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Equilibrium in-fibre standardisation technique for solid-phase microextraction

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Abstract

This note describes a fundamental investigation into solid-phase microextraction (SPME) using a standard loaded into the fibre coating as a means of internal standardisation for the analysis of samples contained in vials. The loading of reproducible amounts of standards into a non-porous SPME fiber was investigated. It was found that spiking low milligram quantities of standards such as benzene, toluene, ethylbenzene, xylenes (BTEX) and/or naphthalene into a few grams of pump oil sealed in a 20 mL vial provided an excellent standard generator. A single solution allowed over a hundred standard loadings with a reproducibility of <4% R.S.D. When a fiber, loaded with the standard(s) was introduced into a sample vial, extraction of analytes into the fiber and desorption of the standard(s) into the sample matrix occur simultaneously. Quantification was then based on the equilibrium distribution of the standards and the analytes between the fibre coating and the sample matrix in the vial. A comparison of equilibration profiles obtained using traditional internal standardisation and the in-fibre approach generally showed the same equilibration behaviour. The developed method was successfully used to correct for matrix effects in the BTEX analysis of a wine sample.

Keywords: Solid-phase microextraction; Calibration; Internal standardisation; Automation

1. Introduction

Internal standardisation is a well known calibration approach in analytical chemistry that is used to improve accuracy and precision of experimental data. It can account for such factors as sample matrix effects, losses during sample preparation and irreproducibility in parameters such as sample injection in gas chromatography (GC) [1]. Solid-phase microextraction (SPME) as a sample preparation and extraction technique is no exception with internal standardisation often used for quantification, particularly when analysing complex samples. However, the addition of an internal standard provides an additional step in sample preparation. For completely automated analysis two robotic arms are required, one to provide the standard spike and the other for SPME. There are also situations in which the addition of an internal standard is not practical such as on-site or in vivo applications.

SPME is a solvent free technique designed for rapid sampling and sample preparation [2,3]. The most common form of the technique uses a fibre coated with a liquid polymeric film, which is exposed to the sample, extracting analytes from it until equilibrium is reached. The amount of analyte absorbed by the coating at equilibrium (n_f) is linearly proportional to the initial concentration in the sample (C_0) by Eq. (1) [2],

$$n_f = \frac{K_{fs} V_f V_s}{K_{fs} V_f + V_s} C_0 \quad (1)$$

where K_{fs} is the fibre/sample distribution coefficient, V_f is the volume of the fibre coating and V_s is the volume of the sample. For analysis in a vial containing headspace this equation

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should be expressed as shown in Eq. (2) [2],

$$n_f = \frac{K_{fs} V_f V_s}{K_{fs} V_f + K_{hs} V_h + V_s} C_0 \quad (2)$$

where K_{hs} and V_h represent the headspace/sample distribution coefficient and the volume of the headspace, respectively.

The fact that SPME is an equilibrium rather than an exhaustive extraction technique means that even after the extraction process has been completed a substantial portion of the analytes usually remain in the matrix. This presents an opportunity for quantification based on internal standardisation, namely that the standard is loaded onto the fibre prior to the extraction step, instead of spiked into the sample. Chen and co-workers [4,5] have recently explored the kinetics of the technique, demonstrating that the absorption and desorption processes are isotropic, which allows for calibration of the rate of absorption using the rate of desorption.

The current investigation aimed to expand on this technique to fundamentally assess the in-fibre standardisation approach with systems reaching equilibrium. The technique was developed for automated sampling from millilitre quantities of liquids in vials and used for the analysis of benzene, toluene, ethylbenzene, xylenes (BTEX) in a wine sample.

2. Theoretical considerations

The equilibrium equation for SPME, most generally described by Eq. (2), is derived from the knowledge that the amount of analyte in the system will remain the same before and after the extraction. This mass balance equation can therefore be expressed by Eq. (3),

$$n_T = n_f + n_h + n_s \quad (3)$$

where n_T is the total number of moles of analyte in the system, and the remaining terms denote the amount of the analyte in the fibre, headspace and sample respectively at equilibrium. Using this form of expressing the mass balance, leads to Eq. (4)

$$n_f = \frac{K_{fs} V_f}{K_{fs} V_f + K_{hs} V_h + V_s} n_T \quad (4)$$

From this equation, it is apparent that no matter where the standard or analyte of interest is initially present in the system, at equilibrium the amount in the fibre should be the same.

A further consideration is the kinetics of the process. For traditional SPME the kinetics for both direct and headspace extraction can be described by Eq. (5) [6,7],

$$\frac{n}{n_f} = 1 - \exp(-at) \quad (5)$$

where n is the moles of analyte in the coating at time t, a is a constant that is dependant on the volumes of the fibre, headspace and sample, mass transfer coefficients, distribution coefficients and the surface area of the fibre. The kinetic processes involved for desorption of analytes from the fibre coating is defined by Eq. (6) [5],

$$q = n_0 \frac{V_s}{K_{fs} V_f + V_s} [1 - \exp(-at)] \quad (6)$$

where q is the moles of the analyte lost from the coating at time t and n_0 represents the moles of the compound originally loaded. For the case of in-vial analysis the moles remaining on the fibre (n) at time t can be expressed as

$$n = n_0 - q \quad (7)$$

From Eqs. (6) and (7) it is apparent that

$$n = n_0 - n_0 \frac{V_s}{K_{fs} V_f + V_s} + n_0 \frac{V_s}{K_{fs} V_f + V_s} \exp(-at) \quad (8)$$

However, the exponential term disappears as time goes to infinity, therefore

$$n_f = n_0 - n_0 \frac{V_s}{K_{fs} V_f + V_s} \quad (9)$$

Substituting Eq. (9) into Eq. (8) and rearranging gives

$$\frac{n - n_f}{n_0 - n_f} = \exp(-at) \quad (10)$$

Comparing Eq. (10) with Eq. (5), it can be concluded that for in-vial analysis the isotropy of absorption and desorption of an analyte from the fibre is still maintained. A similar expression can be derived for headspace analysis with a suitable adjustment in the definition of a.

3. Experimental

3.1. Materials

[$^2H_{10}$]Ethylbenzene (ethylbenzene-d_{10}, 99+%), ethylbenzene, *o*-xylene (98%, HPLC grade), naphthalene (99+%, scintillation grade) and carbon disulfide (99.9+%, HPLC grade) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Benzene (analytical reagent) was from BDH (Toronto, Canada), toluene (Guaranteed Reagent) from EMD (Gibbstown, NJ, USA), [$^2H_8$]naphthalene (naphthalene-d_8, 99%) from Cambridge Isotope Labs (Andover, MA, USA). HPLC grade methanol was obtained from Fisher Scientific (Nepean, Canada), the vacuum pump oil was supplied by BOC Edwards (Wilmington, MA, USA), and the poly(dimethylsiloxane) (PDMS) membrane material was supplied by Specialty Silicone Products (Ballston Spa, NY, USA). PDMS (100 μm) SPME fibres and Tenax TA (80/100) were purchased from Supelco (Bellefonte, PA, USA). Water purified from a Barnstead ultrapure water system (Dubuque, IA, USA) was used throughout. All gases were supplied by Praxair (Kitchener, Canada) and were of ultra high purity. Ten or 20 mL sample vials were used for automated analysis with magnetic crimp caps and PTFE coated silicone septa

(Microliter Analytical Supplies, Suwanee, GA, USA). The dry white wine sample was obtained from a local liquor store.

3.2. GC analysis

Gas chromatography was performed on a Varian (Mississauga, Canada) 3800 gas chromatograph coupled with a Saturn 2000 MS system controlled by computer using Varian Saturn Workstation software (Version 5.51) or with FID (flame ionization detection) using Star Chromatography Workstation (Version 5.51). Automated analysis was performed using a CTC CombiPal autosampler (Zwingen, Switzerland) using the associated Cycle Composer software (Version 1.4.0). The PAL was equipped with a SPME fibre holder, a temperature controlled six vial agitator tray and a fibre conditioning device. Separation was performed using a 30 m × 0.25 mm i.d., 0.25 μm Rtx-5MS fused silica column from Restek (Bellefonte, PA, USA). For analysis of BTEX the column was initially set at 40 °C for 4 min and then ramped at 15 °C/min to 130 °C giving a total run time of 10 min. The injector was set at a temperature of 250 °C and helium was used as the carrier gas at a flow rate of 1 mL/min. For analysis of naphthalene, the column was initially set at 40 °C for 1 min and then ramped at 20 °C/min to 220 °C giving a total run time of 10 min. The temperature of the injector was set at 250 °C and helium was used as the carrier gas at a constant pressure of 12 psi (1 psi = 6894.76 Pa). For both analytes a 1 min desorption time in the GC injection port was used, which was immediately followed by a 2 min bake-out at 250 °C in the autosampler fibre conditioning device.

FID was used at a temperature of 300 °C with gas flows for hydrogen, high-purity air and make-up gas (nitrogen) set at 300, 30 and 25 mL/min, respectively. For the mass spectrometry detection experiments, electron ionisation was used with temperatures of 170, 50 and 260 °C for the trap, manifold and transfer line respectively. A scan range of 70–125 *m/z* was used and quantification was performed using 78 *m/z* for benzene, 91 for toluene, 98 and 116 for deuterated ethylbenzene and 91 and 106 for ethylbenzene and *o*-xylene. For naphthalene, a scan range of 100–160 *m/z* was used and quantification was performed using 128 *m/z* for naphthalene and 136 *m/z* for deuterated naphthalene.

For the automated analysis a sampling temperature of 35 °C was used. The internal standard was loaded onto the fibre by exposure to the headspace of a 20 mL sample vial containing 4.00 g of vacuum pump oil spiked with deuterated ethylbenzene at a concentration of 0.47 mg/g. The loading time was 1 minute with an agitation speed of 500 rpm. The fibre was then immediately exposed to the headspace of a 10 mL vial containing the sample for 5 min, again using a 500 rpm agitation speed. The sample volume used unless otherwise specified in these experiments was 3.0 mL. A 6 min pre-extraction equilibration of the sample was performed in the agitation unit at 500 rpm. Loading of naphthalene onto the fibre was achieved in similar fashion to ethylbenzene except in this case the vial contained 4.0 mg of the standard and 2.00 g of pump oil. All other conditions were the same as in the ethylbenzene experiments unless otherwise specified.

4. Results and discussion

4.1. Preparation of standard solutions

Standards used for constructing calibration curves were prepared by spiking the solution with a stock standard of the target compounds prepared in methanol. Initially this was done after the vial had been capped by means of a 10 or 100 μL syringe. However, using this approach a steady decline in peak areas for the analytes was observed that was related to the amount of time between spiking and sampling. The decline was worse with ethylbenzene and xylene than with benzene and toluene. This suggested the behaviour was caused by absorption of the compounds into the small part of the vial septum silicone layer exposed through addition of the standard spike. To overcome this difficulty it was necessary to spike the solutions prior to capping. To minimise evaporation it was necessary to add the spike below the level of the solution in the vial, a similar approach to that adopted for standard preparation in US Environmental Protection Agency (EPA) method 5021A [8]. This approach was also used when spiking real samples with the analytes.

4.2. Internal standard loading on fibre

The first challenge was to find a method that would allow, automated, fast and reproducible loading of the standard into the fibre. Development of an appropriate method was performed using ethylbenzene as the “standard” for loading.

Sampling from the headspace of a vial containing pure ethylbenzene resulted in unmanageably high loading on the fibre coating even for extremely short absorption times. This was true even when cooled to 5 °C in the sample tray. The use of diluted solutions of ethylbenzene in water to reduce the loading to an acceptable level showed that the mass of ethylbenzene withdrawn from the vial during each loading step was a significant percentage of the total. This made it impossible to reuse a “loading” vial, which is not practical in terms of the number of standard solution vials required for an automated sample list. The use of alternative techniques, such as vials containing ethylbenzene absorbed onto Tenax, or PDMS membrane showed similar problems. Injecting the needle into a headspace of a vial containing pure ethylbenzene, without exposing the fibre coating showed a workable, but not always reproducible loading. This was attributed to the needle sometimes becoming partially blocked with a piece of septum.

Finally, a system was adopted whereby the ethylbenzene was dissolved in vacuum pump oil, to reduce the K_{fs} partition coefficient for the standard into the fibre. Using this method gave an acceptable and reproducible loading with 1 min exposure to the standard solution headspace. This also worked

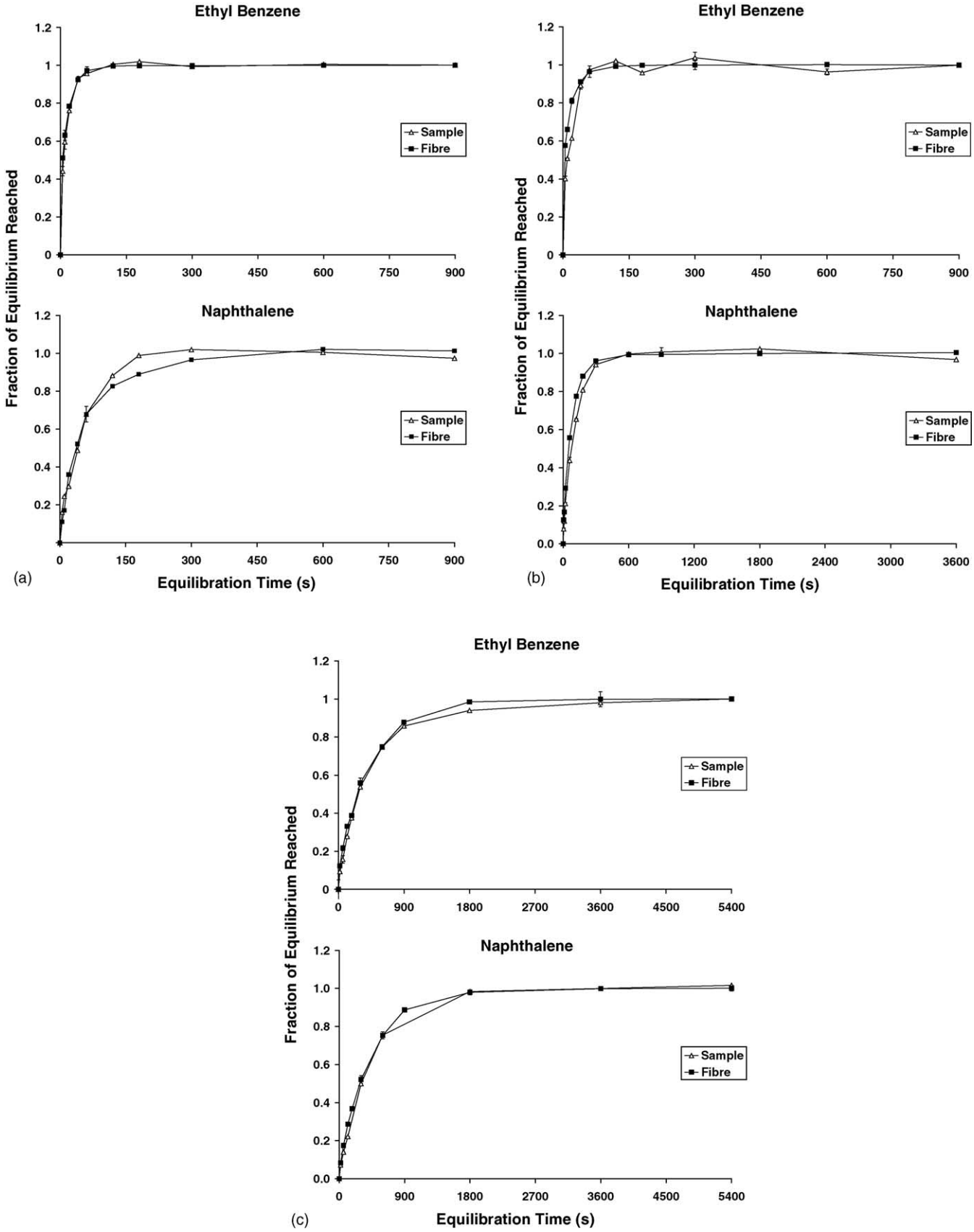

Fig. 1. Comparison of equilibration profiles obtained using standard SPME extraction and the in-fibre standard approach, using ethylbenzene and naphthalene as the test compounds. The graphs show profiles using (a) vials containing only a gas phase (b) headspace sampling from vials containing 3 mL of water, and (c) direct immersion into vials full of water. Agitation speed was set at 500 rpm for all cases. For other conditions see text.

well for naphthalene. The amount loaded into the fiber can be further adjusted by adding different amounts of the standard into the vacuum pump oil and/or exposing the fiber for different times. Using this approach, each loading cycle withdrew only 0.0087% of the ethylbenzene in the vial, making it possible to use the vial for at least 115 injections before 1% of the vial contents will be removed. Reproducibility of the loading step for ethylbenzene, determined by loading the fibre and then immediately desorbing in the GC injection port for analysis was 1.9% for 40 injection cycles. The value was 2.6% over 20 injection cycles for loading followed by equilibration with a vial containing 3 mL of water for 10 min. For naphthalene loading reproducibility was 2.0% for 30 injection cycles, whilst the value was 3.6% over 20 injection cycles for loading followed by equilibration with a vial containing 3 mL water for 10 min. In theory, the naphthalene standard solution can be used at least 300 times before 1% of the vial contents have been removed.

4.3. Comparison of equilibration curves

As a first investigation of the approach, equilibration profiles using traditional SPME and in-fibre standardisation SPME for ethylbenzene and naphthalene were compared when exposed to 10 mL headspace vials (actual volume was determined to be 11.5 mL). The process was tested for vials containing only a gaseous phase, completely filled with water/standard solution and also in a headspace format with vials containing 3 mL of water/standard solution. The results are shown in Fig. 1, with the profiles from the two approaches normalized according to Eqs. (5) and (10), respectively. Under the conditions studied the equilibration time was not influenced by the location of the standard at the beginning of the equilibration process.

4.4. Applications to real sample matrices

To test the in-fibre standardisation method, the technique was used to quantify BTEX in spiked white wine with MS detection. In this case ethylbenzene-d_{10} was used as the internal standard for in-fibre standardisation. Standard solutions in water gave a linear calibration for BTEX over the tested range of 0.09–73 μg/L regardless of whether external calibration or the standard in the fibre approach was used ($r^2 \geq 0.9998$). The recoveries from wine spiked with 7.3 μg/L BTEX calculated against standards prepared in water with both external calibration (by peak area comparison) and the in-fibre standardisation approach (by peak area ratio comparison) are given in Table 1. It demonstrates that the in-fibre technique gives more accurate determination of these compounds than external calibration. With ethylbenzene-d_{10} as internal standard, 99% recovery was obtained for ethylbenzene. The slightly higher deviations from 100% recovery for the other analytes can largely be attributed to differences in the interactions of these compounds with the matrix compared to the internal standard.

Table 1
Calculated recoveries of BTEX from a dry white wine with and without the use of in-fibre internal standardization

Compound	Relative recovery (%) (R.S.D., %; $n = 3$)	
	Using external calibration data	Using internal standard in the fibre
Benzene	77 (4)	98 (2)
Toluene	78 (4)	98 (2)
Ethylbenzene	76 (3)	100 (1)
o-Xylene	69 (2)	91 (1)

5. Conclusions

From these experiments it has been confirmed that the in-fibre standardisation approach works successfully under equilibrium conditions and can be easily automated. The developed procedure requires only a single arm autosampler, unlike SPME with traditional internal standardisation that requires a dual arm system. Equilibration time was not affected by where the standard commenced in the system. The technique was applied to the analysis of BTEX in wine, successfully correcting for matrix effects.

Future work will examine other possibilities for the technique, such as the use of the in-fibre standard to calibrate the variation of sample volume in an enclosed space such as a vial. The method could also be extended to other equilibration extraction techniques, such as liquid-phase microextraction (LPME) or membrane extraction.

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