Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 817 (2005) 3–12
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Review

Bioanalysis of drugs by liquid-phase microextraction coupled to separation techniques

Stig Pedersen-Bjergaard*, Knut Einar Rasmussen

School of Pharmacy, University of Oslo, P.O. Box 1068, Blindern, 0316 Oslo, Norway

Received 1 November 2003; accepted 18 August 2004
Available online 18 September 2004

Abstract

The demand for automation of liquid–liquid extraction (LLE) in drug analysis combined with the demand for reduced sample preparation time has led to the recent development of liquid-phase microextraction (LPME) based on disposable hollow fibres. In LPME, target drugs are extracted from aqueous biological samples, through a thin layer of organic solvent immobilised within the pores of the wall of a porous hollow fibre, and into an μl volume of acceptor solution inside the lumen of the hollow fibre. After extraction, the acceptor solution is subjected directly to a final analysis either by high performance liquid chromatography (HPLC), capillary electrophoresis (CE), mass spectrometry (MS), or capillary gas chromatography (GC) without any further treatments. Hollow fibre-based LPME may provide high enrichment of drugs and excellent sample clean-up, and probably has a broad application potential within the area of drug analysis. This review focuses on the principle of LPME, and recent applications of three-phase, two-phase, and carrier mediated LPME of drugs from plasma, whole blood, urine, and breast milk.

Keywords: Liquid-phase microextraction; Hollow fibres; Drug analysis; Plasma; Whole blood; Urine; Breast milk

Contents

1. Introduction

During the last 10 years, some interest has been focused on the miniaturising of analytical liquid–liquid extractions (LLE). The major idea behind this has been to facilitate automation, to speed up extractions, and to reduce the consumption of organic solvents. Miniaturised liquid–liquid extraction, or liquid-phase microextraction (LPME), was first introduced in 1996, and was based on a droplet of organic solvent hanging at the end of a micro syringe needle [1–4]. The organic micro droplet was placed into the aqueous sample, and the analytes were extracted into the organic droplet (micro extract) based on passive diffusion. Following extraction, the organic droplet was withdrawn into the syringe, the syringe was transferred to a capillary gas chromatograph (GC), and the micro extract was injected into the GC. In addition, LPME was performed in a three-phase system where ionic analytes in their neutral form were extracted from aqueous samples, through a thin layer of an organic solvent on the top of the sample, and into an aqueous micro droplet (micro

* Corresponding author. Tel.: +47 2285 6576; fax: +47 2285 4402.
E-mail address: stig.pedersen-bjergaard@farmasi.uio.no (S. Pedersen-Bjergaard).

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2004.08.034

extract) placed at the tip of a micro syringe [5,6]. In the latter, pH was selected to ionise the analytes to maximise partition coefficients and to prevent back-extraction into the organic phase again. In the three-phase system providing an aqueous micro extract, high performance liquid chromatograph (HPLC) was typically used in the final chromatographic analysis.

In both two- and three-phase LPME based on hanging droplets, high preconcentration may be achieved for analytes with high partition coefficients because they are transferred by passive diffusion from a relatively large sample volume (1–5 ml) and into a micro extract of typically 5–50 μl. In addition, the consumption of organic solvent is low, and especially in the three-phase mode including two simultaneous extractions, excellent clean-up has been observed even from biological samples. Unfortunately, LPME based on hanging droplets is not very robust [7], and the droplets may be lost from the needle tip of the syringe during extraction. This is especially the case when samples are stirred effectively to speed up the extraction process.

In order to develop a more robust format for LPME, Pedersen-Bjergaard and Rasmussen recently introduced an alternative concept for LPME based on the use of disposable low-cost porous hollow fibres made of polypropylene [8–24]. In this LPME device, the micro extract is contained within the lumen of a porous hollow fibre, and consequently, the micro extract is not in direct contact with the sample solution. Analytes are extracted through an organic liquid immobilised within the pores of the hollow fibre before they are trapped in the protected micro extract. Samples may be stirred or vibrated effectively without any loss of micro extract into the sample solution. Thus, hollow fibre-based LPME is a more robust and reliable alternative for LPME. The chemistry of hollow fibre-based LPME is similar to the chemistry used for extraction with supported liquid membranes (SLM) [25–29], but the techniques differ significantly in terms of instrumentation and operation. SLM is a flowing system with a pump, which continuously feed the membrane with fresh sample. Thus, SLM is an instrumental sample preparation technique, and each membrane is normally used for a large number of extractions. On the other hand, in hollow fibre-based LPME, both the sample and the extracting phase are stagnant, the membrane (hollow fibre) is used only for a single extraction, and no instrumentation like pumps are required for the sample processing. Thus, with LPME, a large number of samples may be processed simultaneously for instance in a 96-well system.

In the period from 1999 since the first publication on hollow fibre-based LPME, a few other groups have worked with related concepts in the field of drug analysis [7,19,30–32], and these efforts are reviewed in the present paper together with the work carried out in the authors laboratory. The review is focused on the different extraction principles, on applications of three-phase, two-phase, and carrier-mediated LPME within drug analysis, and on future directions of this promising sample preparation technique.

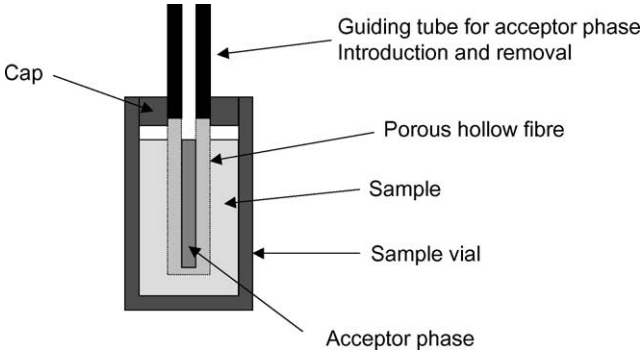

Fig. 1. Principle of LPME.

2. Principle

The basic principle of hollow fibre-based LPME is illustrated in Fig. 1, demonstrating the latest technical set-up used in the authors laboratory. The aqueous sample is filled into a sample vial, and a piece of a porous polypropylene hollow fibre is placed within this sample. The bottom end of the hollow fibre is closed, and the top of the fibre is connected to a guiding tube for a micro syringe to introduce and remove the acceptor phase from the lumen of the fibre. The volume of aqueous sample is typically within 100 μl to 4 ml depending on the application, and the length of the hollow fibre is normally 1.5–8 cm. Before extraction, the hollow fibre has been soaked in an organic solvent to immobilise the solvent in the pores of the wall of the hollow fibre (organic phase), and the lumen of the fibre has been filled with acceptor solution from a micro syringe. Excess solvent on the outside of the fibre has been removed by ultra-sonification. The solvents used as organic phase are immiscible with water and of low volatility to ensure that it remains within the pores during extraction with no leakage to the biological samples. The organic solvent forms a thin layer within the wall of the hollow fibre, which typically has a thickness of 200 μm, and the total volume of organic solvent immobilised in the fibre wall is typically in the range 15–20 μl. For acidic and basic analytes, pH within the sample is adjusted to a value where they are deionised to improve their extractability into the organic phase. During extraction, the fibre is placed in the sample solution within the sample vial. The analytes are transferred by passive diffusion from the aqueous sample, through the organic phase in the pores of the hollow fibre, and further into the acceptor solution placed inside the lumen of the hollow fibre. To speed up this process, extensive agitation or stirring of the sample is applied. After extraction, the acceptor solution is collected by a micro syringe and directly transferred to a chromatographic or electrophoretic system. The acceptor solution may be the same organic solvent as immobilised in the pores of the wall, resulting in a two-phase system where the analyte (A) is collected in an organic phase:

$$A_{Sample} \leftrightarrow A_{Organic\ acceptor}$$