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Quantitative dried blood spot analysis for metallodrugs by laser ablation-inductively coupled plasma-mass spectrometry

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ABSTRACT

A quantitative dried blood spot (DBS) method based on direct sampling by means of laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) is presented. Gadolinium-based contrast agents were used as model metallodrugs with a significant relevance for pharmaceutical applications. Challenges regarding the ablation of the complex blood-filter matrix were characterized and successfully addressed by a thorough adaption of the laser ablation conditions. Especially the laser fluence was optimized with respect to the particle size distribution of the generated aerosol as monitored by an optical particle counter. Thus, generation of micrometer-sized particles could be minimized in favor of smaller particles increasing the transport efficiency of the DBS ablation aerosol to the plasma and the recorded signal stability. Inhomogeneous blood drying on the porous filter paper could be compensated by the addition of an internal standard prior to blood spotting. To preserve the advantages of DBS sampling, such as small blood volumes and minimal invasiveness, the combined use of DBS and a capillary blood sampling system is demonstrated. By placing the internal standard into the capillary prior to blood sampling, a simple workflow usable for clinical application was implemented. The applicability of the developed method, achieving limits of detection and quantification in the low $\mu g\ L^{-1}$ range and covering a linear range of over four orders of magnitude, was demonstrated for blood samples containing different concentrations of the gadolinium contrast agents gadopentetate and gadoterate.

1. Introduction

Since the introduction of dried blood spot (DBS) analysis to newborn screening for inherent metabolic diseases in the 1960s [1], its great potential was recognized in diverse fields of application, such as epidemiological studies [2–8], therapeutic drug monitoring [9–11], and preclinical studies of drug development [12–14]. The advantages over liquid blood analysis lie in the minimally invasive sampling, the sufficiency of small blood volumes as well as simple transport and storage requirements [15–17]. Indeed, the transformation of liquid blood to a solid sample poses some analytical challenges, e.g. the dependency of the DBS spreading on the blood viscosity [18]. Conventional DBS methods comprise punching and extraction steps with subsequent liquid chromatography-tandem mass spectrometry (LC–MS/MS) [19]. To achieve higher throughput and decrease contamination possibilities [20], the application of direct sampling techniques [20], like desorption electrospray ionization (DESI) [21,22] and paper spray (PS) [23,24], has been presented. As a multielemental surface technique with excellent sensitivity, laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) has also demonstrated its capability in various DBS studies for the analysis of essential and toxic elements with a particular focus on lead [25–29]. Indeed, quantification possibilities are limited by the occurrence of inhomogeneous blood spreading on the filter paper and varying ablation efficiencies. Cizdiziel observed a different spreading behavior of real samples and reference materials resulting from the hemolysis of freeze-dried reference materials [27]. To compensate for chromatographic effects, complete DBS desorption by means of a femtosecond laser with a pulse rate of $3{\cdot}10^4$ Hz was presented [30]. Using a nanosecond LA system, complete ablation of very small (0.5 µL) DBS, spotted onto polymer-based filter cards, was proposed [28,31]. Adhering of the blood droplets to the hydrophobic filter surface could prevent the use of high laser fluences, perforating the filter, while however losing the DBS advantages of analyte stabilization and simple transport. As an alternative to complete DBS desorption, Nischkauer et al. used a radial line scan over the entire blood spot to compensate for centro-symmetric drying effects [32]. To address variances in the ablation efficiency, internal standardization using ^{13}C [33] or treatment of the filter cards with a standard solution [29] was

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proposed. Inhomogeneous blood drying can, however, only be fully compensated when adding the internal standard (IS) to the blood prior to spotting.

Goal of this study was the development of a quantitative DBS method for metallodrugs based on direct LA-ICP-MS sampling. As suitable model substances with high medical relevance, gadolinium-based contrast agents for magnetic resonance imaging (MRI) were chosen. Based on the investigation of aerosol characteristics by an optical particle counter, thorough adaption of the laser ablation conditions to the complex blood-filter matrix is presented. For quantification by internal standardization, the combined use of capillary blood sampling systems and DBS filter cards is successfully demonstrated.

2. Materials and methods

2.1. Chemicals and consumables

Chemicals were used in the highest purity available. Nitric acid (69%, Suprapur) as well as gadolinium and europium ICP standard solutions (1000 $mg \cdot L^{-1}$) were purchased from Merck (Darmstadt, Germany). Thulium and rhodium ICP standard solutions (1000 $mg \cdot L^{-1}$) were ordered from SCP Science (Baie D'Urfé, Canada). The contrast agent infusion solutions were obtained from the respective pharmaceutical companies: Magnevist® (gadopentetate, 0.5 $mol \cdot L^{-1}$) from Bayer-Schering Pharma AG (Berlin, Germany) and Dotarem® (gadoterate, 0.5 $mol \cdot L^{-1}$) from Guerbet (Sulzbach, Germany). Triton® X-100 was purchased from Sigma Aldrich (Steinheim, Germany). Throughout all experiments, doubly distilled water generated by an Aquatron Water Still purification system (A4000D, Barloworld Scientific, Nemours Cedex, France) was used. For the collection of small blood volumes, the capillary blood sampling system Minivette® *POCT*, collecting a volume of 50 µL and containing 1.4 µL ethylenediaminetetraacetic acid (EDTA) as anticoagulant, was purchased from Sarstedt (Nümbrecht, Germany). Blood spotting was performed on cellulose-based and chemical untreated Munktell TFN filter cards from Ahlstrom Corporation (Helsinki, Finland). Micro sample vials, providing a volume of 0.3 mL and consisting of polypropylene, were ordered from IVA Analysentechnik (Meerbusch, Germany).

2.2. DBS preparation

2.2.1. Optimization of LA conditions

For DBS preparation, venous whole blood from a healthy volunteer adult, stabilized with EDTA, was utilized. To optimize laser ablation conditions, DBS with a gadolinium concentration of 0.5 $mg \cdot L^{-1}$ were prepared by homogenizing 10 µL of a gadolinium standard solution (5 $mg \cdot L^{-1}$) with 90 µL of whole blood. The DBS were generated by spotting a free falling blood drop of 10 µL onto the filter cards. In all cases, the DBS were allowed to dry for at least 4 h according to the recommendation by the filter card manufacturer.

2.2.2. Combination of DBS and capillary blood sampling systems

To introduce an IS prior to blood spotting onto the filter paper, different capillary blood sampling systems were investigated. The Minivette® *POCT* was chosen for further experiments, as it provides a stamp for the active release of the blood from the capillary. To demonstrate the sampling strategy of a blood droplet from a finger prick, a 70 µL droplet of the whole venous blood was placed onto a hydrophobic plastic microscope slide which allowed for the formation of a single droplet without spreading. By capillary forces, the capillary was filled with the blood when kept in horizontal position. Blood sampling automatically stopped at the blocking filter at the end of the capillary. Prior to blood sampling, 1 µL of a 25 $mg \cdot L^{-1}$ europium solution was added to the capillary blood sampling system by separating the stamp from the capillary. The IS solution was allowed to dry for at least 2 h on a 40 °C heating plate before blood sampling. After blood sampling, the tip of the filled capillary was covered and the IS was allowed to diffuse for a specific period of time while shaking the whole device. Diffusion times of 5, 10, 20, 40 and 60 min were investigated in triplicate using blood with a gadolinium concentration of 0.5 $mg \cdot L^{-1}$. By pushing the stamp in vertical position, the defined volume of the sampled blood could be released into a micro sample vial. The DBS were generated by spotting 10 µL blood onto the filter paper.

2.2.3. Quantification of gadolinium-based contrast agents

To investigate the applicability of the developed method regarding the quantification of contrast agents, human whole blood was spiked with aqueous solutions of gadopentetate and gadoterate, respectively. These two contrast agents were selected as representatives for the two structural classes of linear and macrocyclic contrast agent that are generally in use. For both contrast agents, three concentrations were prepared by diluting the infusion solutions (0.5 $mol \cdot L^{-1}$) with a factor of 1:20, 1:200 and 1:2000. 10 µL of the respective contrast agent solutions were added to 990 µL blood. The final gadolinium concentrations in the blood were validated by ICP-MS using liquid sample introduction via a nebulizer (see 2.5). For quantification by LA-ICP-MS, external calibration using DBS standards with a gadolinium concentration ranging from 0.005 to 50 $mg \cdot L^{-1}$ was applied. The matrix-matched standards were prepared by homogenizing 25 µL of the respective aqueous gadolinium solutions (diluted from the ICP standard solution) with 475 µL of the blood. Subsequently, the blood calibration standards were treated analogous to the samples spiked with the contrast agents. Gadolinium quantification for each contrast agent and concentration was performed in triplicate.

2.3. Sample preparation for validation by ICP-MS

To validate the LA-ICP-MS method for DBS analysis, the gadolinium concentrations of the human blood spiked with the contrast agents were also determined by ICP-MS analysis. The blood samples were diluted with a 0.5% nitric acid solution containing the detergent Triton® X-100 (0.0005% *v/v*) for stabilization. Prior to dilution, europium was added to the samples as IS resulting in a final concentration of 1 $\mu g \cdot L^{-1}$. For external calibration, nine aqueous gadolinium standard solutions, ranging from 0.05 to 20 $\mu g \cdot L^{-1}$, plus blank were used. All standard solutions contained europium in a concentration of 1 $\mu g \cdot L^{-1}$. The gadolinium concentration of each blood sample was determined in triplicate.

2.4. LA-ICP-MS analysis

2.4.1. Instrumentation and setup

For LA-ICP-MS analysis, a commercially available 213 nm laser ablation system (LSX 213 G2+, Teledyne CETAC Technologies, Omaha, NE, USA) equipped with a frequency-quintupled Nd:YAG laser was coupled to a quadrupole-based ICP-MS instrument (Aurora M90 Elite, Bruker, Fremont, CS, USA) providing a 90° ion optics and a collision reaction interface (CRI). The LA system comprised a 2-volume cell (HelEx, Teledyne CETAC Technologies) with wash-out times well below 500 ms and was controlled by DigiLaz software G2+ (Teledyne CETAC Technologies). After DBS preparation, the filter cards were fixed by an double-sided tape on a microscopic glass slide which was clamped into the ablation cell. A linear scan pattern was utilized throughout all experiments. Via Tygon® SE-200 tubing with an inner diameter of 3.2 mm, the generated aerosol was transported to the plasma using helium as carrier gas (800 $mL \cdot min^{-1}$) through the ablation chamber and an additional post-chamber argon flow (400 $mL \cdot min^{-1}$) for enhanced transport efficiencies. To monitor the plasma stability, a thulium solution (50 $ng \cdot L^{-1}$) was simultaneously introduced by a MicroMist nebulizer (Analytik Jena, Jena, Germany) and a Scott-type spray chamber. The nebulizer gas flow was set to 0.7 $L \cdot min^{-1}$. Introduction of the dry LA aerosol was carried out via a modified gas adapter, which was connected to the torch and allowed for coaxial