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# Theoretical and Practical Comparison of Solid Phase Micro - extraction and Liquid - Liquid Extraction with Large Volume Injection for Analysis of Aqueous Samples by Gas Chromatography

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# Theoretical and Practical Comparison of Solid Phase Micro-extraction and Liquid-Liquid Extraction with Large Volume Injection for Analysis of Aqueous Samples by Gas Chromatography

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**Key Words:** sample preparation; solid phase microextraction (SPME); liquid liquid extraction; large volume injection

**Summary**

Sampling methods for gas chromatography have recently received much attention. New techniques, such as solid phase micro-extraction and large volume injection have made the routine analysis of ultra trace impurities in water at the sub-part per billion level possible. The capillary GC analysis of compounds from an aqueous matrix generally requires that the compounds be extracted into a suitable solvent before analysis. In SPME, the fiber coating serves as solvent, whereas, in large volume injection, an appropriate volatile organic solvent is used. Thus, SPME can be directly compared with LVI that employs liquid-liquid extraction prior to injection. Due to its small volume, the SPME fiber forces the method to include a significant concentration of the analyte as it transfers from the aqueous matrix to the fiber. This pre-concentration may not occur in LVI-based methods. Several scenarios for SPME and pre-LVI extractions will be compared for maximum recovery, based on calculations from partition theory and from experimental data. Based upon the above comparisons, and from data on column and detector capabilities available in the literature, it will be possible to theoretically compare ideal detection limits and linear ranges for the two methods. These theoretical calculations will be compared to experimental data for a test mixture of polycyclic aromatic hydrocarbons. Considerations in the practical operation of the two techniques will also be discussed.

## 1 Introduction

### 1.1 Extraction and Sample Introduction Techniques

With the recent proliferation of new sampling methods for capillary gas chromatography, it is common for practitioners to bre faced with difficult choices as to which technique is appropriate for a specific analytical problem. Since its introduction in 1989 [ 1] and its commercialization in 1992, solid phase micro-extraction has generated much attention, including over 100 papers, describing myriad applications. Extraction theory in SPME was described thoroughly by Louch, Matlagh and Pawliszyn in 1992 [2] and the optimization of SPME injection techniques has been addressed by several authors. [3,4] SPME has been shown to be effective for analyzing a wide variety of compounds at ultra trace levels, including volatile organic contaminants [5], polycyclic aromatic hydrocarbons [6] and steroids. [7] For these and many other applications, SPME has proven to be a

viable alternative to classical liquid-liquid or solid phase extractions, with major benefits including reduced solvent consumption and ease of use. The development of electronically controlled pneumatics for GC inlets has led to many improvements in gas chromatography, especially the development of large volume injection. With the advent of large volume injection, and its subsequent commercialization, another route to ultra-trace analysis is available. There are two important configurations of large volume injection capable inlets: a solven vapor exit, which involves injection of the sample onto a retention gap, which is vented prior to sample introduction on the analytical column, and solvent venting, which involves the trapping and thermal desorption of solvent vapor and the analytes within a packed inlet liner. [8,9] When combined with liquidliquid extraction, both of these methods havle also proven capable of analyzing compounds at partper-trilliuon levels from water. In this study, solid phase micro-extraction in which extraction and analysis are accomplished using a single device, is compared to micro-liquid-liquid extraction-large volume injection (MLLE-LVI), in which a liquid-liquid extraction is performed within a sample vial and the organic phase is injected onto the capillary column through a large volume GC inlet.

### 1.2 Theoretical Comparison

Using partition theory, calculation of the maximum possible recovery from any extraction method is straightforward. For SPME, this was described in detail by Louch. et.al., [2] and for liquidliquid extractions, this is described in many classical undergraduate laboratory texts. [ 10] The main requirements for calculating any theoretical extraction recovery are a knowledge of the solubility of the analyte and the volume of both phases. With the extraction recovery calculated, it can be combined with the possible injection volume, detection limits and linear range of the GC to obtain a theoretical range for use of the technique.

### 1.3 Practical Comparison

SPME and MLLE-LVI are significantly different in the apporach to injecting analytes onto the column, with the main differences being the volume of the organic phase and the presence of the liquid solvent in the MLLE procedure. In both cases, classical extraction equilibrium theory guides practice. The lack of liquid solvent is one of the major advantages of SPME, while LVI allows the use of minimal liquid solvent, with a large volume injected onto the column. In SPME, the volume of the organic phase is significantly (3 orders of magnitude) smaller than in MLLE, using 1 mL of organic solvent. Thus, to achieve the same recovery, a higher partition coefficient is needed for SPME. The physical manipulations required for both techniques are similar, with both being possible in manual and automated modes. In order that the physical manipulations are comparable, MLLE will be considerd without any sample concentration prior to injections. Both of these will be taken as “fill the vial, extract and inject” methods.

## 2 Materials and Methods

EPA Method 610 test mixture, 100-2000 mg/ml (Supelco, Bellfonte, PA), was diluted 100 fold with hexane (Aldrich, Milwuakee, WI) and injected using an Optic 2 (Atas, USA, Whittier, CA) inlet, installed into a 5890 Series II gas chromatograph (Hewlett-Packard, Wilmington, DE) equipped with flame ionization detector and 3392 integrator. The carrier gas was helium. The inlet was maintained at 50$^{o}$C for the 4 minute solvent vent time, then ramped at 4$^{o}$C/min to 280$^{o}$C and held throughout the GC run. The inlet liner was packed with 500 mg of Tenax to provide a large surface area for adsorption and evaporation. The GC column was programmed at 50$^{o}$C initial, 10$^{o}$C/min to 300$^{o}$C and held. The column was 30 m by 0.32 mm by 0.25 m SPB-1 (Supelco). 50

L of sample was injected using a gas tight syringe (Hamilton, Reno, NV). Theoretical calculations were performed using Excel 97 (Microsoft, Redmond, WA) on IBM-compatible personal computers.

## 3. Results and Discussion

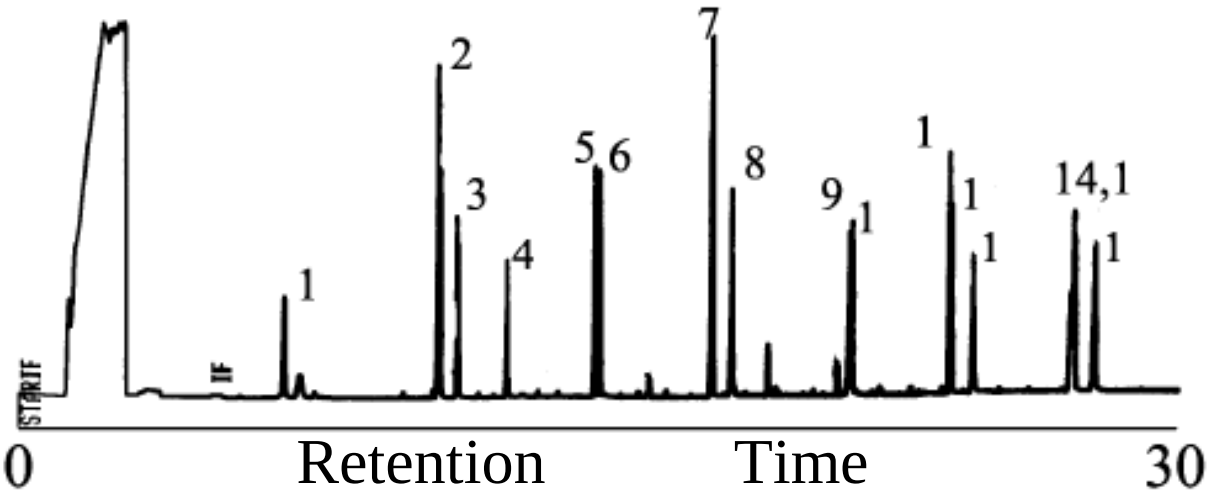


Figure 1 – Solvent elimination LVI chromatogram of EPA 610 Test Mixture. Peak identifications: 1. Naphthalene, 2. Acenaphthylene, 3. Acenaphthene, 4. Fluorene, 5. Phenanthrene, 6. Anthracene, 7. Fluoranthene, 8. Pyrene, 9. Benzo(a)anthracene, 10. Chrysene, 11. Benzo(b)fluoranthene, 12. Benzo(k)fluoranthene, 13. Benzo(a)pyrene, 14. Indeno(1,2,3-cd) pyrene, 15. Dibenzo(g,h)anthracene, 16. Benzo(g,h,i)perylene. Solven vent time 4 min, inlet temperature 50oC (during vent), 4$^{o}$C/min to 280$^{o}$C and hold. Splitless time 1.5 min.

A chromatogram of the EPA method 610 test mixture, diluted 100-fold, obtained using the Optic 2 large volume inlet is shown in Figure 1. Several aspects of any analysis involving large volume injection are illustrated. First, discrimination against early eluting compounds is seen. In this sample, naphthalene is present at 20 ng/L and has the highest concentration, yet it shows the smallest response here. This method can discriminate strongly against higher volatility analytes. Response for thelater eluting components is stronger than would be expected by comparison with the splitless injection of the same analyte mass. These difficulties must be kept in-mind when performeing the recovery comparisons described below.

### 3.1 Theoretical Comparison of Extraction Recovery

In an extraction, the number of moles of analyte transferred from the aqueous phase to the organic phase is given by:

$$n_{org} = {}^{K}(V_{org}/V_{aq})(no_{aq} - n_{org}) \quad (1)$$

where $n_{org}$ is the number of moles transferred to the organic phase, K is the partition coefficient, $V_{org}$ and $V_{aq}$ are the volumes of the organic phase and aqueous phase, $n_{o\ aq}$ is the initial number of moles of analyte in the aqueous phase. To calculate the portion of analyte moles transferred to the organic phase, it is rearranged to give:

$$n_{org}/no_{aq} = [K(V_{org}/V_{aq})] / [\ 1 + {}^{K}(V_{org}/V_{aq})] \quad (2)$$

In the MLLE studies described here, the aqueous and organic volumes are the same, 1 ml, so these terms divide out of the expression, giving:

$$n_{org}/no_{aq} = K / 1+K \quad (3)$$

For SPME, the volume of the thickest available fiber (100 mm polydimethyl siloxane) is $6.6 \times 10^{-4}$ ml. These calculations will assume that the entire fiber volume particiaptes in extracting the analyte, although this may not be the case in practice.

Figure 2 shows the calculation of $n_{org}/no_{aq}$ for MLLE and SPME, based upon partition coefficient. It is seen for MLLE that nearly exhaustive extractions are seen, even for relatively low partition coefficients. A value of $n_{org}/no_{aq} = 1$ indicates an exhaustive extraction. 95% recovery is seen for K equal to about 20. For SPME, extraction does not become exhaustive until K is approximately $10^4$-$10^5$ and may never be exhaustive due to limitations in fiber capacity. Direct comparisons, however, are difficult to make, unless an actual sampling situation is considered.

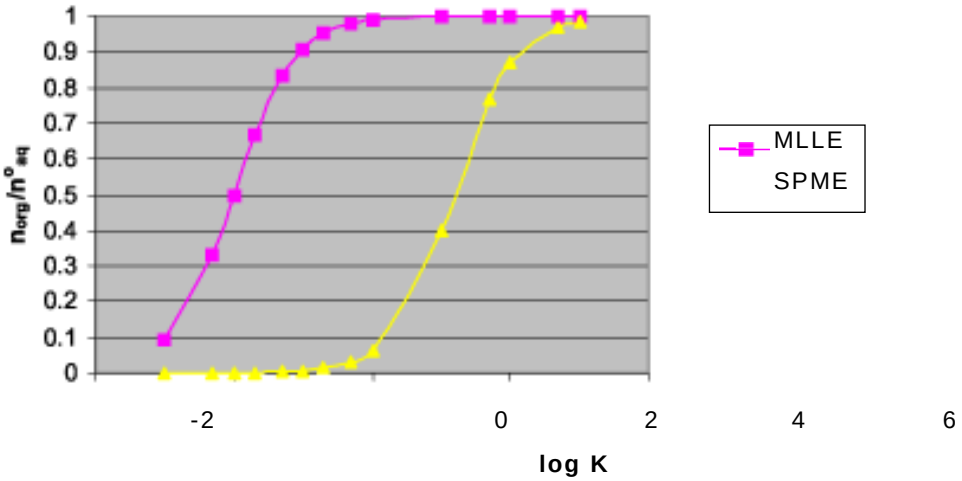


Figure 2 – Theoretical Extraction Recovery for MLLE and SPME.

Partition coefficient data for SPME has been generated by several workers [5,6,11]. SPME has also been used to estimate octanol-water partition coefficients. Partition coefficients for several of the PAH in the EPA method 610 test mixture were determined by Potter and Pawliszyn [6] and are given in Table 1, below.

| Compound | Log K (SPME) | Log K (octanol-water) |
|---|---|---|
| Naphthalene | 3.01 | 3.01-3.59 |
| Anthracene | 4.10 | 4.54 |
| Benz(a)anthracene | 4.96 | 5.61 |
| Benzo(a)pyrene | 4.86 | 6.44 |

Table 1 – Partition Coefficients for Several PAH. Data from reference 6.

If a 1 ng/ml sample of each PAH in water is assumed, then the molar mass can be used to determine the number of moles present. The number of moles extracted is calculated using equations 2 and 3 and is then converted back to mass to give the final mass extracted by each technique. The SPME partition coefficents shown above areused for the SPME case, while the octanol-water partition coefficients are used for MLLE, although these may be a conservative estimate, based upon our choice of toluene as solvent for these extractions. The $K_{ow}$ values might more closely mimic those obtained for these compounds in hexane.

Figure 3 shows the results of this calculation for the PAH shown in Table 1. Again, it appears that MLLE can extract a larger quantity of material, but for these compounds, which have very high partition coefficients, both techniques offer nearly exhaustive extraction from a a 1 ml sample containing 1 ng of analyte. When injection volume is considered, SPME is clearly superior in this case, as the SPME extraction sllows the entire extract to be injected in a single step, whereas injection volume in MLLE-LVI is limited to 50-100 l, or 5-10% of the total extract.

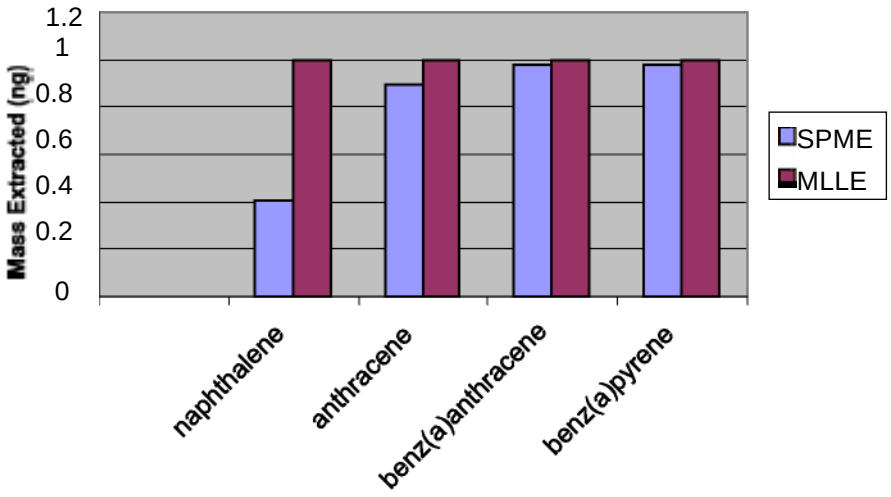


Figure 3 – Theoretical MLLE and SPME reciveries for Polycyclic Aromatice Hydrocarbons. Calculated for extraction from 1 ml of a 1 ng/ml aqueous solution.

The above analysis describes the situation where the partition coefficient is large (>1000). The calculation is easily extended to describe a wide range of partition coefficients. Figure 4 shows the same calculation for partition coefficients ranging from 1-$10^6$. It is seen that MLLE extracts considerably more analyte as the partition coefficients decrease.

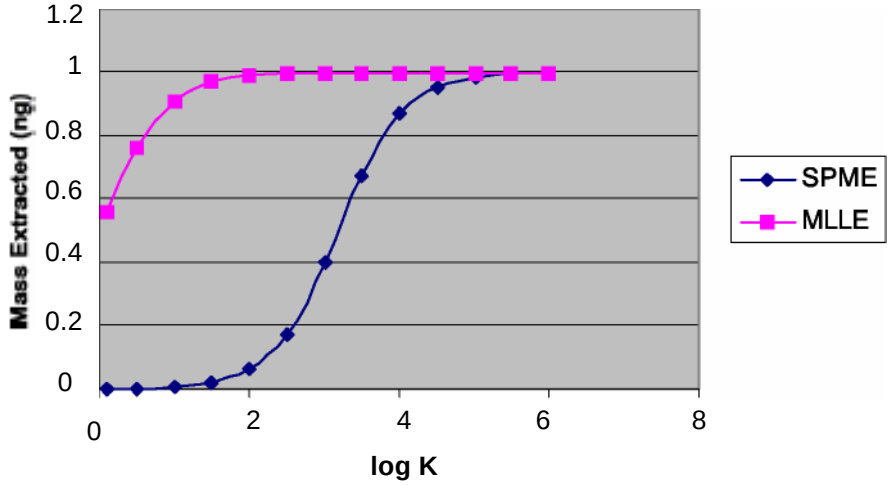


Figure 4 – Mass of Analyte Extracted From 1 ng/ml Aqueous Sample by SPME and MLLE

### 3.2 Injection Volume Consideration

In Figure 4, the volume of analyte extracted by MLLE and SPME, as it relates to the partition coefficient, K, is shown. The actual amount of analyte transferred to the Gc column, however, depends upon th e portion of the extract that can be injected during a single injection. For the SPME extraction, the entire extract is placed into the GC inlet and essentially 100% desorption and transfer to the column can be assumed. For the MLLE –LVI case, the extract has a volume of 1 mL and the maximum injection volume is 50-100 L, or 5-10% of the extract.

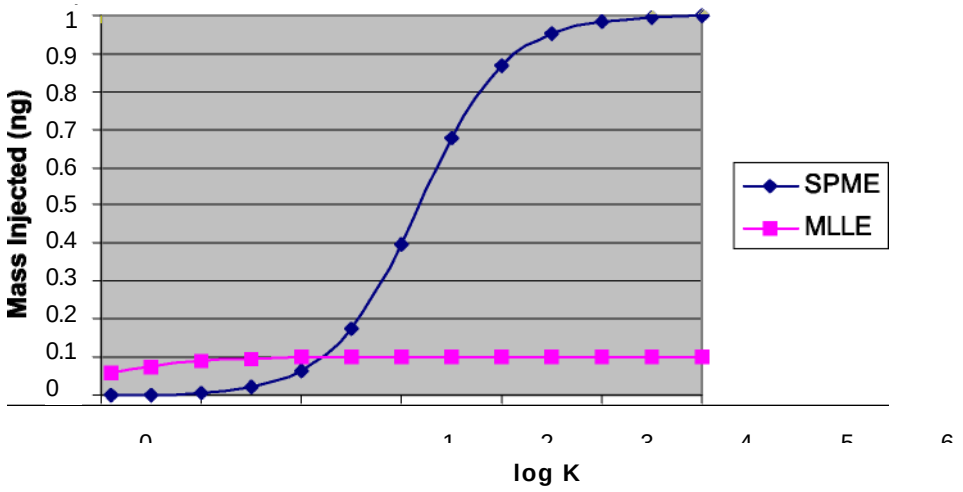


Figure 5 – Mass of Analyte Injected in MLLE and SPME Calculated from Partition Coefficients. Assume 100 L LVI with MLLE.

In figure 5, the mass of analyte injected onto the column is shown for both techniques, again assumeing a 1 ng/mL aqueous sample and a range of partition coefficients from 0.1-$10^6$. A 100 mL injection volume is assumed for the large volume injection and 100% recovery of both injections onto the GC column is assumed. The curves in Figure 5 intersect at approximately logK = 2.3, or K = 200. Thus, SPME can be expected to provide more sample on column for those compounds with K > 200, while MLLE provides more on column for those with K < 200.

Returning to the example of the PAH test mixture, it is seen form Figure 1 that in the MLLE-LVI injection, the injection conditions play a significant role in the final response. Note the discriminaiton against naphthalene and the strong repsonses for the last eluting compounds. For this sample, it can be expected that an SPME injection will less discrimination against the early

elutinng componnents, but will discriminate against the later eluting compounds. Thus, in the development of any method involving these extraction techniques, the injection parameters must be carefully considered, as described in references 3,4 8 and 9.

## 4. Conclusions

The fundamental partition theory involved in solid phase micro-extraction and micro liquid liquid extraction have been compared. It has been shown that in nearly all cases, MLLE results in a larger quantity of analyte being extracted from the aqueous sample. However, the difference in Gc injection procedures requires that only a small portion of the MLLE sample can be redily injected in a single injection step. For a typical trace sample, it has been shown that SPME will povide more analyte on column in cases where the extractionpartition coefficient exceeds 200, where MLLE will provide more sample for cases where K < 200. This will have a strong prcticval influence on the choice of analytical method for a given problem. This choice was illustrated using a sample of polycyclic aromatic hydrocarbons, which show, from partition coefficients, superior extraction and injection by SPME.

## 5. Acknowledgments

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