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Dynamic hollow-fibre liquid phase microextraction of dinitrophenols from human plasma: Optimization of an extraction flow system using experimental design methodology

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ABSTRACT

The utility of a dynamic hollow-fibre liquid phase microextraction method (optimized using a four-variable experimental design and response surface modelling) for extracting dinitrophenolic compounds from human plasma samples was evaluated. The investigated variables were donor phase salt concentration (10–400 mM), donor phase pH (2–6), acceptor phase pH (7–12), and donor/acceptor phase flow rates (30/7.5 to 70/17.5 μL min^{-1}). Four dinitrophenol pesticides were used as model substances at concentrations of 0.1 μg mL^{-1} in spiked human plasma samples. Extraction efficiencies ranging from 42 to 77% with RSDs below 9 were achieved with the optimized method. The flow rate and acceptor pH were shown to strongly affect the extraction efficiency for all compounds, while the donor phase pH and salt concentration had minor effects. With a well-defined acceptor phase pH and flow rate the system exhibited high robustness. The limits of quantification for the investigated compounds, using the presented extraction method followed by liquid chromatography/electrospray ionization mass spectrometry in selected ion monitoring mode, ranged from 0.05 to 0.1 μg mL^{-1} plasma.

1. Introduction

In this study we use a miniaturized version (wall thickness 50 μm, inner diameter 280 μm) of the XT-extractor [1,2], a liquid membrane-based system for dynamic hollow-fibre liquid phase microextraction (HF-LPME), previously designed by Jonsson et al. [1]. The XT-extractor had larger hollow-fibre dimensions (wall thickness 100 μm, inner diameter 330 μm). The name is due to a cross (X)-connector and a tee (T)-connector and a PTFE tubing with a hollow fibre mounted in between. The version presented in this work is similar, but has two T-connectors instead of X and T. Miniaturization offers several obvious advantages, including the scope to extract analytes from samples with smaller volumes and to reduce solvent consumption. The utility of the minituarized system for extracting nitrophenol pesticides from aqueous standard solutions was subsequently evaluated [2], but no attempt was made in the cited study to optimize the system for extracting plasma samples.

HF-LPME resembles classical liquid–liquid extraction, but the liquid phases in HF-LPME are separated by a hollow fibre, typically made of polypropylene, with pores in which an organic solvent of sufficiently low vapour pressure (e.g. undecanone or dihexyl ether [3]) is immobilized by capillary forces, thereby forming a liquid membrane. Thus, the system can be used for a form of three-phase liquid–liquid–liquid extraction, known as supported liquid membrane extraction (SLM), of either acidic or basic compounds. The analytes in the sample (donor phase, or simply donor) are neutralized and extracted across the organic liquid membrane into the extracting solvent (acceptor phase, or simply acceptor). Generally the efficiency is highest for compounds with log P values in the range −1 to +3 [4]. HF-LPME based extraction procedures for basic compounds in stagnant systems have been reported by Bårdstu et al. [5] and Pedersen-Bjergaard et al. [6].

Nitrophenolic pesticides, used as model compounds in the present study, are acidic and have log P values ranging between 1.7 and 5.6. This group of compounds were used globally in agriculture and as raw industrial materials before they were banned both by the EU and the American Environmental Protection Agency (US EPA) [7,8]. However, they are still used in several countries outside the EU and US, and are widely spread in the environment because of their high water solubility [7,8].

Tremp et al. identified and quantified nitrated phenols in exhausts from motor vehicles and studied the transfer process of these compounds between air and water bodies [9]. Morville et al. [10] have recently presented measurements to show variations of concentration and the role of traffic in the emission of phenols and nitrophenols to the atmosphere.

In the early 1930s dinitrophenols were used as weight loss drugs, but this was shown to pose serious health risks [11]. Although the

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use of 2,4-dinitrophenol (DNP) as drug is illegal nowadays it can still be purchased for weight loss. It is sold under names as Sulfo black, Nitro Klenup or Caswell No. 392 without warnings [12]. The enlarged interest in weight loss drugs has caused an increase in case reports [13]. Recently, there have been several fatalities due to the use of 2,4-dinitrophenol (DNP), sold over the Internet as a dietary supplement for bodybuilders or as weight loss drug [12,14]. Exposure limits for the substances set to 10 $\mu g\,mL^{-1}$ serum and 5 $\mu g\,mL^{-1}$ whole blood have been proposed by the EU [15].

Recently, several on-line and off-line microextraction techniques were reviewed by Hyötyläinen and Riekkola [16], and Lambropoulou et al. [17] have reviewed several liquid-phase microtechniques specifically for extracting pesticides, mostly applied to pesticides in aqueous matrices. In addition, a static HF-SLM method for detecting dinitrophenolic compounds in water samples has been developed and optimized by Lezamiz and Jönsson [18], and an HF-SLM method for extracting short-chain fatty acids from human serum, followed by GC/FID detection, has been developed by Zhao et al. [19]. For detection of DNP Leftwich et al. has presented a UV method for serum from a man poisoned after crop spraying [20] and Miranda et al. used a GC/MS a method for serum from a 17-yerar-old female and a 28-year-old male using DNP as weight loss drug [12]. In the latter case reported admission blood levels were 36 and 28 $mg\,L^{-1}$, respectively, and fatal [12].

The aim of the study presented here was to optimize the efficiency of the dynamic micro-HF-LPME system for extracting nitrophenols from human plasma, using LC/ESI-MS to detect the analytes, due to its high selectivity and lack of any need for derivatization prior to analysis.

2. Experimental

2.1. Chemicals and material

All standard compounds, i.e. 4,6-*o*-dinitrocresol (DNOC), 2-sec-butyl-4,6 dinitrophenol (Dinoseb), 2-*tert*-butyl-4,6 dinitrophenol (Dinoterb), 2,4-dinitrophenol (DNP) and 3-methyl-4-nitrophenol (IS) (all of 98% purity) were purchased from Sigma–Aldrich (Seelze, Germany). Dihexyl ether (97%) and acetonitrile of analytical grade were from Sigma–Aldrich (Steinheim, Germany).

Sodium hydrogen carbonate, sulphuric acid, sodium hydroxide and sodium chloride were obtained from Merck (Darmstadt, Germany), formic acid (98–100%) from Scharlau Chemie S.A. (Barcelona, Spain) and normapure acetone from VWR International (Lutterworth, UK). Plasma was obtained from Karolinska Hospital (Stockholm, Sweden). The polypropylene hollow fibre (Accurel PP, 50/280, porosity 40%, i.d. 280 μm) used to construct the microextraction system was purchased from Membrana GmbH (Wuppertal, Germany), the polyetheretherketone (PEEK) T-connectors, low-pressure polyacetal fittings, o-rings and PTFE tubing (1/16″ 0.5 mm i.d.) from Vici Jour (Schenkon, Switzerland), the cyanoacrylate glue from Casco AB (Stockholm, Sweden), the fused-silica capillary (TSP 100245) from Polymicro Technologies Inc. (Phoenix, AZ, USA) and the TL-105 column heater from Timberline Instruments Inc. (Boulder, CO, USA).

Water used to prepare aqueous solutions was purified using a Milli-Q system (Millipore, Billerica, MA, USA).

2.2. The extractor and extraction procedure

The extractor is easy to assemble from cheap, commercially available parts. A 12-cm hollow fibre glued to a fused-silica capillary at both ends was placed in PTFE tubing, which was then connected to two T-connectors. The extractor was placed vertically and was filled with the organic phase (dihexyl ether) to saturate the pores of the hollow fibre. The extractor was then placed in a column heater and connected to a syringe pump with two syringes to control the flow of the donor and acceptor, respectively. The donor flow rate was always set four times higher than the acceptor flow. The flow rate of both donor and acceptor was measured ($n = 6$, RSD = 1 and RSD = 7, respectively).

Plasma was kept at −20 °C until use, and was then thawed at room temperature. Portions of 1 mL plasma and 0.5 mL of aqueous standard solutions were mixed on a mixing table for 30 min. After mixing, 0.9 mL water, 0.1 mL isopropanol and two drops of sulphuric acid were added to each plasma sample, which was then ultrasonicated for 1 min. Plasma samples were kept at room temperature during the day of analysis. Blank plasma samples were treated identically, except that 0.5 mL of ultra pure water was added instead of the standard solution.

The plasma sample pretreatment and extraction system have been recently described in detail [2].

2.3. LC/ESI-MS

The analytes were separated from other constituents of 5-μL portions of the samples by high-performance liquid chromatography using an LC 10 AD vp chromatograph with a SIL-10 AD vp autoinjector (Shimadzu, Japan) and a Hypersil-Gold C_{18} column (50 mm × 2.1 mm i.d., 3 μm particle size; Thermo, Cheshire, UK). The mobile phase (flow rate 200 $\mu L\,min^{-1}$, total run time 18 min) consisted of 20% A for 5 min, a step change to 50% A, held for 10 min, followed by a step change to 20% A, held for 3 min, where A was acetonitrile and the balance was provided by Milli-Q water (both acidified with 0.01% formic acid). An ESI triple-quadrupole API 2000 mass spectrometer from Applied Biosystems (MDS SCIEX, Foster City, Canada) was then used in negative mode to monitor the quasi-molecular ions $[M-H]^-$ of the analytes (m/z 152 for IS, 183 for DNP, 197 for DNOC and 239 for both Dinoseb and Dinoterb). The following settings were used: desolvation temperature, 350 °C; declustering potential, −40 V; focusing potential, −400 V; entrance potential, −10 V; ion spray voltage, −4500 V; nebulizer gas, 10 psi; curtain gas, 20 psi; ion source gases, 20 psi. Reconstructed ion chromatograms (RICs) for extracted human plasma samples are shown in Fig. 1. A volumetric internal standard (3-methyl-4-nitrophenol) and single-point calibration with an external standard solution were used to calculate the extraction efficiency.

2.4. Optimization and experimental design

A full-factorial experimental design, as described in Table 1, was set up to evaluate the influence of the following four controlled variables on the extraction efficiency: donor pH (2 and 6, adjusted with sulphuric acid), salt concentration in the donor phase (10 and 400 mM sodium chloride), flow rates of the acceptor and donor phases (30/7.5 and 70/17.5 $\mu L\,min^{-1}$), and acceptor pH (7 and 12, adjusted with sodium hydroxide). In all experiments the acceptor solution contained 60mM sodium hydrogen carbonate and the donor flow rate was four times higher than the acceptor flow rate. The experimental design included 17 permutations of conditions, obtained by varying each of the investigated variables at the lower and upper levels (designated − and + in Table 1, respectively). Four experiments were performed for each of these permutations of conditions, and an additional set of 12 replicate experiments were performed with central settings of the variables (designated 0).

The design was subsequently complemented with 8 further permutations of experimental conditions to complete a central composite face (CCF) design, with one experiment per permutation. The CCF design allows a quadratic model for extraction efficiency to be fitted as a function of the four controlled variables, thereby improving the optimization. The experimental design was set up