

Determination Of Chlorinated Phenols By Supercritical Fluid Extraction (SFE)
— Coupled Liquid Chromatography (LC) System

Liu, M.H., Kapila, S., Puri, R.K. and A. F. Yanders

Environmental Trace Substances Research Center, University of Missouri, Columbia,
Missouri 65203.

INTRODUCTION

Supercritical fluids with low viscosities and relatively high densities are efficient solvents for a number of compounds. These fluids can be made selective solvents through changes in density brought about by relatively simple temperature and pressure manipulations. Due to the rapid equilibration periods and variable solvating strengths 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 70-100% have been obtained for nonpolar and moderately polar analytes such as PCBs, chlorinated pesticides and phenols. 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).

A tandem SFE-LC system for determination of chlorinated phenols in various solid matrices has been fabricated in our laboratory. The system permits direct introduction of supercritical fluid extracts into the LC, allowing quantitation down to the sub-parts per million (ppm) levels without any sample cleanup step. The system performance compares favorably with the traditional methodologies in terms of both the analysis speed and the selectivity. In addition, the system permits determination of the absolute solubilities of a variety of substances in supercritical fluids.

EXPERIMENTAL

The SFE-LC system consisted of a pneumatic amplifier (Model AAD-30, Haskel Engineering Corp, Burbank, CA), a pressure surge tank, extraction vessels and a liquid chromatograph. The pressure surge tank and extraction vessels were placed in a thermostated water bath. Extraction vessels with internal volumes ranging from 15 - 150 ml were used. These vessels were designed to operate at pressures up to 400 atm, and were fabricated at the Science Instrument Shop, University of Missouri - Columbia. Three-way stainless steel valves were purchased from High Pressure Equipment Company, Erie, PA. The vessel selection valve (V-3) and sampling loop valves (V-1 and V-2) were obtained from Rheodyne, Inc., Cotati, CA.

A 20 μ l loop (V-1) was used for introducing samples during the routine LC mode of operation, whereas a 50 μ l 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 Corp., Norwalk, CT) and a UV/VIS detector (Model LC-85, Perkin-Elmer Corp.). To prevent formation of CO₂ bubbles, two restrictors were attached at the end of the detector and the vent tube. The selection of restrictors was based on the pressure limit of the detector cell, mobile phase flow rate and composition. Low volume pressure transducers (void volume \sim 10 μ l) were installed in the system for the pressure measurement.

The separation of chlorinated phenols was accomplished by reverse phase chromatography with a 250 x 4.6 mm i.d. stainless steel column with 5 μ C-18 bonded silica packing (Supelcosil, Supelco, Inc., Supelcopark, PA). The mobile phase composition was changed from 100%A (94% water, 5% acetonitrile and 1% acetic acid) to 100%B (99% acetonitrile and 1% acetic acid) 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. 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. For comparative purposes soil and wood shavings samples were also extracted by conventional liquid solvent 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.

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 operation of the LC in the independent mode. The off-line experiment 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 one hour 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 CO₂) at the exit end of the chromatographic column and the detector cell. Higher diffusivities of solute molecules in the CO₂ band also led to peak broadening. The band broadening problem was addressed through the modification of the solvent gradient, which entailed a longer initial hold and lowering of acetonitrile concentration in the eluent A from 30% to 5%. These changes allowed the CO₂ band to elute away from most solutes of interest. The problem of CO₂ bubble formation was eliminated by installing a restrictor at the outlet of the detector. In the present study, 25 cm long restrictors (50 μ i.d.) were used; these resulted in an over-

pressure of 850 psi, which was found to be adequate for preventing bubble formation. The chromatographic performance of the LC system remained largely unchanged after SFE coupling except for peaks co-eluting with CO₂ or immediately after it.

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. It should be pointed out that detection limit and linear range in the system are interrelated and are dependent on a number of parameters which include sample size, extraction vessel volume and sampling loop volume.

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

System performance was found to be decidedly superior for complex matrices which contain high levels of interfering compounds, e.g., wood samples with high pigment content. The analyses of chlorinated phenols in such matrices are exceedingly difficult due to the presence of interferents. By contrast, the supercritical fluid extracts were decidedly cleaner with considerably less pigment load. All of the phenols could be quantitatively determined to the sub ppm level.

CONCLUSION

Quantitative recoveries for chlorinated phenols can be obtained with CO₂ under near critical conditions. The CO₂ extract can be easily and directly introduced into a LC system for rapid determination of these analytes.

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