Talanta 143 (2015) 394–401

Contents lists available at ScienceDirect

Talanta

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

Vortex-assisted liquid–liquid–liquid microextraction (VALLLME) technique: A new microextraction approach for direct liquid chromatography and capillary electrophoresis analysis

Ahmad Makahleh, Hui Fang Yap, Bahruddin Saad *

School of Chemical Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia

ARTICLE INFO

Article history:
Received 29 January 2015
Received in revised form
2 May 2015
Accepted 4 May 2015
Available online 11 May 2015

Keywords:
Vortex-assisted liquid–liquid–liquid microextraction
High performance liquid chromatography
Capillary electrophoresis
Carvedilol
Biological fluids

ABSTRACT

A new, rapid and sensitive microextraction technique named vortex-assisted liquid–liquid–liquid microextraction (VALLLME) is proposed. The complete extraction process involves two steps. First, a vortex-assisted liquid–liquid microextraction (VALLME) procedure was used to extract the analytes from a relatively large volume of sample (donor phase) to a small volume of organic solvent (intermediate phase). Next, a micro-vortex-assisted liquid–liquid extraction (μ-VALLE) was used to extract the target analytes from the intermediate phase to a smaller volume of aqueous solution (acceptor phase). The final extract (acceptor phase) can be directly injected into the high performance liquid chromatography or capillary electrophoresis units without any further treatments. The selection of the intermediate phase and the manipulation of pH are key parameters that ensure good extraction efficiency of the technique. The proposed technique has been successfully applied for the determination of carvedilol (used as model analyte) in biological fluid samples. The optimum extraction conditions were: toluene as intermediate phase (150 μL); pH of the donor phase, 9.5; vortex time of the VALLME, 45 s (maximum speed, 2500 rpm); 0.1 M HCl (15 μL) as acceptor phase; vortexing time of the μ-VALLME, 75 s (maximum stirring speed, 2500 rpm) and salt concentration in the donor phase, 5% (w/v). Under these conditions, enrichment factors of 51- and 418-fold for VALLME step and VALLLME procedure, respectively, were achieved.

1. Introduction

The last decade witnesses the phenomenal growth of microextraction techniques as innovative ways towards the minimization of organic solvent consumption, as well as to meet the key goals of green analytical chemistry [1–3]. A plethora of liquid phase microextraction (LPME) techniques such as the single drop microextraction (SDME), hollow fiber-liquid phase microextraction (HF-LPME), dispersive liquid–liquid microextraction (DLLME) and vortex assisted liquid–liquid microextraction (VALLME) have been developed [1]. Teething problems of the SDME technique lead to its replacement with the HF-LPME in order to protect the suspended drop (acceptor phase, AP) [4]. The two- and three-phases HF-LPME have been developed [5], the latter is of much significance due to its direct applicability for high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) [5–7] analyses. Although the long extraction time (~30–40 min) has to a significant extent been reduced by using the electromembrane approach [5], the low reproducibility has motivated scientists to seek for alternative non-fiber microextraction techniques [1,8]. Of these, the DLLME, first introduced in 2006 has gained considerable interest [8].

The DLLME technique is based on the use of a dispersive solvent (e.g., acetone, methanol, acetonitrile, etc.) in order to improve the dispersion process and thus increasing the interfacial area between the organic phase (AP) and aqueous sample (donor phase, DP) that is fundamental for the rapid extraction [9]. However, the use of dispersive solvents may decrease the partitioning and the mass transfer of the analytes into the extraction solvent, thereby reducing the enrichment efficiency [10]. The use of high density and toxic organic solvents (e.g. carbon tetrachloride, chloroform, dichloromethane, etc.) is another disadvantage of this technique [8,11,12]. Moreover, the two phase extraction procedure normally requires time-consuming evaporation step when HPLC or CE analyses are to be performed [11–13].

* Corresponding author. Tel.: +604 653 4049; fax.: +604 657 4854.
E-mail addresses: makahleha@yahoo.com (A. Makahleh), hfang_0929@hotmail.com (H.F. Yap), bahrud@usm.my (B. Saad).

http://dx.doi.org/10.1016/j.talanta.2015.05.011
0039-9140/

In order to overcome the drawbacks of the DLLME technique, an alternative dispersive technique named VALLME has been introduced [10]. In this technique, an organic solvent (e.g., 1-octanol, hexane, chloroform, hexanoic acid, etc.) is dispersed into the aqueous sample using vortex agitation to form a mild emulsification (fine droplets) without involving any dispersive solvent [10,14–17]. The droplets that are formed are able to accelerate the extraction due to the large surface area and shorter diffusion distance compared to the DLLME technique [10]. Both high and low density organic solvents can be used, depending on the affinity of the target analytes. The VALLME technique was successfully applied for the extraction of diverse analytes such as alkyl phenols [10,16], haloanisoles and halophenol [17], pesticides [18–21], herbicides [22], phthalate esters [14,15], polychlorinated biphenyls [23], furfurals [24], perfluorooctanesulfonate [25] and aliphatic amines [26]. In order to improve the extraction efficiency and/or phase separation, several modifications such as the addition of surfactant [14,18,19,22], ultrasound agitation [21], aeration [27], centrifugation [15,25,26] and salt addition [24] were used.

As organic solvent is used as the acceptor phase in VALLME and DLLME (both are two phase extraction procedures), gas chromatography (GC) naturally lends itself as the most preferable analytical technique [14,18,20,21,23]. Although the organic extracts have been directly analyzed using reversed phase HPLC, poor reproducibility of retention times and resolution are sometimes observed [10,15,16,24–27]. The non-compatible nature of the organic extracts and the reversed phase HPLC resulted in long retention of the organic solvent in the column. Strategies to overcome this problem include diluting the organic extract with methanol, extension of the HPLC analysis time and conditioning of the column after the elution of the peaks [10,15,16,24–27]. These attempts leading to low extraction yield, are not only time consuming but also consume large amounts of mobile phase [10,15,16,24]. The lack of selectivity of the two phase VALLME procedure also poses interferences when applied to real samples [15,16,24,25].

Thus, the present study is dedicated to the development of a new technique, herein referred to as vortex-assisted liquid–liquid–liquid microextraction (VALLLME). The technique involves two major steps: (i) VALLME to extract the analyte from aqueous (DP) to organic phase (intermediate phase) and (ii) a micro-vortex assisted liquid–liquid extraction (μ-VALLE) procedure to extract the analyte in the intermediate phase back to an aqueous phase (AP). A major hallmark of the technique is that the extracts are aqueous-base and thus can be directly analyzed using reversed phase HPLC and CE.

Carvedilol (Fig. 1), 1-(9H-carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy)ethyl]amino]-2-propanol, is a beta (β1,β2) and alpha (α1) blocker with antioxidant activities [28]. It is widely used as a clinical cardioprotective agent for hypertension [29] and heart failure treatment [30]. Carvedilol is highly bound to protein in plasma (98%) [30]. In urine, 2–4% of the dosage is excreted unchanged [31]. Numerous methods have been reported for the determination of carvedilol in biological samples; these include protein precipitation (PP) [32,33], liquid–liquid extraction (LLE) [34–37], solid phase extraction (SPE) [38,39], stir bar sorptive extraction (SBSE) [40] and DLLME [31]. However, PP, LLE and SPE methods suffered from many drawbacks such as consumption of large amounts of organic solvents and sample, time consuming and involvement of multi-extraction steps [32,34,38]. Although SBSE and DLLME generally provided better enrichments compared to the traditional extraction methods, carryover effect, the requirement of professional coating skills of the stir bar, the use of toxic organic solvents, long analysis time and prone to interferences are the main disadvantages of the reported methods [31,40].

In the present study, the method development of the new VALLLME technique is described and its analytical usefulness is demonstrated for the determination of carvedilol (as a model analyte) in biological fluids.

2. Experimental

2.1. Chemicals and reagents

Carvedilol was kindly donated by Hikma Pharmaceuticals (Amman, Jordan). Chemicals and reagents used were obtained from the following sources: HPLC-grade methanol ($\geq$99.96%) and phosphoric acid (85%, w/w), Merck (Darmstadt, Germany); HPLC-grade acetonitrile (99.99%) and toluene, Fisher Scientific (Milwaukee, WI, USA); hydrochloric acid (37%, w/w), Quality Reagent Chemicals (QReC, Auckland, New Zealand); sodium chloride, A.R. Bendosen (Selangor, Malaysia); 1-heptanol ($\geq$99.9%) and butyl acetate ($>$99.0%), Fluka (Buchs, Switzerland); sodium hydroxide ($\geq$98.0%), R&M Marketing (Essex, UK); *n*-octane ($>$99%), Acrōs (Geel, Belgium); dihexyl ether (97.0%), *n*-heptane (99.0%), 1-hexanol ($\geq$99%), 1-octanol ($\geq$99%), sodium phosphate monobasic monohydrate and sodium phosphate tribasic dedecahydrate, Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water (resistivity, 18.2 MΩ cm^{-1}), produced by Millipore water purification system (Molsheim, France), was used throughout for the preparation of solutions. Human urine sample was obtained from a healthy student volunteer. Plasma sample was donated by the Centre for Drug Research, Universiti Sains Malaysia, Penang, Malaysia. Sodium chloride solution (20%, w/v) was prepared by dissolving NaCl (20 g) in water (100 mL).

2.2. Instrumentation

A Hitachi LC-6200 intelligent pump (Tokyo, Japan) equipped with a Hitachi L-4250 UV–vis detector (Tokyo, Japan) was used for the separation and determination of carvedilol. Sample was injected to the instrument via a Rheodyne 7125 injection valve (Cotati, CA, USA), with a 10 μL loop. The separation was carried out using ODS-3 Hypersil C18 column (250 mm × 4.6 mm, 5 μm) purchased from Thermo Fisher Scientific (Waltham, MA, USA). The detection wavelength was set at 285 nm. A PowerChrom data acquisition was obtained from eDAQ (Denistone East, Australia) and performed with PowerChrom software (version 2.6.11) for processing and analyzing of the data. A mixture of acetonitrile and sodium phosphate buffer (10 mM, pH 4.0) (50:50 or 70:30, v/v, for VALLME and VALLLME, respectively) was used as mobile phase at the flow rate 1.0 mL min^{-1}. The mobile phase was filtered using nylon membrane filter (0.22 μm) from Agilent Technologies (Waldbronn, Germany) and degassed for 15 min before use.

CE analysis was performed using $HP^{3D}CE$ system (model 7100) equipped with diode array detector (model G7100A) from Agilent Technologies (Waldbronn, Germany). The adopted electrophoretic conditions were: capillary, uncoated bare fused-silica (50 μm i. d. × 56 cm) purchased from Agilent Technologies (Waldbronn, Germany); injection time, 40 s (hydrodynamically for 50 mbar); voltage, 30 kV; background electrolyte (BGE), 50 mM sodium phosphate buffer (pH 4.0) and detection wavelength, 285 nm [41]. The new capillary was conditioned by flushing with 1.0 M NaOH (15 min), followed by 0.1 M NaOH (20 min) and water (20 min). Between the injections, the capillary was preconditioned by 0.1 M NaOH (5 min), water (5 min) and BGE (5 min). All solutions were filtered using nylon membrane filter (0.22 μm) from Agilent Technologies (Waldbronn, Germany) before use.

2.3. Minimization of the matrix effect in plasma

In order to minimize the matrix effects of plasma, a simple and rapid pretreatment method has been used. Methanol (2 mL) was