

Immobilized Liquid Extraction of Drinking or Waste Water as a New Sample Preparation Technique for GC/LC Analysis

ABSTRACT

Traditionally, techniques used for the extraction of organic compounds from drinking and waste water samples employed in EPA and Standard Methods are based either on liquid/liquid extraction or liquid/solid extraction employing solid phase extraction (SPE) disks or cartridges. Liquid/liquid extraction is the less desirable of the two because it involves considerable time, labor, and expense. SPE reduces solvent consumption, but still requires sample concentration, and is impractical when large quantities of particulates are present in a sample.

This study investigates Immobilized Liquid Extraction (ILE) as an alternative technique for preparing aqueous environmental samples for analysis by gas or liquid chromatography. ILE is a form of liquid/liquid extraction in which the “organic” phase is immobilized on the surface of an ILE device. Though the ILE method may be applied to a number of procedures involving the extraction of organic compounds from aqueous matrices (drinking water, blood serum, wastewater, etc.), the scope of this study is strictly environmental, specifically relating to EPA methods 508, 525, 8270 and 8082. The effectiveness of the ILE method was evaluated in the extraction and subsequent analysis of polychlorinated biphenyls (PCBs) and other semivolatiles from spiked water samples. Method detection limits, reduced sample sizes and solvent usage, complex matrix effects, and enhanceable selectivity are discussed.

INTRODUCTION

To best evaluate the viability of ILE as a potentially effective EPA method for preparing aqueous environmental samples, we performed a series of experiments to determine method detection limits, extraction efficiency and data precision.

ILE shares many of its fundamental principles with liquid/liquid extraction (LLE), solid phase micro extraction (SPME), and stir bar sorptive extraction (SBSE). The extraction is performed by an equilibrium process that depends on the analyte's partition ratio between the aqueous phase and the immobilized organic phase ($K_{PDMS/W}$), and on the phase ratio (β). As the ratio of the volume of organic phase relative to the volume of the sample (i.e., the phase ratio) increases, extraction efficiency increases. The partition ratio is analyte-specific, and defined as the concentration of an analyte in the PDMS relative the concentration of the same analyte in an equilibrated sample (note: the partition ratio of PDMS/Water is approximately equal to the partition ratio for octanol/water). From only

this information one can estimate the expected efficiency of the extraction of any compound.

As stated earlier, the “organic,” or extracting, phase is immobilized on the surface of an ILE device. This surface may be the inside of an autosampler vial, the inside surface of a bottle or vial cap, the inside surface of a disposable pipette or tip, or a number of other possibilities.

ILE Caps were chosen for this study. The septum of each ILE Cap is coated with a thin layer of a non-extractable homogeneous sorptive polymer. In this case, the most commonly used hydrophobic elastomer, polydimethylsiloxane (PDMS), was used, although selectivity may be altered or enhanced by using fluoro, phenyl or cyano siloxanes.

The polymer coating acts as the extraction medium, into which a targeted compound, or analyte(s), partition(s) from the sample matrix. Optionally a co-solvent may be used to enhance extraction speed and selectivity. The analyte(s) are then back-extracted from the polymer into a small amount of suitable solvent, and the resultant extract is ready for analysis by gas or liquid chromatography (in this experiment, all extracts were analyzed by a GCMS).

MATERIALS & METHODS

Instrumentation

Extracts were generated using the 24mm Immobilized Liquid Extraction (ILE) Cap. Each ILE cap septum is coated with a 508 micron thick layer of polydimethylsiloxane, resulting in a 122 μL “organic” phase. The appropriate volume of organic phase is determined by sample size, and caps with different volumes of polymer may be available to ensure the best results for samples of a variety of sizes.

Analysis of the extracts was performed by a Hewlett Packard 5971 GC-MS with a splitless injection port in scan mode and a 30 m x 0.25 mm i.d. x 0.25 μm VB-5 column. All samples were injected manually, which may be a source of additional uncertainty in recovered data.

Method

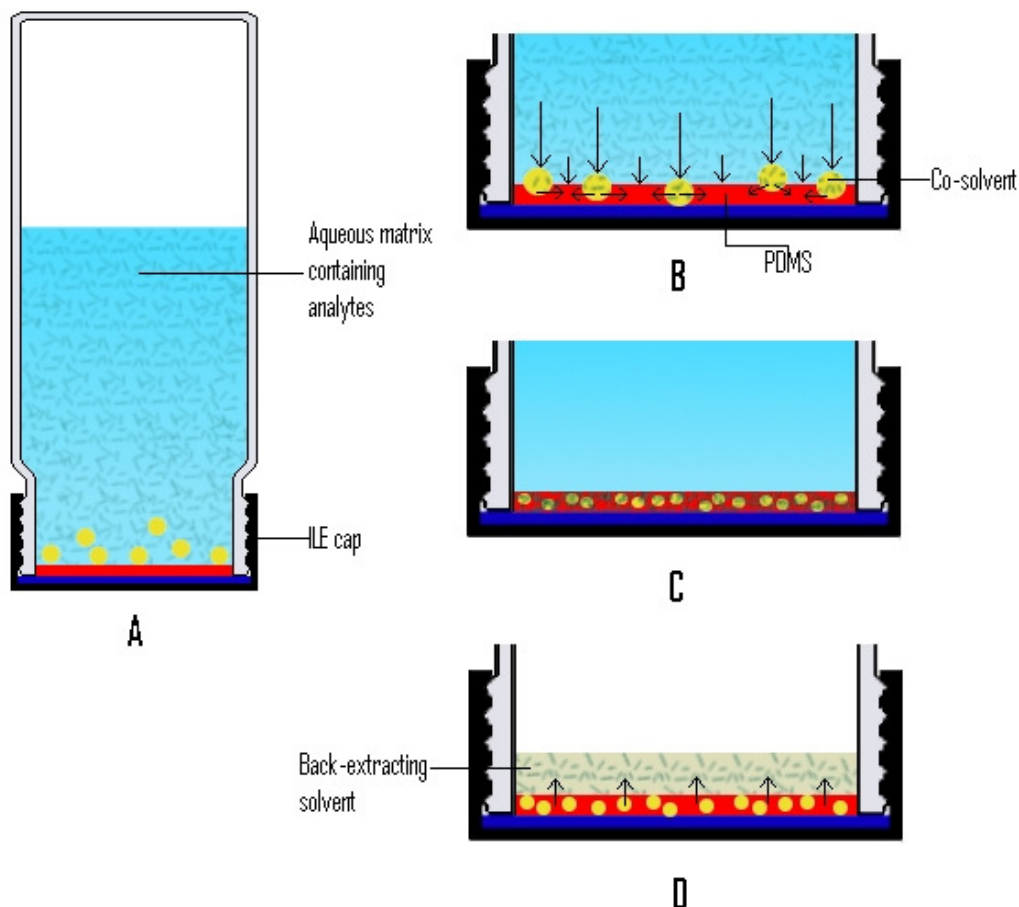
For each extraction, an ILE cap was placed on a 250 mL bottle containing a 100 mL water sample that was spiked with known quantities of one or more analytes. All internal standards and analytes of interest were present in each sample at concentrations of 0.5 $\mu\text{g/L}$ (other than pentachlorophenol, which was at a concentration of 2.0 $\mu\text{g/L}$). A 100 μL aliquot of methylene chloride was added to each sample to add selectivity to and enhance the partitioning of analytes from the sample into the polymer (this co-solvent extraction step is described and evaluated in greater detail in the discussion section). The sample-containing vessel was then agitated by a mechanical wrist-shaker for 1 hour in a manner

such that the cap came in repeated contact with the sample. This agitation disrupts the sample-polymer interface, thereby accelerating partitioning from the sample into the PDMS.

Upon completion of the extraction, the analyte-laden cap was removed and placed on a 10 mL conical bottom vial containing 100 μ L of acetone. The vessel was then agitated for 30 minutes to accelerate partitioning from the methylene chloride swelled PDMS into any solvent that exceeds the swelling capacity of the polymer. The resultant extract was then analyzed by gas chromatography coupled with mass spectrometry.

The complete extraction procedure is detailed in Figure 1 below. **(A)** The initial sample containing analytes and co-solvent is inverted to allow contact between the sample and polymer. **(B)** When adding a co-solvent to the ILE process, the rate of extraction is enhanced by increasing both total solvent surface area and volume. Analytes are first rapidly sorped into the co-solvent followed by concurrent sorption of the co-solvent and analytes into the polymer phase. The resultant partition ratio will be analogous to that of an aqueous phase and a mixed solvent system. **(C)** The polymer layer, swollen with a water immiscible co-solvent contains partitioned analyte(s). **(D)** The back-extraction process consists of the partitioning of the analyte(s) into a suitable solvent from the co-solvent swollen polymer.

Figure 1: ILE Cap Procedure



Calibration

Calibration spike solutions were composed by diluting appropriate aliquots of Accustandard M-525.2-SV-ASL in a 50:50 mixture of acetone and methylene chloride such that the concentrations of analytes of interest would correlate to a 100mL sample that is extracted with one-hundred percent efficiency. Calibration spikes were then analyzed by GCMS, and the MS response of the quantitation ion of each analyte in the spike was then related to the MS response of the quantitation ion of the internal standard with the nearest retention time, and an average response factor (RF) was assigned to each compound of interest (Equation 1). This response factor was later used to calculate the observed concentration, and subsequently, the extraction efficiency of a processed sample.

Equation 1: Response Factor

$$RF = \frac{(A_x) \cdot (Q_{is})}{(A_{is}) \cdot (Q_x)}$$

Where:
 A_x = integrated abundance of the quantitation ion of the analyte
 A_{is} = integrated abundance of the quantitation ion internal standard
 Q_x = quantity of analyte in calibration spike (μg)
 Q_{is} = quantity of internal standard in calibration spike (μg)

Data Collection

The observed concentration of an identified compound in an extracted sample was calculated by relating the MS response of the quantitation ion of said compound to the MS response of the quantitation ion produced by a compound used as an internal standard (Equation 2).

Equation 2: Observed Concentration Calculation

$$C_x = \frac{(A_x) \cdot (Q_{is})}{(A_{is}) \cdot RF \cdot V}$$

Where:
 C_x = concentration of analyte in water sample ($\mu\text{g/L}$)
 A_x = integrated abundance of the quantitation ion of the analyte
 A_{is} = integrated abundance of the quantitation ion internal standard
 Q_{is} = total quantity of internal standard added to water sample (μg)
 V = original water sample volume (L)
 RF = mean response factor of analyte from the initial calibration

Additionally, standard deviations and method detection limits (MDL) were determined for all compounds of interest.

RESULTS

Table 1 (below) displays a cross section of analytes of varying volatility, mass, and polarity. This data indicates that ILE has the capability to reproducibly extract a variety of semi-volatile analytes in the sub-nanogram/Liter range from 100 mL drinking and waste water samples.

Table 1: Calculating Method Detection Limits

Compound	True Conc. (µg/L)	Mean Observed Conc. (µg/L)	Relative Standard Deviation (%)	Mean Method Accuracy (% of True Conc.)	MDL (µg/L)
Acenaphthalene	0.5	0.37	8.3	73.9	0.17
Benzo(b)fluoranthene	0.5	0.48	6.8	95.6	0.14
Benzo(g,h,i)perylene	0.5	0.50	28.2	99.9	0.57
2-Chlorobiphenyl	0.5	0.49	6.3	97.1	0.13
2,3- Dichlorobiphenyl	0.5	0.57	1.2	113.1	0.02
Dibenz(a,h)anthracene	0.5	0.48	4.7	96.4	0.09
2,2,4,4,5,6-Hexachlorobiphenyl	0.5	0.53	8.8	106.4	0.18
Indeno(1,2,3-cd)pyrene	0.5	0.52	1.7	104.9	0.03
Pyrene	0.5	0.47	8.5	93.3	0.17
2,4,5- Trichlorobiphenyl	0.5	0.55	10.9	110.7	0.22

DISCUSSION

Smaller Sample Sizes, Reduced Solvent Usage, Matrix Effects

Current EPA-approved methods for analyzing semivolatiles in water require the use of an entire 1 L sample in order to provide data that is within the parameters that are required by the EPA (MDLs, precision, etc.). The ILE method requires only 100 mL of a 1 L sample to provide precise results and sufficient detection limits. This is possible because the ratio of extract volume to sample volume is identical to that associated with current EPA-approved methods. In current methods, a 1 mL extract is formed from a 1 L sample, whereas in ILE, a 100 µL extract is composed from a 100 mL sample (both result in a concentration factor of 1,000).

There are a number of advantages involved with this reduction in required sample size. First, in the case of operator error or equipment malfunction, the entire sample is not lost.

Additionally, an analyst has the capability to archive an analyzed sample or to analyze the sample under similar conditions to permit verification of data. Last, a sample may be analyzed using a range of co-solvent / polymer combinations to enhance or alter selectivity.

The ILE procedure provides many other distinct advantages over currently accepted and practiced methods for environmental sampling. Both SPE, and especially LLE, involve complex, labor-intensive, multi-step procedures in which each step can introduce errors or loss of analytes. The ILE procedure is composed of two non laborious steps. Additionally, solvent usage is decreased by factors greater than 100 and 1000 relative to SPE and LLE, respectively.

Many significant problems associated with the SPE method are avoided by ILE caps. ILE caps are immune to clogging by dirty samples, particulates, and viscous samples, and analyte breakthrough is avoided because ILE is an equilibrium reaction. Similarly, problems involved with LLE, such as the formation of emulsions, are avoided.

Co-Solvent Extraction & Sample Cleanup

In this series of experiments, a small amount of methylene chloride was added to each sample as a co-solvent to enhance partitioning from the sample into the polymer extraction medium. Compounds that may have a relatively low affinity for the PDMS may partition into the methylene chloride, which is effectively absorbed into the polymer, causing a sponge-like swelling effect. This co-solvent may be any organic solvent that is both absorbed by the polymer and is immiscible in water.

Different co-solvent/polymer combinations will yield different results, as many analytes may have a greater or lesser relative affinity for different solvents and/or polymers. For example, the appropriate combination of solvent and polymer may enhance selectivity for isolating (non)-halogenated analytes, PCBs from other interferants, or polar from non-polar analytes.

The addition of a co-solvent to a sample may also be omitted. Data collected from extractions both with and without co-solvent assistance suggests that some analytes may have a significantly greater affinity for methylene chloride than for either acetone or the PDMS. Because PDMS is capable of absorbing a much greater quantity of methylene chloride than acetone, analytes that retain a high concentration in the methylene chloride through the back-extraction process may yield a lesser MS response than expected. An analyst may also strategically determine his or her back-extracting solvent to enhance or alter specificity as he or she desires.

Additional specificity may be obtained by adding a rinsing or solvent cleanup step prior to the back-extraction of analytes from the polymer coating. This step is composed of rinsing the cap with a water miscible aqueous phase to essentially remove, or clean up, unwanted analytes (this step is common in SPE).

CONCLUSIONS

ILE is a promising new method for extracting organic compounds from aqueous matrices, and has terrific potential for environmental applications. The method consists of a simplified procedure that requires minimal operator effort, reduces solvent usage and allows for the analysis of smaller samples without compromising limits of detection and data precision. The possibilities to alter selectivity through strategic combinations of solvents and polymers will allow an experienced analyst to zero in on a compound, or type of compounds, with incredible ease. Further studies investigating the potential of the method in environmental and other applications are in progress.

REFERENCES

1. U.S. Environmental Protection Agency, 1995. *Method 525.2 – Determination of Organic Compounds in Drinking Water by Liquid-Solid Extraction and Capillary Column Gas Chromatography / Mass Spectrometry*. Office of Research and Development