Journal of Chromatography A, 1556 (2018) 29–36

Contents lists available at ScienceDirect

Journal of Chromatography A

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

A comprehensive study of a new versatile microchip device based liquid phase microextraction for stopped-flow and double-flow conditions

María Ramos Payán[a,*], Elia Santigosa Murillo[b], Jordi Coello[b], Miguel Ángel Bello López[a]

[a] Department of Analytical Chemistry, Faculty of Chemistry, c/Prof. García González s/n, 41012, Seville, Spain
[b] Department of Analytical Chemistry, Universitat Autónoma de Barcelona, 08193 Bellaterra, Barcelona, Spain

ARTICLE INFO

Article history:
Received 12 February 2018
Received in revised form 19 April 2018
Accepted 23 April 2018
Available online 30 April 2018

Keywords:
Microextraction
Miniaturization
Sample preparation
Microfluidic
Drugs
Urine sample

ABSTRACT

A new geometry for a versatile microfluidic-chip device based liquid phase microextraction was developed in order to enhance the preconcentration in microfluidic chips and also to enable double-flow and stopped-flow working modes. The microchip device was combined with a HPLC procedure for the simultaneous determination of two different families as model analytes, which were parabens and non-steroidal anti-inflammatories (NSAIDs): Ethyl 4-hydroxybenzoate (Et-P), Propyl 4-hydroxybenzoate (Pr-P), Butyl 4-hydroxybenzoate (Bu-P), IsoButyl 4-hydroxybenzoate (iBu-P), salycilic acid (SAC), ketoprofen (KET), naproxen (NAX), diclofenac (DIC) and ibuprofen (IBU) in urine samples. The new miniaturized microchip proposed in this work allows not only the possibility of working in double-flow conditions, but also under stagnant conditions (stopped-flow) (SF-μLPME). The sample (pH 1.5) was delivered to the SF-μLPME at 20 μL min^{-1} while keeping the acceptor phase (pH 11.75) under stagnant conditions during 20 min. The highest enrichment factors (between 16 and 47) were obtained under stopped-flow conditions at 20 μL min^{-1} (sample flow rate) after 20 min extraction; whereas the extraction efficiencies were within the range of 27–81% for all compounds. The procedure provided very low detection limits between 0.7 and 8.5 μg L^{-1} with a sample volume consumption of 400 μL. Parabens and NSAIDs have successfully been extracted from urine samples with excellent clean up and recoveries over 90% for all compounds. In parallel, the new device was also tested under double flow conditions, obtaining good but lower enrichment factors (between 9 and 20) and higher extraction efficiencies (between 45 and 95) after 7 min extraction, consuming a volume sample of 140 μL.

The versatile device offered very high extraction efficiencies and good enrichment factor for double flow and stopped-flow conditions, respectively. In addition, this new miniaturized SF-μLPME device significantly reduced costs compared to the existing analytical techniques for sample preparation since this microchip require few microliters of sample and reagents and it is reusable.

1. Introduction

Liquid Phase microextraction (LPME) is a very well-known and popular technique used for the extraction of acid and basic drugs based on the passive diffusion of the analytes from the sample (donor solution) into an acceptor solution, through a membrane (which support an organic solvent into its porous). LPME has been applied to many different fields, considering biological, pharmaceutical, environmental, food, toxicology analysis, among others [1–7]. The transport phenomena based on passive diffusion depends not only on the nature of the analytes, and the optimal parameters (as phase's composition, organic solvent, stirring speed, flow rate, etc), but also on the geometry of the system used for LPME. Another popular technique based liquid phase microextraction, named electromembrane extraction (EME), has also been frequently used since it improves the extraction of compounds in many cases due to an external electrical field created to both sides of the support liquid membrane [8–16]. However, EME also offers some limitations since its requirement is the use of a

* Corresponding author at: Department of Analytical Chemistry, Faculty of Chemistry, c/Prof. García González s/n, 41012, Seville, Spain.
E-mail address: ramospayan@us.es (M.R. Payán).

https://doi.org/10.1016/j.chroma.2018.04.051
0021-9673/

suitable and conductor organic solvent for carrying out the extractions. Both techniques have been widely used for the determination of pharmaceutical drugs either in biological samples (urine) or water samples [17–23] due to the great concern that exists regarding their contribution as emergent pollutants in the environment. Also, parabens have been studied due to the concern about their endocrine disrupting potential [24–29]. This has required the use of powerful, fast and sensitive techniques that offer better limits of quantification.

Up to date, parabens and non-steroidal antiinflamatories haven been determined by traditional LPME and EME procedures resulting in very good enrichment factors [1–4,17,19,20,30–33]. Those procedures allowed good enrichment factors but low extraction efficiencies. In the last years, liquid–liquid extraction has been miniaturized into microfluidic devices in order to address the limitations from traditional procedures and these chip devices are becoming an attractive alternative due to the many advantages that it presents [34–42]. The microchip devices for sample treatment have two channels that allow working in two different ways based on the flow rate of each phase: double-flow or stopped-flow conditions. In double-flow conditions, both phases (sample and acceptor) are moving at some flow rate. However, in stopped-flow conditions, the acceptor phase keep stagnant while the sample solution is used at some flow rate. Recent microchip devices based LPME, have been demonstrated to work only under double-flow conditions but not under stopped-flow conditions in a single step since the latter required to collect several extracts for its direct injection into HPLC. This was due to the low sample volume available in the acceptor channel ($\sim 2\,\mu L$) [41,42] and consequently, the analysis time increased and the reproducibility decreased when an enrichment factor was necessary prior to the sample analysis. On the other hand, the devices did not allowed high preconcentration factors although the sample flow rate was significantly increased under double-flow conditions.

Based on the current limitations of microfluidic devices for microextraction procedures, the aim of this work was to develop a new versatile and effective microfluidic device in order to overcome the limitations from previous microfluidic devices, increasing the preconcentration and allowing working under stopped-flow conditions compatible with direct analysis.

Based on geometry aspects, an increase of the depth channel would increase the volume capacity contained in the channel but it could decrease the transport phenomena by passive diffusion since the analytes are farther away from the membrane. Microfluidic systems that follow a laminar regimen do not carry agitation, so diffusion can be slow if the distance between the analytes and extraction solvent is increased. Moreover, an increase of the channelís width would increase the contact surface between the sample and the analytes, however, very wide channels could destabilize the laminar flow and affect the membrane stability.

In this work, we present for the first time a new versatile and effective microfluidic chip based LPME which allow the possibility of working under two different working modes (double-flow or stopped-flow conditions). The microchip was applied to the simultaneous determination of two different families in urine samples. This way, a comprehensive study between both different working conditions was carried out. The microchip decreased the sample volume and time of analysis since no collecting samples were needed for direct injection. The proposed stopped-flow device (SF-μLPME) is the easiest microfluidic chip for the simultaneous extractions of different drugs resulting in higher enrichment factors with lower cost instrumentation, simple handling, reusability and is still considered a “green method” by keeping low organic solvent (<5 μL) consumption. The proposed device has been successfully applied to urine samples.

2. Experimental

2.1. Chemicals and solutions

Ethyl 4-hydroxybenzoate (Et-P), Propyl 4-hydroxybenzoate (Pr-P), Butyl 4-hydroxybenzoate (Bu-P), IsoButyl 4-hydroxybenzoate (iBu-P), salicylic acid (SAC), ketoprofen (KTP), naproxen (NPX), diclofenac (DIC), ibuprofen (IBU),1-octanol, dihexyl ether, 2-nitrophenyl octhyl ether (NPOE), formic acid, sodium hydroxide, chloride acid,sodium chloride and methanol were purchased from Fluka–Sigma–Aldrich (Madrid, Spain). 100 mg L^{-1} stocks solutions were prepared in methanol except SAC, DIC and IBU that were prepared in Milli-Q Plus water (Elga, purelab option S-R 7-15 (Madrid, Spain). All working dilutions were prepared using ultrapure water from a Milli-Q Plus by adequate dilutions from stored at 4 °C. A membrane (Celgard 2500) of 25 μm thickness, 55% porosity, and 0.21 μm x 0.05 μm pores was obtained from Celgard (Charlotte, NC, USA).

2.2. Fabrication of the microfluidic-chip device

Fig. 1 shows a scheme of the microfluidic device based liquid phase microextraction. This microfluidic device has been re-designed and modified in order to overcome the limitations and disadvantages from previous microfluidic devices. The optimal poly(methyl methacrylate(PMMA) device consisted of two symmetrical patterned plates with one channel of 23 mm length, 120 μm depth and 3 mm width each. Six holes of 3 mm and two holes of 1.35 mm diameter were drilled for assembling and fixing in/outlets Teflon tubes in each PMMA plate, respectively. A flat polypropylene membrane piece of 27 mm length × 5 mm width separated the acceptor phase (channel 1) and the donor phase (channel 2). Firstly, the membrane was placed over one channel and impregnated with 4 μL of dihexyl ether. Once the extracting solvent was immobilized along the membrane by capillary forces, the channels were aligned and the device was closed using six small crews. The final size of a microfluidic device for one single extraction was 47 × 29 × 6 mm, however by increasing the size of both PMMA plates, an arbitrarily large number of extraction channels can be implemented and independently addressed. Also, the microchip-device can be opened any time when exchange membrane is needed.

A laser cutter (Epilog Mini 24–30 W) was used to fabricate this chip. The best quality was obtained using a writing speed of 40%, power of 33%, a resolution of 1500 and a frequency of 5000.

Inlets Teflon tubes (acceptor and donor inlets) were connected to two separate micro-syringe pumps (Cetoni GmbH, Korbussen, Germany). The sample (pH 1.5) was pumped into the microfluidic device at 20 μL min^{-1} while keeping the acceptor phase (pH 11.75) constant. The microfluidic device was also tested under double-flow conditions as described below, in order to compare different working modes. The acceptor phase was collected using a micropipette and was directly injected into a HPLC for analysis.

2.3. Chromatographic conditions

An Agilent 1100 series (Barcelona, Spain) liquid chromatography equipped with a G1312A Bipump and an autosamplerG1313A for 5 μL of sample injection was used as HPLC system. The column used for the separation of the nine compounds was a LiChroCART® 75-4 Purospher® STAR RP-18e 3 μm (75 mm × 4.0 mm i.d.) (VWR, Barcelona, Spain) proceeded by a guard column Kromasil1 100 Å, C18, 5 μm (20 mm × 4.6 mm i.d.) (Scharlab S.L., Barcelona, Spain).

The mobile phase consisted of 0.1% formic acid (pH 2.6) (component A) and methanol (component B) at a flow rate of 0.5 mL