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Mass Spectrometry Hot Paper

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Direct Mass Spectrometry Analysis of Biofluid Samples Using Slug-Flow Microextraction Nano-Electrospray Ionization**

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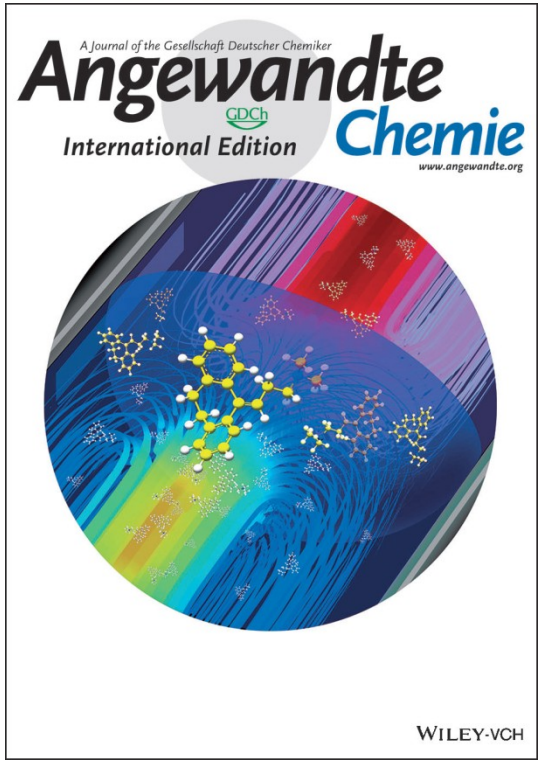
Abstract: *Direct mass spectrometry (MS) analysis of biofluids with simple procedures represents a key step in the translation of MS techniques to clinical and point-of-care applications. The current study reports the development of a single-step method using slug-flow microextraction and nano-electrospray ionization for MS analysis of organic compounds in blood and urine. High sensitivity and quantitation precision have been achieved in the analysis of therapeutic and illicit drugs in 5 μL samples. Real-time chemical derivatization has been incorporated for analyzing anabolic steroids. The monitoring of enzymatic functions has also been demonstrated with cholinesterase in wet blood. The reported study encourages the future development of disposable cartridges, which function with simple operation to replace the traditional complex laboratory procedures for MS analysis of biological samples.*

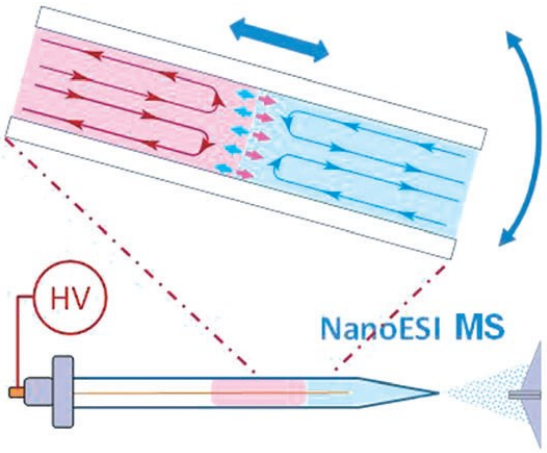


Rahul Narayanan
29/11/2014

Introduction

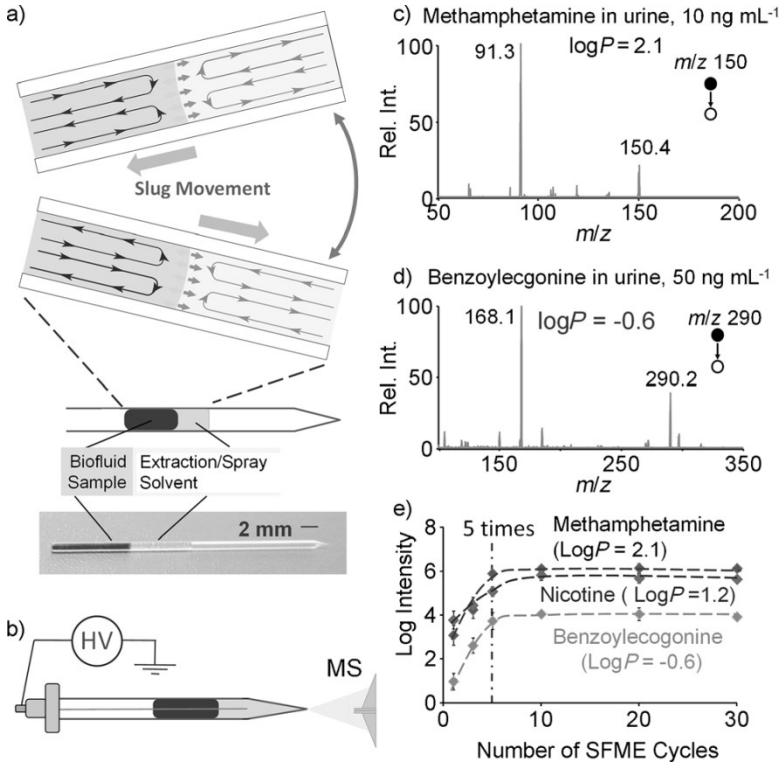
- Mass spectrometry (MS) has been demonstrated to be a powerful tool for chemical and biological analysis. The high specificity, high sensitivity, and high precision in quantitation are achieved traditionally in the laboratory by eliminating the matrix effect through sample extraction and chromatographic separation prior to the MS analysis.
- The development of ambient ionization, especially with the recent demonstration of using a paper spray, has indicated a promising future for direct MS analysis with high quantitation performance but with highly simplified procedures that consume ultra small amounts of samples.
- This would be extremely important for the translation of the MS analysis to field applications, especially point-of-care (POC) diagnosis. The underlying principle for a successful development along this direction is to minimize the sample consumption and to achieve high efficiency in an integrated process for analyte extraction and ionization.

***This paper*.....**



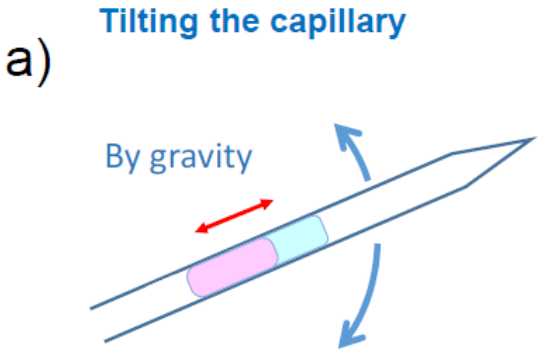
- A new method that uses slug-flow microextraction (SFME) and nanoESI (electrospray ionization) to perform a one-step analysis of biofluid samples has been devoloped.
- Excellent sensitivity and high quantitation precision have been obtained with blood and urine samples of only 5 μL.

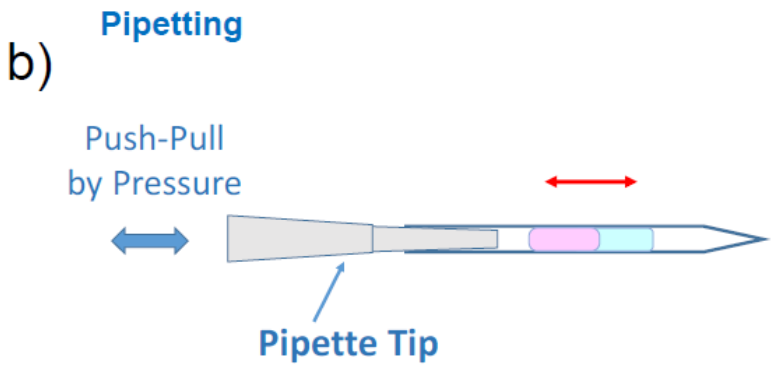
Results



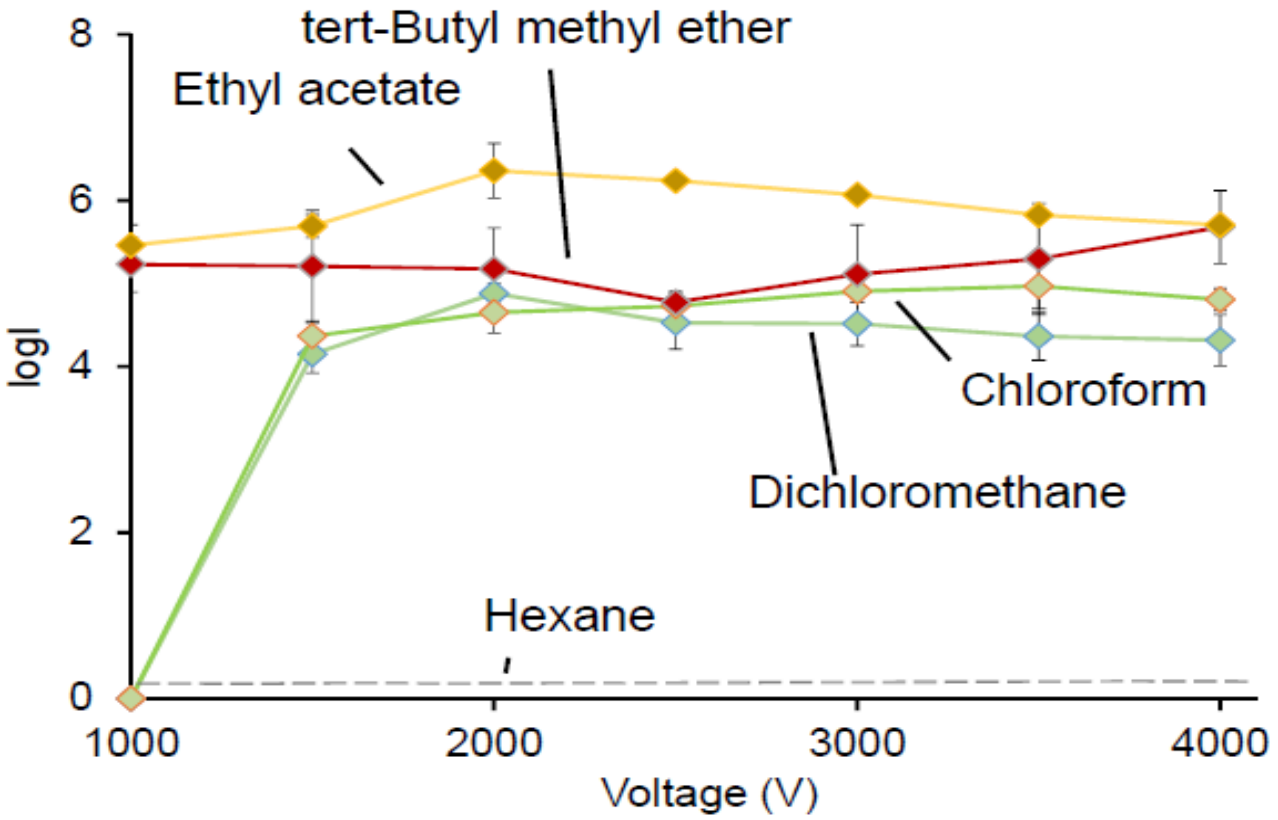
a) In-capillary sample extraction using slug-flow microextraction and b) the subsequent MS analysis with nanoESI. MS/MS spectra for c) 10 ngmL1 methamphetamine in 5 mL urine and d) 50 ngmL1 benzoylecgonine in 5 mL urine. e) Impact of the number of SFME cycles on the extraction of the analytes, intensities of the MS/MS product ions monitored for methamphetamine (m/z 150!91), nicotine (m/z 163!130), and benzoylecgonine (m/z 290!168), each at 50 ngmL1 in urine samples. 2 kV used for nanoESI.

The movements of liquid plugs inside the capillary could be created in two ways, by a) gently tilting capillary up and down, or b) adding a push-and-pull force by air pressure through a pipette. The pipetting volume was set to 10 µL for this purpose.



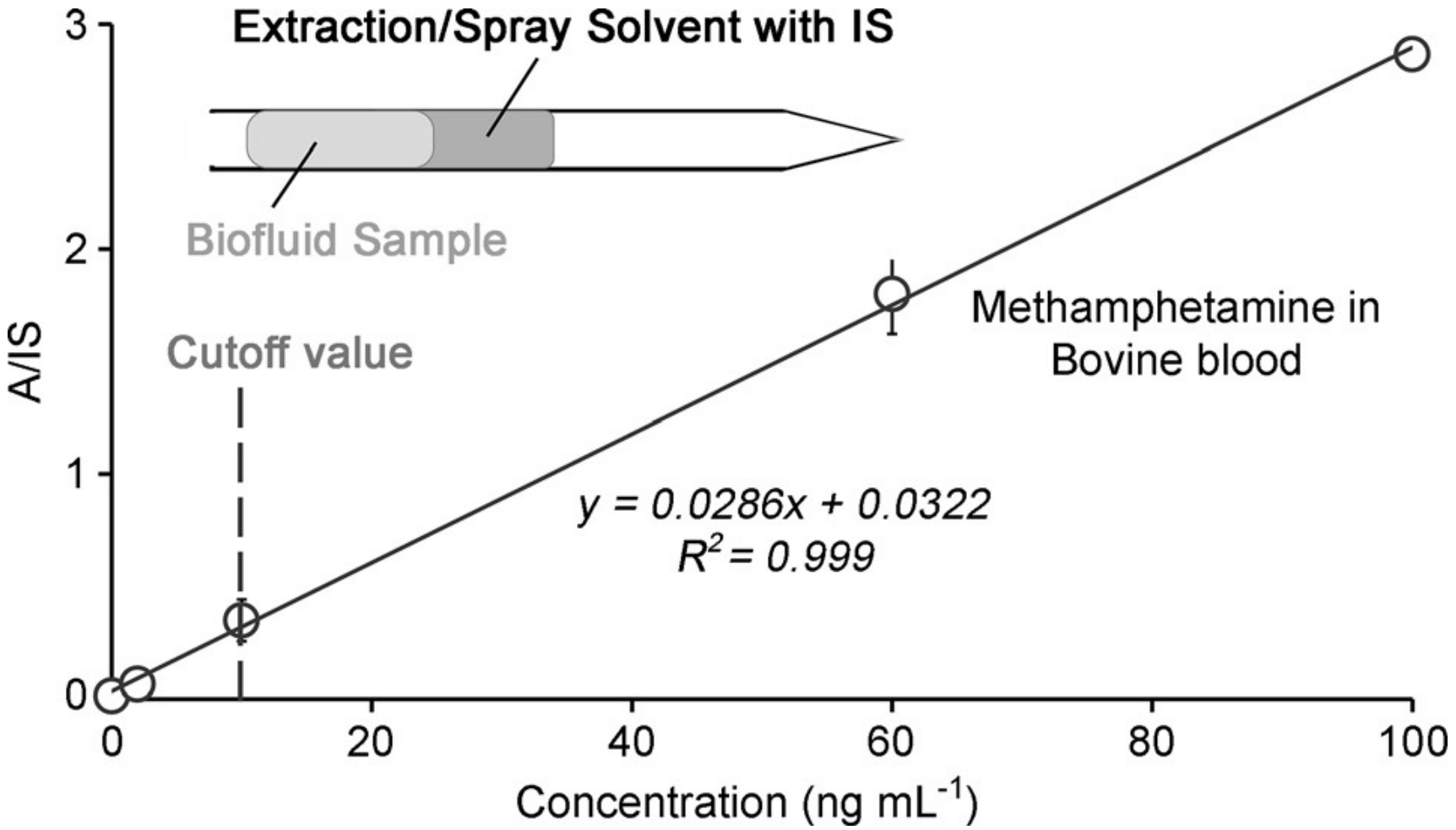


Results



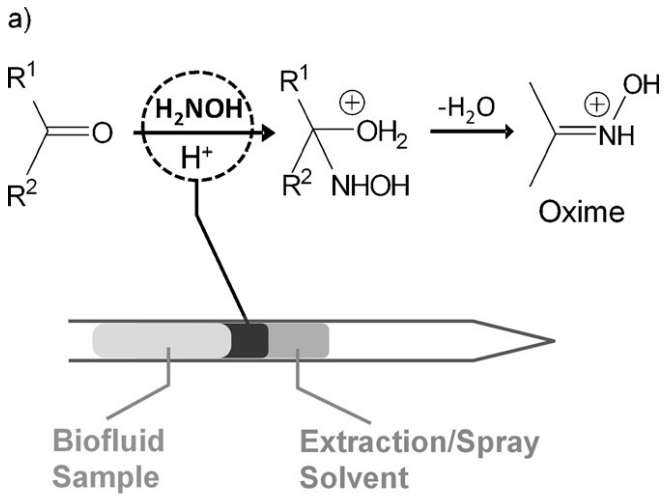
Comparison of different organic solvents for extraction phase used in SFMEnanoESI. For each test, the whole bovine blood spiked with 5 ng mL-1 methamphetamine was diluted 10 times with water; 5 µL sample was then used to prepare the sample plug and 5 µL of one organic solvent was used for the extraction plug. SRM (single reaction monitoring) with a transition m/z 150 □91 was used to record the intensity of product ion m/z 91 from protonated methamphetamine m/z 150, while the spray voltage is varied.

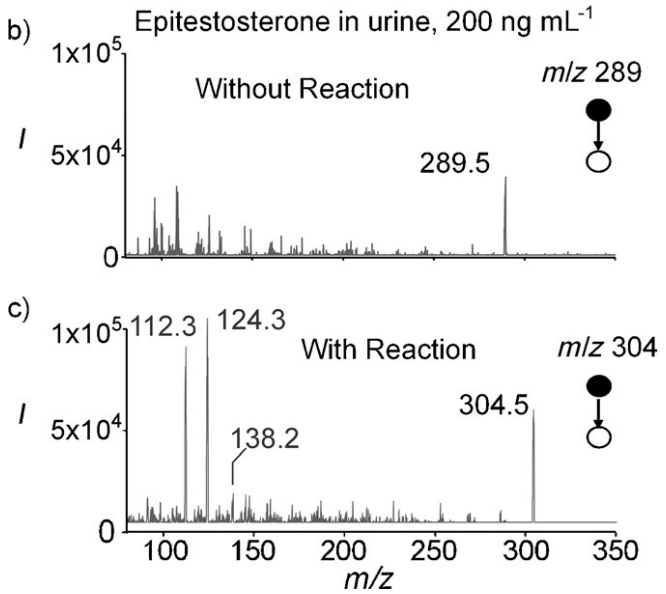
Results

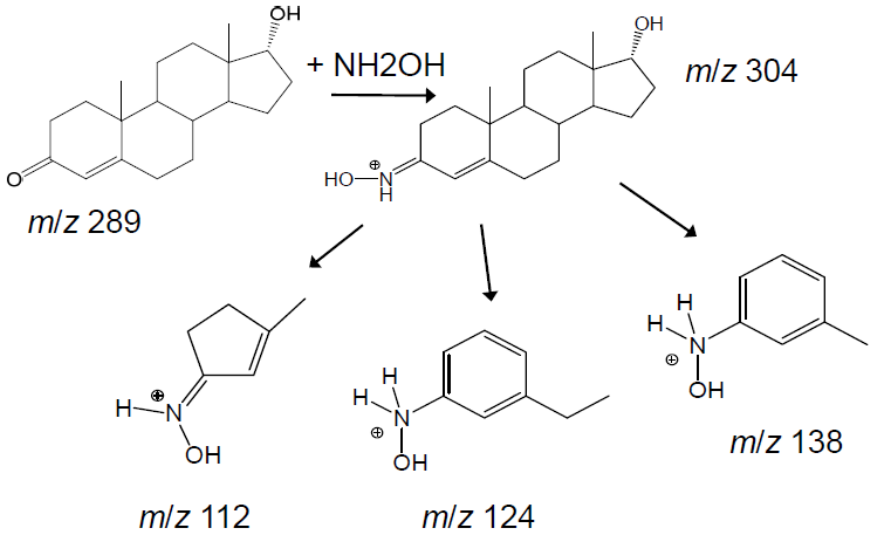


Quantitative analysis of whole blood spiked with methamphetamine(1–100 ngmL1). The blood samples were diluted 10 times to decrease the viscosity. [D8]Methamphetamine (2 ngmL1) in ethyl acetate was used as the extraction solvent.

Results





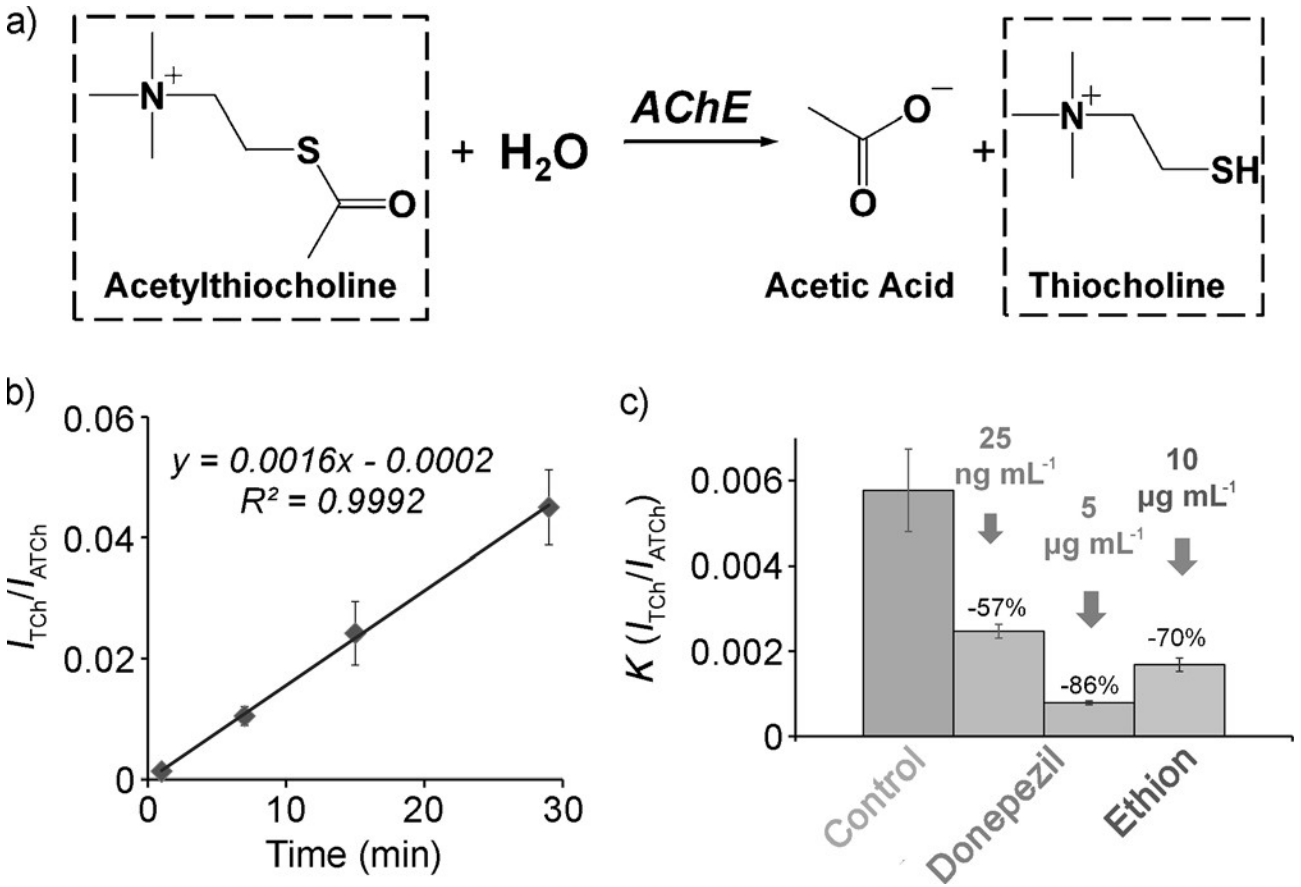


Derivatization and MS/MS fragmentation pathways of epitestosterone.

Steroids	Molecular Ion (m/z)	Product Ion (m/z)	Monitored Ion (*m/z*)
Epitestosterone	289	304	124
6-Dehydrocholestenone	383.6	398.1	203.9
5α –Androstan-3β, 17β –Diol-16-one	307	322	102
Stigmastadienone	429	411.7	83.4

a) Reactive SFME-nanoESI with a reagent plug injected between the biofluid sample and the extraction solvent. MS/MS spectra from b) direct and c) reactive SFME-nanoESI analysis of 200 ngmL1 epitestosterone in synthetic urine. 5 mL water containing 50 mm hydroxylamine was used as the liquid reagent plug.

Results



a) The reaction scheme of the enzymatic conversion of acetylthiocholine (ATCh) into thiocholine (TCh) catalyzed by cholinesterase (ChE). b) Progression curve of ATCh digestion determined by SFME-nanoESI. The incubation was for 30 min and catalyzed by blood cholinesterase (ChE) at room temperature. Acetylthiocholine iodide was added as the enzyme substrate into human whole blood at a final concentration of 1.8 mgmL1 before incubation. The intensity ratios of product ions from thiocholine (m/z 102!61) and enzyme substrate (m/z 162!103) were monitored using MRM; c) Evaluation of blood ChE with different levels of enzyme inhibition. The ChE activity in the blood sample was determined by SFME-nanoESI after 5 min incubation.

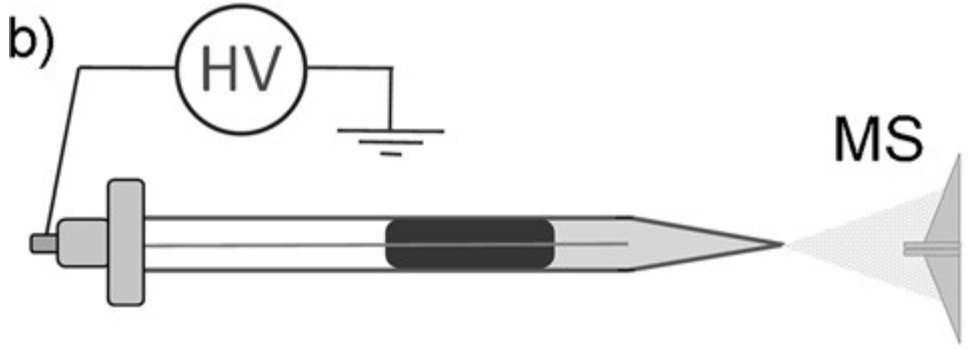
Conclusions

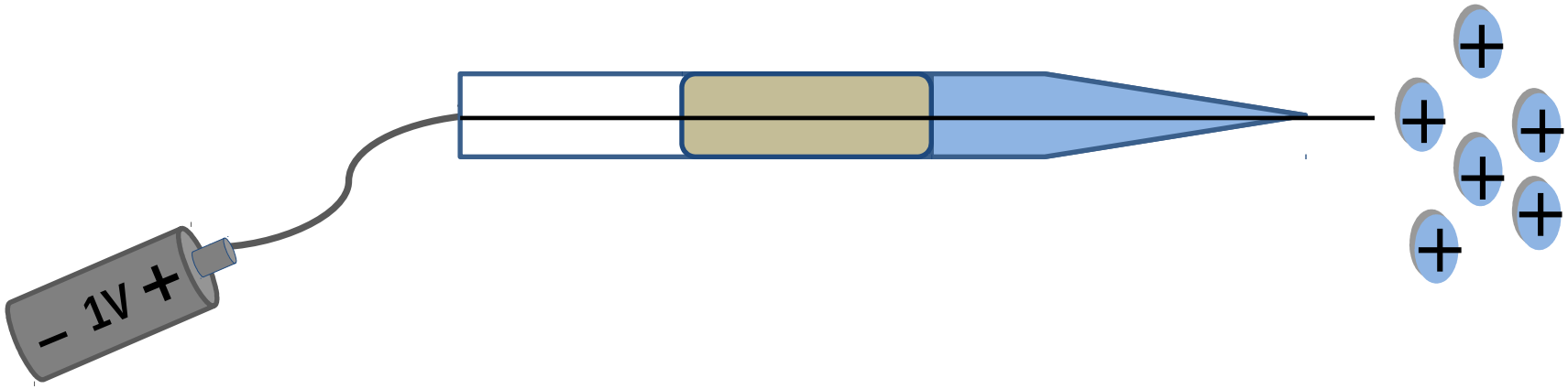
- The combination of slug-flow microextraction with nanoESI enabled a highly sensitive direct analysis of organic compounds in biofluids. Multiple types of processes for sample treatments, which traditionally require complex setups in the laboratory, can now be incorporated into a onestep analysis with an extremely simplified operation procedure.

- The extraction process can be turned on and off by controlling the movements of the sample and extraction plugs. This allows an online monitoring of the chemical and biological reactions in a biofluid sample of only 5 µL.

- With the increasing interest in the translation of MS technologies to clinical applications, this development has a profound implication on designing disposable sample cartridges with adequate function for direct analysis. This could ultimately lead to an elimination of the traditional laboratory procedures that require complex setups and expertize.

- Its implementation with miniature mass spectrometers would produce a powerful solution for POC diagnosis.


b)
HV
MS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Thank You!