Published in final edited form as:

Talanta. 2018 November 01; 189: 451–457. doi:10.1016/j.talanta.2018.07.027.

In-Capillary Microextraction for Direct Mass Spectrometry Analysis of Biological Samples

Yue Ren^{a,b}, Wenpeng Zhang^{a,b,*}, Ziqing Lin^b, Lane R. Bushman^c, Peter L. Anderson^c, and Zheng Ouyang^{a,b,*}

^a State Key Laboratory of Precision Measurement Technology and Instruments, Department of Precision Instrument, Tsinghua University, Beijing, 100084, China.

^b Weldon School of Biomedical Engineering and Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA.

^c Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado Denver, Aurora, CO 80045, USA.

Abstract

Slug flow microextraction-nanoESI (SFME-nanoESI) was originally explored as a single-step sampling ionization method for MS analysis of biofluids. In this work, a comprehensive study and development of the SFME has been carried out. Revers-phase SFME was developed to analyze chemical compounds in oil samples. A three-phase SFME system was introduced as a suitable approach for analyzing polar compounds in biofluids. The impacts by the capillary inner diameter, solvent and sample properties were also investigated, leading the use of fused silica capillaries for performing SFME.

Graphical abstract

Keywords

Slug flow microextraction; mass spectrometry; biofluid; polar compounds

*Corresponding authors: Wenpeng Zhang (zhan2360@purdue.edu), Zheng Ouyang (ouyang@tsinghua.edu.cn).

Conflict of interest

Z. O. is the founder of the PURSPEC Technologies Inc.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Mass spectrometry (MS) is an effective tool for chemical analysis, due to its high sensitivity and specificity for general purpose analysis. The development of novel sampling ionization methods has been proven to have a tremendous impact on the expansion of MS applications[1,2]. The introduction of electrospray ionization (ESI)[1,3] and atmospheric pressure chemical ionization (APCI)[4,5] enabled the coupling of mass spectrometry with liquid chromatography (LC) for interrogation of complex samples. This approach has been well-developed and become the gold standard for complex mixture analysis[6,7]. The requirement of equipment and expertise for this approach, however, limits its utilization outside of laboratories.

To effectively apply MS analysis outside of traditional analytical laboratories, such as point-of-care (POC) applications, it is highly desirable to develop alternative approaches that use simplified protocols and require less user training. Starting with desorption electrospray ionization (DESI)[8] and direct analysis in real time (DART) [9], ambient ionization methods have developed into a major field of research for mass spectrometry. Sample processing can be minimized for analysis of complex samples using an effective ambient ionization method[2]. Some successful applications have also been demonstrated by Laser ablation electrospray ionization,[10] electrochemical electrospray ionization[11], coated blade spray[12,13], electrospray-assisted laser desorption ionization[14], paper spray[15] and extraction spray[16]. These achievements suggest a promising direction in technical development for direct MS analysis of complex samples. Although several works have been done by DESI[17] and paper spray[18,19], direct MS analysis of biofluid is still inefficient and remains challenging.

For the purpose of direct analysis of biofluids in liquid form, a slug flow microextraction-nanoelectrospray ionization (SFME-nanoESI) method was explored in our previous work. In the initial study[20], the concepts of online purification, derivatization, quantitative analysis and enzymatic reaction monitoring have been demonstrated. With accelerated liquid-liquid extraction, relatively hydrophobic compounds in blood, plasma or urine samples were well analyzed by nanoESI MS. However, the analysis of hydrophilic compounds (e.g. $\log P < -2$) was limited by extremely low extraction efficiency.

In this work, we introduced new concepts to develop the in-capillary microextraction and to extend its application in the analysis of hydrophilic compounds in biofluids and oil samples. The influence of other factors related to capillaries and the extraction solvents were also investigated.

2. Material and Methods

All the experiments, if not otherwise specified, were carried out using a TSQ Quantum Access Max mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). The binary solvent study for blood analysis was performed on a QTRAP 4000 mass spectrometer (Sciex, Toronto, Canada). The bovine blood was purchased from Innovative Research Inc (Novi, MI, USA). The synthetic urine was purchased from CST Technologies Inc (Great

Neck, NY, USA). Tenofovir diphosphate and stable-labelled internal standard were purchased from Moravak Biochemicals, Inc (Brea, CA, USA). The blood cell lysate was prepared by lysing 0.25 mL of whole blood with 5 mL of 70% methanol/water solution (v/v). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). Borosilicate glass capillary (i.d. 0.86 mm) and fused silica tubing (i.d. 0.25 and 0.53 mm) for the characterization experiment were purchased from Sutter Instrument (Novato, CA, USA) and Polymicro Technologies (Phoenix, AZ, USA), respectively.

Raw samples were spiked with the chemicals of interest to prepare simulated samples for method evaluations. For three-phase SFME, plugs of extraction solvents (e.g. methanol/water), bridge solvents (e.g. hexane) and biofluid were injected into a glass or Teflon tubing sequentially. For reverse-phase SFME of oil samples, plugs of extraction solvents and oil were injected into a glass tubing. For quantitative analysis, internal standards were used, which were pre-mixed with extraction solvents before SFME. Unless otherwise specified, 50 extraction cycles were applied for sample processing in SFME. The moving of plugs of liquid inside capillary was driven by a pipette, the volume of which was set at 10 μ L. During SFME, pulling and pushing the liquid plugs were realized by pushing and releasing the pipette gun, the frequency is about 1 pushing and releasing per second. After SFME, the extraction solvents were transferred into a pulled borosilicate glass capillary, and a wire electrode was introduced to apply high voltage ($\sim$ 1.8 kV) for nanoESI MS analysis. For online SFME, the SFME process was performed in a pulled borosilicate glass capillary, into which the extraction solvents were injected before biofluid. After SFME, plugs of both extraction solvents and biofluid were pushed to the end of the pulled tip, and a wire electrode was introduced to apply high voltage ($\sim$ 1.8 kV) for nanoESI MS analysis.

3. Results and Discussion

3.1. Development of three-phase SFME for analysis of polar compounds

Previously we developed a fast in-capillary microextraction method for direct MS analysis of biofluid samples. Figure 1a shows the process of a typical SFME method. A disposable glass capillary of 0.86 mm i.d. with a pulled tip was used to perform the entire sampling and ionization process. Two adjacent liquid plugs of organic solvents and biofluid samples were injected into the capillary. Liquid-liquid microextraction of the analytes from the biofluid into the organic solvent is significantly improved by inducing movement of the two liquid plugs through an air-pressure induced push-pull force. After the extraction process, the organic solvent plug can be simply pushed to the tip of the capillary, and then a stainless-steel wire inserted for nanoESI MS analysis. For biofluids, immiscible solvents such as ethyl acetate were used as the extraction solvents, which showed extremely low extraction efficiency toward hydrophilic compounds due to their low solubility in these solvents.

In this work, an improved SFME method was explored to extract polar analytes from polar biofluid samples, which obviously could not be readily achieved with two immiscible phases as previously used for SFME. Here we propose a three-phase SFME system, as shown in Figure 1b. Three plugs of liquid were injected into a capillary, including an organic phase, to bridge the biofluid sample and a polar extraction solvent. During SFME, the extraction could be accelerated with the slug flows induced by the movements of the liquid plugs.

Water-immiscible solvents such as ethyl acetate and hexane are required for direct SFME of biofluid samples. In a typical three-phase SFME system of biofluid-hexane-methanol/water, most of salts, cell debris and proteins in biofluid can be blocked by the hexane because of insolubility. Hexane may only extract very small amount of polar compounds such as amino acids in each SFME cycle because of limited solubility, the extracted amino acids can be transferred into the plug of methanol/water very quickly due to the fast mass transfer induced by slug flow and good solubility of methanol/water to amino acids. By repeating SFME cycles (50–300 cycles), amino acids can be enriched into the plug of methanol/water step-by-step. The material of the capillary is an important factor since it determines the affinity of inner wall of capillary toward sample solutions and solvents. Glass capillaries were proven to be effective for SFME of different kinds of drugs, so we used glass capillaries with i.d. of 0.86 mm for the initial studies of three-phase SFME. However, it was found that the plug of urine mixed partially with the extraction solvent, probably due to strong affinity between borosilicate and polar solvents such as water and methanol. Capillaries made of different materials were tested and the hydrophobic property of the capillary wall was found to be critical to maintain the three-phase system while moving the liquid plugs. Therefore, a Teflon tubing with i.d. of 0.86 mm, instead of glass capillaries, was used for three-phase SFME. Good stability and extraction efficiency were found utilizing the Teflon tubing.

This SFME system was first tested for analysis of amino acids ($\log P < -4$) in urine samples[21]. Hexane of 10 μL was used as the organic bridge and methanol/water (50/50, v/v) solvent of 10 μL was used as the polar extraction phase. Since the surface of the Teflon tubing is hydrophobic, slug flow induced by surface interaction was much slower. As a result, more SFME cycles were required to reach the liquid-liquid extraction equilibrium. The effect of SFME cycles on extraction efficiency of amino acids was investigated by using synthetic urine samples spiked with 200 ng/mL phenylalanine (m/z 166 $\rightarrow$ 120), glutamine (m/z 147 $\rightarrow$ 130) and arginine (m/z 175 $\rightarrow$ 70). As shown in Figure 2, the three-phase SFME did not reach equilibrium until around 300 cycles. However, most of analytes were extracted in the first 50 cycles. As shown in Figure 3a and 3b, with 50 SFME cycles, glutamine and arginine in urine samples were well extracted and detected through nanoESI MS. With increased extraction efficiency and good ionization, limits of detection (LODs) of the three-phase SFME method were better than 2 ng/mL toward these amino acids. It should be noted that the direct MS analysis of amino acids can be difficult. Water immiscible organic solvents are not suitable for extraction of amino acids in biofluid. Methanol or acetonitrile were often used for de-protein before analysis. We pretreated the phenylalanine-d5 (10 ng/mL) spiked urine sample by adding methanol (1/2, v/v), direct nanoESI MS analysis was performed after filtration. However, phenylalanine-d5 (m/z 171 $\rightarrow$ 125) was not detected because of high concentration of salts in the sample. While by the three-phase SFME using hexane as the organic bridge and methanol/water (50/50, v/v) solvent, phenylalanine-d5 was well detected, indicating that most salts were excluded from the extraction solvent by the plug of hexane.

Incorporation of internal standards is important for direct MS analysis methods. For the SFME-nanoESI MS method, we have developed an efficient approach to incorporate IS into extraction solvent. With this approach, it is not necessary to mix very small volumes of

solvents together, which often brings in poor reproducibility. For three-phase SFME, the IS can be incorporated into the extraction solvents by the same method, where the volume ratio of the analyte to internal standard is determined by the volumes of the sample and the extraction solvent. For the evaluation of extraction efficiency, phenylalanine-d5 (m/z 171→125) of 200 ng/mL was added as the analyte and tyrosine-d2 (m/z 184→138) of 200 ng/mL prepared in methanol/water (50/50, v/v) was used as the IS. Hexane of 10 μ L was used as the organic bridge and methanol/water (50/50, v/v) solvent of 10 μ L was used as the polar extraction phase. After extraction, the extraction phase was mixed with IS in a ratio of 1:1 for MS analysis. The intensity ratio of analyte and IS was recorded (R_e). For comparison, phenylalanine-d5 of 200 ng/mL and tyrosine-d2 of 200 ng/mL were mixed in methanol/water (50/50, v/v) and analyzed directly by MS. The intensity ratio of analyte and IS was recorded (R_0). The recovery of the three-phase SFME method was calculated by the ratio of R_e/R_0 and found to be 25.2%. By changing the concentration of phenylalanine-d5, a quantitative curve was developed, with R^2 of 0.9973 (Figure 3c).

The three-phase SFME was also tested for analysis of tenofovir-diphosphate (TFV-DP) [22], a therapeutic drug for HIV and a very hydrophilic compound ($\log P = -4.6$), in whole blood cell lysate at a concentration of 45 ng/mL. The volume of the cell lysate sample used was 10 μ L; hexane of 10 μ L was used as the bridging solvent and water of 10 μ L was used as the polar extraction solvent. With SFME, the subsequent nanoESI MS/MS analysis produced a spectrum as shown in Figure 3d. The characteristic fragment ions at m/z 149 and 79 were observed with S/N better than 50. The nonpolar bridge added between the two polar phases allowed adequate transfer of the analyte compounds but efficiently blocked substances, such as salt and cell debris, which could interfere with the nanoESI and MS analysis.

3.1. Development of reverse-phase SFME for analysis of oil samples

In the application demonstrated previously for SFME, the analytes in aqueous phase samples such as biofluids were extracted into organic phase for subsequent MS analysis. The principle of SFME, which is based on the partitioning of two immiscible phases of different polarities, should be applicable for analysis of samples of low polarities as well, such as oils. In this study, a reverse-phase SFME system was also developed to explore this possibility.

A polar solvent, such as water/methanol mixture that is suitable for nanoESI, was used as the extraction solvent for performing SFME-nanoESI analysis of vegetable oils (Figure 4a). Extraction solvent and oil sample, each 5 μ L, were used for performing the SFME. As shown in Figure 4a-b, the spectral pattern in positive MS analysis mode varied significantly with the change in mixing ratio of water and methanol. Abundant signals were obtained for triacylglycerol and diacylglycerol with the extraction solvent of methanol/water at 8/2 (v/v) (Figure 4b). Using the same solvent condition, fatty acids were effectively observed in negative MS analysis mode after SFME (Figure 4c). In this application, SFME serves as a fast and direct sampling method for these neutral lipids. SFME method is faster and easier to control through extraction cycles and extraction solvents, it is also convenient to handle small volume of samples.

Reverse-phase SFME can also be applied for analysis of nonpolar pesticides in the vegetable oils. Generally, pesticide residues are hydrophobic and hard to be separated from oil sample.

In this study, vegetable oil samples containing atrazine at 200 ng/mL was analyzed using reverse-phase SFME-nanoESI with methanol/water 1/9 (v/v) in consideration of good extraction and ionization of atrazine. The MS/MS spectrum recorded is shown in Figure 5a, with a high S/N ratio obtained. The partitioning coefficient of atrazine is $\log P = 2.5$, which means the concentration in the polar extraction solvent was significantly lower than that for the original oil sample; however, as previously discovered for the normal-phase SFME of non-polar compound benzoylecgonine[20], the optimization of the ionization condition could significantly enhance the overall sensitivity for the analysis of the target analytes. As shown in Figure 5b, higher organic solvent ratios (methanol/water 9/1, v/v) are favorable to the ionization of atrazine by nanoESI. However, this solution also dissolved a lot of vegetable oils, which can suppress the ionization of atrazine significantly. By using higher ratios of water (methanol/water 1/9, v/v), atrazine can be separated with vegetable oils, bringing in increased ionization. The analytical sensitivity can also be improved by adding acid into the extraction solvents.

3.3. Improvement of SFME-based MS analysis by other strategies

Apart from three-phase SFME for hydrophilic compounds and reverse-phase SFME for oil samples, there are also some other strategies which can be used to increase the performance of SFME methods.

In the initial test, SFME was performed using glass capillary typically used for nanoESI, with an i.d. of 0.86 mm[20]. In this work, the feasibility of using capillaries of smaller inner diameters, such as fused-silica capillaries, was investigated. Figure 6a schematically shows the implementation of SFME with fused-silica capillaries. The use of fused-silica capillaries improves the practicality of SFME by potentially improving the coupling capability to other on-line techniques. The use of fused-silica capillaries improves the practicality of SFME by potentially improving the coupling capability to other analytical techniques. As a comparison with the i.d. 0.86 mm borosilicate capillaries previously used, this study utilized fused-silica capillaries of i.d. 0.53 and 0.25 mm to investigate the effect of diameters. Bovine whole blood samples were prepared by spiking therapeutic drugs amitriptyline and lidocaine at their typical therapeutic levels of 50 ng/mL and 4 μ g/mL, respectively. The blood samples were diluted by 10 times, which was found to be helpful for performing the SFME due to the decrease of the sample viscosity. EtAc was used as the extraction solvent, which was found to be also adequately friendly for nanoESI. The volume of the two plugs were maintained equally with the plug length of 3 cm. The plug movement was also controlled with a displacement of 3 cm in each cycle. Results of amitriptyline and lidocaine analysis in bovine blood samples showed that chemical extraction could be achieved through SFME using fused-silica capillaries (Figure 6b and 6c).

However, the extraction efficiency could be affected by the inner diameter of the capillary. By using fused-silica capillaries of smaller internal diameters, it is expected that more SFME cycles are needed to reach the extraction equilibrium, since the interfacial area is significantly decreased, and the narrowing of the tubing could have additional impact on the induction of the turbulent flows. This issue can be addressed by applying more SFME cycles. A comparison study was done with diluted blood samples containing lidocaine at 4

Author Manuscript

$\mu\text{g/mL}$, the volumes of both diluted blood sample and ethyl acetate were 5 μL . The intensities of the extracted lidocaine were acquired after 5 SFME cycles for borosilicate capillary of i.d. 0.86 and fused-silica capillaries of i.d. 0.53 and 0.25 mm, as shown in Figure 6d. SRM (single reaction monitoring) transition m/z 235 $\rightarrow$ 86 was used for monitoring. As expected, a much lower intensity was observed for the i.d. 0.25 mm fused capillary (Figure 6d-e). Surprisingly, the decrease from i.d. 0.86 mm to 0.53 mm did not have a significant impact on the SFME efficiency. Data were acquired for i.d. 0.25 mm capillary to characterize the extraction efficiency as a function of SFME cycles. As indicated by the curve shown in Figure 6e, extraction equilibrium could be reached after 20 SFME cycles for capillary of i.d. 0.25 mm. It is worth noting that the sample volume handled with the i.d. 0.25 mm was less than 1.5 μL , which is meaningful for analysis of ultra-low amounts of biological samples.

Author Manuscript

The extraction efficiency of SFME toward target analytes should also be dependent on the relative chemical properties of the sample and the extraction solvent. The extraction efficiency can certainly be dramatically altered by changing the extraction solvent, but also by adjusting the sample properties. Extensive polymer peaks were obtained in positive nanoESI mode after SFME-nanoESI MS, corresponding to nonylphenol species normally present as contaminants[23,24]. For a comparison, 0.1% of acetic acid was spiked into the blood sample, which was then analyzed using the same SFME-nanoESI protocol. A peak of heme at high intensity and a group of phospholipids were observed in the mass spectrum (Figure 7). The appearance of heme is presumably due to the unfolding of the plasma protein under an acidic condition[25]. The suppression of the nonylphenol species and the presence of lipid signals were due to significant shifts of the extraction equilibrium during SFME, which were driven by the change of their charge status with the increase of proton strength in the sample matrix.

Author Manuscript

Author Manuscript

The partition of the analytes between the sample and extraction phases determines the extraction efficiency of the SFME. For direct analysis applications, it is preferred to use the extraction solvent directly for nanoESI. However, an extraction solvent with ultimately high extraction efficiency might not be optimal for ionization or overall analysis results. A balance needs to be maintained when selecting the relative polarity of the extraction solvent for SFME. By using pure solvent, the choice of the polarity is fairly limited. In order to analyze fatty acids in bovine blood samples, a series of binary solvent systems were made by mixing ethyl acetate and hexane at different ratios of 10:0, 8:2 and 2:8 (v/v). Hexane has been widely used for lipid extraction due to its low polarity; however, ethyl acetate was required to assure adequate ionization efficiency for subsequent nanoESI. The MS spectra recorded for different solvent used for SFME-nanoESI in negative mode are shown in Figure 8. The profile of fatty acids varied with the ratio change of the extraction solvent, with an optimal result obtained at ethyl acetate/hexane of 8/2 (v/v). With high percentage of hexane, abundant peaks at m/z 500–600 was detected, which are fatty acid dimers.[26] It should be noted that these fatty acid dimers were most likely produced in the process of electrospray. Results showed that ethyl acetate/hexane of 8/2 (v/v) was suitable for analysis of fatty acids in blood samples in consideration of both extraction and ionization efficiency.

4. Conclusions

In summary, we designed solvent systems for the in-capillary microextraction methods to meet the needs of direct analysis. Three-phase SFME was developed for analysis of extremely polar compounds in POC settings and reverse-phase SFME was also developed as a simple protocol for oil analysis. With investigation of key factors of SFME, the results also identified that adjusting sample and solvent properties are efficient means for the analysis of various analytes in biofluid samples using SFME.

Acknowledgement

The authors would like to thank financial supports from the National Natural Science Foundation of China (Project 21627807) and the National Institutes of Health (Project R44GM119584, R01AI122932 and 1R01AI122298).

References

- [1]. Fenn JB , Mann M , Meng CK , Wong SF , Whitehouse CM , Electrospray ionization for mass-spectrometry of large biomolecules, *Science* 246 (1989) 64–71.2675315
- [2]. Cooks RG , Ouyang Z , Takats Z , Wiseman JM , Ambient mass spectrometry, *Science* 311 (2006) 1566–1570.16543450
- [3]. Bruins AP , Covey TR , Henion JD , Ion spray interface for combined liquid chromatography/atmospheric pressure ionization mass spectrometry, *Anal. Chem* 59 (1987) 2642–2646.
- [4]. Thomson BA , Atmospheric pressure ionization and liquid chromatography mass spectrometry - Together at last, *J. Am. Soc. Mass Spectrom* 9 (1998) 187–193.
- [5]. Carroll DI , Dzidic I , Stillwell RN , Haegele KD , Horning EC , Atmospheric pressure ionization mass spectrometry. Corona discharge ion source for use in a liquid chromatograph-mass spectrometer-computer analytical system, *Anal. Chem* 47 (1975) 2369–2373.
- [6]. Adaway JE , Keevil BG , Therapeutic drug monitoring and LC-MS/MS, *J. Chromatogr. B* 883–884 (2012) 33–49.
- [7]. Ombrone D , Giocaliere E , Forni G , Malvagias S , la Marca G , Expanded newborn screening by mass spectrometry: New tests, future perspectives, *Mass. Spectrom. Rev* 35 (2016) 71–84.25952022
- [8]. Takats Z , Wiseman JM , Gologan B , Cooks RG , Mass spectrometry sampling under ambient conditions with desorption electrospray ionization, *Science* 306 (2004) 471–473.15486296
- [9]. Cody RB , Laramée JA , Durst HD , Versatile new ion source for the analysis of materials in open air under ambient conditions, *Anal. Chem* 77 (2005) 2297–2302.15828760
- [10]. Nemes P , Vertes A , Laser ablation electrospray ionization for atmospheric pressure, in vivo, and imaging mass spectrometry, *Anal. Chem* 79 (2007) 8098–8106.17900146
- [11]. Liu PY , Zheng QL , Dewald HD , Zhou R , Chen H , The study of electrochemistry with ambient mass spectrometry, *TRAC Trend. Anal. Chem* 70 (2015) 20–30.
- [12]. Gomez-Rios GA , Pawliszyn J , Development of coated blade spray ionization mass spectrometry for the quantitation of target analytes present in complex matrices, *Angew. Chem. Int. Ed* 53 (2014) 14503–14507.
- [13]. Piri-Moghadam H , Ahmadi F , Gomez-Rios GA , Boyaci E , Reyes-Garcés N , Aghakhani A , Bojko B , Pawliszyn J , Fast quantitation of target analytes in small volumes of complex samples by matrix-compatible solid-phase microextraction devices, *Angew. Chem. Int. Ed* 55 (2016) 7510–7514.
- [14]. Peng IX , Loo RRO , Shiea J , Loo JA , Reactive-electrospray-assisted laser desorption/ionization for characterization of peptides and proteins, *Anal. Chem* 80 (2008) 6995–7003.18683952
- [15]. Wang H , Liu JJ , Cooks RG , Ouyang Z , Paper spray for direct analysis of complex mixtures using mass spectrometry, *Angew. Chem. Int. Ed* 49 (2010) 877–880.

- [16]. Ren Y , Liu JJ , Li LF , McLuckey MN , Ouyang Z , Direct mass spectrometry analysis of untreated samples of ultralow amounts using extraction nano-electrospray, *Anal. Methods* 5 (2013) 6686–6692.
- [17]. Siebenhaar M , Küllmer K , de Barros Fernandes NM , Hüllen V , Hopf C , Personalized monitoring of therapeutic salicylic acid in dried blood spots using a three-layer setup and desorption electrospray ionization mass spectrometry, *Anal. Bioanal. Chem* 407 (2015) 7229–7238.26168972
- [18]. Shi R-Z , El Gierari ETM , Faix JD , Manicke NE , Rapid measurement of cyclosporine and sirolimus in whole blood by paper spray-tandem mass spectrometry, *Clin. Chem* 62 (2015) 295.26430072
- [19]. Li L , Chen T-C , Ren Y , Hendricks PI , Cooks RG , Ouyang Z , Mini 12, miniature mass spectrometer for clinical and other applications—introduction and characterization, *Anal. Chem* 86 (2014) 2909–2916.24521423
- [20]. Ren Y , McLuckey MN , Liu JJ , Ouyang Z , Direct mass spectrometry analysis of biofluid samples using slug flow microextraction nano-electrospray ionization, *Angew. Chem. Int. Ed* 53 (2014) 14124–14127.
- [21]. Khamis MM , Adamko DJ , El-Aneed A , Mass spectrometric based approaches in urine metabolomics and biomarker discovery, *Mass. Spectrom. Rev* 36 (2017) 115–134.25881008
- [22]. Marrazzo JM , Ramjee G , Richardson BA , Gomez K , Mgodini N , Nair G , Palanee T , Nakabiito C , van der Straten A , Noguchi L , Hendrix CW , Dai JY , Ganesh S , Mkhize B , Taljaard M , Parikh UM , Piper J , Masse B , Grossman C , Rooney J , Schwartz JL , Watts H , Marzinke MA , Hillier SL , McGowan IM , Chirenje ZM , Tenofovir-based preexposure prophylaxis for hiv infection among African women, *New Engl. J. Med* 372 (2015) 509–518.25651245
- [23]. Tan BLL , Mohd MA , Analysis of selected pesticides and alkylphenols in human cord blood by gas chromatograph-mass spectrometer, *Talanta* 61 (2003) 385–391.18969198
- [24]. Chen YC , Sun MC , Qualitative determination of trace quantities of nonyl phenyl polyethylene glycol ether in water based on solid-phase microextraction combined with surface-assisted laser desorption/ionization mass spectrometry, *Rapid Comm. Mass Spectrom.* 16 (2002) 1243–1247.
- [25]. Sogbein OO , Simmons DA , Konermann L , Effects of pH on the kinetic reaction mechanism of myoglobin unfolding studied by time-resolved electrospray ionization mass spectrometry, *J. Am. Soc. Mass Spectrom* 11 (2000) 312–319.10757167
- [26]. Dill AL , Eberlin LS , Costa AB , Ifa DR , Cooks RG , Data quality in tissue analysis using desorption electrospray ionization, *Anal. Bioanal. Chem* 401 (2011)1949–1961.21789488

Highlights

- Fast sampling of small volumes of raw biofluid for direct mass spectrometry analysis
- Three-phase SFME for efficient extraction of polar compounds in biofluid
- Reverse-phase SFME for efficient extraction of compounds in oil samples
- Improvement of SFME-MS performances by optimizing conditions

Fig. 1. Schematic of a) normal SFME using an immiscible organic phase for extraction of biofluids and b) three-phase SFME, following with analysis by nanoESI MS.

Fig. 2.

Impact of the number of SFME cycles on the extraction of the analytes, intensities of the MS/MS product ions monitored for 200 ng/mL phenylalanine (m/z 166 $\rightarrow$ 120), glutamine (m/z 147 $\rightarrow$ 130) and arginine (m/z 175 $\rightarrow$ 70) in urine.

Fig. 3.

Analysis of hydrophilic compounds using three-phase SFME-nanoESI: MS/MS spectra of a) arginine and b) glutamine, 2 ng/mL, prepared in synthetic urine. The volume 10 μ L urine samples. Hexane of 10 μ L was used as the organic bridge and methanol/water (50/50, v/v) solvent of 10 μ L was used as the polar extraction phase; c) Calibration curve of phenylalanine quantitation, tyrosine-d2 of 200 ng/mL was used as the internal standard. d) MS/MS spectrum of TFV-DP 45 ng/mL in blood cell lysate. The volume of the cell lysate sample was 10 μ L. Hexane of 10 μ L was used as the organic bridge and water of 10 μ L was used as the polar extraction solvent.

Fig. 4. Analysis of a vegetable oil sample using reverse-phase SFME with H₂O and MeOH of different ratios as extraction solvent: a) shows the MS spectrum of the oil sample extracted using H₂O; b) and c) demonstrate the MS spectra of the vegetable oil SFME processed by MeOH/H₂O of 8/2 (v/v).

Fig. 5.

a) MS/MS spectrum of atrazine, 200 ng/mL, prepared in vegetable oil acquired by reverse-phase SFME with MeOH/H₂O of 1/9 (v/v). b) Intensities of atrazine in MeOH/H₂O of 9/1, MeOH/H₂O of 9/1 mixed with vegetable oil and MeOH/H₂O of 1/9 (v/v).

Fig. 6.

a) SFME using fused capillaries for sample extraction and MS analysis. MS/MS spectra of therapeutic drugs in whole blood, b) amitriptyline at 50 ng/mL and c) lidocaine at 4 $\mu\text{g/mL}$. d) Signal intensities recorded for lidocaine in blood samples after SFME using different capillaries, 5 extraction cycles, SRM transition m/z 235 $\rightarrow$ 86. e) SRM signal intensity of lidocaine as a function of SFME cycles, fused silica capillary of i.d. 250 μm .

Fig. 7. Analysis of whole blood sample using SFME-nanoESI with altered solvent/sample conditions: a) Mass spectra of whole blood without acid, b) Mass spectra of whole blood with adding 0.1% acetic acid.

Fig. 8.
Mass spectra of whole blood extracted by extraction solvents of different polarity.
Borosilicate capillary of i.d. 0.86 mm with pulled tip used for SFME-nanoESI MS.