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On-site calibration method based on stepwise solid-phase microextraction

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Abstract

A stepwise solid-phase microextraction (SPME) method was developed for on-site calibration of SPME for volatile organic compounds analysis. In this approach, a 75-μm Carboxen–polydimethylsiloxane coated fibre was loaded with a standard prior to exposure to samples of interest. Extraction time for the target analytes can be controlled independently from that of the standard, and the response factors for the target analytes can be adjusted accordingly. A good reproducibility of the response factors for BTEXs (benzene, toluene, ethylbenzene and xylenes) was obtained with stepwise SPME. Satisfactory results were obtained by using this method for quantitative analysis of BTEXs in the air of a gas station when tetrachloroethylene was used as a standard. The introduction of standard via the stepwise SPME procedure makes SPME more useful in field applications. It can be used to detect leaks, contaminations and losses from loading of a standard onto a fibre to introduction of the fibre to an analytical instrument. However, this method cannot be used for compensation of sample matrix effects.
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1. Introduction

As a traditional and most commonly used tool, calibration curves are very helpful for quantitative analysis but have their drawbacks [1–3]. First, calibration curves are only accurate for the exact conditions under which they were obtained, so calibration with internal standards is also required in many cases. Second, in some cases. such as the gas chromatographic (GC) analysis of volatile organic compounds (VOCs), an injection of a series of standard solutions into the GC injector/column is typically required to obtain a calibration curve. Since the organic solvent is the overwhelming main component of standard solutions, its chromatographic peak is generally much larger than those of the analytes, which makes it difficult or even impossible to determine very volatile analytes with retention times close to that of the solvent. The injection of an organic solvent into the GC system also has a negative effect on the sensitivity and lifetime of certain detection systems such as electron-capture detection (ECD) and mass spectrometry (MS) systems. For most modern GC systems this negative effect of solvents on detectors can be eliminated by setting a ''solvent delay'' program, however, it might deteriorate the chromatographic peaks of some of the target VOCs.

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Solid-phase microextraction (SPME) is a solvent-free technique that has been developed to combine sampling and sample preparation into one step [4,5]. With commercialization of a series of SPME fibre coatings and development of SPME field samplers, this method has been demonstrated to be suitable for different types of analytes and samples in both laboratory and field applications [6–8]. Calibration of the GC system with solvent injection is still the main technique for SPME–GC analysis when mass-response calibration curves are needed. However, there is a problem when responses obtained with SPME are smaller than the limit of detection (LOD) of solvent injection, which is readily understandable considering the strong back ground from the solvents. In such cases, the calibration curves have to be extrapolated, which introduces extra uncertainties into the results. A solution to this problem is to generate calibration curves with SPME, which involves (1) preparation of standard gases (or gas mixtures) using National Institute of Standards and Technology (NIST) traceable, certified permeation tubes in gas sampling chambers [9] or by microwave assisted evaporation [10]. (2) Utilizing a SPME fibre to extract and deliver standards to the analytical instruments. The procedure is the same as in general SPME, and will not be discussed further here, but it is worthy to note that this method provides an alternative to calibration and obviously has its own advantages.

SPME fibre/coatings extract analytes by absorption or adsorption. Consequently, variations in the SPME fibre's status or the extraction conditions. such as the temperature of the environment will affect extraction efficiency. Moreover, when SPME is used in the field, possible losses of extracts from fibres will occur during transport or storage. Thus calibration with an internal standard is desirable to ensure accuracy of SPME. It is not difficult to prepare internal standards for gaseous, liquid or solid samples of small size. However, for field analysis, the analytes are usually collected under natural conditions and analytical targets are huge and open systems such as urban air, river and lake water. In such cases it is a challenge to introduce an internal standard.

Recently, 75-μm Carboxen–polydimethylsiloxane (CAR–PDMS) coating fibres provided by Supelco (Bellefonte, PA, USA) have attracted great attention due to their extraordinary capacity to extract and store VOCs [7,8]. The SPME mechanism of CAR–PDMS coating is classified as adsorption. Particularly, the molecular sieve Carboxen fraction of this coating acts as a trap for volatiles, which can be desorbed only at high temperature. According to the report by Shirey et al. [7], even very volatile organic compounds could remain on a CAR–PDMS fibre without considerable losses through a 3-day storage. Studies conducted in our laboratory also proved that the CAR–PDMS coating is the best choice for field samplers for sampling and storing VOCs [8]. The special behaviours of this coating suggest that it is possible to develop a ''stepwise SPME'' method, with which standards can be introduced for calibration of the SPME–GC analysis of VOCs for large samples without the necessity of spiking standards into samples. This so-called stepwise SPME consists of two separate SPME procedures: (a) a standard is first extracted on a SPME fibre/coating; and (b) the standard-loaded fibre is successively used to extract the target analytes. The objective of this work is to realize the idea of stepwise SPME for analysis of VOCs in ambient air. Obviously, the approach to be developed is not equivalent to the classic internal standard or standard addition calibration, for in the latter standard material is added into the sample prior to sampling.

For field application of stepwise SPME, BTEXs (benzene, toluene, ethylbenzene and xylenes) were selected as the target compounds since they have been well investigated as indicator substances (markers) for human exposure to VOCs and to petroleum-related compounds [11]. It is known that traffic is the main source of BTEXs in ambient air because of the releases of gasoline and diesel from diverse vehicles and gas stations, etc., while both contamination from the outside as well as human activities in homes such as smoking, cooking, heating, etc., contribute to the amount of BTEXs in indoor air.

2. Experimental

2.1. Preparation of standard gases or gas mixtures

Several methods have been developed for the preparation of standard gases [9,10]. Two methods

Table 1
Preparation of different standard gases and gas mixtures

Compound	Preparation method used	Chemicals used	Concentration of standard gas (μg/l)
BTEXs (benzene, toluene, ethylbenzene, *p*-xylene, *o*-xylene)	Permeation from permeation tubes at controlled temperature	NIST-traceable, certified permeation tubes, Kin-Tech Labs.	Benzene: 3.91 Toluene: 2.74 Ethylbenzene: 0.414 *p*-Xylene: 2.41 *o*-Xylene: 0.483
1,3-Dichlorobenzene	Microwave-assisted evaporation	0.5-μl pure liquid, Caledon Labs., Georgetown, Canada	650 (diluted to 6.5 for use)
1,1,2-Trichloroethane	Microwave-assisted evaporation	0.5-μl pure liquid, Caledon Labs.	720 (diluted to 7.2 for use)
Tetrachloroethylene	Microwave-assisted evaporation	0.5-μl pure liquid, Caledon Labs.	810 (diluted to 8.1 for use)

were employed in this work to prepare the required standard gases or gas mixtures as follows (Table 1).

2.1.1. Preparation of a BTEX gas mixture using NIST permeation tubes [9]

A standard gas mixture of BTEXs was generated with NIST-traceable, certified permeation tubes (Kin-Tech Labs., La Marque, TX, USA), in a custom built, flow-through gas chamber where sampling took place. The temperature of the sampling chamber was controlled at 25 °C. Airflow-rate was at 300 ml/min.

2.1.2. Microwave-assisted generation of gas standards of VOCs [10]

A domestic microwave oven (1000 W, Model MW5490W, Samsung, South Korea) and 1-l gas sampling bulbs (Supelco) were used for preparation of standard gases and gas mixtures of the investigated VOCs with different concentrations. The inner walls of the glass bulbs were deactivated by silanization and the bulbs were cleaned with nitrogen flushing before use. For the preparation of standard gases or gas mixtures of 1,3-dichlorobenzene, 1,1,2-trichloroethane and tetrachloroethylene, a clean piece of glass wool (ca. 10 mg) was set inside the sampling port of the bulb each time and was moistened with deionized water (15 μl). Water was used to absorb microwave energy and then to prompt evaporation of compounds that are poor absorbers of microwave energy. The port of the glass bulb was sealed with a PTFE-faced silicon rubber septum through which a certain volume of the liquid target

compound (or a mixture of several compounds) was injected onto the glass wool. Finally, the bulb was placed into the microwave oven to receive microwave radiation for 90 s. The power output was always set to its maximum level. After cooling the bulb to room temperature, SPME of the standard gas was performed through the septum of the sampling port.

2.2. SPME devices and the stepwise SPME procedure

SPME fibre coatings and conventional SPME samplers used were provided by Supelco. A SPME device for field application was designed and built in our laboratory, which is an improvement over the two-leaf SPME device reported previously [8] and will be described in another paper. The coatings utilized included 75-μm CAR–PDMS, 85-μm polyacrylate (PA), 100-μm PDMS and 65-μm PDMS–divinylbenzene (DVB).

The stepwise SPME procedure was conducted as follows: first, a SPME fibre was exposed to the tetrachloroethylene standard gas in a bulb for 2 min, then the fibre was withdrawn into the needle and a Thermogreen septum (LB-2, Supelco) was used to seal the tip of the needle. When using the field SPME sampler, no separate sealing septum is needed [8]. The tetrachloroethylene-loaded fibre was then exposed for a few minutes to a BTEX standard gas mixture in a sampling chamber or to a real air sample. Finally, the SPME fibre was transferred to a

GC injector to simultaneously desorb the standard and the analytes.

2.3. GC–flame ionization detection (FID) analysis of analytes

A Varian Model 3500 GC equipped with an FID system was employed for sample analysis. A SPB-5 capillary column (30 m×0.25 mm, 1 μm) from Supelco was used and hydrogen as carrier gas was set at 30 p.s.i (ca. 207 kPa). The column was programmed as follows: 35 °C initial, held for 1 min, ramped to 135 °C at 10 °C/min and held for 1 min. The detector was maintained at 280 °C. For PA, PDMS and PDMS–DVB fibres, the injector was controlled at 250 °C and the desorption time was 1 min CAR–PDMS fibres were desorbed for 2 min at 300 °C.

3. Results and discussion

3.1. Stability of VOCs on SPME coatings after exposing the coatings to zero air

When an SPME coating loaded with VOCs is exposed to pure air, a portion of the extracts will transfer into the air and trying to reach an equilibrium distribution between the coating and gas phases. The release of extracts from the coating is controlled by multiple factors, including the prop-

erties of the compounds and the coatings, the temperature of the environment and differences in the concentrations of the compounds in the coating phases and in the samples or the environment. Table 2 shows that the remains of several VOCs (BTEXs included) on the 85-μm PA, 100-μm PDMS and 65-μm PDMS–DVB coatings ranged from 0 to 91.5% after 1 min exposure of the coatings to zero air at room temperature. However, the 75-μm CAR–PDMS coating could store as much as 89.9–97.2% of the extracts even through a 6-min exposure to zero air under the same conditions (Table 3). No losses could be found for most of the extracts on the 75-μm CAR–PDMS coating when the exposure time was less than 4 min. Thus, it is possible to create a stepwise SPME procedure with the CAR–PDMS coating—that is, this coating can be used to extract a standard in the first step and then transfer the fibre to extract other compounds while the previously extracted standard still remains on the coating.

3.2. Selection of an internal standard for BTEXs analysis with stepwise SPME

1,3-Dichlorobenzene, 1,1,2-trichloroethane and tetrachloroethylene were tested, respectively, as internal standards for BTEX analysis with a 75-μm CAR–PDMS coated fibre. It can be seen in Table 2 that the CAR–PDMS coating has a strong affinity towards these compounds and their retention on the CAR–PDMS coating was similar to that of BTEXs.

Table 2
Remains of VOCs on SPME fibres after 1-min exposure of the fibres to zero air[a]

Compound	Remains of VOCs (%)[b]			
	CAR–PDMS (75 μm)	PA (85 μm)	PDMS (100 μm)	PDMS–DVB (65 μm)
Benzene	98.7	ND[c]	2.0	46.4
Toluene	99.6	ND[c]	4.5	72.1
Ethylbenzene	99.9	ND[c]	10.0	86.4
p-Xylene	100.2	ND[c]	9.1	88.2
o-Xylene	100.0	ND[c]	24.1	85.4
1,3-Dichlorobenzene	100.5	ND[c]	34.8	91.5
1,1,2-Trichloroethane	99.8	ND[c]	6.4	74.5
Tetrachloroethylene	100.2	ND[c]	9.6	77.4

[a] SPME of VOCs: 1 min; VOC concentrations: see Table 1.
[b] Compared with the corresponding data obtained by 1-min extraction without exposure to zero air.
[c] ND: Not detectable.

Table 3
Effect of exposure time on the remains of BTEXs on the 75-μm CAR–PDMS fibre[a]

Compound	Remains of BTEXs (%)[b]				
	2-min extraction– 2-min exposure	3-min extraction– 3-min exposure	4-min extraction– 4-min exposure	5-min extraction– 5-min exposure	6-min extraction– 6-min exposure
Benzene	98.6	99.0	97.7	96.1	89.9
Toluene	99.5	99.2	100.3	101.2	93.6
Ethylbenzene	100.7	99.1	100.9	97.7	94.5
p-Xylene	99.8	100.3	103.1	95.7	95.2
o-Xylene	100.6	99.9	99.2	95.5	93.7
1,3-Dichlorobenzene	99.3	100.8	100.1	98.6	97.2
1,1,2-Trichloroethane	98.8	99.7	98.1	96.7	93 5
Tetrachloroethylene	100.1	101.3	98.5	97.2	94.1

[a] Concentrations of the compounds: see Table 1.
[b] Compared with the corresponding data obtained by 2-, 3-, 4-, 5- and 6-min extractions without exposure to the air, respectively.

Considering their chromatographic behaviour, tetrachloroethylene is the best internal standard for BTEX analysis, because its chromatographic peak is positioned in the middle of, but well separated from, those of BTEXs. Further investigation demonstrated that, under the selected conditions, loading of tetrachloroethylene on a SPME fibre did not affect the SPME of BTEXs, and vice versa (Table 4). In other words, displacement of tetrachloroethylene by BTEXs is not likely to happen, unless the concentrations of BTEXs are very high, in which case an internal standard with a large distribution constant need to be selected. Tetrachloroethylene as an internal standard for BTEX analysis has another advantage: the background concentration of tetrachloroethylene is generally very low for the main sources of BTEXs, such as petroleum. However, tetrachloroethylene also has its drawbacks: it is a halogenated compound and the GC–FID response for it is not as sensitive as for BTEXs.

3.3. Response factors for BTEXs with tetrachloroethylene as an internal standard

For chromatographic analysis, the response factor (F) can be defined as [1]:

$$\frac{A_x}{C_x} = F \cdot \frac{A_s}{C_s} \tag{1}$$

where A_x and A_s are peak areas of analyte X and the internal standard, while C_x are C_s concentrations of

Table 4
Comparison of separated and stepwise SPME of tetrachloroethylene and BTEXs[a]

Compound	Separated SPME				Stepwise SPME			
	Tetrachloroethylene		BTEXs		Test 1		Test 2	
	Peak area (counts)	Peak area (counts)	Peak area (counts)	Peak area (counts)	Peak area (counts)	Response factor (*F*)	Peak area (counts)	Response area (*F*)[b]
Benzene	–	–	50 655	50 379	50 059	9.83	49 741	9.96
Toluene	–	–	43 082	41 779	43 008	12.0	42 032	12.0
Ethylbenzene	–	–	5005	5086	5059	9.38	4987	9.43
p-Xylene	–	–	27 854	28 254	28 343	9.02	27 925	9.07
o-Xylene	–	–	5623	5410	5554	8.83	5455	8.85
Tetrachloroethylene	10 973	10 545	–	–	10 684	1	10 471	1

[a] A 75-μm CAR–PDMS fibre was used. Separated SPME: 2 min; stepwise SPME: 2 min + 2 min; concentrations of the compounds: see Table 1.
[b] Response factor (*F*): calculated according to Eq. (1) using tetrachloroethylene as the internal standard.

analyte X and the internal standard after they have been mixed together. In the stepwise SPME procedure described above, the standard had not been mixed with the analytes before they were extracted onto the SPME fibre, therefore C_s stands for the concentration of tetrachloroethylene in the standard gas and C_x for concentration of individual BTEX compounds in the samples.

For stepwise SPME–GC–FID analysis of BTEXs, the response factors were measured and presented in Table 4. The time for SPME of BTEXs was 2 min, equal to that for SPME of the standard. The response factors in duplicate tests were very similar in almost all cases, reflecting that stepwise SPME is feasible and that it is a practical method for the introduction an internal standard for SPME–GC analysis of BTEXs with the CAR–PDMS coated fibre.

3.4. Effect of extraction time on response factors for stepwise SPME

Since in stepwise SPME the standard and BTEXs are not extracted at the same time, the extraction time of BTEXs and the standard can be controlled independently. Obviously, the extraction time will significantly affect the response factors. This is one of the special features of stepwise SPME distinguishing it from the conventional use of internal standards in which the analytes and the internal standard are extracted simultaneously. The response factors varied linearly with the extraction time of BTEXs in the range of 1–5 min when the extraction time of tetrachloroethylene (standard) was kept at 2 min. The linear equations obtained were as follows:

Benzene: $F = 4.265t + 1.301$, $R^2 = 0.9979$ (2a)

Toluene: $F = 5.776t + 0.402$, $R^2 = 0.9981$ (2b)

Ethylbenzene: $F = 4.663t - 0.031$,

$$R^2 = 0.9996 \qquad (2c)$$

p-Xylene: $F = 4.623t - 0.247$, $R^2 = 0.9993$ (2d)

o-Xylene: $F = 4.767t - 0.703$, $R^2 = 0.9963$ (2e)

where F is the response factor and t is the extraction time in minutes for BTEXs.

It is essential that the concentrations of both the standard and the analytes be within the linear range. GC–FID has a large linear response range ($\sim 10^7$) to VOCs, and so does SPME with the CAR–PDMS coating.

3.5. Field application—analysis of BTEXs in the air of a gas station

VOCs (BTEXs were of interest) were sampled at a local gas station using a laboratory-made field sampler with a 75-μm CAR–PDMS fibre on a clear day with an outdoor temperature of ca. 24 °C. A glass bulb holding the standard gas of tetrachloroethylene (8.1 μg/l) was carried to the field and SPME of tetrachloroethylene was performed prior to that of BTEXs. Sampling time was 2 min for the standard and 4 min for the gas station air. The sample was analyzed in our laboratory within 10 min. The chromatogram obtained is shown in Fig. 1. Identification of the individual BTEXs is based on their retention times as well as GC–MS analysis using a Hewlett-Packard 6890 GC system equipped with a 5973 MS system (Agilent Technologies, Palo Alto, CA, USA). According to a preliminary study, tetrachloroethylene was not found in the gas station air. Separation of the standard and the BTEXs from the other components of the extracts is very good, only peak 4 might contain m- and p-xylenes, which could not be separated from each other under the selected chromatographic conditions. Finally, using the peak areas (A_s and A_x), the standard concentration (C_s), and the response factors (F) given by Eqs. (2a)–(2e) the concentrations of BTEXs in the air were calculated according to Eq. (1) and shown in Table 5.

4. Conclusion

A stepwise SPME method was developed to introduce an internal standard for SPME–GC analysis of BTEXs in field applications. CAR–PDMS proved to be the only suitable coating for stepwise SPME due to its strong affinity and large capacity towards VOCs. Tetrachloroethylene was selected as an internal standard because its GC retention time compared with those of BTEXs was appropriate, its
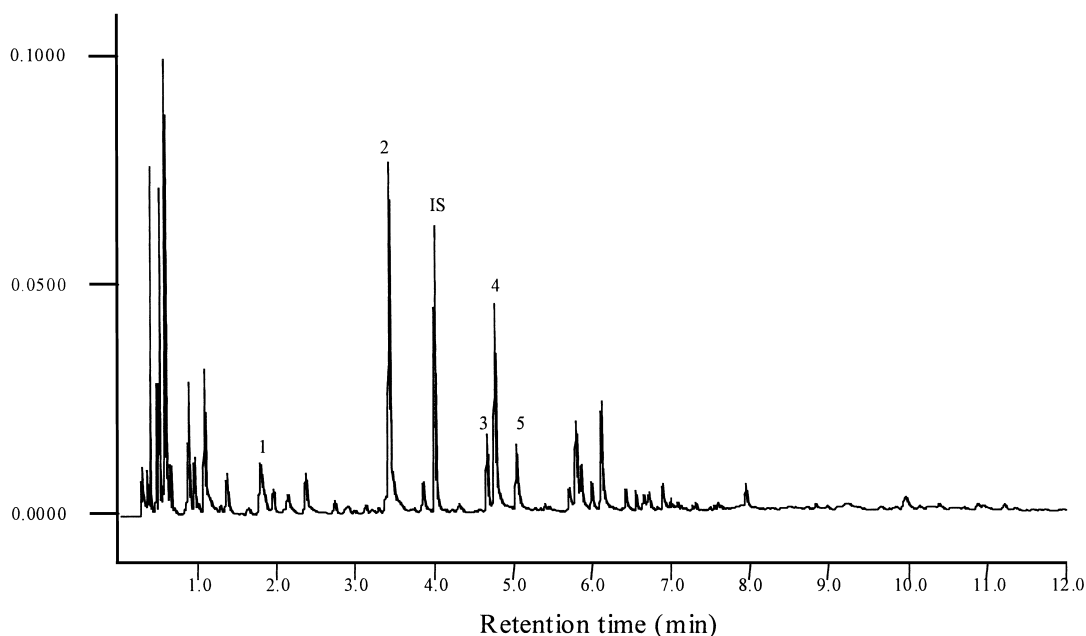
Fig. 1. Chromatogram of the VOCs sampled from a gas station with stepwise SPME: 1: benzene; 2: toluene; 3: ethylbenzene; 4: *m*-, *p*-xylenes; 5: *o*-xylene; I.S.: internal standard (tetrachloroethylene). SPME conditions: coating: 75-mm CAR–PDMS; tetrachloroethylene concentration: 8.1 μg/l; SPME of tetrachloroethylene: 2 min; SPME of analytes: 4 min.

adsorption in the CAR–PDMS coating was similar to that of BTEXs and its background concentration in the main BTEXs contamination sources was very low. Using the developed method, analytical results can be calibrated without a necessity to spike a standard material into samples, making SPME more advantageous in field applications. However, since

the standard is not directly added into the sample and SPME of the standard and the analytes is conducted separately, this method may not account for the matrix effects of air on the SPME of BTEXs. This approach is also suitable to detect problems with fibre storage, such as leaks, which will result in analyte and standard losses. Further development of

Table 5
BTEXs concentrations in the gas station air determined with stepwise SPME–GC–FID[a]

Compound	Extraction time (min)	Response factor (F)[b]	Peak area (counts)	Concentration (μg/l)[c]
Tetrachloroethylene	2	1	10 146	8.1
Benzene	4	18.4	4642	0.20
Toluene	4	23.5	16 337	0 56
Ethylbenzene	4	18.6	3236	0.14
p-Xylene	4	18.2	11 103	0.49[d]
o-Xylene	4	18.4	4364	0.19

[a] A field sampler with a 75-μm CAR–PDMS fibre was used.
[b] Response factors were calculated according to Eqs. (2a)–(2e) with tetrachloroethylene as the internal standard.
[c] BTEXs concentrations were calculated according to Eq. (1). Tetrachloroethylene was not found in the air sample itself.
[d] It was calculated according to Eq. (2e) but might include the content of *m*- and *p*-xylenes.

this technology can include chemical immobilization of compounds, which will facilitate production of certified standards.

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