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Dried blood spots and parallel artificial liquid membrane extraction—A simple combination of microsampling and microextraction

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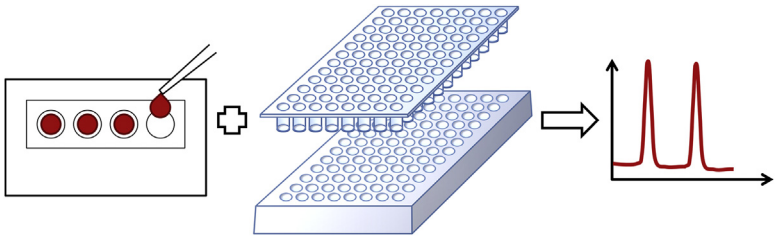
HIGHLIGHTS

- Dried blood spots and parallel artificial liquid membrane extraction were combined for the first time.
- Desorption of the model analytes from the DBS were performed under alkaline or acidic, aqueous conditions.
- Desorption and extraction of the analytes across the supported liquid membrane occurred simultaneously.
- The extracts were clean, with no detected phospholipids.
- Validation data were in accordance with the European Medicines Agency (EMA) guidelines.

GRAPHICAL ABSTRACT



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ABSTRACT

In this paper, parallel artificial liquid membrane extraction (PALME) was used for the first time to clean-up dried blood spots (DBS) prior to ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). Fundamental studies exploring amongst others desorption from the DBS in alkaline or acidic aqueous conditions, total extraction time and absolute recoveries were executed. Desorption and PALME were performed using a set of two 96-well plates, one of them housing the sample and the other comprising the supported liquid membrane (SLM) and the acceptor solution. In one procedure, amitriptyline and quetiapine (basic model analytes) were desorbed from the DBS using 250 μL of 10 mM sodium hydroxide solution (aqueous), and subsequently extracted through the SLM consisting of 4 μL of 1% trioctylamine in dodecyl acetate, and further into an acceptor solution consisting of 50 μL of 20 mM formic acid. In a second procedure, ketoprofen, fenoprofen, flurbiprofen, and ibuprofen (acidic model analytes) were desorbed from the DBS into 20 mM formic acid, extracted through an SLM with dihexyl ether, and further into an acceptor solution of 25 mM ammonia. Within 60 min of PALME, both basic and acidic model analytes were effectively desorbed from the DBS and extracted into the acceptor solution, which was injected directly into the analytical instrument. Recoveries between 63 and 85% for the six model analytes were obtained. PALME provided excellent clean-up from the DBS samples, and acceptor solutions were free from phospholipids. Linearity was obtained with $r^2 > 0.99$ for five of the six analytes. Accuracy, precision and UHPLC-MS/MS matrix effects were in accordance with the European Medicines Agency (EMA) guideline. Based on these experiments, PALME shows great potential for future

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processing of DBS in a short and simple way, and with the presented setup, up to 96 DBS can be processed within a total extraction time of 60 min.

1. Introduction

In dried blood spot (DBS) analysis, small volumes of whole blood from finger or heel pricks are spotted onto a filter paper and dried before extraction of the analytes [1–5]. There has been a growing interest for the technique over the years, owing to its many advantages over the traditional, and more invasive, blood sampling by venepuncture. The use of DBS was first described by Ivar Christian Bang [6], and achieved its breakthrough in the 1960s, when Guthrie and Susi introduced it in measurements of phenylalanine in newborns for the detection of phenylketonuria [7]. The use of DBS is especially advantageous in this subpopulation, as newborns have a limited blood volume. Today DBS is used in screenings for several inherited metabolic diseases. Over the last years, the use of DBS has also become attractive in the quantification of drug substances in therapeutic drug monitoring (TDM), pharmacokinetics (PK), and toxicokinetics owing to development of highly sensitive LC-MS/MS instrumentation [3–5]. Apart from the advantages of small blood volumes and less invasive sampling, DBS also offers easy storage and transportation, providing that samples are stable at room temperature. In addition, the exposure to infectious diseases for people handling the samples is reduced to a minimum [2–5]. There are, however, some challenges related to the use of DBS. The assay sensitivity is limited when the analyte concentration is low, due to the small sample size. Differences in hematocrit between individuals lead to different spreading of the blood on the filter paper. This, together with the possibility for non-homogenous distribution of the analytes within the DBS sample, may affect the measured analyte concentration [3–5].

The classical DBS procedure starts with application of 10–25 μL of whole blood to the DBS card (often based on filter paper), before the sample is dried for at least 2 h. The spot is then punched and the analytes are usually desorbed in a polar organic solvent mixed with water, and finally the mixture is centrifuged. The supernatant is typically analysed by LC-MS/MS, either directly or after evaporation and reconstitution [3,4]. Both procedures provide limited sample clean-up, and the desorption efficiency of analytes from the DBS cards may be low [8]. Poor sample clean-up is a serious issue, because dirty samples may cause ion-suppression and contamination of the mass spectrometer. Liquid-liquid extraction (LLE) or solid phase extraction (SPE) can be performed after the traditional desorption to improve sample clean-up. However, the procedure tends to be time-consuming, with a more complex workflow [2,4]. To address this challenge, a new approach to clean-up from DBS was investigated in the present work, using a recently published extraction method termed parallel artificial liquid membrane extraction (PALME).

PALME was introduced in 2013 as a novel extraction technique [9]. The technique is an extension of liquid-phase microextraction (LPME) into 96-well format. Two 96-well plates, one donor- and one acceptor plate, are used to perform the extractions. The performance and extraction principle are described elsewhere [9]. PALME is performed with commercially available 96-well plates, and the extraction procedure offers a simple workflow. The potential for automation with pipette robots is therefore high. PALME offers a high degree of sample clean-up, and can be considered a contribution to “green chemistry”, as the use of organic solvent per sample is low (3–5 μL). Previously, set-ups for extraction of basic, acidic, and polar analytes have been investigated [9–11]. PALME has also been found to offer efficient clean-up of phospholipids from plasma samples [12]. In the previous work with PALME, the extractions have been performed from plasma, whole blood or water samples [9,11,13]. As PALME is a microextraction technique it was interesting to investigate extractions from the microsampling technique DBS. Thus, miniaturization could be accomplished in both the sampling and extraction procedure.

In this project, PALME and DBS were combined for the first time. The aim of this proof-of-principle study was to investigate the abilities of PALME for simultaneous desorption and clean-up from DBS. Two basic model analytes; amitriptyline and quetiapine, and four acidic model analytes; ketoprofen, fenoprofen, flurbiprofen, and ibuprofen, were selected for the experiments. An optimization of one method for the basic, and one method for the acidic model analytes was performed, and finally the methods were evaluated.

2. Experimental

2.1. Chemicals

Amitriptyline hydrochloride, quetiapine hemifumarate, ketoprofen, fenoprofen calcium salt hydrate, flurbiprofen, ibuprofen, trioctylamine, dodecyl acetate, dihexyl ether, formic acid, and trichloroacetic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). The internal standard solution of 100 μg mL^{-1} quetiapine-d8 hemifumarate in methanol was also from Sigma-Aldrich (St. Louis, MO, USA). Sodium hydroxide was from VWR (Leuven, Belgium). Methanol and ammonia solution 25% were from Merck (Darmstadt, Germany). Purified water was obtained from a Millipore Milli-Q water purification system (Millipore, Molsheim, France).

2.2. Standard solutions

Stock solutions of amitriptyline and quetiapine with a concentration of 1 mg mL^{-1} were prepared in methanol, and stock solutions of ketoprofen, fenoprofen, flurbiprofen, and ibuprofen with a concentration of 5 mg mL^{-1} were prepared in ethanol. The stock solutions were used to spike drug-free whole blood.

2.3. Biological matrices

Drug-free human whole blood was obtained from a healthy volunteer at the School of Pharmacy, University of Oslo, and stored at −18 °C.

2.4. Blood sampling

5, 10 or 20 μL of whole blood spiked with the model analytes was applied to Whatman® FTA® DMPK-C cards (GE Healthcare, Piscataway, NJ, USA), and dried at room temperature for at least 2 h. When using 5 or 10 μL blood, the entire spot was cut out using a pair of scissors, before sample preparation. From 20 μL spots, 3 mm discs were punched out using a Harris Uni-Core puncher and Cutting Mat from Whatman (GE Healthcare, Buckinghamshire, UK).

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