In vivo solid-phase microextraction swab sampling of environmental pollutants and drugs in human body for nano-electrospray ionization mass spectrometry analysis

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ABSTRACT

In vivo sampling and sensitive detection of environmental pollutants and drugs in human body play a crucial role in understanding human health. In this study, in vivo solid-phase microextraction (SPME) swab was fabricated using a SPME fiber and a medical cotton swab for noninvasive sampling and extraction of environmental pollutants and drugs in human oral cavity, nasal cavity and on skin surface. After sampling, SPME was coupled with nano-electrospray ionization mass spectrometry (nanoESI-MS) for desorption, ionization, and detection of the extracted analytes. As a result, limit of detection (LOD) and limit of quantification (LOQ) of nicotine in oral fluid were found to be 1.0 pg/mL (S/N ≥ 3) and 4.0 pg/mL (S/N ≥ 10), respectively. Linear dynamic signal responses of nicotine exhibited excellent linearity (R² = 0.9996) in human oral fluid ranging from 0.1 to 50 ng/mL. The coefficient of variation (CV) values of SPME swab for five measurements from sample vials and human body were 5.1–6.7% and 22.7–32.6%, respectively. Rapid analysis of a single sample could be completed within 10 min. Overall, our results demonstrated that SPME swab-MS is a promising noninvasive method for enhanced detection of analytes in human body.

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1. Introduction

The development of new analytical methods that integrate noninvasive sampling, high sensitivity, and quantitation performances for rapid analysis of human samples can undoubtedly
Solid-phase microextraction (SPME) is one of the most powerful sample pretreatment techniques [19]. Different types of SPME have been successfully developed for enhanced detection of target analytes from human samples [20–26]. Direct coupling of SPME with MS has been increasingly developed because of their respective advantages [27–34]. Among coupling strategies of SPME with MS, implementation of SPME-nanoESI-MS is an attractive strategy for fast, sensitive and quantitative measurement of target analytes in complex samples, because the combination of SPME with nanoESI-MS was considered to be ideal method which integrated high desorption/ionization efficiency with low solvent consumption and a long time of continuous stable spraying events that allow multiple MS analysis [18,24,35–38]. Particularly, direct coupling of biocompatible SPME fibers such as C18-SPME fiber with nanoESI-MS have been emerged for fast analysis of raw human samples, due to its excellent recovery, stability and reproducibility in complex matrices [39]. Typically, SPME fiber was used for sampling of pre-collected samples in a sample vial or bottle [19,20]. Unlike in vivo SPME sampling of animals [2,40,41], noninvasive sampling of human body with SPME fiber is a challenging task due to feasibility and safety concern. Alternatively, pioneering work on noninvasive SPME sampling of target analytes from human body such as oral cavity and skin surface using thin-film SPME has been made [42–44]. Extracted analytes on thin-film SPME could be further analyzed by conventional gas chromatography (GC) or liquid chromatography (LC) couple with MS [42–45]. Unlike the SPME fiber that can be inserted into nano-capillary, thin-film SPME poses a challenge to couple with nanoESI-MS. Therefore, further development of new noninvasive SPME method coupled with nanoESI-MS for fast extraction and sensitive detection of target analytes from human body is highly needed.

In this study, by combining the advantages of medical swab (for noninvasive sampling), biocompatible C18-SPME fiber has a sharp needle and an extraction phase of C18-coating. The extraction phase was conditioned in a mixture of pure water and methanol (1:1, vol/vol, 1.5 mL) for 30 min before sampling. The coating part (1.5 cm) of SPME fiber can be easily pulled into and pushed out the needle tip. The whole needle can also be easily inserted into the nanoESI capillary (Fig. 1a and g). Fig. 1b shows that each common medical swab has a cotton ball. Fig. 1c shows conditioned SPME fiber was directly inserted into the cotton ball of medical swab via metal needle to fabricate the SPME swab. In SPME swab, the SPME fiber and metal needle tip were completely coated by the cotton ball. For sampling oral fluid and oral cavity, the SPME swab was directly used (Fig. 1d). Prior to sampling of internal surface of nasal cavity (Fig. 1e) and human skin surface (Fig. 1f), the cotton ball of SPME swab was pre-wetted by pure water. Next, the SPME fiber was pulled out from medical swab and was then rinsed in a sample vial containing pure water (1.0 mL) for removing matrix components. Subsequently, the fiber was inserted into a nanoESI capillary that prefilled with desorption solvent for desorption and was then mounted on a commercial nanoESI-MS (Q Exactive, Thermo Fisher Scientific, Bremen, Germany) for MS analysis (Fig. 1g). Finally, a high voltage (2.0 kV) was applied onto nanoESI capillary, and analytes were ionized via nanoESI process. All MS analysis was performed under parallel reaction monitor (PRM) mode. As such, full MS and MS/MS spectra of target analytes were parallelly recorded for at least 1 min. Followed our previous optimized protocol for a fast analysis [36], the extraction time, rinsing time, and desorption time were selected at 5.0 min, 0.5 min and 3 min, respectively; and desorption solvent of analytes was methanol/water (4.0 μL, 95/5, vol/vol, with 0.1% formic acid and 12 mM ammonium acetate). Internal standard (IS) in desorption solvent (10 ng/mL) was pre-loaded into nanoESI capillary [46]. Details of sample preparation, sampling procedure, medical swab spray-MS [12] (Fig. S1), and conventional ESI-MS were given in the Supplementary Material. All volunteers have signed informed consents to collect their human samples.
2.3. Safety remarks

The needle tip of SPME fiber had to be coated by the swab ball in order to avoid stabbing human body. The sharp needle of SPME fiber can also be easily pre-flattened before sampling (Fig. S2).

3. Results and discussion

3.1. Characterization of SPME swab

To investigate the applicability of SPME swab for sampling of liquid sample, the water absorption of SPME swabs was measured by immersing SPME swabs in pure water (Fig. S3). The data showed that 0.50 mL and 0.18 mL of pure water could be steadily absorbed by normal SPME swab and tiny SPME swab, respectively. There is no significant difference between medical swabs and SPME swabs for absorbing water ($p > 0.05$). These results indicated that SPME swab is suitable for quantitative analysis of raw liquid samples, due to a steady volume of water absorption in sampling process. The background ions of medical swab and SPME swab were also investigated (Fig. S4), showing some characteristic background ions that could be further subtracted. To characterize SPME swab-nanoESI-MS, detection of nicotine with a concentration of 1.0 ng/mL in human oral fluid (0.5 mL) was performed using SPME swab. As a result, mass spectrum was rapidly recorded with cleaning up the matrices by SPME swab (Fig. 2a). It is clearly observed the peak at $m/z$ 163.12 that was corresponding to the protonated nicotine and was identified by MS/MS experiments (inset of Fig. 2a) and authorized nicotine (Fig. S5). A comprehensive comparison shows that there is similar extraction time between SPME swab and SPME fiber (Fig. S6). In the SPME swab sampling of liquid sample, the cotton ball of medical swab was rapidly wetted and thus analytes could be extracted onto SPME fiber. For a fast analysis, the extraction efficiency of analytes for 5 min is acceptable for improving detection of analytes and reducing the complex matrices. Longer extraction time, e.g., 20 min, is beneficial for enhanced detection of analytes at extremely low concentration if necessary. Therefore, a single sample analysis can be completed within about 10 min, including extraction (5 min), rinsing (0.5 min), desorption (3 min), and ionization/detection (1 min).

![Fig. 1. Schematic illustration of coupling of SPME-swab with nanoESI-MS: a) nanoESI capillary and SPME fiber, b) medical swabs, c) SPME swabs, d) dry SPME swab for sampling human oral cavity, e) prewetted SPME swab for sampling of human nasal cavity, f) prewetted SPME swab for sampling of skin surface, g) direct coupling of SPME fiber with MS via nanoESI capillary for desorption/ionization of analytes.]

The limit of detection (LOD) and limit of quantitation (LOQ) of SPME-swab-nanoESI-MS for detecting nicotine in oral fluid in sample vials were experimentally measured. The signal/noise ratios ($S/N$) were obtained by comparing the intensity of spiked samples to that of blank samples (Figs. S7a and S7c). MS/MS experiments were parallelly conducted to confirm the detectable analytes at extremely low concentration (Figs. S7b and S7d). The LOD ($S/N > 3$) and LOQ ($S/N \geq 10$) were found to be 1.0 pg/mL and 4.0 pg/mL,
respectively (Fig. S7).

The calibration curve for quantitation was constructed with eight sets of triplicate oral samples (0.5 mL) containing different levels of the nicotine in human oral fluid (0.1, 0.5, 1, 5, 10, 20 and 50 ng/mL) in sample vials, as shown in Fig. 2b. The calibration curve obtained for nicotine exhibited excellent linearity ($R^2 = 0.9996$), demonstrating the applicability of the SPME swab method in quantitative analysis of real human samples. The precision and accuracy of SPME swab were also evaluated by quantitation of nicotine in oral fluid spiked with nicotine of low (0.4 ng/mL), middle (4 ng/mL) and high (40 ng/mL) concentrations. Five replicated samples of each concentration were analyzed, and the data were averaged for comparison. The accuracies were obtained in the range of 95.0 – 102.0% (n = 5) with CV values at 5.1 – 6.7% (n = 5) (Table S2), showing excellent reproducibility from different SPME swabs sampling. Moreover, to compare the performances of medical swab with SPME swab, experiments were conducted for detecting drugs of abuse in oral fluid, which is of paramount importance in drug analysis [12,13]. Human oral fluid spiked with drugs of abuse, i.e., cocaine, ketamine, alprazolam and venlafaxine, were analyzed by SPME swab-MS (Fig. S8). Compared to medical swab, the detection limits were significantly improved about two orders of magnitude by SPME swab (Table S2). In this work, the SPME fiber was inserted into the medical swab making enhanced sensitivity of analytes. These data showed good analytical performances for enhanced detection of target analytes in human body by SPME swab-nanoESI-MS.

3.2. Direct sampling of human oral cavity

To investigate the applicability of in vivo SPME swab for noninvasive sampling of analytes in human oral cavity, medical swab and SPME swab were simultaneously put into an oral cavity of a healthy volunteer (23-year-old, female, nonsmoker) who was exposed to secondhand smoke via mouth breathing for 5 s. Mass spectrum of oral fluid was recorded using SPME swab (Fig. S9a). The peak of nicotine at m/z 163.12, a well-known marker for environmental tobacco smoke, was raised among abundant signals, showing the nicotine residue in human oral cavity. MS/MS spectrum of the peak at m/z 163.12 (Fig. S9b) was identical to authentic nicotine (Fig. S5). By contrast, no significant characteristic peaks of nicotine in full mass spectrum and MS/MS spectrum were observed by use of medical swab touch spray-MS analysis (Figs. S9c and S9d). These results showed that enhanced detection of nicotine in human oral cavity was achieved by SPME swab.

Furthermore, quantitative detection of nicotine in human oral cavity could also be achieved by using the calibration curve (Fig. 2b), because the sampling volume of oral fluid is stable at 0.5 mL. Quantitative detection of nicotine from same individual (female, 23 years old, nonsmoker) was performed to investigate the behaviors of passive smoking and active smoking in this study. It is found that nicotine in oral cavity was detected at 0.31 ± 0.09 ng/mL for passive smoking (n = 5, CV = 29.0%, Fig. S10a) and 30 ± 6.8 ng/mL for active smoking (n = 5, CV = 22.7%, Fig. S10b), respectively. Although the extracted ion chromatogram of analyte was not very steady over time, the internal standard was used to compensate variations (Fig. S10). It was noted that the CV values obtained from human oral cavity are much larger than those obtained from sample vials (CV: 5.1 – 6.7%, n = 5). The inhomogeneity of raw samples is a significant source of variabilities when SPME sampling of real objects [47] such as human oral fluid in this work. In addition, it is fact that the amount of nicotine passively or actively breathed in by the volunteer is also difficult to be unchanged. Although there are some variabilities in SPME swab sampling of human body, the sensitivity was significantly improved. These results demonstrated that noninvasive sampling and quantitative detection of environmental pollutants in human oral cavity by SPME swab-nanoESI-MS is feasible.

3.3. Direct sampling of skin surface

Pre-wetted SPME swab was further developed for wiping of analytes on human skin surface. For example, dried sweat on human forehead of healthy volunteer (female, 23 years old) was sampled and analyzed by SPME swab-MS after she drinking a cup of coffee in 45 min, peaks at m/z 195.09 and m/z 181.07 (Fig. 3a) were identified as caffeine and its metabolites by MS/MS experiments (insets of Fig. 3a). It is reported that isobaric theophylline, paraxanthine and theobromine are the metabolites of caffeine in human body [48]. There is a potential limitation for distinguishing these compounds from raw complex samples, because of the isobaric interference. However, the detection of these compounds could be confirmed with authentic compounds (Fig. S11). Furthermore, signal responses of caffeine (m/z 195.09) and its metabolites (m/z 181.07) at different parts of human body, including forehead, back, and arm, were obtained by pre-wetted SPME swab sampling (Fig. S12). Higher responses of caffeine and its metabolites were recorded from human forehead, probably forehead skin is much more efficient release of metabolites than other skins tested. The different responses recorded from the skin surface might give hints about the molecular distribution of human metabolites [49]. Enhanced detection of environmental pollutants and drugs that were pre-deposited on human skin was further examined by SPME swab (Fig. S13). Comparison of signal responses of target analytes obtained by SPME swab and medical swab demonstrated that signal responses of analytes on human skin surface were improved about two orders of magnitude by use of SPME swab (Fig. S14). These results further elucidated that analytes on human skin surface could be rapidly dissolved and extracted onto SPME fiber using pre-wetted SPME swab, greatly extending the application of SPME fiber for surface analysis.

Unlike large area on human skin surface, there is small area at internal surface of human nasal cavity. A tiny pre-wetted SPME swab was used for noninvasive sampling of drugs on the internal surface of human nasal cavity (Fig. 1e). Fig. 3b shows a mass spectrum obtained from rapid detection of target xylometazoline (a common nasal drug-aerosol) in nasal cavity by pre-wetted tiny SPME swab after the drug aerosol was pre-spraying in the air to nasal cavity. The base peak of protonated xylometazoline at m/z 245.20 and MS/MS spectrum of protonated xylometazoline indicated the successful detection of target analytes in nasal cavity. Another nasal drug aerosol (oxymetazoline) was also successfully detected by using tiny pre-wetted SPME swab (Fig. S15), confirming the applicability of tiny pre-wetted SPME swab. By using tiny pre-wetted medical swab, signal responses of these analytes were found to be less than 2% relative to signal responses obtained by SPME swab (Fig. S16), showing that SPME swab is particularly useful in the cases where analytes in a small space at low concentration. The variability of SPME swab for surface sampling was also investigated by detecting xylometazoline (0.1 ng/mL, IS: oxymetazoline at 10 ng/mL) in nasal cavity (Fig. S17) and caffeine (0.1 ng/mL, IS: theophylline at 10 ng/mL) on clean human skin surface of healthy volunteers (female, 23 years old) (Fig. S18) for five measures from same individual. The CV values of analyte-to-IS ratios were obtained to be 25.8% (n = 5) and 32.6% (n = 5) from human nasal cavity and human skin surface, respectively. The variation is mostly likely due to manual wiping, sample inhomogeneity, and the fact that sample surfaces may differ from person to person, and even from spot to spot. Alternatively, safe internal standard that can be spiked in human body and automated
SPME sampling [50] could be used to reduce the variation of surface sampling procedure for semi-quantification purpose. Further optimization and investigation of SPME swab for quantitative analysis are being studied.

4. Conclusions

In summary, we have developed a simple yet powerful in vivo SPME swab method that integrated medical swab and commercial bio-SPME fiber. Owing to the advantage of medical swab for non-invasive sampling, SPME swab rather than the SPME fiber was used to extract analyte in human body. After sampling, SPME fiber was directly coupled with a nanoESI-MS for detecting trace amounts of analytes (e.g., environmental pollutants, drugs and metabolites) in human body. The development of SPME swab not only greatly extended the use of SPME fiber for surface analysis but also for noninvasive analysis. Acceptable analytical performances of SPME swab, including sensitivity, reproducibility, accuracy and precision, were achieved. Significant applications of SPME swab for rapid analysis of human samples were also demonstrated, showing that SPME swab is potentially an attractive analytical tool to monitor, characterize, and quantify environmental pollutants, chemical residues and metabolites from human body. Furthermore, SPME swab allows easy operation and simple fabrication. This makes SPME swab suitable for coupling to portable mass spectrometers, resulting in platforms based on SPME-nanoESI-MS, and toward on-site analysis of complex samples with good sensitivity [51].

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Fig. 3.** Mass spectra obtained by pre-wetted SPME swab for surface sampling: a) skin surface of human forehead after intake of a cup of coffee in 45 min, insets show the MS/MS spectra and structures of caffeine and its metabolites; b) internal surface of human nasal cavity pre-sprayed with xylometazoline aerosol, insets show the MS/MS spectra and structure of xylometazoline.
CRediT authorship contribution statement

Lin Wu: Investigation, Visualization, Methodology. Zi-Cheng Yuan: Investigation, Validation. Zeng-Ming Li: Resources. Zhengyu Huang: Supervision. Bin Hu: Conceptualization, Methodology, Validation, Resources, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2020.05.022.

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