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C_{18}-attached membrane funnel-based spray ionization mass spectrometry for quantification of anti-diabetic drug from human plasma☆

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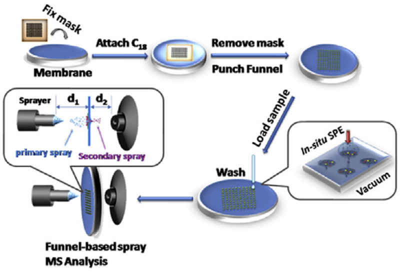
HIGHLIGHTS

- Sorbent attached membrane funnel based spray platform was used for drug determination in human plasma.
- The matrix suppression effect of human plasma was largely eliminated.
- The method was applied to determine repaglinide in plasma volunteers.
- Membrane funnel-based spray is promising for analysis of biological samples.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, sorbent-attached membrane funnel-based spray ionization mass spectrometry was explored for quantitative analysis of anti-diabetic drugs spiked in human plasma. C_{18}-attached membrane funnel was fabricated for *in situ* extraction and clean-up to alleviate matrix suppression effect in the ionization process. Repaglinide was used as a target analyte of anti-diabetic drugs. Under optimal working conditions, good linearity ($R^2 > 0.99$) was obtained in the concentration range of 1–100 ng mL^{-1}. The method detection limit of target drugs spiked in the human plasma was around 0.30 ng mL^{-1}. Through the application of an isotope-labeled internal standard, the signal fluctuation caused by residual background matrices was largely alleviated and the precision of measurement (RSD) was below 15%. The recovery of repaglinide for 5, 25, and 100 ng mL^{-1} of spiked human plasma matrixes ranged from 87% to 112%. The developed method was successfully applied to determine repaglinide in plasma volunteers who orally received a dose of drug association. Our results demonstrated that membrane funnel-based spray is a simple and sensitive method for rapid screening analysis of complex biological samples.

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1. Introduction

In the last decade, the development of new ambient ion (AI) sources for mass spectrometry analysis has attracted much attention [1,2]. Pioneer AI techniques include desorption electrospray ionization (DESI) [3] and direct analysis in real time (DART) [4], which directly analyze samples with minimal or no sample preparation processes. For example, DESI has been used to quantify drugs in biological fluids, such as urine and plasma [5,6]. The advantage of this method is its simplicity and high throughput (i.e., 96 samples/3 min). DART has been used to quantify small drugs (e.g., verapamil) in untreated rat plasma [7]. The most attractive feature of DART is its instant response, which is useful for real-time monitoring analysis. In recent years, extractive electrospray ionization [8] and paper spray [9,10] have drawn special interest and were used for quantitative analysis of various types of molecules from a complex matrix. As a complementary alternative to traditional liquid chromatography-mass spectrometry-based method, direct mass spectrometric analysis is simple, efficient, and offers significant advantages in sample throughput and cost reduction. Owing presumably to the lack of component separation, these techniques suffer from matrix suppression effect [11,12]. The signals of target compounds are largely suppressed because of the involvement of matrix molecules in the ionization process. In addition, the non-uniform distribution of the target species in the sample limits the reliability of the analytical results.

Some ambient ion sources were coupled with clean-up techniques, such as protein precipitation [6], liquid-liquid extraction [13], solid phase extraction (SPE) [14–16], or solid phase microextraction (SPME) [17]. The matrix suppression effect was alleviated and sensitivity was also improved with the use of these clean-up techniques. The offline clean-up process slowed down the analysis and restricted the throughput. Significant sample loss might also occur during sample handling and transfer procedure. Recently, thin-film and stainless steel mesh-based SPME fibers were coupled with DART in transmission mode (TM) for *in situ* enrichment and high-sensitivity MS analysis of target analytes from complex matrices [17–19]. Inspired by the TM-DESI [20–22], our group has demonstrated a novel ambient ion source named membrane funnel-based spray ionization source. In membrane funnel-based spray, charged solvent droplets formed in primary electrospray were directed toward the membrane funnels to dissolve the deposited analytes. In the funnel-based spray source, the straight alignment of TM-DESI was preserved, whereas the geometry of the sample plate was different from that of the TM-DESI. In the funnel-based spray, the samples were confined in a small region with high surface concentration and direct desorbed spray toward the central orifice. The typical TM-DESI used nebulizing gas to assist the desorption process, which was avoided in funnel-based spray ionization. In addition, the ion transfer process is an electrostatically driven process instead of a predominant momentum transfer process as in DESI. This process has been used to analyze different classes of compounds, including pharmaceuticals, agricultural chemicals, oligosaccharides, polymers, peptides, and proteins [23]. Through the attachment of different sorbents, the SPE sorbent funnel was successfully fabricated and applied in the spray analysis of trypsin-digested bovine serum album in solution in the presence of high concentrations of chaotropic reagents and phosphopeptides in the trypsin-digested solution of casein complex biological samples [24].

This work studies the feasibility of sorbent-attached membrane funnel-based spray ionization MS for the quantification of anti-diabetic drugs from human plasma. Repaglinide, which is an orally administered anti-diabetic drug that helps reduce blood sugar levels to treat type 2 diabetes, is selected as the target analyte. To reduce the ion suppression effect caused by interfering substances in the human plasma, a miniaturized sample processing platform (funnel arrays) that allows online SPE was developed. This platform permitted the effective removal of matrix components with minimal sample loss. The sensitivity, linearity, dynamic range, precision, and accuracy of this method were evaluated.

2. Experimental

2.1. Chemicals and materials

All chemicals were obtained commercially and were used without further purification. Repaglinide and human plasma were purchased from Sigma Aldrich (St. Louis, MO. USA). Repaglinide-d_5 was obtained from Santa Cruz Biotechnology (Dallas, Texas. USA). The chemical structure of repaglinide is shown in Fig.S1. HPLC-grade methanol and water were purchased from Merck KGaA (Darmstadt, Germany) and Labscan (Bangkok, Thailand),respectively. HPLC-grade acetic acid was purchased from Riedel-de Haën (Seelze, Germany). Parafilm M® laboratory film (Pechiney Plastic Packaging, Chicago, IL, USA) was used as the substrate material for the membrane funnel, and 51 μm Teflon tape with silicone adhesive (CS Hyde Company, Lake Villa, IL, USA) was used to fabricate SPE sorbent funnel. C_{18}powder with a particle size of 50 μm was obtained by disassembling a 3 mL Grace Pure™ SPE C_{18}-Low cartridge (500 mg, Grace, Deerfield, IL). The commercially available tabletFulaidi® (Repaglinide Tablets, 0.5 mg) was obtained from the local medicine market.

2.2. Sorbent-attached membrane funnel-based spray-MS

All experiments were carried out using Fourier transform ion cyclotron resonance mass spectrometer (FTICR-MS) systems (9.4 T SolariX, Bruker Instrument Int., Billerica, MA) equipped with a membrane funnel-based spray ion source (Fig. S2). The funnel-based spray ion source was home-built, and the detailed information was described elsewhere [22–24]. The fabrication of sorbent funnel is described in the supplementary information. The FTICR-MS was used on the selected ion monitoring (isolation by quadrupole) by recording the accurate *m/z* of the precursor ions of the target compounds. Fig. 1 shows the flowchart of C_{18}-attached funnel fabrication, *in situ* SPE, and funnel-based spray analysis. The operating parameters of the ion source were as follows: spray voltage of 3–6 kV; solvent flow rate of 2.5–15 μL/h; drying gas temperature of 250 °C; funnel diameter of 35–40 μm; funnel-to-sprayer distance (d_1) of 0.5 mm; and funnel-to-MS inlet distance (d_2) of 0.45 mm. These parameters were further optimized to obtain suitable working conditions. Data acquisition was performed by summing up 10 scans. The mass spectra were recorded using 1 M memory.

2.3. Standard solutions

Stock solutions of repaglinide and repaglinide-d_5 were prepared at a concentration of 1 mg mL^{-1} in $MeOH-H_2O$ (1:1, v/v). All the stock solutions were stored in amber bottles at 4 °C. Working standard solutions for calibration and quality control (QC) samples were freshly prepared from stock solution as needed through appropriate dilution of stock solution. All dilutions were in $MeOH-H_2O$ (1:1, v/v with 0.1% acetic acid). The internal standard (IS)