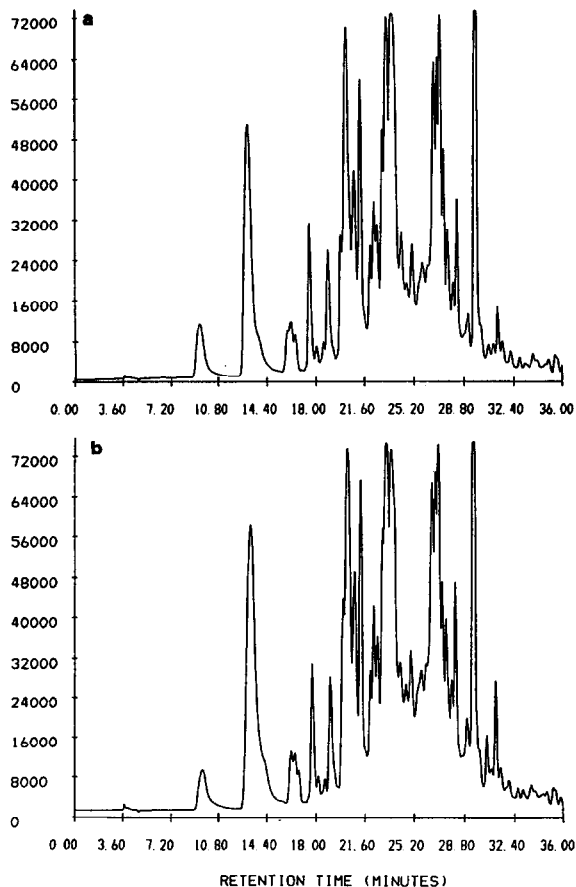
Fig. 6. (a) Chromatogram of liquid solvent extract of a wood shaving sample. (b) Chromatogram of liquid solvent extract of a wood shaving samples spiked with chlorinated phenols; spike concentration 20 ppm. *y*-Axes represent response in arbitrary units.
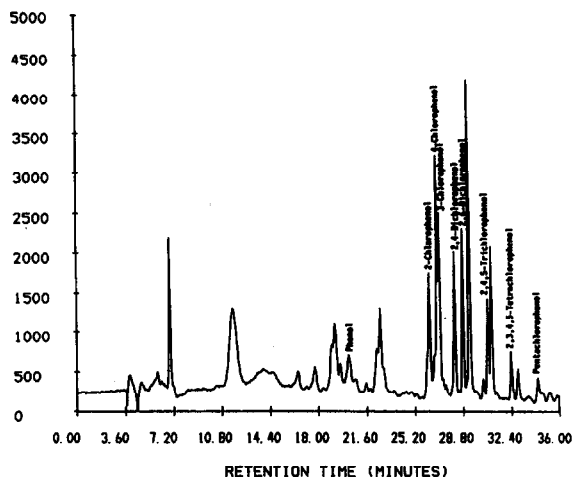
Fig. 7. Chromatogram of supercritical fluid extract of a wood shavings sample spiked with chlorinated phenols. Spike concentration 20 ppm. y-Axis is response in arbitrary units.

matrices are exceedingly difficult due to the presence of interferents. The chromatography of a wood shaving extract obtained by Soxhlet extraction, followed by extraction with a base, neutralization and black extraction into methylene chloride is shown in Fig. 6a. The complexity of the chromatographic data is self evident and prevented quantitation of spiked phenols (Fig. 6b). By contrast, the supercritical fluid extracts were decidedly cleaner with con-

siderably less pigment load. Results obtained with SFE–LC system for wood shaving samples are shown in Fig. 7; all of the phenols could be quantitatively determined to sub ppm level. A summary of spike recoveries and detection limits are given in Table I.

CONCLUSIONS

Quantitative recoveries for chlorinated phenols can be obtained with CO_2 under near critical conditions. The CO_2 extract can be easily and directly introduced into a LC system for rapid determination of these analytes.

ACKNOWLEDGEMENTS

TABLE I

DETECTION OF PHENOLS BY SFE–HPLC IN WOOD SAMPLE

Components	Recovery (%)[a]	Detection limit (in 15 g sample) (ppm)
Phenol	106.4	2.5
2-Chlorophenol	93.3	1.0
4-Chlorophenol	94.6	1.0
3-Chlorophenol	96.1	1.0
2,4-Dichlorophenol	100.7	1.0
2,6-Dichlorophenol	101.1	1.0
Trichlorophenol	85.2	1.0
Tetrachlorophenol	85.0	2.5
Pentachlorophenol	84.7	3.3

[a] Based on the reference standard spiked on the glass beads for SFE.

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