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Calibration of solid-phase microextraction for quantitative analysis by gas chromatography

Gangfeng Ouyang [1], Yong Chen, Lucie Setkova, Janusz Pawliszyn *

Faculty of Science, Department of Chemistry, University of Waterloo, 200 University Avenue West, Waterloo, Ont., Canada N2L 3G1

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Abstract

Calibration of SPME by the injection of liquid standard solution is a common method for SPME quantitative analysis by gas chromatography. The feasibility of this calibration method relies on the assumption that sample transfer efficiencies are the same for both the liquid injection and the SPME fiber injection. Sample transfer efficiencies for liquid injection and SPME fiber injection were studied in this paper. The results showed that the sample transfer efficiency for liquid injection was affected by several factors, such as the dimensions of the liner, the presence of the wool, and the temperature of the injector. The sample transfer efficiency for SPME fiber injection was affected by the cross-sectional area of the space between the column and the liner, the carrier gas flow rate, and the length of the column inside the liner. An equation was proposed to estimate the sample loss rate for SPME injection. It was found that the use of a direct injection (DI) liner and program temperature vaporizing provides high sample transfer efficiencies, for both liquid injection and SPME fiber injection. When a common SPME straight liner is used, large outer diameter (o.d.) pre-column will help to achieve high sample transfer efficiency.

Keywords: Calibration; Solid-phase microextraction; Gas chromatography; Liquid injection; SPME fiber injection; Sample transfer efficiency; Direct injection (DI) liner; SPME liner

1. Introduction

SPME is used as a simple, high efficient, and reliable sample preparation technique for the analysis of a variety of organic compounds [1–4]. In SPME, a thin layer of polymeric coating that is coated on the outside of a fused silica rod is used to extract analytes. The small volume of the extraction phase allows for the combination of sampling, sample preparation, and sample introduction into one step. It also differs from other extraction techniques in that only small portion of analytes is removed from the sample matrix, which results in unique calibration approaches in SPME for quantitative analysis.

Equilibrium extraction is the most frequently used method [5]. When a sample volume is very small, exhaustive extraction might occur in SPME and can be used for calibration. To shorten long equilibrium extraction times, and/or address the displacement effects that occur when porous coatings are used, extraction can be interrupted before equilibrium, and calibration is still feasible if the agitation and the extraction time remain constant [6]. While performing derivatiziation/SPME, when the reaction is the rate-limiting step, the first-order reaction rate constant can be used for calibration [7]. The last approach is diffusion-based calibration method for rapid reaction rate [8–10]. Most of the calibrations can be conducted in the same manner as other extraction techniques, assuming that the amounts of analytes extracted on the fiber are linearly proportional to the detector response. However, for some calibration approaches, it is required that the absolute amounts of the analytes are known, which are most often calibrated by the injection of liquid standards.

* Corresponding author. Tel.: +1 519 888 4641/885 1211; fax: +1 519 746 0435.
E-mail address: janusz@uwaterloo.ca (J. Pawliszyn).
[1] Permanent address: School of Chemistry and Chemical Engineering, Sun Yat-sen University, Guangzhou 510275, PR China.

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For both liquid injection and SPME fiber injection, usually more than 95% of the sample is supposed to be transferred into the capillary column with splitless injection. However, the rate of sample transferred into the column for liquid injection is affected by many factors, such as the dimensions of the liner, the presence of the wool, the temperature of the injector, etc. [11–18]. The sample transfer efficiency for SPME fiber injection is also affected by the diameter of the inject insert, the fiber desorption position and time in the injector, the carrier gas flow rate, the cross-sectional area of the space between the column and the liner, the length of the column inside the liner, etc. [19–21].

The expansion associated with the solvent vaporization will cause analyte loss when liquid standards are injected by common injection methods, especially with high temperature liquid injection [22]. However, SPME is a solvent-free sample preparation and sampling technique, and the sample transfer efficiency for SPME fiber injection is affected by different factors. When liquid injection is used to calibrate SPME fiber injection, the sample transfer efficiency of liquid injection might be different from that of SPME fiber injection if the analytes are not completely transferred into the GC column. The best scenario is, of course, that the analytes are completely transferred into the GC column for both the liquid injection and the SPME fiber injection. When this is difficult to achieve, the analytes should be transferred into the GC column at the same rate to ensure correct calibration.

In this paper, the effects of the inner diameter (i.d.) of the liner, the temperature of the injector, wool, and solvent types on sample transfer efficiencies of liquid injection were investigated. The sample transfer efficiencies were obtained by comparing the results with on-column injection results. For SPME fiber injection, the effects of the outer diameter (o.d.) of the column, the length of the column inside the liner, and the carrier gas flow rate on the sample transfer efficiency were investigated. The sample transfer efficiencies were estimated by comparing the results with the SPME fiber injection using a direct injection (DI) liner (SPI or Drilled Uniliner®) (Fig. 1).

2. Experimental

2.1. Materials and reagents

Naphthalene, acenaphthene, fluorene, anthrancene, fluoranthene, pyrene were purchased from Supelco (Bellefonte, PA, USA). Benzene, toluene, ethylbenzene, *o*-xylene were purchased from Sigma–Aldrich (Mississaga, Ont., Canada). HPLC grade methanol was purchased from Fisher Scientific (Nepean, Ont., Canada). HPLC grade acetone was purchased from EMD (Gibbstown, NJ, USA). Hamilton Model 701 10 μL syringe, On-column syringe, fiber holder, and 100 μm poly(dimethylsiloxane) (PDMS) fibers were obtained from Supelco (Oakville, Ont., Canada). N_2, H_2, He, and air were supplied by Praxair (Kitchener, Ont., Canada) and were of ultra high purity.

2.2. Instrument

Gas chromatography was performed on three instruments. One is a Varian (Mississauga, Canada) 3800 gas chromatograph coupled with a Saturn 2000 MS system controlled by computer using Varian Saturn Workstation software (ver. 5.51). The second one is a Varian (Mississauga, Canada) 3800 gas chromatograph coupled with flame ionization detection (FID) using Star Chromatography Workstation (ver. 5.51). Both the Varian GC–FID and GC–MS were equipped with a

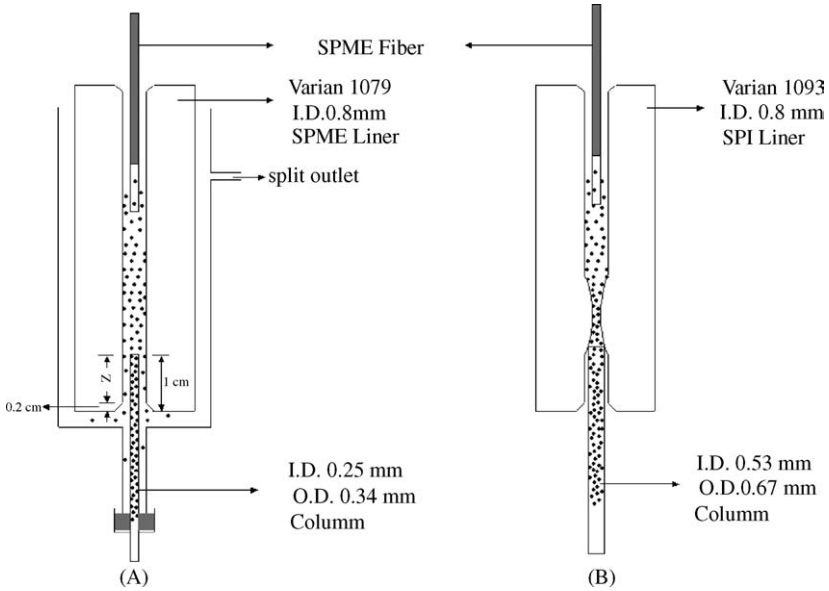

Fig. 1. Comparison of SPME liner with SPI liner for SPME injection. (A) SPME linear; (B) SPI linear.

1079 injector and fitted with a SPB-5 column (30 m, 0.25 mm i.d., 0.25 μm film thickness) (Suplco). The third one is an Agilent 6890 GC coupled with a Leco® Pegasus III TOF-MS. It was equipped with a split/splitless injector and fitted with a RTX-5 column (10 m, 0.18 mm i.d., 0.20 μm film thickness).

For Varian GC, when perform liquid injections, the oven was initially set at 40 °C for 2 min and then ramped at a rate of 30 °C/min to 250 °C, which was held for 21 min. When perform SPME fiber injections, the oven was set at 50 °C for 1 min and then increased to 120 °C at a rate of 20 °C/min. The carrier gas (helium) rate was set to 1 mL/min. The injector was programmed to return to split mode 5 min after liquid injection and 2 min after SPME fiber injection, if it was not equipped a SPI liner. The 1079 injector temperature was set at 250 °C for both SPME fiber injection and high temperature liquid injection. For on-column injection and program temperature vaporizing (PTV) injection, the injector was set at 40 °C and then increased to 250 °C at a rate of 200 °C/min.

For Agilent GC, the split/splitless injector was set at 250 °C, oven was set at 40 °C, isothermal, and carrier gas (helium) rate was set at 1 mL/min. The total analysis time was 3 min.

2.3. Liquid injections

One hundred parts per million polycyclic aromatic hydrocarbons (PAHs) methanolic solution, including naphthalene, acenaphthene, fluorene, anthrancene, fluoranthene, pyrene, was used for liquid injection experiments. The SPME liner injection and SPI liner injection experiments were performed by a CTC CombiPal autosampler (Zwingen, Switzerland) using the associated Cycle Composer software (ver. 1.4.0). For all liquid injection experiments, the liquid sample was withdrawn from the needle into the glass barrel (sample followed by 2 μL air), and then rapidly introduced to the injector. The sample volume introduced into the injector was 1 μL. (The real sample volume withdrawn into the barrel was 1.9 μL. For PTV injection, 1 μL of sample was introduced into the injector and 0.9 μL of sample remained in the needle. For high temperature injection, 1.3 μL of sample was introduced into the injector and 0.6 μL sample remained in the needle). To ensure the sample volumes introduced into the injector were equal, the sample volumes introduced into the injector for both automatic injection (Hamilton 701 10 μL syringe) and manual injection (On-column syringe) were calibrated with six injections under the same GC conditions.

1. On-column injection (Fig. 2A). A Varian 1093 SPI liner, which was installed upside down, was used for on-column liquid injection. An i.d. 0.53 mm × 20 cm uncoated pre-column was tightly connected to the SPI liner. On-column injections were performed by manual injection with a 10 μL on-column syringe with a 7.5 cm × 0.21 mm o.d. needle.
2. Regular injection (Fig. 2B), i.d. 0.8 mm (SPME liner), 2.0 mm and 3.4 mm liner for Varian 1079 injector were used for regular injection.
3. SPI liner injection (Fig. 2C). Varian 1079 injector was equipped with an i.d. 0.8 mm Varian 1093 SPI liner for liquid PTV injection (SPI liners for the 1079 injector are not commercially available. The only difference between the 1093 liner and the 1079 liner is that their outer diameters are different). The liner was tightly connected with an i.d. 0.53 mm pre-column to ensure that the bottom of the liner was sealed by the column. Liquid samples were injected by the CTC CombiPal autosampler with a Hamilton 701 10 μL syringe. Sometimes split peaks

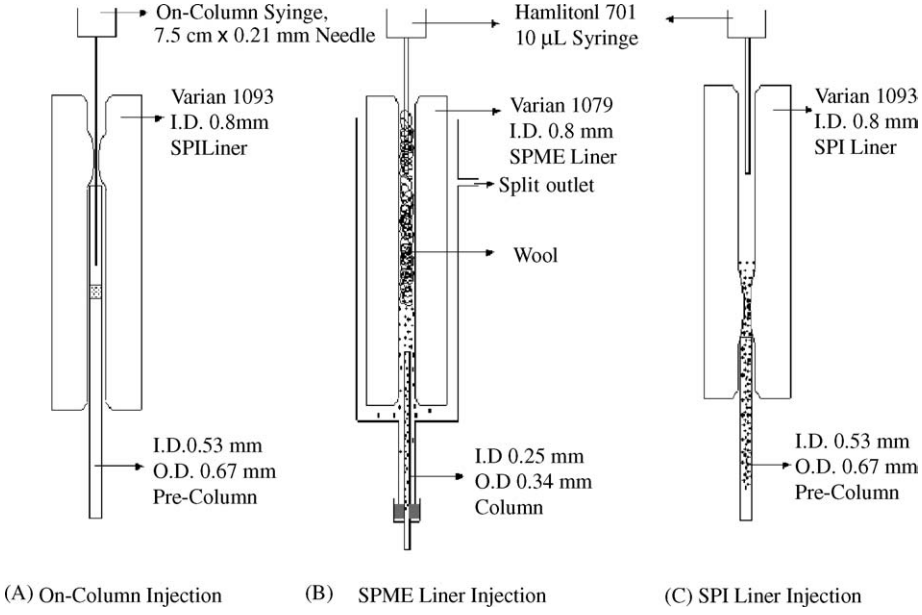

Fig. 2. Comparison of different liquid injection methods.

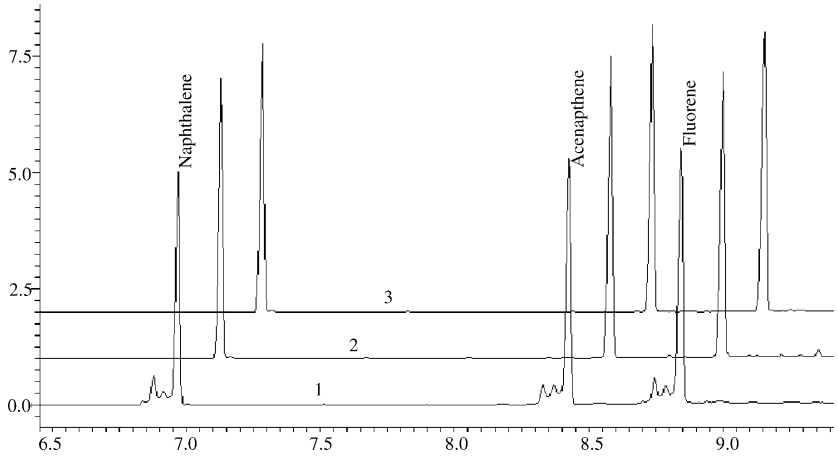

Fig. 3. Comparison of peak shapes for SPI liner liquid injection: (1) 20 cm pre-column, the line was not filled with wool; (2) 100 cm pre-column, the line was not filled with wool; and (3) 20 cm pre column, the line was filled with wool.

were observed, which could be eliminated by the use of a liner filled with wool or a longer pre-column (Fig. 3) [12,18,23,24].

2.4. SPME fiber injection

Two hundred parts per billion BTEX (benzene, toluene, ethylbenzene, and *o*-xylene) aqueous solution was used for SPME injection experiments. A SPME holder with 100 μm PDMS fiber was used for the headspace solid-phase microextraction.

For Varian GC, the SPME fiber injections were performed by manual operation: 25 mL of the 200 ppb BTEX aqueous solution was filled in a 40 mL vial capped with a phenolic screw cap and PTFE-coated silicone septa (Supelco), and a 1" (2.54 cm) PTFE-coated stirring bar (Supelco) was used to agitate the solution at 1200 rpm (VWR Scientific). The solution was agitated for 15 min before a PDMS 100 μm fiber was used to sample. The headspace extraction time and GC desorption time were each 2 min. The more detailed procedures for this experiment are described previously in the litereature[19]. An i.d 0.8 mm SPI liner was used for SPI liner SPME fiber injection experiments (Fig. 1B) and i.d 0.8 mm SPME liner was used for the other SPME injection experiments (Fig. 1A). To ensure reproducible results, experiments were performed on both the GC–FID and the GC–MS.

For Agilent GC, the SPME fiber injections were performed by a CTC Analytics CombiPal autosampler using the associated Cycle Composer software: 3 mL of the 200 ppb BTEX aqueous solution was filled in a 10 mL vial capped with a phenolic screw cap and PTFE-coated silicone septa. The temperature was set at 50 °C. The solution was agitated for 5 min at 500 rpm before a PDMS 100 μm fiber was used to sample. The headspace extraction time was 2 min and GC desorption time was 1 min. An i.d. 0.8 mm SPME liner and an i.d. 1.0 mm Drilled Uniliner® liner were used for SPME fiber injections.

3. Results and discussion

3.1. Liquid injection

The effects of the liner i.d., wool, injector temperature, and solvent type on detector response were studied, respectively. The relative ratios of the peak areas were obtained by comparing the results with on-column injection.

3.1.1. Liner i.d. and wool

The effects of liner i.d. and wool on the sample transfer efficiency were determined by program temperature vaporizing (PTV) injection. The results are presented in Table 1. The

Table 1
The effect of wool and liner i.d. on relative peak areas ($n = 6$, liquid injection)

		Naphthalene (RSD %)	Acenaphthene (RSD %)	Fluorene (RSD %)	Anthrancene (RSD %)	Fluoranthene (RSD %)	Pyrene (RSD %)	Average
On-column		1 (1.8)	1 (1.2)	1 (1.5)	1 (2.0)	1 (1.9)	1 (1.5)	1
i.d. 0.8 mm SPME liner	With wool	0.95 (0.2)	0.93 (0.2)	0.94 (0.2)	0.92 (0.5)	0.92 (0.3)	0.90 (0.2)	0.93
	Without wool	0.65 (6.5)	0.64 (9.1)	0.64 (9.5)	0.60 (12.2)	0.60 (14.5)	0.59 (14.2)	0.62
i.d. 2.0 mm liner	With wool	0.84 (0.5)	0.83 (0.8)	0.85 (0.5)	0.85 (0.4)	0.85 (0.4)	0.84 (0.3)	0.84
	Without wool	0.73 (6.0)	0.74 (0.7)	0.71 (1.0)	0.63 (3.0)	0.62 (3.9)	0.61 (3.6)	0.67
i.d. 3.4 mm liner	With wool	0.79 (2.8)	0.77 (3.8)	0.76 (3.1)	0.70 (1.2)	0.67 (1.5)	0.66 (2.0)	0.72
	Without wool	0.76 (4.6)	0.73 (0.5)	0.76 (1.2)	0.75 (1.0)	0.76 (1.9)	0.75 (1.7)	0.75

Table 2
Relative peak areas for ptv injection and high temperature (250 °C) injection ($n = 6$, liquid injection)

	Naphthalene (RSD %)	Acenaphthene (RSD %)	Fluorene (RSD %)	Anthrancene (RSD %)	Fluoranthene (RSD %)	Pyrene (RSD %)	Average
On-column	1 (1.8)	1 (1.2)	1 (1.5)	1 (2.0)	1 (1.9)	1 (1.5)	1
i.d. 3.4 mm liner, PTV, with wool	0.79 (2.8)	0.77 (3.8)	0.76 (3.1)	0.70 (1.2)	0.67 (1.5)	0.66 (2.0)	0.72
i.d. 3.4 mm liner, high temperature, with wool	0.56 (2.8)	0.66 (2.0)	0.69 (1.7)	0.72 (2.9)	0.74 (3.0)	0.75 (2.8)	0.69

results illustrate that the best transfer efficiency, except on-column injection, was obtained using the smallest i.d. liner with wool. However, wool exhibited different effects for different i.d. liners. When the liner was filled with wool, the liner with the smaller i.d. provided the highest sample transfer efficiency. When the liner was not filled with wool, the larger the liner i.d., the higher the sample transfer efficiency. These findings can be explained by the following observations. When the liner is filled with wool, and the sample is evaporated with a PTV method, the vapor of the sample is unlikely to backflush. In addition, the linear flow rate of the carrier gas is higher for smaller i.d. liners with smaller dead volume, so a higher sample transfer efficiency can be expected with liners that possess smaller i.d.s. On the other hand, when an empty liner is used (no wool), the vapor of the sample is more likely to backflush for liners that possess smaller i.d.s. In addition, the sample solution could be dispersed inside the liner, or even be injected directly onto the bottom of the injector body for cold injection if there is no wool inside the liner and fast injection is used. Therefore, the sample transfer efficiency obtained with the 0.8 mm i.d. liner without wool was low, and exhibited poor RSD %. For the i.d. 3.4 mm liner, the sample transfer efficiencies of anthrancene, fluoranthene and pyrene were even smaller when the liner was filled with wool than when it was empty. This might be due to the increase of the dead volume in the liner and the adsorption of these analytes by the glass wool.

3.1.2. Injector temperature

Because high temperature injection needs a large injector chamber [14], an i.d. 3.4 mm liner (the largest i.d. liner available for Varian 1079 injector) was used for the injection experiments to determine the effect of injector temperature on sample transfer efficiency. Table 2 illustrates the results of the relative ratios of peak areas for PTV injection and high temperature injection. For high temperature injection experiments, the actual sample volume introduced into the injector was 1.3 μL, and the relative peak areas were calibrated to 1 μL. The results show that the sample transfer efficiencies were lower than 70%, although the largest i.d. liner for this injector was used. Similar results were obtained by Gebhart et al. [22].

On-column injection exhibited the best transfer efficiency because the sample was injected directly into the column, and with a program temperature vaporizing the sample is less likely to backflush. However, there are some drawbacks associated with on-column injection. On-column injections require a special on-column syringe with long and small o.d needles, a special inlet liner, a large i.d. column, and the approach is unsuitable for sample injection with a common autosampler.

Sample transfer efficiency for liquid injection was already higher than 90% when an i.d. 0.8 mm SPME liner with wool was used (Table 1). The reason that not all analytes were transferred into the column was probably due to the loss of sample by diffusion through the interspace between the column and the inner wall of the liner (Fig. 2B). To verify the assumption, a SPI liner was used for liquid PTV injection (Fig. 2C). The comparison of transfer efficiency for the SPI liner injection and on-column injection is presented in Table 3. The results suggest that the sample transfer efficiencies for the SPI liner injection were close to those of the on-column injection experiment.

3.1.3. Solvent type

Lee et al. [25] discussed the effects of solvents on the response factors for PAH solutions, and found that the sample transfer efficiencies were different for different solvents (because high temperature injection method was used, the volume of vapor produced by evaporating 1 μL of sample varies, depending on the molecular weight and density of the solvent [11]). It is, thus, important to further investigate the effects of solvents on the sample transfer efficiency and response factor.

Methanol, acetone, and toluene were used to prepare 100 ppm PAHs standard solutions, and the response factors of 1 μL of the standard solutions were determined by PTV SPI liner injection method. The relative ratios of the peak area, compared with on-column injection, for different solvents are summarized in Table 4, which suggest that there is not a big

Table 3
Comparison of transfer efficiency with spi liner injection and on-column injection ($n = 6$, liquid injection)

	Naphthalene (RSD %)	Acenaphthene (RSD %)	Fluorene (RSD %)	Anthrancene (RSD %)	Fluoranthene (RSD %)	Pyrene (RSD %)	Average
On-column	1 (1.8)	1 (1.2)	1 (1.5)	1 (2.0)	1 (1.9)	1 (1.5)	1
SPI liner	0.97 (1.4)	0.97 (1.6)	0.99 (1.2)	0.99 (1.0)	0.98 (0.8)	0.99 (1.0)	0.98

Table 4
Relative peak areas of liquid injection with different solvents

	Naphthalene (RSD %)	Acenaphthene (RSD %)	Fluorene (RSD %)	Anthrancene (RSD %)	Fluoranthene (RSD %)	Pyrene (RSD %)	Average
Methanol ($n=6$)	0.97 (1.4)	0.97 (1.6)	0.99 (1.2)	0.99 (1.0)	0.98 (0.8)	0.99 (1.0)	0.98
Acetone ($n=3$)	0.95 (2.4)	1.00 (1.6)	0.98 (2.6)	0.99 (1.2)	0.99 (2.5)	0.98 (1.8)	0.98
Toluene ($n=3$)	0.95 (0.7)	0.97 (0.1)	0.98 (1.9)	0.96 (0.5)	0.96 (0.6)	0.94 (0.3)	0.96

difference between the response factors for different solvents by PTV SPI liner injection. This is because almost all of the analytes were transferred into the column when the PTV SPI liner injection method was used. Therefore, the solvent will not affect the response factors of the analytes. However, different solvent will obtain different transfer efficiency of the analytes when the injection method is not a PTV method and the liner is not a DI liner.

3.2. SPME fiber injection

The results of the relative ratios of the peak areas for SPME fiber injection (Varian GC) with a SPME liner and a SPI liner are summarized in Table 5. For SPME liner injection, the column i.d. was 0.25 mm and the o.d. was 0.34 mm. The length of the column inside the liner was 1 cm. It was found that about 9–16% of the analytes (BTEX) was lost during the SPME liner injection, compared with SPI liner injection.

The losses of the analytes due to diffusion could be estimated by:

$$n_L = RCt \quad (1)$$

where n_L is the amount of the lost analytes (g), R is the diffusion rate (cm^3/s), C is the sample concentration at the entrance of the column (g/cm^3), and t is the diffusion time (s). Diffusion rate R can be calculated by:

$$R = D\left(\frac{A}{Z}\right) \quad (2)$$

where D is the diffusion coefficient of the analytes (cm^2/s), A is the cross-sectional area for the space between the liner and the column (cm^2), and Z is the diffusion distance (cm). Combining Eqs. (1) and (2) leads to:

$$n_L = DCt\left(\frac{A}{Z}\right) \quad (3)$$

The total amount of analytes n_T can be calculated by:

$$n_T = Ctv \quad (4)$$

where C is the sample concentration at the column entrance (g/cm^3), and t is the time for transferring the sample into the column (s), which is equal to the diffusion time, and v is the sample flow rate (cm^3/s). Therefore, the rate of loss of the analytes is:

$$\frac{n_L}{n_T} = \frac{DA}{Zv} \quad (5)$$

Eq. (5) implies that the rate of loss of the analytes is affected by A, the cross-sectional area of the space between the liner and the column, which is determined by the liner i.d. and the column o.d., Z, the diffusion distance, which is determined by the length of the column inside the liner (Fig. 1A), v, the sample flow rate, which is determined by the flow rate of the carrier gas, and D, the diffusion coefficient, which can be estimated by [26]:

$$D = 10^{-3}\frac{T^{1.75}[(1/m_{gas})+(1/m)]^{1/2}}{P[\overline{V}_{gas}^{1/3}+\overline{V}^{1/3}]^2} \quad (6)$$

where T is the absolute temperature (K) of the injector, m_{gas} is the molecular mass of the carrier gas, m is the molecular mass of the chemical of interest, P is the gas phase pressure (atm), $\overline{V}_{gas}$ is the molar volume of the gas, and $\overline{V}$ is the molar volume of the chemical of interest. For example, for the analysis of BTEX, the injector temperature was 250 °C, the carrier gas was helium, the liner i.d was 0.8 mm, the column i.d was 0.25 mm and the o.d was 0.34 mm, the length of the column inside the liner was 1 cm ($Z=0.8$ cm), the flow rate of the carrier gas was 1 mL/min (0.0167 cm^3/s), and the head pressure was 6.7 psi (absolute pressure is about 1.5 atm). Thus, the calculated rate of sample loss will be about 10% for BTEX; this value is close to the experimental results.

To investigate the effects of the factors in Eq. (5) on the sample transfer efficiency for SPME fiber injection, experiments were conducted for columns with different o.d, different lengths of the column inside the liner, and different carrier gas flow rates.

Table 5
Relative peak areas of different length column inserted liners ($n=6$, SPME injection)

	Benzene (RSD %)	Toluene (RSD %)	Ethylbenzene (RSD %)	*o*-Xylene (RSD %)	Average
SPI liner (GC–MS)	1 (2.1)	1 (1.5)	1 (3.2)	1 (2.8)	1
SPI liner (GC–FID)	1 (1.6)	1 (1.6)	1 (1.8)	1 (2.0)	1
$Z_1=0.8$ cm (GC–MS)	0.85 (3.5)	0.84 (3.0)	0.88 (2.8)	0.86 (3.6)	0.86
$Z_1=0.8$ cm (GC–FID)	0.85 (2.1)	0.88 (1.7)	0.90 (3.4)	0.91 (2.7)	0.89
$Z_2=0.4$ cm (GC–MS)	0.62 (4.1)	0.68 (4.2)	0.73 (6.2)	0.72 (5.8)	0.69
$Z_2=0.4$ cm (GC–FID)	0.58 (2.8)	0.67 (2.3)	0.69 (3.0)	0.71 (3.5)	0.66

Table 6
Relative peak area of different o.d. column inserted liners ($n = 6$, SPME injection)

	Benzene (RSD %)	Toluene (RSD %)	Ethylbenzene (RSD %)	*o*-Xylene (RSD %)	Average
Column 1, $Z = 0.4$ cm (GC–MS)	0.62 (4.1)	0.68 (4.2)	0.73 (6.2)	0.72 (5.8)	0.69
Column 1, $Z = 0.4$ cm (GC–FID)	0.58 (2.8)	0.67 (2.3)	0.69 (3.0)	0.71 (3.5)	0.66
Column 2, $Z = 0.4$ cm (GC–MS)	0.80 (6.4)	0.83 (4.2)	0.86 (6.6)	0.87 (3.9)	0.84
Column 2, $Z = 0.4$ cm (GC–FID)	0.73 (2.1)	0.80 (1.3)	0.81 (3.0)	0.84 (2.2)	0.80
Column 1, $Z = 0.8$ cm (GC–FID)	0.85 (2.1)	0.88 (1.7)	0.90 (3.4)	0.91 (2.7)	0.89
Column 2, $Z = 0.8$ cm (GC–FID)	0.91 (1.2)	0.94 (1.7)	0.97 (1.8)	0.98 (1.8)	0.95

Table 7
Relative peak area of different column flow rates ($n = 6$, SPME injection)

	Benzene (RSD %)	Toluene (RSD %)	Ethylbenzene (RSD %)	*o*-Xylene (RSD %)	Average
$v = 1$ mL/min, $Z = 0.4$ cm (GC–FID)	0.58 (2.8)	0.67 (2.3)	0.69 (3.0)	0.71 (3.5)	0.66
$v = 2$ mL/min, $Z = 0.4$ cm (GC–FID)	0.77 (2.1)	0.84 (4.3)	0.83 (2.1)	0.86 (2.6)	0.83
$v = 1$ mL/min, $Z = 0.8$ cm (GC–FID)	0.85 (2.1)	0.88 (1.7)	0.90 (3.4)	0.91 (2.7)	0.89
$v = 2$ mL/min, $Z = 0.8$ cm (GC–FID)	0.93 (0.8)	0.94 (1.3)	0.96 (3.5)	0.96 (1.8)	0.95

Table 8
Relative peak area of agilent 6890 GC–MS by using different liners ($n = 6$, SPME injection)

	Benzene (RSD %)	Toluene (RSD %)	Ethylbenzene (RSD %)	*o*-Xylene (RSD %)	Average
Drilled Uniliner®	1 (0.8)	1 (0.6)	1 (0.5)	1 (0.6)	1
SPME liner	0.83 (1.1)	0.85 (1.6)	0.91 (2.6)	0.92 (2.1)	0.88

Table 5 summarizes the relative ratios of the peak areas for different lengths of the column inside the liner. The column i.d. was 0.25 mm, the o.d. was 0.34 mm, and the flow rate of the carrier gas was 1 mL/min. It was found that when Z was changed from 0.8 to 0.4 cm, the analyte rate of loss increased to 28–42%, which demonstrated that the rate of loss is higher when the length of the column inside the liner is shorter.

Table 6 summarizes the relative ratios of the peak areas for different o.d. columns inside the liner. Column 1 is the separation column, with an i.d. 0.25 mm and an o.d. of 0.34 mm. Column 2 is a pre-column, with a length of 20 cm, an i.d of 0.53 mm, and an o.d. of 0.67 mm. When column 1 was inserted into the SPME liner (i.d. 0.8 mm), the cross-sectional area A for the space between the liner and column was about 0.4 mm^2. When column 2 was inserted into the liner, the cross-sectional area A was about 0.15 mm^2 (Fig. 4). The results of the injection experiments illustrate that the analyte rate of loss is lower when the cross-sectional area A of the space between the liner and the column is smaller.

Table 7 summarizes the relative ratios of the peak areas for the different column flow rates. An i.d. 0.25 mm column was used for the experiments. The results illustrate that the analyte rate of loss is lower when the column flow is increased (because the peak areas will be affected by the column flow rate, the relative ratios of the peak areas for the 2 mL/min column flow rate were obtained by comparing the peak areas of the 2 mL/min SPME liner injection with those of the 2 mL/min SPI liner injection).

The experiments of SPME fiber injection were also performed on an Agilent 6890 GC by using an i.d. 0.8 mm SPME liner and an i.d. 1.0 mm Drilled Uniliner® liner (Cheomatographic Specialties Inc., Brockville, Ont., Canada. Hole in Drilled Uniliner makes direct injection possible with EPC-equipped Agilent 6890 GCs). Table 8 summarizes the relative

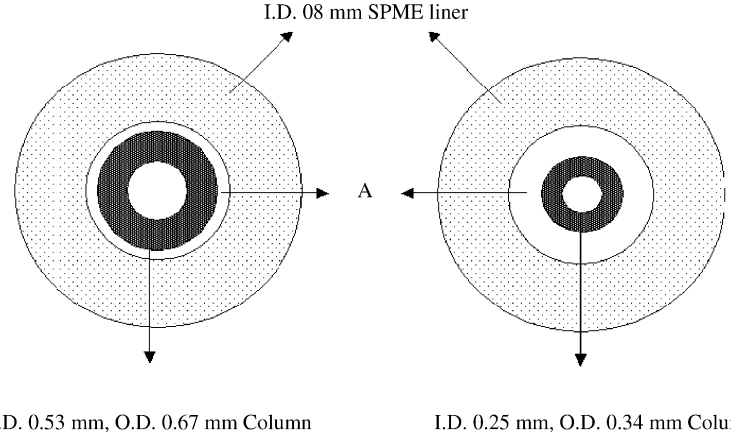

Fig. 4. Comparison of the cross-sectional area of the space between the column and the liner when different o.d. column inside a SPME liner.

Table 9
Average sample transfer efficiencies for liquid injection and SPME injection with i.d. 0.8 mm SPME liner

	Injection method			
	Liquid injection		SPME injection	
	With wool, PTV, column o.d. 0.34 mm	With wool, PTV, column o.d. 0.67 mm	Z = 0.8 cm, column flow 1 mL/min, column o.d. 0.67 mm	Z = 0.8 cm, column flow 2 mL/min, column o.d. 0.34 mm
Average sample transfer efficiency	0.93	0.94	0.95	0.95

ratios of the peak areas for the different liners. The results also illustrate that DI liner (Drilled Uniliner®) provides higher sample transfer efficiency than SPME liner.

4. Conclusions

Calibration is an important step for quantitative analysis. To ensure proper calibration of SPME by the injection of liquid standards, factors affecting both liquid injection and SPME injection were investigated.

The sample transfer efficiencies were obviously affected by the dimensions of the liner and the presence of wool. PTV injection using a small i.d liner with wool can result in good sample transfer efficiency. It was found that less than 70% of the analytes (the results obtained by Gebhart et al. [22] were less than 60%) was transferred into the column when high temperature liquid injection method was used, which suggests that high temperature liquid injection may not be suitable for SPME calibration. No obvious difference in the response factors was found when methanol, acetone, and toluene were used as the solvent.

For SPME injection, the sample transfer efficiency was affected by the carrier gas flow rate, the outer diameter of the column, and the length of the column inside the liner. The sample transfer efficiency would be higher if the column flow was higher, and/or the space between column and liner was smaller, and/or the length of the column inside the liner was longer.

For both liquid injection and SPME injection, the cross-sectional area between the column inserted into the liner and the inside of the liner is the most important factor in sample transfer. The best way to avoid the deviation for SPME quantitative analysis caused by the calibration with liquid injection is if the analytes are completely transferred into the GC column, and the DI liner (SPI, Uniliner®, etc.) is the best choice to achieve this objective in the current study. Sample introduced into the DI liner can obtain high sample transfer efficiency, for both liquid injection and SPME fiber injection, and can be performed with a common autosampler and a regular syringe for liquid injection.

When DI liners are not available for the injectors, Table 9 summarizes the average sample transfer efficiencies for liquid injection and SPME injection when a SPME liner was used. The average sample transfer efficiencies were close to 95%, for both liquid injection and SPME injection. The close sample transfer efficiencies suggest that it is feasible to calibrate SPME injection by liquid injection with common SPME liners.

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