Journal of Chromatography B, 980 (2015) 28–33

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Calibration of pre-equilibrium HF-LPME and its application to the rapid determination of free analytes in biological fluids

Baiqiu Xu[a], Min Chen[a,b], Jinxing Hou[a], Xi Chen[c], Xu Zhang[d], Shufen Cui[a,*]

[a] Department of Biological Applied Engineering, Shenzhen Key Laboratory of Fermentation, Purification and Analysis, Shenzhen Polytechnic, Shenzhen 518055, China
[b] School of Chemistry and Chemical Engineering, Fuzhou University, Fuzhou 510275, China
[c] State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen 361005, China
[d] Verschuren Centre for Sustainability in Energy and the Environment, Cape Breton University, Sydney B1P 6L2, Canada

ARTICLE INFO

Article history:
Received 27 August 2014
Accepted 21 December 2014
Available online 30 December 2014

Keywords:
Calibration
Hollow fiber liquid-phase microextraction (HF-LPME)
Concentration curve method
Time constant α
Free concentration
Fast analysis

ABSTRACT

This study establishes a novel calibration method for pre-equilibrium hollow-fiber liquid-phase microextraction (PE-HF-LPME), where the time constant of the extraction of the analyte from sample matrix to the extraction phase (organic solvent) is obtained from a simple concentration curve. Comparing to the traditional kinetic calibration method, where the time constant was obtained from the extraction time profile, the new calibration approach shows improved accuracy and precision. More importantly, deuterated standards are not required in the new method, thus significantly improving its cost-effectiveness and extending its applicability to a wide range of analytes lack of deuterated analogs serving as internal standards. In addition, mass spectrometry is not necessary for the quantification of analytes with the new calibration method, which may further extend the applicability of PE-HF-LPME to some laboratories without mass spectrometers. This study has been substantiated with both theoretical and experimental evidences. Further, the feasibility of the method for real biological samples was demonstrated by measuring the free concentration of flunitrazepam in urine and plasma samples and its drug–protein binding ratio in plasma. The results showed that the method had a short analysis time and was easily implemented with high accuracy and good reproducibility.

1. Introduction

Drugs usually exist in one of two forms after entering the bloodstream. One form is as a bound drug with weak binding to albumin proteins if acidic or to 1-acid glycoprotein (AGP) if alkaline. The other form is a free drug. Free drugs in plasma lead to the pharmacological effects. The concentration of a free drug should be in equilibrium with the receptor sites and is thus closely related to the efficacy of or adverse reactions to the drug. Additionally, only free drugs can be metabolized and excreted by organisms, thus being regarded as the bio-available portion of the total amount of drug within biological tissue [1]. Therefore, free drugs have drawn increasing attention and become one of the most popular research focuses in pharmacokinetics and therapeutic drug monitoring in recent years [2].

Hollow fiber liquid-phase microextraction (HF-LPME) plays an important role in extracting, separating, enriching, and concentrating trace components from complex samples [3]. The hollow fiber has a unique porous structure that allows small drug molecules to pass through while blocking biomacromolecules both with and without bound drug molecules [4–6]. It has been reported that measuring the free drug concentration and drug–protein binding rate can be achieved by taking advantage of this structural feature of hollow fibers [7–9]. An automated HF-LPME procedure was developed to endow operative simplicity and time-effectiveness [10]. But all of the studies mentioned above used the equilibrium extraction method. However, the relatively long equilibrium-extraction time is the primary downside to HF-LPME [4]. For drug analyses, a long extraction time affects the equilibrium between the free and bound drugs, which could create a false measurement of the biological samples and affect the analytical accuracy and precision. Furthermore, a long analysis time may lead to the loss of extraction solvents. Additionally, the matrix of biological samples, such as plasma, will change due to the metabolism of enzymes and other factors.

* Corresponding author. Tel.: +86 75526003546; fax: +86 75526003546.
E-mail address: shufencui@163.com (S. Cui).

http://dx.doi.org/10.1016/j.jchromb.2014.12.022
1570-0232/

Shaking, stirring, or diluting high-viscosity samples, such as plasma, or applying a voltage to the extraction phase can reduce the HF-LPME time [3–7,9–15]. However, these methods suffer from increased instrumental and operating difficulty. Moreover, all of these methods improve the mass-transfer rate in the sample matrix, which is not the rate-limiting step of HF-LPME for polar or semi-polar analytes with small partition coefficients.

Alternatively, pre-equilibrium HF-LPME can be used to achieve rapid extraction of analyte from sample matrices, suitable for complex samples with high concentration of analyte that may saturate the extraction phase in equilibrium extraction and/or with unstable analytes or matrix components, but accurate quantification of the pre-equilibrium extraction is challenging, because during the pre-equilibrium stage of the extraction, a small variation of experimental conditions such as temperature, agitation and sampling time, may result in significant experimental error. To overcome such issues, a kinetic calibration method was first introduced by Chen et al. for solid-phase microextraction (SPME) [16], where the extraction of an analyte from the sample matrix to the extraction phase was calibrated with the desorption of the deuterated standard from the extraction phase to the sample matrix. Since the extraction and desorption occur under the same conditions, the method compensates for the variation of the experimental conditions, thus improve the accuracy and precision of the measurement, showing broad applications for environmental monitoring, in vivo pharmacokinetics, and drug analysis [16–30]. The concept of kinetic calibration was introduced to HF-LPME recently [31,32], but there are two challenges limited the application of the kinetic calibration method. First, this method is based on the use of deuterated compounds serving as the calibrants, which are not always available for analytes and much more expensive even if they are available. In addition, mass spectrometry is required to differentiate the analytes and their deuterated analogs, while mass spectrometers are expensive to purchase and run in many laboratories, especially in some developing countries. Therefore, it would be desirable to develop a kinetic calibration using regular (non-deuterated) standards.

In this study, a kinetic calibration method that does not require a deuterated analyte was developed to calibrate the pre-equilibrium HF-LPME, where the time constant is obtained by a concentration profile with back extraction (desorption) in regular standard solutions rather than the time-profile used in the traditional kinetic calibration method with deuterated standards. This feasibility of the new pre-equilibrium calibration method was exemplified by a rapid analysis of free flunitrazepam in biological samples. This study will benefit the development of quantitative HF-LPME methods for faster and more accurate analysis of free analytes in complex biological samples.

2. Theoretical considerations

In HF-LPME, kinetics for extraction and back-extraction have the similar equations as shown in Eqs. (1) and (2), respectively [31,32].

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

$$\frac{Q - q_e}{q_0 - q_e} = \exp(-at) \tag{2}$$

where a is the time constant, which is used to describe how quickly the equilibrium can be reached; n is the amount of analyte in the extraction phase at sampling time t; n_e is the amount of the extracted analyte at equilibrium; 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; q_0 is the amount of pre-added standard in the extraction phase and q_e is the remaining amount of analyte in the extraction phase at equilibrium. Meanwhile, n_e and q_e are the functions of the initial sample concentration C_0 and preload q_0, respectively, as shown in Eqs. (3) and (4) [31].

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

$$q_e = \frac{K_{es}V_e}{K_{es}V_e + V_s}q_0 \tag{4}$$

where K_{es} is the distribution coefficient of the analyte between the extraction phase and the sample matrix. V_e and V_s are the volume of the extraction phase and the sample, respectively.

Based on Eqs. (1)–(4), the amount of analyte extracted (n) and the standard remaining in the extraction phase (Q) at sampling time t can be expressed as Eqs. (5) and (6), respectively.

$$n = \frac{K_{es}V_eV_s[1 - \exp(-at)]}{K_{es}V_e + V_s}C_0 \tag{5}$$

$$Q = \frac{K_{es}V_e + V_s\ \exp(-at)}{K_{es}V_e + V_s}q_0 \tag{6}$$

Thus, if we keep sampling time t constant, then there will be linear concentration profiles in Eqs. (5) and (6).

For practical use, from Eq. (6), a plot of Q versus q_0 should yield a straight line with a slope of $\frac{K_{es}V_e+V_s[\exp(-at)]}{K_{es}V_e+V_s}$ which was used for the determination of time constant t (since K_{es}, V_e, V_s and sampling time t are all known parameters), then, the value of the time constant a can be used to obtain the initial concentration of the analyte in sample matrix by Eq. (5). This new kinetic calibration method was much easier for the experiment operation and only the accurate measurement of n (extraction) and Q (back-extraction) was required (q_0 is the spiked quantities and there is no need to measure it).

3. Experimental

3.1. Chemicals and supplies

Flunitrazepam (1 mg/mL in methanol) and [$^2H_7$]flunitrazepam (0.1 mg/mL in methanol) were obtained from Cerilliant (Round Rock, TX, USA). Methanol (HPLC grade) was purchased from Merck KGaA (Darmstadt, Germany). 1-Octanol (HPLC grade) and *p*-xylene (HPLC grade) were purchased from Alfa Aesar (Lancs, England). Analytically pure grade dipotassium phosphate, monopotassium phosphate, and sodium chloride were purchased from Shanghai Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Helium (99.999%) with ultra-high purity was purchased from ShenTe Co. (Shenzhen, China). Plasma was purchased from Ruite Bio-tec Co., Ltd. (Guangzhou, China). Twenty-four hour urine samples were collected from healthy female volunteers on the day when performing experiments and stored in a refrigerator (4 °C) when not in use. Water was purified with a Millipore SAS 67120 Molsheim (Millipore Simplicity, France).

Polyvinylidene fluoride hydrophobic hollow fibers (200 μm wall thickness, 1.2 mm internal diameter, 0.2 μm pore size) (PVDF 200) were purchased from Tianjin Polytechnic University (Tianjin, China). Hamilton Model 701N 10 μL syringes (26s gauge, cone tip) were purchased from Hamilton (Reno, NV, USA). A Kunshan KQ3200DE ultrasonic cleaner (Kunshan Ultrasonic Instruments Company, Kunshan, China) was used for hollow fiber pre-washing (100.00 kHz frequency). Ten-milliliter screw vials with magnetic crimp caps and PTFE coated silicone septa (Agela, Delaware, USA) were used for the analysis. Vortex Mixers (Anpel Scientific Instrument, Shanghai, China) was used for agitation in HF-LPME.