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Kinetic Calibration for Automated Hollow Fiber-Protected Liquid-Phase Microextraction

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Recently, a kinetic calibration method was developed for the quantification of microextraction. In this study, we proved that the sample volume and sampling time do not affect the feasibility of the calibration method, theoretically. The new theoretical considerations of the kinetic calibration method were validated through the investigation of the kinetics of the absorption and desorption processes of hollow fiber-protected liquid-phase microextractrion (HF-LPME). The kinetic calibration method for HF-LPME was successfully used to correct the matrix effects in the carbaryl analysis of a red wine sample. This research extends the kinetic calibration approach to fast sampling and some in-vial analyses, whereby the sample volume is not much larger than the product of the distribution coefficient and the volume of the extraction phase. HF-LPME technique was successfully automated with a CTC CombiPal autosampler, and a new device was designed for the automation of HF-LPME in this study. All steps of the HF-LPME technique, including the filling of the extraction solvent, sample transfer and agitation, withdrawing the solvent to a syringe, and introducing the extraction phase into the injector, were automated by a CTC autosampler. The fully automated HF-LPME technique is more convenient and more accurate. The good reproducibility of the fully automated HF-LPME technique eliminates the need for an internal standard to improve the analytical precision. The automated HF-LPME technique can be also used to obtain the distribution coefficient between the sample matrix and the extraction phase. The distribution coefficients of carbaryl and ^{13}C-carbaryl between 1-octanol and red wine, at 25 °C, were obtained with this technique.

Liquid−liquid extraction is a classic technique for sample enrichment and isolation in analytical chemistry, but it is time-consuming and requires the use of large amounts of high-purity organic solvent, which is generally expensive and toxic. Solid-phase microextraction (SPME) was developed to address the need for rapid sampling and sample preparation.[1] As a fast, simple, sensitive, solvent-free sampling and analysis technique, SPME has been widely used for volatile organic compounds (VOCs) and semi-VOCs analysis.[2,3]

More recently, following the development of the SPME technique, liquid-phase microextraction (LPME), also known as a solvent microextraction technique, has been developed as a novel sample preparation and enrichment technique for chromatography and electrophoresis. LPME is a fast, simple, inexpensive sample preparation technique and combines extraction, concentration, and sample introduction into one step. Similar to SPME, the developed methods for LPME include direct-immersed LPME,[4−6] headspace LPME (HS-LPME)[7−9] and hollow fiber-protected LPME (HF-LPME).[10−12] These LPME techniques have been applied to environmental and biological analyses.[13−20]

Traditional calibration methods, such as external standard, internal standard, or standard addition methods, can be used for the quantification of SPME and LPME, but each calibration method presents different advantages and disadvantages. The external calibration method is more suitable for simple matrixes, such as air or clean water, and if there are matrix effects, a blank sample matrix is necessary. The standard addition method is not practical if the number of target analytes is large, due to the need for extensive sample preparation. The internal standard method can be used to compensate for the matrix effect, but the standard compound must be different from the analytes and well-resolved

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in the separation. Standard addition and internal standard methods also require the delivery of a standard. This is incompatible in some sampling situations, such as with on-site (field) or in vivo investigations.

Recently, a new kinetic calibration technique has been developed to extend the calibration method for microextraction.[21−23] The kinetic calibration method uses the desorption of standards, which are preloaded in the extraction phase, to calibrate the extraction of analytes. When the standard is an isotopically labeled analogue of the target analyte, the information from the desorption process, such as time constant a, can be directly used to estimate the concentration of the target analyte. The newly developed calibration technique has been successfully used in on-site and in-vial SPME investigations.[22−24] The feasibility of this calibration method for HS-LPME has also been demonstrated and successfully used to correct the matrix effects in the BTEX analysis of an orange juice sample.[25]

In previous studies, the derivation of the kinetic calibration method assumed $V_s \gg KV_e$,[22] where V_s is the volume of sample matrix, V_e is the volume of extraction phase, and K is the distribution coefficient of analyte between the sample matrix and extraction phase, which means this calibration approach is just suitable for field sampling or in-vial sampling of compounds with low distribution coefficients between the extraction phase and the sample matrix. In this study, we proved that the sample volume and sampling time do not affect the feasibility of the kinetic calibration method, theoretically. This extends the technique for use in fast sampling situations and for some in-vial analyses, when the sample volume is not much larger than the product of K and V_e, such as with HF-LPME.

Semiautomated HF-LPME techniques have been reported previously, but only one or two steps of HF-LPME were automated.[26−28] In this study, the HF-LPME technique was successfully fully automated with a CTC CombiPal autosampler. The automated HF-LPME technique was subsequently used to investigate the kinetics of the absorption and desorption processes of HF-LPME, and the isotropic nature of the absorption and desorption processes was demonstrated. The kinetic calibration method for HF-LPME was successfully used to correct the matrix effects in carbaryl analysis of a red wine sample.

THEORETICAL CONSIDERATIONS

For two-phase HF-LPME, the amount of analyte in the system will remain the same before and after extraction. At equilibrium, this mass balance equation can therefore be expressed by eq 1,

$$n_T = n_e + n_s \tag{1}$$

where n_T is the total number of moles of the analyte in the system and n_e and n_s are the amount of analyte in the extraction phase and the sample at equilibrium, respectively. Thus, eq 1 leads to eq 2,[2]

$$n_e = \frac{K_{es}V_e}{K_{es}V_e + V_s}n_T \tag{2}$$

where V_e and V_s are the volume of the extraction phase and the sample, respectively. K_{es} is the distribution coefficient of the analyte between the extraction phase and the sample. Equation 2 can be expressed by eq 3,

$$n_e = \frac{K_{es}V_eV_s}{K_{es}V_e + V_s}C_0 \tag{3}$$

where C_0 is the initial concentration of the analyte in the sample. According to eq 3, if n_e, the amount of analyte in the liquid extraction phase at equilibrium, was obtained, then C_0, the initial concentration of the analyte in the sample, can be calculated with K_{es}.

The kinetic process for the absorption of HF-LPME can be described with eq 4,[29]

$$n/n_e = 1 - \exp(-at) \tag{4}$$

where n is the amount of analyte in the extraction phase at time t, and a is a constant that is dependent on the volumes of the extraction phase, headspace and sample, mass-transfer coefficients, distribution coefficients, and the surface area of the extraction phase. The kinetic process of the desorption of the standard from the extraction phase is defined by eq 5,[22]

$$q = q_0\frac{V_s}{K'_{es}V_e + V_s}[1 - \exp(-at)] \tag{5}$$

where K'_{es} is the distribution coefficient of the standard between the extraction phase and the sample, q is the amount of standard lost from the extraction phase for sampling time t, and q_0 is the amount of preadded standard in the extraction phase. Let $Q = q_0 - q$, and Q is the amount of the standard remaining in the extraction phase after exposure of the extraction phase to the sample matrix for the sampling time, t. Thus, for the desorption process, eq 5 can be expressed by eq 6,[23]

$$\frac{Q - q_e}{q_0 - q_e} = \exp(-at) \tag{6}$$

where q_e is the amount of standard remaining in the extraction phase at equilibrium. If the desorption and absorption processes occur simultaneously, the constant a should be similar for the analytes and the labeled compounds (^{13}C, deuterated, etc.) or compounds with similar physicochemical properties. Then, eq 4 and eq 6 can be combined to

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$$\frac{n}{n_e} + \frac{Q - q_e}{q_0 - q_e} = 1 \quad (7)$$

As q_e can be calculated with the distribution coefficient of the standard between extraction phase and sample,

$$q_e = \frac{K'_{es} V_e}{K'_{es} V_e + V_s} q_0 \quad (8)$$

then eq 7 can be expressed by eq 9.

$$n_e = \frac{q_0 n V_s}{(K'_{es} V_e + V_s)(q_0 - Q)} \quad (9)$$

When $K'_{es} \approx K_{es}$, eq 3 and eq 9 can be combined to

$$C_0 = \frac{q_0 n}{K_{es} V_e (q_0 - Q)} \quad (10)$$

Equation 10 indicates that the sample volume and sampling time do not affect the determination of C_0, the initial concentration of the analyte in the sample. This illustrates that the kinetic calibration technique can be used for fast on-site or in vivo sampling, and some in-vial analysis, when the sample volume is not much larger than the product of the distribution coefficient and the volume of the extraction phase. For example, it could be used when the distribution coefficient of the analyte between the extraction phase and the sample matrix is very large.

EXPERIMENTAL SECTION

Chemicals and Supplies. Carbaryl (97%), and 1-octanol (HPLC, 99+%) were purchased from Sigma-Aldrich (Mississaga, ON, Canada). Carbaryl (ring $^{13}C_6$, 99%) was purchased from Cambridge Isotope Laboratories Inc. (Andover, MA). HPLC grade methanol was purchased from BDH (Toronto, ON, Canada). Hamilton model 701N 10-μL syringes (26s gauge, cone tip for CTC autosampler) were purchased from Hamilton (Reno, NV). The Accurel KM polypropylene hollow fiber (200-μm wall thickness, 1.2-mm internal diameter, 0.2-μm pore size) was purchased from Membrana (Wuppertal, Germany). The 10-μL screw vials with magnetic crimp caps and PTFE-coated silicone septa (Supelco, Oakville, ON, Canada) were used for the automated analysis. Water was purified with a Nanopure filter (Barnstead, Dubuque, IA). Ultrahigh-purity helium was purchased from Praxair (Kitchener, ON, Canada). The red wine sample was purchased from a local store.

Instrument. A Varian 3800 GC/Saturn 2000 ITMS system fitted with a SPB-5 column (30-m, 0.25-mm i.d., 0.25-μm film thickness) (Supelco, Mississauga, ON, Canada) was used for the analysis. Helium was used as the carrier gas and was set at 1 mL/min. The column temperature was maintained at 50 °C for 2 min, programmed to increase by 30 °C/min to 280 °C, and then held for 5.33 min. The total run time was 15 min. An i.d. 0.8-mm SPME liner packed with glass wool was used for the 1079 injector. The injector was set to 50 °C and then programmed to increase by 200 °C/min to 250 °C to obtain good sample-transfer efficiency.[30] The split outlet was opened after 5 min with a split ratio of 100:1. The MS system was operated in the electron ionization (EI) mode and tuned to perfluorotributylamine. The EI was set to turn on at 7.3 min (after the elution of the solvent). A mass scan from 100 to 250 was used, and quantification was performed using m/z 144 for carbaryl and m/z 150 for $^{13}C_6$-carbaryl.

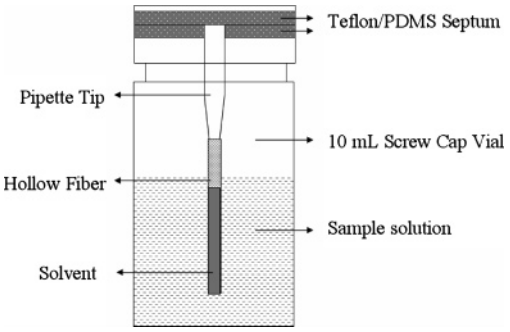

Figure 1. Schematic diagram of the HF-LPME device for automation.

New HF-LPME Device and Extraction Procedure. To fully automate HF-LPME, a new HF-LPME device was developed (Figure 1). The hollow fiber was ultrasonically cleaned in acetone. After the fiber was dried, it was then cut into 1.8-cm lengths. The top of a pipet tip (0.5−10 μL, VWR, Mississauga, ON, Canada) was cut off, and the remaining tip (~2.8 cm long) was used as a needle guide of the device. The hollow fiber was fixed in the pipet tip by heat, and the end of the hollow fiber was sealed by mechanical pressure. The practical length of the fiber, after fixing to the pipet tip and sealing, is ~1.5 cm. Two Teflon/PDMS septa were used for the HF-LPME device. One septum was drilled a small hole in the center and the needle guide with the fiber was fixed on the septum. The other septum was used for seal.

Figure 2 illustrates the automated HF-LPME procedures. All of the extraction procedures, including filling the extraction solvent, sample transfer and agitation, withdrawing the solvent to syringe, and introducing the extraction phase into injector, were autoperformed by a CTC CombiPal autosampler (Zwingen, Switzerland) using the associated Cycle Composer software. A 1000 μg/mL stock solution of carbaryl was prepared in methanol. The 250 ng/mL standard solutions of carbaryl were prepared daily by spiking the stock solution in pure water with a CTC autosampler.

The extraction of the analyte in the sample and the desorption of the internal standard were performed in a 10-mL vial that contained 4 mL of 250 ng/mL carbaryl aqueous solution for the determination of the absorption and desorption profiles. The extraction phase, 20 μL of pure 1-octanol or 25 μg/mL $^{13}C_6$-carbaryl 1-octanolic solution, was filled into the hollow fiber with a cone tip 10-μL syringe by an CTC autosampler. To avoid the production of air bubbles, the needle tip of the syringe was carefully set to close the bottom of the hollow fiber (~1 mm above the bottom of the hollow fiber) with the use of the software and then the extraction solvent was slowly released from the syringe. After the hollow fiber was filled with 20 μL of extraction solvent, the 10-mL sample vial was autotransferred from the sample tray to the vortex

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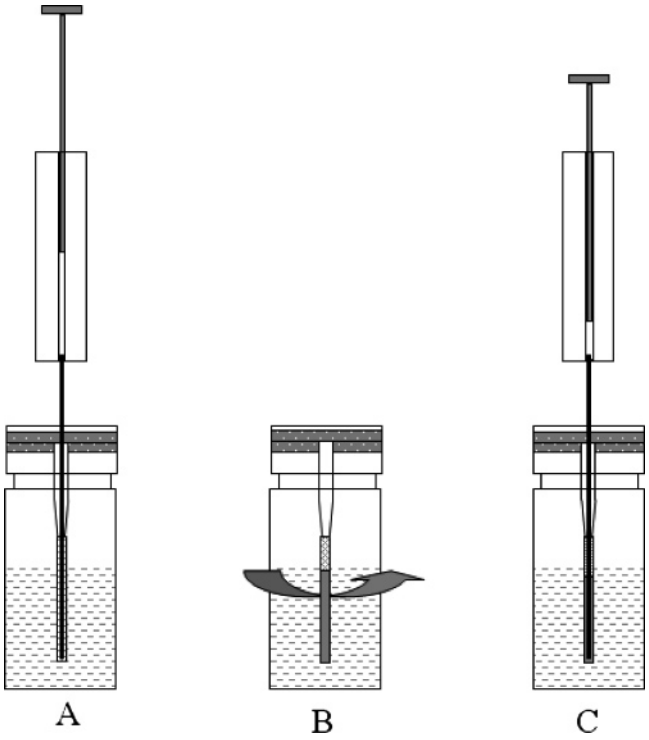

Figure 2. Schematic diagram of the automated HF-LPME procedures. (A) Filling of extraction solvent; (B) agitation; (C) withdrawing the solvent to syringe and injection to GC.

agitator with a temperature controller and shaken at 750 rpm and 25 °C. Following different extraction times, the sample vial was transferred from the agitator to the sample tray, 2 μL of extraction solvent was slowly withdrawn into the syringe, and 1 μL was introduced into the GC/MS for analysis.

Quantification. According to eq 10, the amount of the extracted analyte and the standard need to be known when performing the kinetic calibration. An external standard calibration method was used for quantification of the analyte and the standard. The solvent used for the standard solution preparation will affect the sample transfer into the GC column.[25,31] To avoid the effect of solvent on the quantification, the standard solutions for the calibration were also prepared with 1-octanol. Five standard solutions were prepared, and the concentrations are between 1 and 100 μg/mL. The 1-μL standard solution was direct injected into GC/MS for analysis. The conditions of GC/MS for the analysis of standard solutions are the same as the automated HF-LPME. Each concentration of the standard solutions was analyzed three times. Good precision (RSD < 5%) and linearity (^{2} > 0.995) were obtained for the calibration curves with the 1-octanolic standard solutions.

For the calibration of red wine sample, Q, the amount of the standard remaining in the extraction phase, and n, the amount of extracted analyte, are quantified with calibration curves, K_{es}, the 1-octanol/red wine distribution coefficient of analyte, is determined by automated HF-LPME technique, then C_0, the initial concentration of the analyte in the sample, can be calculated by eq 10, since q_0, the amount of preadded standard in the extraction phase, and V_e, the volume of the extraction phase, have been known.

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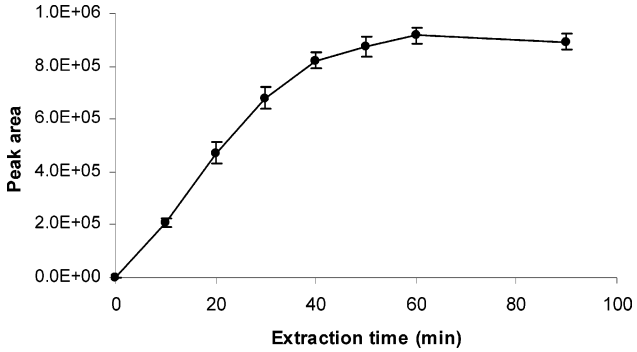

Figure 3. Extraction time profile of carbaryl 250 ng/mL aqueous solutions at 25 °C for the automated HF-LPME procedure.

RESULTS AND DISCUSSION

Reproducibility and Stability of Automated HF-LPME. The reproducibility and stability of the newly developed, fully automated HF-LPME technique required experimental validation.

Figure 3 illustrates an extraction time profile of a carbaryl 250 ng/mL aqueous solution at 25 °C for the automated HF-LPME technique. There are seven experimental points in Figure 3, and each point was repeated three times. In total, 21 HF-LPME devices were used and the samples were autoanalyzed by the autosampler one by one. The RSDs for three repeated extractions are less than 8.7%, even without a standard in the extraction phase to improve the precision. The reproducibility is better when the system reached equilibrium. For the carbaryl 250 ng/mL aqueous solution, the equilibrium time was ~60 min and the RSD for the results at 60 and 90 min were less than 5%. It is believed that the poor reproducibility observed prior to equilibrium was caused by little differences in the handmade devices.

Although the inner diameter of the needle guide for the device is very small and the devices are handmade, every device still worked well. The solvent can be filled into the hollow fiber exactly, and all devices were kept in good condition after the experiment was finished. All procedures were performed by an autosampler. This study proved that the newly developed HF-LPME device is stable and reliable, and the fully automated HF-LPME technique can be used for the following studies.

Isotropy of the Absorption and Desorption for HF-LPME. The kinetics of absorption and desorption for automated HF-LPME were studied to validate that the desorption of a standard from the extraction phase into the sampling matrix is isotropic to the absorption of the analyte into the extraction phase from the sample matrix, under the same conditions.

The desorption time profile of $^{13}C_6$-carbaryl and the absorption time profile of carbaryl were determined simultaneously. The $^{13}C_6$-carbaryl was dissolved in 1-octanol (25 μg/mL), and then 20 μL of the $^{13}C_6$-carbaryl 1-octanolic solution was added into the hollow fiber inside the sample vial. The extraction times were from 10 to 60 min. Figure 4 presents the values of $(Q - q_e)/(q_0 - q_e)$ calculated from the resulting desorption time profile and the values of n/n_e calculated from the resulting absorption time profile at 25 °C. The data are the average values of the results of three times repeated experiments (RSD less than 7%). The sum of $(Q - q_e)/$

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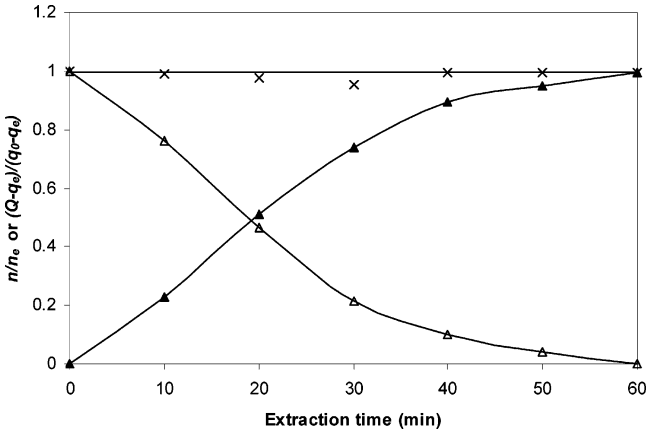

Figure 4. Desorption time profile of $^{13}C_6$-carbaryl and absorption time profile of carbaryl at 25 °C for the automated HF-LPME procedure. ▲, carbaryl; △, $^{13}C_6$-carbaryl; × , sum of $(Q - q_e)/(q_0 - q_e)$ and n/n_e.

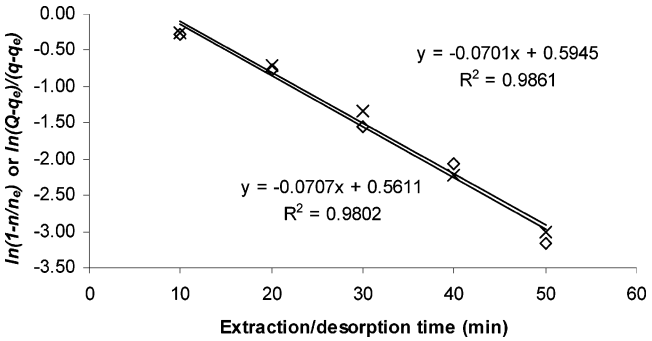

Figure 5. Desorption and adorption time profile of $\ln(Q - q_e)/(q_0 - q_e) - t$ or $\ln(1 - n/n_e) - t$ at 25 °C for the automated HF-LPME procedure. ◇, $^{13}C_6$-carbaryl; ×, carbaryl.

$(q_0 - q_e)$ and n/n_e at any time are close to 1. Comparing the time constant a of the absorption and the desorption clearly illustrates the isotropism of absorption and desorption, as the time constant a should be the same. Figure 5 illustrates the desorption and absorption time profiles at 25 °C where $\ln(Q - q_e)/(q_0 - q_e)$ or $\ln(1 - n/n_e)$ is used as the y-axis, because $\ln(Q - q_e)/(q_0 - q_e)$ and $\ln(1 - n/n_e)$ should change linearly with desorption/absorption time and the slope is $-a$, according to eqs 4 and 6. Figure 5 showed that the time constant a for the absorption of carbaryl (0.0701) is very close to the time constant a for the desorption of $^{13}C_6$-carbaryl (0.0707). Both Figures 4 and 5 illustrated that the desorption of the standard from the extraction phase into the sampling matrix is isotropic to the absorption of the analyte into the extraction phase from the sample matrix, under the same experimental conditions.

Application to a Real Sample Matrix. To test the kinetic calibration of the HF-LPME method and prove that the sample volume will not affect the feasibility of the calibration method, the calibration method and fully automated HF-LPME technique were used to quantify carbaryl in a spiked red wine sample with GC/MS.

1. Determination of 1-Octanol/Red Wine Distribution Coefficient. As an equilibrium standardization technique, the extraction phase/sample distribution coefficient should be known when the kinetic calibration method is applied to HF-LPME to estimate the concentration of analyte in a sample solution. When 1-octanol is used as the extraction phase, the extraction phase/water distribution coefficient (K_{ow}) for most analytes can be found in the literature. If the method is used for the analysis of red wine, the distribution coefficient between 1-octanol and red wine should be determined. The distribution coefficient of the analyte between the extraction phase and the sample matrix can be obtained by two methods: direct determination with a spiked sample or using the standard addition method, as described previously.[25]

Table 1. Distribution Coefficients of Carbaryl and $^{13}C_6$-Carbaryl at 25 °C

	carbaryl	$^{13}C_6$-carbaryl
log K_{ow} [a]	2.36	
K_{ow}(log K_{ow})[b]	254 (2.41)	263 (2.42)
RSD (%), $n = 3$	3.6	4.8
$K_{octanol/wine}$ (log $K_{octanol/wine}$)[b]	154 (2.19)	160 (2.20)
RSD (%), $n = 3$	4.2	4.1

[a] Reference 32. [b] Determined by automated HF-LPME technique.

In previous studies, it has been demonstrated that the automated HS-LPME can be used for the determination of the distribution coefficient between the extraction phase and the sample.[25] Similar to automated HS-LPME, automated HF-LPME is also an equilibrium technique and can be used for the determination of the distribution coefficient. To illustrate that the automated HF-LPME technique can be used to determine the distribution coefficient between the extraction phase and the sample, the K_{ow} of carbaryl and $^{13}C_6$-carbaryl at 25 °C were determined by the automated HF-LPME technique and the K_{ow} of carbaryl was compared with the literature value (Table 1). Twenty microliters of a 25 μg/mL $^{13}C_6$-carbaryl 1-octanolic solution was used as the extraction phase, and 4 mL of a 250 ng/mL carbaryl aqueous solution was used as the sample solution. The speed of agitation was set to 750 rpm, and the temperature was controlled at 25 ± 1 °C. To ensure that the system can reach equilibrium, the extraction time was set to 90 min. K_{ow} for carbaryl was determined by extraction, and K_{ow} for ^{13}C-carbaryl was determined simultaneously by desorption. The results illustrated that the K_{ow} of carbaryl at 25 °C was close to the literature value, which demonstrated that the automated HF-LPME technique can be used for the determination of distribution coefficients of the analytes between the extraction phase and the sample matrix.

The determination of $K_{octanol/wine}$ for carbaryl and $^{13}C_6$-carbaryl at 25 °C was similar to the aforementioned method; however, the sample was changed to a 250 ng/mL carbaryl red wine solution. Table 1 presents the $K_{octanol/wine}$ of carbaryl and $^{13}C_6$-carbaryl. The results show that the distribution coefficients of carbaryl and $^{13}C_6$-carbaryl between 1-octanol and red wine are lower than the distribution coefficients of carbaryl and $^{13}C_6$-carbaryl between 1-octanol and water. The results in Table 1 also show that the distribution coefficients of carbaryl and $^{13}C_6$−Carbaryl are very close, both in the 1-octanol/red wine system and in the 1-octanol/water system, which demonstrates that the assumption for eq 10 is correct.

2. Analysis of Carbaryl in Red Wine. The recoveries of the 250 ng/mL carbaryl-spiked red wine samples, using the automated HF-LPME technique and the external calibration approach (using standards prepared in water) or the kinetic calibration

Table 2. Calculated Recoveries of Carbaryl from Red Wine, with and without Kinetic Calibration

	relative recovery (%) (RSD, %; $n = 3$)	
analyte	using external calibration	using kinetic calibration
carbaryl	50 (5.6)	108 (5.1)

approach, are given in Table 2. For both the external and kinetic calibration approaches, the extraction times were 10 min when the temperature was controlled at 25 ± 1 °C. The results demonstrate that the kinetic calibration approach produces a more accurate measure of carbaryl in red wine than the external calibration method.

CONCLUSION

In previous studies, the derivation of the kinetic calibration method assumed $V_s \gg KV_e$,[22], which suggests that this calibration approach is just suitable for field sampling or in-vial sampling of compounds with low distribution coefficient between the extraction phase and the sample matrix. However, in this study, we proved that the sample volume and sampling time do not affect the feasibility of the kinetic calibration method, theoretically. This therefore extends the application of this technique to fast on-site or in vivo sampling and some in-vial analyses when the sample volume is not much larger than the product of K and V_e.

The HF-LPME technique was selected to validate the new theory, and 1-octanol and carbaryl were selected as the extraction phase and the analyte, respectively. The sample volume used (4000 μL) was comparable to the product of K and V_e (254 × 20 = 5080 μL). The isotropy of the desorption of the standard and the absorption of the analyte was demonstrated. The results illustrated that the kinetic calibration method can be used for microextraction without considering the sample volume and sampling time experimentally. The kinetic calibration method for HF-LPME was successfully used to correct for the matrix effects in the carbaryl analysis of a red wine sample.

In this study, a hollow fiber-protected LPME technique was successfully automated with the use of a CTC CombiPal autosampler and a new device was designed for the automation of HF-LPME. The fully automated HF-LPME technique is more accurate and more convenient. The good reproducibility of the fully automated HF-LPME technique eliminates the need for an internal standard to improve the method precision. It can be expected that improved reproducibility would be obtained if a commercial assembly of this new HF-LPME device were to become available.

Similar to the automated HS-LPME technique, the automated HF-LPME technique can also be used to obtain the distribution coefficient between the sample matrix and the extraction phase. The distribution coefficient of carbaryl between 1-octanol and water at 25 °C was determined with the automated HF-LPME technique, and the determined value was close to the published literature value. The distribution coefficients of carbaryl and $^{13}C_6$-carbaryl between 1-octanol and red wine at 25 °C were also determined with the automated HF-LPME technique, and the results were subsequently used for the carbaryl analysis of the red wine sample. Because the CTC CombiPal autosampler is equipped with a temperature-controlled agitator, the distribution coefficient of the solvent/sample at different temperatures can be easily obtained for various target analytes. Thus, similar to automated HS-LPME, the automated HF-LPME technique can also be used to obtain the ΔH, the molar change in enthalpy of the analyte when it moves from the sample to the extraction phase, by determining the distribution coefficients at different temperatures.[25] However, it should be noted that the temperature cannot be too high, because the extraction solvent in the hollow fiber may then be lost.

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