Biocompatible-Solid Phase Microextraction (SPME)-nano-electrospray ionization (nano-ESI): an unexploited tool in bioanalysis

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Abstract

In recent years, different geometrical configurations of SPME have been directly coupled to mass spectrometry, resulting in benefits such as diminishing matrix effects, improvement of detection limits, and considerable enhancement of analysis throughput. Although SPME fibers have been used for years, their potential for quantitative analysis when directly combined with mass spectrometry has not been explored to its full extent. In this study, we present the direct coupling of biocompatible-SPME (Bio-SPME) fibers to mass spectrometry via nano-ESI emitters as a powerful tool for fast quantitative analysis of target analytes in biofluids. Total sample preparation time does not exceed 2 min and, by selecting an appropriate fiber length and sample vessel, sample volumes ranging between 10 and 1500 µL can be used. Despite the short extraction time of the technique, limits of detection in the sub-nanogram per millilitre with good accuracy ($\geq$ 90 %) and linearity (R^2>0.999) were attained for all the studied probes in PBS, urine, and whole blood. Given that Bio-SPME-nano-ESI efficiently integrates sampling with analyte extraction/enrichment, sample clean-up (including elimination of matrix effects in the form of particles), and ionization, our results demonstrated that it is an advantageous configuration for bioanalytical applications such as therapeutic drug monitoring, doping in sports, and pharmacological studies in various matrices.

Introduction

The ability to scrutinize samples without sample pre-treatment, or with minimum sample preparation, is the key feature of ambient mass spectrometry (AMS) that has massified its use around the globe[1,2], encompassing a variety of applications, from monitoring chemical transformations[3], to discerning between healthy and cancerous tissues at the surgery room[4]. However, no technique is perfect, and predictable limitations of pure AMS for analysis of complex matrices (*e.g.* blood and urine), such as ionization suppression, poor sensitivity at trace levels, and narrow linear dynamic range, have been the driven force toward the development of methods that efficiently integrate sample clean-up, analyte extraction/enrichment, and ionization[5–10]. This new-fangled era of mass spectrometry (MS), where sample preparation devices are directly coupled to MS instrumentation, has given rise to a growing branch of innovative research where micro- and nano-extraction approaches excel. Some of the most relevant methods recently developed in this field include the use of micro-solid phase extraction (SPE)[6,11,12], slug-flow microextraction (SFME)[5], single drop microextraction (SDME)[13], liquid phase microextraction (LPME)[14,15], membrane extraction[9,16], and polymer monolith microextraction (PMME)[10]. Solid Phase Microextraction (SPME), a world-wide recognized green sample preparation technique for GC[17] and LC[18–24] applications, was certainly not the exception[25,26]. Indeed, direct coupling of SPME to MS instrumentation is not a new trend; it has been explored for almost two decades[26–28], earlier than the publication of most popular AMS methods[26,28–32] (Section 1, Supporting Information). Currently, diverse geometrical configurations of SPME have been coupled to mass spectrometry for a broad range of applications including food, environmental, and bioanalytical[7,8,27,33]. Surprisingly, to the best of

our knowledge, few have exploited the genuine potential of the best known configuration of SPME: the fiber[34].

As a matter of fact, most direct couplings of SPME fibres reported up to date are based on Chen *et al.* work[26,28,35]. In this configuration, fibers are placed on a desorption chamber filled with a "large" amount of solvent (*i.e.* $V_{des} \geq 70$ µL) with high affinity for the analytes of interest, preceding its introduction to the eluent flow entering an atmospheric pressure ionization (API) source (either ESI[36]or APCI[37]). Although initial attempts to couple SPME fibers to MS using small desorption volumes were performed more than 10 years ago ($V_{des} \leq 10$ µL, via nano-ESI[38] or substrate spray[32]), recent endeavors have rather focused on either developing new SPME-fiber based substrates[8,33,39] or desorbing SPME fibers on a large desorption/ionization chambers where the enrichment factor provided by SPME was not fully exploited[34]. With the aim of taking advantage of the pre-concentration offered by SPME, this work continues the approach initially proposed by Walles *et al.*[38] In essence, it is intended to demonstrate that by using biocompatible-SPME (Bio-SPME) fibers[19,34,40–42] together with small desorption volumes[33,43] (i.e. $V_{des} \leq 4$ µL, in nano-ESI emitters[5,44–46]) remarkably low detection limits and satisfactory figures of merit can be attained with exceedingly short sample preparation times[7,8]. Although non-biocompatible coatings can be interfaced to nano-ESI sources in cases where the matrix does not represent major complexity (*e.g.* drinking water), the present work exclusively focuses on bioanalytical applications where the use of biocompatible devices is essential. Thus, it is important to highlight that, from the SPME perspective, biocompatibility relates to the use of coatings that do not induce or cause toxic reactions to the system under study. Therefore, fouling or adsorption of proteins does not occur when the extraction phase surface is exposed to the complex biological media[20,47,48]. This attractive feature of Bio-SPME makes it ideal for nano-ESI applications

considering that matrix components that can potentially block the nanospray opening, are not introduced into the emitter. In addition, given that SPME extracts via free-concentration and that a washing step is carried out after extraction, introduction of compounds such as phospholipids and salts into the system is minimized, therefore reducing the chances of ion suppression or enhancement [17,47]. In summary, direct coupling of Bio-SPME fibers to mass spectrometry via nano-ESI emitters is herein presented as a useful tool for screening and quantitative analysis of small molecules present in samples of bioanalytical relevance

Experimental Section

Materials and Supplies

The following compounds were selected as model analytes to evaluate BioSPME-nanoESI: cocaine, diazepam, salbutamol, codeine, oxycodone, methadone, amitriptyline and imatinib. Deuterated analogues of each analyte were used for correction of intra- and inter-experiment variability. Further details regarding compound suppliers, properties, and SRM transitions are provided in Table S1 of the supplementary information. All LC-MS grade solvents (acetonitrile, methanol, and water) used in experiments were purchased from Fischer Scientific. Biocompatible SPME mixed mode probes (*i.e.* C_{18}-SCX particles, 45 μm thickness, 15 mm coating length) were kindly provided by Supelco (Bellefonte, PA, USA). The phosphate-buffered saline solution (PBS) (pH 7.4) was prepared according to the procedure provided in the supplementary information. Pooled whole blood from healthy donors in potassium (K2) ethylenediaminetetraacetic acid (EDTA) was purchased from Bioreclamation IVT (Baltimore, MA, USA). Urine samples were collected from two healthy volunteers (one female and one

male). Collection of urine from healthy volunteers for this particular study was under the approval of the Office of Research Ethical Board of University of Waterloo).
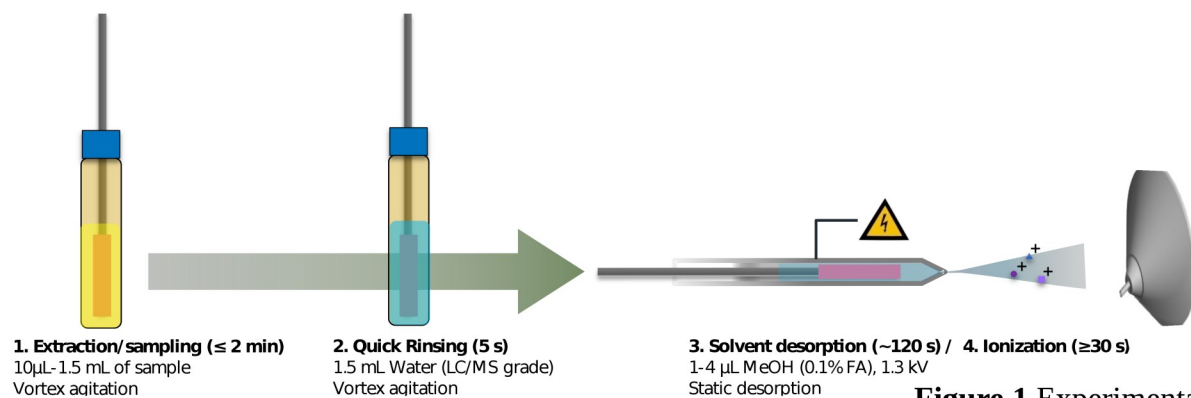
SPME-MS interface for nano-ESI

All the experiments herein described were performed on a triple quadrupole mass spectrometer TSQ Vantage (Thermo Scientific, San Jose, USA). The nano-electrospray emitters Econotip (Econo10, 1.0/0.58 OD/ID, mm) and coated Glasstip (1.0/0.58, OD/ID, mm; 1 and 2 μm tip), were obtained from New Objective Inc. (Woburn, MA, USA). An in-house ionization source was built at the machine and electronic shop of the University of Waterloo (see Figure S1, Supplementary Information) to accurately position nano-ESI emitters in front of the mass spectrometer. This system contained a holder, in which a ball-end clamping screw enabled straightforward connection of high voltage (HV) to the nano-ESI emitter, and ready and fast replacement of emitters between experiments (Figure S1). In order to demonstrate the simplicity of our system and the suitability of replicating our approach in an easy way, commercially available emitters and fibers were utilized in this study. However, custom-made coatings[42,47] and emitters[44,46] enabling tuning of protocols for given applications are also expected to be used in the near future.

Experimental set-up for Bio-SPME-nano-ESI

The established analytical workflow consisted of four main steps: extraction/pre-concentration, rinsing, desorption, and ionization (Figure 1). First, a preconditioned Bio-SPME fiber was inserted in a vial containing the sample matrix (*e.g.* $V_{ext} \sim 10$–1500 μL), and quick extraction/enrichment of the analytes was performed by agitating the sample at high speed

(agitation at 3200 rpm, t≤ 2 min). Next, the fiber was rinsed in a vial containing LC/MS grade water (t ≤ 15 s) to remove matrix components that could potentially adhere to the coating surface. Subsequently, the fiber was introduced into an emitter prefilled with desorption solution. Lastly, and after allowing some time for the analytes to be desorbed in the selected solvent, a high electrical field between the emitter and the mass spectrometer was applied, and analytes were ionized via electrospray mechanisms[5,29,45]. Although extraction on SPME fibers can also be performed under static conditions (*e.g.* during in-vivo tissue analysis[41]), high-speed agitation with vortex was selected in this manuscript to achieve low detection limits without compromising the total analysis time (*i.e.* faster analyte enrichment of the analytes on the SPME coating). However, it is worth emphasizing that our group is currently working toward the implementation of SPME-nano-ESI as a strategy for the fast and quantitative in vivo measurement of compounds of interest in tissue under static conditions.

1. Extraction/sampling (≤ 2 min)
10µL-1.5 mL of sample
Vortex agitation

2. Quick Rinsing (5 s)
1.5 mL Water (LC/MS grade)
Vortex agitation

3. Solvent desorption (~120 s) / 4. Ionization (≥30 s)
1-4 µL MeOH (0.1% FA), 1.3 kV
Static desorption

Figure 1 Experimental set-up for Bio-SPME extraction from complex matrices and desorption–ionization using nano-ESI-MS/MS.

Results and Discussion

Rediscovering SPME-MS: Bio-SPME-nano-ESI, a new era

When optimizing SPME-nano-ESI desorption/ionization conditions, several factors should be taken into consideration. Some of the most important parameters include a) the chemistry of the

target molecule, which dictates its affinity for the coating and for the desorption solution; b) the kinetics of analyte desorption into a particular solvent; c) the geometrical characteristics of the emitter and its ionization efficiency[8,45,46]; and d) the spatial position of the emitter in regards to the mass spectrometer entrance. In regards to the SPME extraction phase, mixed mode was selected for this study due to its better performance when extracting polar analytes compared to C18. Emitter selection was performed according to its internal volume (*i.e.* as small as possible to enhance the enrichment factor[49], but large enough that the fiber could freely move in and out of the emitter without being damaged), spray-current stability, intra- and inter-experiment reproducibility, and its cost. Among the studied emitters described in the experimental section, Econo10 provided the best compromise in terms of inter-analysis reproducibility/stability (Table S2) and price (*i.e.* ~ 6 US dollars per analysis). All experiments herein reported were conducted with the use of the aforementioned glass-coated emitters (1.0/0.58 mm, OD/ID) filled with few microliters of acidified methanol (*i.e.* 0.1% formic acid), unless otherwise stated. Desorption volumes were set according to the length of the coating, always ensuring that the whole coating was completely immersed into the solvent (*e.g.* 4 µL for 15 mm fibers and 1 µL for 4 mm fibers). Considering the small tip size of these emitters (1 ± 0.5 µm), and aiming to prevent clogging and instable spray-currents, desorption solvent was filtered and degasified with helium prior to analysis.

It is important to highlight that a slightly dry Bio-SPME fiber (for instance, due to excessive delay time preceding its introduction into the emitter) could be *per se* a significant source of bubbles that can distort the Taylor-cone formation and consequently, electrospray ionization[50]. Based on our experience, if the delay-time between the fiber rinsing step and its insertion into

the emitter is longer than 30 seconds, bubble generation inside the emitter (Figure S2) can be anticipated as a result of microscopic dry spots/pores on the coating. This could be related to the intrinsic low-wettability of the C_{18}-SCX particles used to manufacture mixed-mode Bio-SPME fibers[24]. Consequently, for the experiments described in this manuscript, stable electrospray was guaranteed by using short fiber transition times from the rinsing vial to the nano-ESI emitter ($t_{trans} \leq 15$ s). Indeed, when the affinity of the coating for the target analyte is high (*i.e.* large fiber constant, K_{fs}[17,20,49]), as well as the analyte hydrophobicity[21,41,47], fibers can be transported to the emitter inside the rinsing vessel with minimal/negligible analyte loss[24], thus preventing bubble formation dry surface. An alternative to solve this issue would be the use of coatings with better water-wettability[24], such as HLB (m-divinylbenzene and n-vinylpyrrolidone copolymer), and/or substrates with lower thermal conductivity, so that the evaporation rate of the water is slower, allowing coating particles to remain wet for longer times[51].

Given the slow flow rates inherent of nano-ESI (*i.e.* 20-80 nL min^{-1}; depending on solvent composition, voltage applied, and emitter tip architecture[52,53]), 4 µL of desorption solvent is enough to perform at least four instrumental replicates per fiber from a single emitter (Figure S3). Desorption time ($t_{des} \leq 5$min) was selected in such a way that the monitored ion signal was reproducible between consecutive replicates (*i.e.* RSD ≤ 15 %, n =4, calculated using the area under the curve for each repetition, as shown in Figure S3 and Table S3). In essence, steady signal among experimental replicates means that the partitioning equilibrium between the fiber coating and the desorption solvent was reached and, consequently, it could be assumed that the amount of analyte in the desorption solvent was not statistically changing over the time. However, this does not necessarily mean that total desorption of the extracted analyte has been

achieved. The amount of analyte desorbed from the fiber is certainly dependent on the strength

and, in this particular approach, on the volume of the desorption solvent [43]. Undeniably, fiber

desorption is the bottleneck step in the entire analytical process herein proposed. Given that

desorption time is selected based on the desorption kinetics of the analyte under study[43] and that

this depends, in addition to the affinity of the analyte for the coating, on the coating thickness

and the agitation/thermal conditions in the desorption vessel, our current efforts are focused on

the development of thinner fiber coatings (*e.g.* thickness $\leq$ 10 µm; mono or dual-layer

coatings[7,49]), a heated desorption chamber (*i.e.* to decrease K_{fs}[43] and increase the diffusion

coefficient of the analytes[54]), and a fiber vibration system, such that the desorption step keeps

pace with the entire analytical workflow.

Bio-SPME-nano-ESI-MS/MS: an unexploited tool

Not long ago, sample-preparation techniques directly coupled to MS were considered avoidable,

intricate, and exceedingly laborious[2]. In addition, for some microextraction techniques such

SPME, the quantitation capabilities and throughput of analysis were questioned due to the low

analyte recoveries and the long extraction times needed to achieve practical detection limits[8]. In

defiance of what is normally believed, recent developments have demonstrated that different

geometries of SPME not only can perform quantitative analyses from complex matrices at trace

levels (*i.e.* pg mL^{-1} levels), but also in short periods of time ($t_{ext} \leq$ 1 min)[7,8]. To date, the

quantitation potential of Bio-SPME fibers has not been exploited to its maximum[34,42,47], provided

that during the desorption (inherent of SPME–LC methods), analytes are significantly diluted

and non-efficiently ionized[43]. As a proof of concept, we demonstrated that Bio-SPME-nano-ESI

can reach limits of quantitation (LOQs) of 34 and 100 pg mL^{-1} upon 1 min extraction from 1500

µL of PBS spiked with cocaine and diazepam, respectively (calibration functions were constructed on the basis of the signal ratio of the analyte and its isotopologue (A/Is) in three independent experiments; Figure 2). Furthermore, exceptional linearity in the range of 50 pg mL^{-1} up to 1 µg mL^{-1}, and outstanding accuracy (*i.e.* 87-98 %) at three different levels (*i.e.* 0.3, 7.5 and 200 ng mL^{-1}) were attained (see Table S4, Supplementary Information). Certainly, higher concentration levels are not a limitation for Bio-SPME fibers. Thus, in cases where the affinity of the coating for the analyte(s) is high, and analytes are present at concentrations larger than 100 ppb, shorter extraction times (≤ 1 min) could be used. Although the best combination of desorption/ionization conditions for SPME-nano-ESI (*e.g.* strength of desorption solution, emitter size, and fiber thickness) was not investigated in this manuscript, our results are simply exceptional[17,34,47]. Certainly, Design of Experiment (DOE)[12] would aid not only to improve current results, but also to decrease total analysis time.
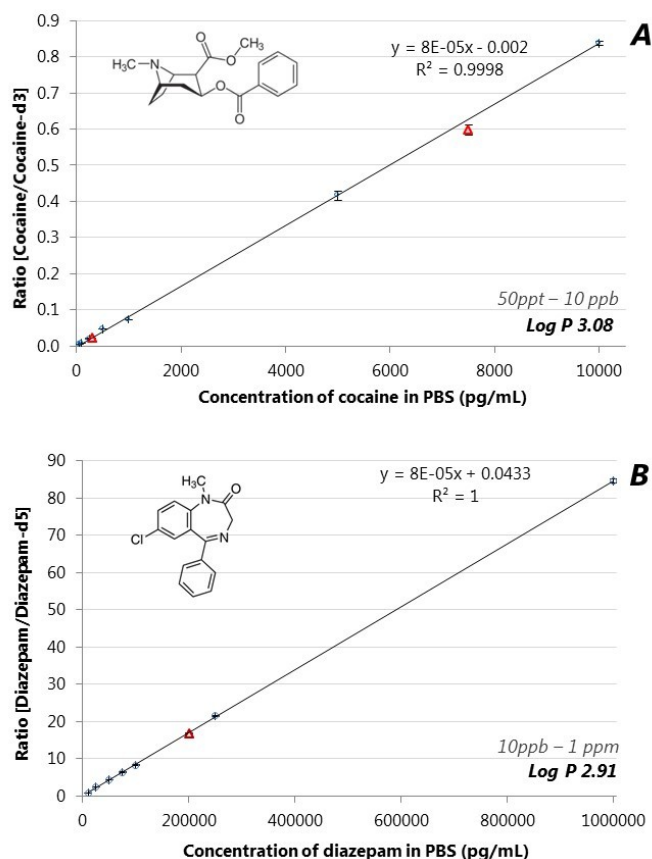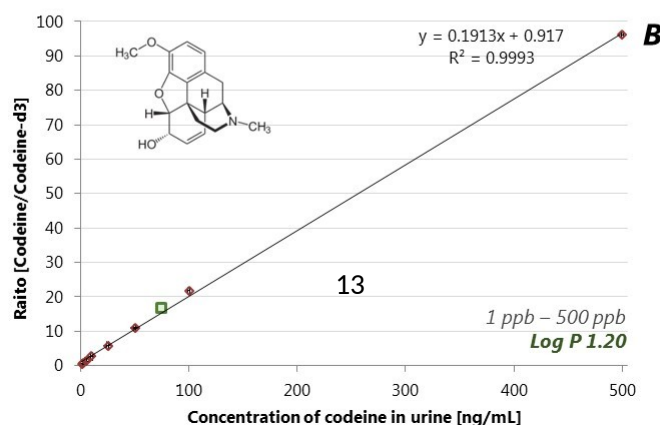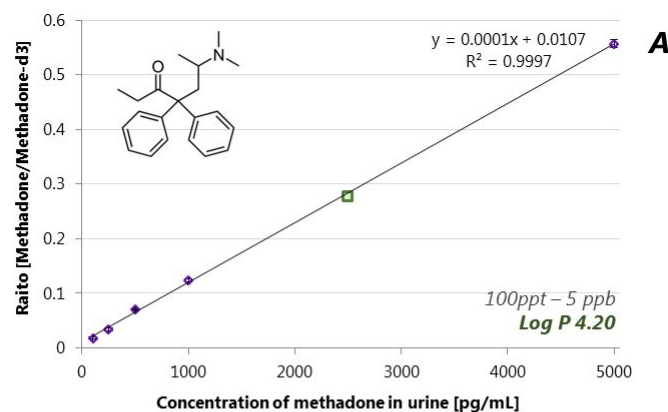
Figure 2 A. Quantitative analysis of PBS spiked with cocaine (50 pg ml^{-1} to 10 ng mL^{-1}) and its isotopologue [D$_3$] cocaine (12 ng mL^{-1}). **B.** Quantitative analysis of PBS spiked with diazepam (10 ng ml^{-1} to 1 µg mL^{-1}) and its isotopologue [D$_5$] diazepam (12 ng mL^{-1}). Bars represent the standard deviation of analyses for three replicates with independent fibers and nano-ESI emitters. Red triangles represent the accuracy levels evaluated for both compounds.

Analysis of controlled substances in urine samples

Due to its non-invasive sample collection nature and the typically large sample volumes available, urine is the most traditional matrix employed when monitoring abuse of illicit drugs[55] or doping in sports[56]. Since the amount of parent drug excreted in urine is typically low (*e.g.* parent drug could be metabolized by the liver), analytical methods capable of providing sensitive analysis in the sub-ng per millilitre levels are needed[11,22]. Recently, Boyacı *et al.*[22] and Reyes-Garcés *et al.*[21,24] demonstrated that different geometrical formats of SPME were capable of meeting the Minimum Required Performance Levels (MRPL) set by the World Anti-Doping Agency (WADA) for the analysis of prohibited substances in urine. As a proof-of-concept, we present the application of Bio-SPME fibers coupled to nano-ESI-MS/MS for the concomitant determination of salbutamol, codeine, oxycodone, and methadone in urine.

100ppt – 5 ppb
Log P 4.20
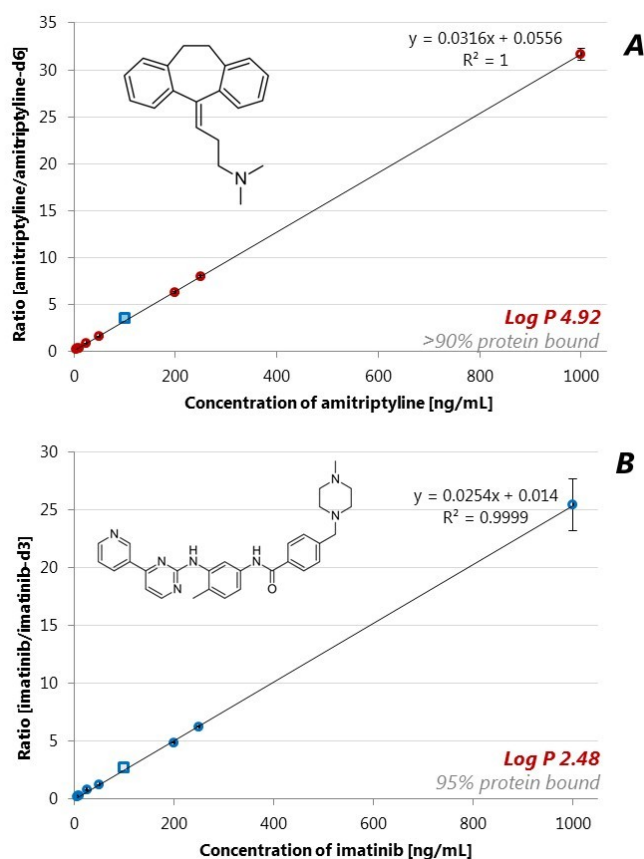
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1 ppb – 500 ppb
Log P 1.20

Figure 3 A. Quantitative analysis of urine spiked with methadone (100 pg ml^{-1} to 5 ng mL^{-1}) and its isotopologue [D$_3$] methadone (10 ng mL^{-1}). **B.** Quantitative analysis of urine spiked with codeine (1 ng ml^{-1} to 500 ng mL^{-1}) and its isotopologue [D$_3$] codeine (12 ng mL^{-1}). Bars represent the standard deviation of analyses for three replicates with independent fibers and nano-ESI emitters. Green squares represent the accuracy levels evaluated for both compounds.

As can be seen in Figure 3 and Table S5, an extraction time of 1 minute from 700 μL of urine was sufficient to achieve LOQs ranging between 100 and 500 pg mL^{-1}. Not only were LOQ values below MRPL levels (*i.e.* 50-100 ng mL^{-1})[22,55], but also rewarding correlation coefficients (>0.999) were observed for all probes in the range evaluated (*i.e.* 100 pg mL^{-1} up to 500 ng mL^{-1}; see Figures 3 and S4). Since SPME derives its sensitivity and selectivity from the physicochemical/geometrical characteristics of the coating used, current research is directed towards the development of thinner coatings with greater affinity for the target analytes (*e.g.* HLB[21]), with aims to provide lower limits of detection without compromising total analysis time[8]. Although this possibility was not evaluated in this manuscript, we foresee that SPME-

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nano-ESI in combination with tandem-mass/high-resolving-power instruments could be used for simultaneous screening of multiple controlled substances in a single analysis[7,8,21,22,24].

Bio-SPME-nanoESI as a tool for Therapeutic Drug Monitoring (TDM)

Nowadays, in the era of personalised medicine, the development of bioanalytical methods that are capable of rapidly quantifying systemic concentrations of drugs that have a low therapeutic index or narrow therapeutic range is crucial. Indeed, such methods should provide not only equal or better performance than the existing approaches (*e.g.* immunoassays and liquid chromatography-MS) in terms of accuracy and linear dynamic range, but also lower cost per sample and simpler operation. In order to fulfil these objectives, techniques such SFME[5] and SPE-Paper Spray (SPE-PS)[6] have been recently introduced as exciting alternatives for point-of-care TDM of diverse analytes in blood and plasma, with minimal sample consumption and reasonable sample preparation. Although SPME has also been used in TDM applications[47,57], depending on the physicochemical properties of the analyte and its affinity for the extracting particles[47], relatively long sample preparation times (*i.e.* $t_{ext} \geq 10$ min) and moderately large sample volumes ($V_s \geq 1$ mL) were needed to achieve quantitative results via LC-MS/MS[21]. In view of this, Bio-SPME-nano-ESI is herein introduced as a simpler and faster approach for the quantitation of target analytes in whole blood samples. Given that SPME extracts via free concentration[47], analytes largely bound to plasma proteins would be expected to provide extremely low extraction recoveries (*i.e.* worst-case scenario for SPME)[21]. Thus, aiming to evaluate the method under "extreme" conditions, two probes with protein-binding larger than 90%, amitriptyline and imatinib[58], were selected. Unlike SFME and SPE-PS, when using Bio-SPME fibers, neither sample dilution[5], nor sample drying[6] is required.

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Figure 4 A. Quantitative analysis of whole blood spiked with amitriptyline (100 pg ml^{-1} to 5 ng mL^{-1}) and its isotopologue [D$_6$] amitriptyline (10 ng mL^{-1}). **B.** Quantitative analysis of whole blood spiked with imatinib (1 ng ml^{-1} to 50 ng mL^{-1}) and its isotopologue [D$_3$] imatinib (12 ng mL^{-1}). Bars represent the standard deviation of analyses for three replicates with independent fibers and nano-ESI emitters. Blue squares represent the accuracy levels evaluated for both compounds.

In comparison with the analysis of urine or PBS, additional rinsing steps[24] were needed to remove clusters of macromolecules that lingered on the coating surface during the extraction process, which could have potentially clogged the nano-ESI emitter. Thus, for blood analysis, the analytical process was as follows: first, a pre-conditioned fiber (*i.e.* methanol/water, 1:1) was rinsed 10 seconds in LC-MS water prior to sampling in order to minimize attachment of proteins and cells on the coating/wire surface[24]. Then, extraction was performed by immersing the fiber for 2 min in the vial containing the sample, and subsequently the fiber was rinsed for 5 seconds

on a new vial containing LC-MS grade water. Afterwards, the fiber coating was carefully cleaned with a Kimwipe tissue and rinsed for another 5 seconds on a new vessel containing LC-MS grade water. Finally, the wet-fiber was inserted on the nano-ESI emitter for desorption/ionization (Figure S5 summarizes the modified analytical procedure). Given the small tip size of the emitter used for urine and PBS analyses (*i.e.* ~ 1± 0.5 µm), the possibility of clogging while performing instrumental replicates (*i.e.* multiple ionizations from the same emitter) was taken into consideration; thus, whole blood experiments were performed using emitters with a slightly larger tip size (*i.e.* ~ 2 ± 1 µm; BG-10-58-2-AP-20). When using these emitters, plugging was never observed. Certainly, nano-ESI devices with larger emitter tips (*e.g.* 4-20 µm) would provide more robust analysis, especially when considering these for unattended high-throughput applications[59]. Although the technique has yet to be perfected, still requiring that certain parameters be optimize, herein we demonstrated that limits of detection (LODs) in the sub-nanogram per millilitre range were achieved for amitriptyline and imatinib when performing 2 min extraction from 300 µL of whole blood (see Table S6). In addition, great accuracy (*i.e.* 91-93% at 100 ng mL^{-1}, Figure 4) and linearity were attained for both probes in the range assessed. Hence, due to the speed of analysis, the suitability of performing extraction/enrichment on-site, and the simplicity of the method, BioSPME is proposed as an ideal tool for fast correlation of drugs to their therapeutic efficacy or toxicity while treating a patient[19,40,41].

Towards targeted analysis in small blood volumes

Until now, the use of SPME fibers for quantitative analysis of target analytes in limited sample volumes ($V_s \leq 50$ µL) of biofluids, such as blood, has remained overall unexplored. Challenges

involving getting reliable quantitative data at practical concentration ranges while keeping a simple sampling/sample preparation protocol have hindered the implementation of SPME in such cases. Chiefly, analytes with high protein binding coefficients typically provide extremely low recoveries by SPME; therefore, it is difficult to achieve useful quantitation limits by LC-MS/MS, unless long extraction times or larger coating surface areas are used[20,47]. Further, classical SPME fibers, with a coating length of 10-15 mm, are too long to be entirely in contact with small sample volumes unless a miniature vessel, such as a glass capillary or vial conical insert, is used for such purpose[60]. Finally, there are currently no appropriate protocols in place that ensure both total contact between fiber coating and sample[60], and efficient desorption/transmission of analytes into the mass spectrometer. To the best of our knowledge, the use of SPME fibers for the quantitation of target analytes in volumes below 10 μL has only been described in work conducted by Zhu and collaborators[60].

Therefore, in order to ensure that the entire fiber remained immersed in the sample, glass vials with a fused-in conical insert and fibers with a coating length of 4 mm were used (as shown in Figure S6) for extraction from 20 μL of whole blood spiked with amitriptyline at concentrations of clinical relevance (*i.e.* 5-250 ng mL^{-1}). As can be seen in Figure 5, notable linearity, great accuracy, and good signal were obtained in the concentration range evaluated. Although this application is just a proof-of-concept demonstrating the quantitation capabilities of Bio-SPME-nano-ESI, we anticipate its use in forensic and clinical applications where only minimal sample volumes are available. Currently, our group is workings on the development of miniature devices that allow for the analysis of sample volumes below 10 μL (*i.e.* single cells[33,61], small pieces of tissue[62], and biofluids) without sacrificing analysis time or the extraction capabilities of SPME. Unlike other sampling devices coupled to nano-ESI[63,64], mini-SPME devices truly

collect analytes of interest based on their affinity towards the extraction phase, while

minimizing or removing potential interferences that might cause suppression/enhancement.
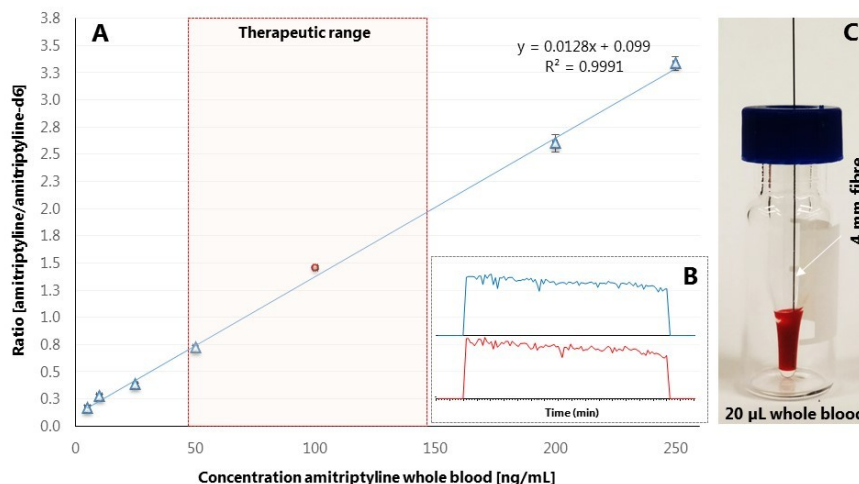
Figure 5 A. Quantitative analysis of whole blood spiked with amitriptyline (5 ng ml^{-1} to 250 ng mL^{-1}) and its isotopologue [D$_6$] amitriptyline (100 ng mL^{-1}). Sample volume is 20 µL with 2 min extraction/enrichment using 4 mm mix-mode Bio-SPME. Bars represent the standard deviation of analyses for three replicates with independent fibers and nano-ESI emitters. **B.** Ion chronogram of amitriptyline (top) and [D$_6$] amitriptyline (bottom) for an acquisition time of 45 s. **C.** SPME sampling from 20 µL of whole human blood using a 300 µL glass insert vial.

Conclusions

In this work, the Bio-SPME-nano-ESI platform was shown to rapidly and accurately determine

total concentrations of target compounds in complex matrices. Furthermore, the suitability of

these biocompatible probes to extract, identify, and quantify analytes present in small sample

volumes was demonstrated for the first time. Through the selection of appropriate experimental

conditions, the entire analytical process was completed in less than 7 min per sample with

outstanding figures of merit. In addition to the abovementioned advantages, the Bio-SPME

approach has a built-in clean-up step, which allows for the incidence of capillary plugging to be

substantially reduced. In light of the results herein presented, we foresee the combination of

SPME with nano-ESI as a rapid diagnosis tool for *in vivo* and *in situ* analyses of endogenous and exogenous substances in biological fluids[42] and tissue[41] samples. Certainly, the direct coupling of SPME-nanoESI to miniature mass spectrometers (MMS)[65,66] should bring a new dimension to what we know until now as on-site analysis in clinical, environmental, and forensic applications. Indeed, SPME-nanoESI and MMS, in combination with robotic platforms[67] is projected to be an ideal analytical tool for non-assisted time-resolved mass spectrometry applications[68] such monitoring at remote locations. Furthermore, in near future, Bio-SPME fibres in combination with Ion Mobility Spectrometry will be a key combination towards the analysis of compounds otherwise difficult to resolve by MS without LC[69].

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Associated content

Supporting information

The supporting information includes the following: materials and supplies; details about selective reaction monitoring transitions used to quantify each model compound; inter-emitter device reproducibility results; figures of merit for analysis of PBS, blood, and urine; pictures of the ion source and of vials used for sampling of small sample volumes. This information is available free of charge via the internet at http://pubs.acs.org/.

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