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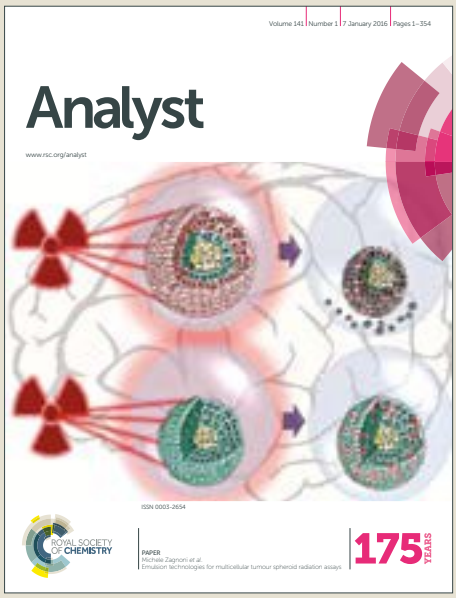

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ARTICLE

Solid phase microextraction coupled to mass spectrometry via microfluidic interface for rapid therapeutic drug monitoring

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Tranexamic acid (TXA) is an antifibrinolytic used during cardiac surgery that presents high inter-patient variability. High plasma concentrations have been associated with post-operative seizures. Due to the difficulties with maintaining acceptable concentrations of TXA during surgery, implementation of a point-of-care strategy for testing TXA plasma concentration would allow for close monitoring of its concentration during administration. This would facilitate timely corrections to the dosing schedule, and in effect tailor treatment for individual patient needs. In this work, a method for the rapid monitoring of TXA from plasma samples was subsequently carried out via biocompatible solid-phase microextraction (Bio-SPME) coupled directly to tandem mass spectrometry via a microfluidic open interface (MOI). MOI operates under the concept of a flow-isolated desorption volume and was designed with aims to directly hyphenate Bio-SPME to different detection and ionization systems. In addition, it allows the desorption of Bio-SPME fibers in small volumes while it concurrently continues feeding the ESI with a constant flow to minimize cross-talking and instabilities. The methodology was used to monitor six patients with varying degrees of renal dysfunction, at different time points during cardiac surgery. MOI proves to be a reliable and feasible tool for rapid therapeutic drug monitoring. Affording total times of analysis as low as 30 seconds per sample in its high throughput mode configuration while the single sample turn-around time was 15 minutes, including sample preparation. In addition, cross-validation against a standard thin film solid phase microextraction using liquid chromatography coupled to tandem mass spectrometry (TFME-LC-MS/MS) method was performed. Bland-Altman analysis was used to cross-validate the results obtained by the two methods. Data analysis demonstrated that 92 % of the compared data pairs (n = 63) were distributed within the acceptable range. The data was also validated by the Passing Bablok regression, demonstrating good statistical agreement between these two methods. Finally, the currently presented method offers comparable results to the conventional liquid chromatography with acceptable RSDs, while only necessitating a fraction of the time. In this way, TXA concentration in plasma can be monitored in a close to real time throughput during surgery.

Introduction

Tranexamic acid (TXA), classified as an antifibrinolytic agent, is a synthetic lysine analogue that works by inhibiting plasminogen and thus prevents its conversion to plasmin, an enzyme that degrades fibrin in blood clots.[1]. This medication is used to stop or reduce bleeding in a wide variety of haemorrhagic conditions.[2] For instance, it is used in a number of high-risk operations such as cardiac surgery, liver transplantation, as well as in emergency rooms to treat cases of haemorrhaging as a result of trauma.[1,2] Its safety profile provides significant advantages over older generation antifibrinolytic agents such as aprotinin and ecallantide, which have been linked to increased mortality, increased post-operative bleeding, increased blood transfusion requirements, and kidney failure.[2] Although no major risk factors have been reported in relation to the use of TXA in clinical applications, increased incidences of post-operative seizures in cardiac surgical patients have been reported in association with its increased clinical use in high doses.[1,2] A number of investigations directed at reaching the appropriate target concentrations unveiled high inter-patient variability for TXA, in that therapeutic levels varied greatly from patient to patient, often exceeding the target concentrations of both high dose and low dose dosing schedules.[2,3] Recently, Jerath *et al.* carried out a study demonstrating that exceedingly high TXA plasma levels – beyond 100 µg/mL – may be attributed to poor drug clearance from the system as a result of kidney dysfunction or failure.[4] The above investigation was carried out by measuring levels of TXA in the plasma of patients presenting

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Electronic Supplementary Information (ESI) available: [This file is comprised of TXA dosing schedule, a scheme of the high-throughput configuration, detailed experimental SPME-LC-MS/MS and Bio-SPME-MOI-MS/MS protocols and mass spectrometric parameters. Calibration and validation specifications together with some LC-MS/MS validation data is also included herein]. See DOI: 10.1039/x0xx00000x

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varying stages of kidney damage who were undergoing cardiac surgery with the use of TXA.[5] These findings aided in the development of a new dosing regimen that accommodates patients who suffer from renal insufficiency. In this context, the development of technologies geared at fast and precise point-of-care (POC) analysis play a vital role in further advances in personalized medicine.[6] Such developments are especially critical in cases where the inter-patient variability is high, necessitating accurate regulation of dosage to produce the desired therapeutic effect.

In this context, the direct coupling of solid-phase microextraction (SPME) to ambient mass spectrometry (MS) has emerged as a solution that efficiently integrates the sample preparation step with analysis.[7–9] Indeed, several clinical applications reported to date have demonstrated the ability of this platform to both minimize overall time of analysis as well as mass spectrometer contamination.[10–13] In this work, a novel recently developed MOI that directly couples SPME to tandem mass spectrometry (MS/MS) in order to measure TXA is presented.[14] This interface allows for efficient analyte transmission into the electrospray ionization (ESI) interface; hence, allowing a dramatic reduction in acquisition time when compared to the traditional liquid chromatography (LC) protocol (time scale on order of seconds by MOI versus time scale on order of minutes by LC).[15] Furthermore, after extraction, analytes collected onto the SPME devices are eluted for a period of 5 seconds into an open microfluidic desorption chamber which subsequently rapidly introduces the plug of desorbed analytes into the ESI interface and allows for improved sensitivity in comparison to other SPME-MS approaches.[16] In light of the recently reported connection between TXA use and post-operative seizures, and considering the well-documented sizable inter-patient variability of this drug, the currently presented technology was applied for the high-throughput monitoring of TXA in plasma samples taken from patients presenting different stages of renal damage undergoing cardiac surgery. Assessments carried out at various time-points, from five minutes after the bolus was injected, up until 72 hours post-surgery. The technology was also cross-validated against a previously validated thin film solid phase microextraction (TFME) with the use of LC-MS/MS method.[17] The methodology showed turnaround times of 15 minutes per sample for single-injection analysis (i.e. one sample at the time), and less than 30 seconds per sample for high-throughput analysis (i.e. extraction from a 96-well plate). These results demonstrate the capabilities of this technique towards close to real-time analysis of TXA, while evidencing its great potential for therapeutic drug monitoring in a wide range of clinical applications.

Experimental methods and materials

Chemicals and materials

Tranexamic acid (trans-4-(aminomethyl)cyclohexanecarboxylic acid), the internal standard cis-4-aminocyclohexanecarboxylic acid, LC-MS grade formic acid, HPLC grade acetonitrile and methanol as well as chemicals used to prepare phosphate buffered saline (PBS), namely sodium chloride, potassium chloride, potassium phosphate monobasic, sodium phosphate dibasic were purchased from Sigma-Aldrich (Oakville, ON, Canada). Deionized water was obtained from a Milli-Q Reference A+ water purification system (Fisher Scientific). LC-MS grade water, used to prepare PBS as well as the stock solutions, was purchased from Fisher Scientific (Ottawa, ON, Canada). Human plasma (sodium citrate) was obtained from Lampire Biological Laboratories Inc. (Pipersville, PA, USA). HLB Supel™-Select particles (~ 60 µm) used for thin-film solid phase microextraction (TFME) were kindly provided by Supelco (Bellefonte, PA, USA), while HLB particles (~ 5 µm) used to fashion Bio-SPME fibres were kindly provided by Waters Corporation (Winslow, UK). Polypropylene 96-well 2 mL deep plates were purchased from Fisher Scientific (ON, Canada).

Human plasma samples

All procedures employed in this study, including the retrieval of samples from patients, were approved by the Research Ethics Boards of the Toronto General Hospital/ University Health Network and the University of Waterloo. Each patient signed an informed consent while recruited to participate in the study. Plasma samples were obtained from patients undergoing elective high or low risk cardiac surgery under cardiopulmonary bypass (CPB). For high risk (HR) cardiac surgery the dose administered was as outlined in the Blood Conservation Using Antifibrinolytics in a Randomized Trial (BART) study[18] whereby 30 mg/kg bolus of TXA infused over a 15 min span post induction of anaesthesia (period 1) followed by an infusion of TXA at a dose of 16 mg/kg·hr (period 2) until the sternotomy was closed with a 2 mg/kg load in the pump prime. For low risk (LR) cardiac surgery, the dose was as per institutional practise at Toronto General Hospital as outlined by Jerath *et al*[5] whereby patients received a bolus dose of 50 mg/kg post induction of anaesthesia. The sampling protocol, which is schematically demonstrated in supplementary information S1 – figure S1, was as outlined in previous research.[17] Blood samples were first collected at baseline and then at 5 min and 10 min during period 1 which was the administration of a bolus dose (single dose of TXA given to the patient by injection into a blood vessel). During period 2, whereby TXA was infused, samples were collected post sternotomy (chest opening), immediately before and after the start of CPB, and at 30 min intervals during CPB for up to four sampling points. Samples were collected again after CPB and prior to chest closing. Finally, in period 3 - the post operation (postop) period - samples were collected at 1 hr, 2 hr, 4 hr, 8 hr, 12 hr, 24 hr, 48 hr, and 72 hr. A total, 114 samples were expected to be collected from 6 patients, with each of the 19 samples obtained, processed in triplicate. However, only 86 samples were obtained from 6 patients undergoing cardiac surgery for reasons undisclosed.

High throughput analysis with concept-96 unit

The concept-96, shown in supplementary information S2 – figure S2, is a software-operated system that automatically performs each step of the SPME protocol: pre-conditioning, extraction, rinsing, and desorption.[19] It houses a robotic arm, where either the 96 blades or fibres can be immobilized. These devices are compatible with 96-well plates, which can be stationed in their respective compartments on the unit. The system allows for preparation of up to 96 samples at once. After completing the sample preparation workflow, well plates can be deposited directly into the auto sampler of the LC system.

Sample preparation

All samples, including human plasma samples used to create the matrix-matched external calibration curve, were prepared as follows: 250 µL of sample was placed in a 2 mL well, and diluted with 750 µl of a PBS solution containing the internal standard. The dilution of samples (1:3) was supported for this application due to the high therapeutic levels of TXA in the samples, as well as negligible binding of TXA to plasma proteins (~ 3%).[20] Extractions from the prepared samples were carried out with TFME in brush format and Bio-SPME fibres; both compatible with the 96-well plate format. Please see

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supplementary information S3 for more information on preparation of standards and calibration curve.

Instrumental analysis:

SPME-LC-MS/MS

The TFME brush was prepared using a spray protocol developed in-house.[21] The device was coated with 60 µm HLB particles, and characterized by final coating dimensions of 2 cm length and 0.3 mm thickness.[22] Collected patient samples were prepared for analysis in 96-well plates, which were then mounted on their respective stations on the Concept-96. The SPME protocol for the LC-MS/MS method was as follows: pre-conditioning of the coating was carried out in 1.5 mL 50:50 (v/v) methanol/water for 15 min at 1500 rpm; extraction was performed at room temperature (25 °C) from 1 mL of biological matrix for 5 min at 1500 rpm; a 10 s agitated rinse (1500 rpm) was carried out in 1 mL 90:10 (v/v) water/methanol; and desorption was completed in 1 mL 3:3:4 (v/v/v) acetonitrile/methanol/water for 10 min at 1500 rpm. The desorption plate was then mounted into the autosampler and 10 µL of resultant extracts were withdrawn for LC-MS/MS analysis. Chromatography and mass spectrometry details can be found in supplementary information S4 and table S1. Chromatograms of the typical signals for blank plasma and patient samples are shown in supplementary information S4 – figures S3 and S4 respectively.

Bio-SPME-MOI-MS/MS

Bio-SPME fibres were prepared on a nitinol support in accordance to a dipping method developed in our laboratory.[12] The final dimensions of the fibres were 4 mm coating length and 20 µm of thickness, using 5 µm HLB particles. Parameters for analysis via MOI-MS/MS were as follows: extraction time was 15 min, followed by two 5 s rinsing steps with water, then desorption for 5 seconds at the MOI device, which was directly coupled to the mass spectrometer API 4000 triple quadrupole (AB SCIEX, Concord, Ontario). The operational duty cycle and performance of this interface is described in detail elsewhere.[14,15,23] The LC pump employed for fluid delivery was a 200 Series Perkin Elmer (Santa Clara, CA, USA), while ionization was produced with the use of a TurboIon™ spray source. Conditions required for the desorption step consisted of an equilibrium between the pump flow at (350 µL·min^{-1}) and the electrospray ionization (ESI) aspiration. Essentially, the MOI device is designed with two sections[14] as shown in the second step of the analytical workflow in figure 1. The top section, which functions as the SPME desorption chamber, consists of a Teflon cylinder with two holes connected by a channel of a smaller diameter.[14] The connection between the open ambient desorption chamber and the electrospray needle employed in this device was inspired by the design of the open-port interface reported by Van Berkel et al.[9,13–15,23] Succinctly, the procedure involves the employment of two co-axial tubes that allow for solvent delivery through the gap formed between these two tubes. Once the solvent reaches the top of the coaxial tubes, it is aspirated towards the MS by means of the Venturi effect produced by the ESI source. ESI parameters were as follows: positive ion mode; nitrogen gas set at GS1 = 90, GS2 = 70; collision gas (CAD) = 6; curtain gas = 25; heated nebulizer temperature = 300 °C; and electrospray voltage = 5000 V. More information can be found in the supplementary information S5 and table S2. Chromatograms of the typical signals for blank plasma and patient samples are shown in supplementary information S5 – figures S5 and S6 respectively. The solvent employed in the MOI-MS/MS system was methanol with 0.1% v/v formic acid. These conditions provided better sensitivity for this direct-to-MS application than acetonitrile or the desorption conditions used for TFME-LC-MS/MS.

Results and discussion

SPME-MOI as a tool for rapid analysis of clinical samples

Given that tranexamic acid is known to present high inter-patient variability, concentration levels for TXA should be closely monitored in patients over the course of treatment. In this regard, previous studies have demonstrated the emergence of a two-group stratified

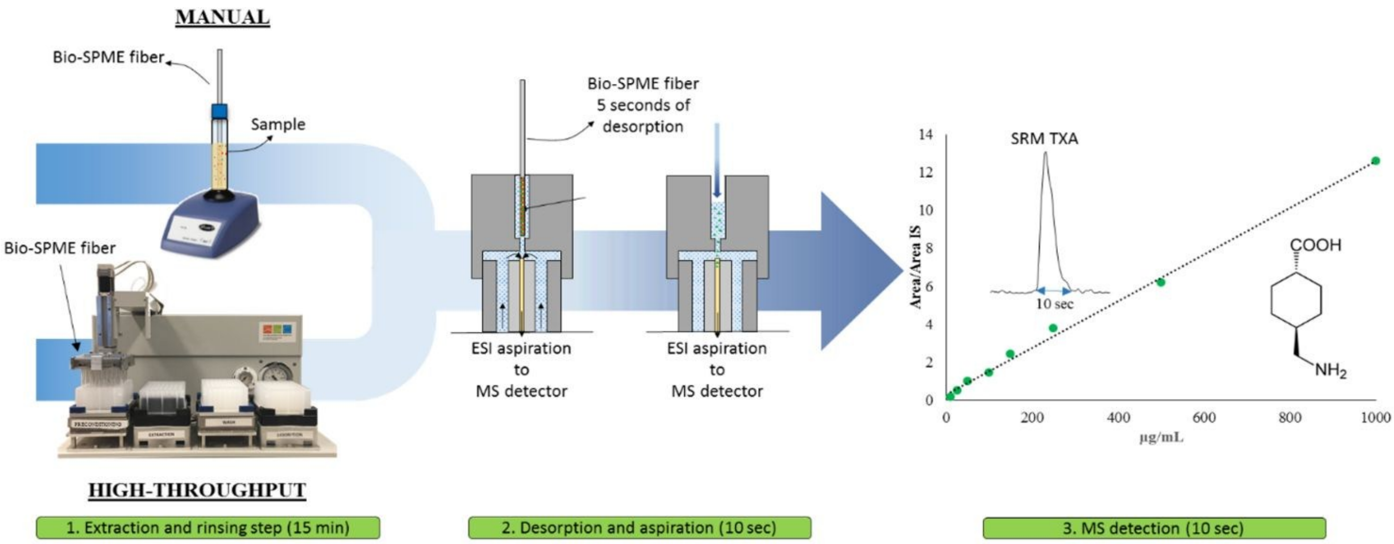

Figure 1: Analytical workflow for Bio-SPME-MOI-MS/MS

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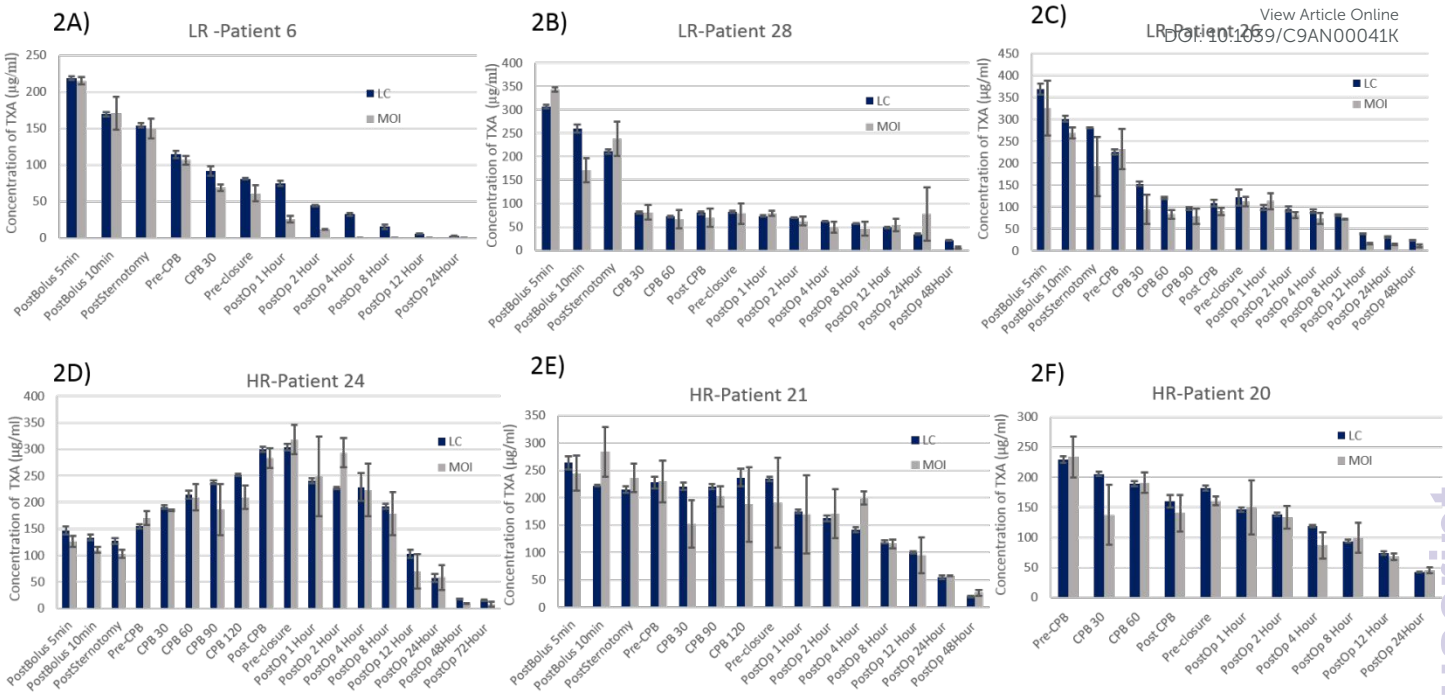

Figure 2: Patient profiles of 6 patients from two different risk groups. Figures 2A, 2B and 2C show profiles for patients who underwent low risk cardiac surgery with Blood Conservation Using Antifibrinolytics in a Randomized Trial (BART) dosing schedule. Figures 2D, 2E and 2F show profiles of patients who underwent high risk cardiac surgery with a dosing schedule as per institutional practise at the Toronto General Hospital. Results obtained via SPME-LC-MS/MS is represented by bars in dark blue while results obtained via SPME-MOI-MS/MS are represented by grey bars.

trend of TXA plasma concentration for patients receiving the same therapeutic treatment.[2] For the confirmatory high throughput study performed by Jerath *et al*,[5] patients were first categorized based on the type of cardiac surgery endured – low risk (LR) or high risk (HR). Within each group, patients were further categorised by healthy renal function and on the basis of degree of chronic renal dysfunction, stages 1 – 5; stage 1 being normal or increased glomerular filtration rate (GFR) and stage 5 being kidney failure.[5] As the use of TF-SPME technology for monitoring TXA in plasma has already been validated in previous research[17,20,24] against traditional methods such as ultrafiltration (UF) and plasma protein precipitation (PPP) which is routinely used for analysis in clinical applications, the same technology, with a few modifications was employed for this high throughput study. Given that all patients within the same risk category (high risk or low risk) were submitted to the same dosing schedule, the drastic differences observed in patient profiles as seen in figure 2 (for example high risk patients in figure 2D, 2E and 2F, patients with more severe chronic renal dysfunction (CRD) experience elevated and persistently high TXA concentrations – figure 2D and 2E – in comparison to patients with normal kidney function – figure 2F) endorses not only the need for revision and adjustment of the current TXA dosing schedule for cardiac surgical patients, but also the necessity of rapid sample analysis technology to facilitate POC testing. The former would generally contribute towards improving the recovery of patients post operation, particularly those with renal impairment, while the latter would better navigate clinical practitioners in personalizing treatment peri-operatively. Herein, we explain how Bio-SPME-MOI-MS/MS can be used as an alternative technology for rapid high throughput analyses of clinically relevant samples containing TXA. For instance, Figure 1 shows two sample preparation workflows that can be used with this technology. The first approach, the single injection strategy, allows for turnaround times under 15 minutes. The second approach, namely the high throughput workflow, as illustrated in Figure 1, allows for simultaneous extraction of TXA from up to 96 plasma samples. Hence, total sample preparation time drops to less than 10 seconds per sample, with total analysis times of approximately 30 seconds per sample given that the operator needs to manually place each Bio-SPME device on the MOI. As such, this method not only offers the selectivity provided by mass spectrometry and the efficient sample clean-up afforded by SPME but also facilitates rapid sample throughput due to semi-automation via the Concept-96. The Bio-SPME-MOI-MS/MS method produced a coefficient of determination (R^2) of 0.997 and a linear dynamic range over more than two orders of magnitude (refer to figure 1), from 25µg/mL – 1000 µg/mL, comparable to the TFME-LC-MS/MS method. However, an LOQ of 25 µg/mL was obtained for the MOI-MS/MS as opposed to the LOQ of 5 µg/mL obtained for LC-MS/MS. These differences reflect the differences observed in recoveries for the target compound, which were dependent on the geometry utilized for analysis, whereby a 1% recovery was experienced for the TFME method in comparison to a 0.1 % recovery for the Bio-SPME method. Nonetheless, the range of concentrations that are expected to be encountered during TXA administration are well above these LOQs, with the target therapeutic level estimated to be 100 µg/mL. The method was validated in human plasma at a working concentration of 100 µg/mL, with an inter-day precision of 20 %, a corresponding average accuracy of 91 % (n = 15), and an intra-day precision of 20 %, corresponding to an average accuracy of 95 % (n = 5) (See supplementary information S6 – figure S7).

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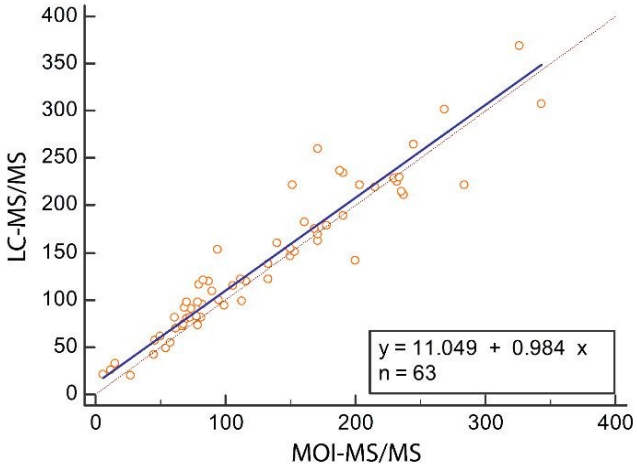

Figure 3: Passing-Bablok regression from data from LC-MS/MS vs MOI-MS/MS

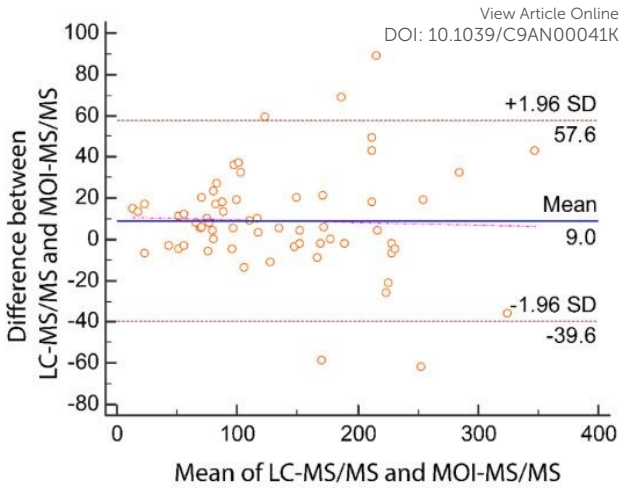

Figure 4: Bland-Altman plot of data pairs (n=63) of LC-MS/MS and MOI-MS/MS.

As a result, figure 2 shows the relatively close reproducibility of patient profiles by Bio-SPME-MOI-MS/MS in comparison to the trend generated by TFME-LC-MS/MS from both sets of patients undergoing high-risk (HR) or low-risk (LR) cardiac surgical procedures. Each bar plot shows the comparison of MOI (represented by grey bars) vs LC (represented by dark blue bars) for the concentration of tranexamic acid in plasma at each time point over the course of surgery. The profiles generated by Bio-SPME-MOI-MS/MS are very similar to profiles obtained by TFME-LC-MS/MS in that the same trend for a particular individual was observed. For instance, HR-Patient 24 had much higher TXA levels between CPB-30 (30 minutes after the start of cardiopulmonary bypass) and post-op-4hour (4 hours post operation after removal of CPB and cessation of TXA IV infusion) compared to the concentrations prior to Pre-CPB (start of surgery before cardiopulmonary bypass was implemented). In other patients however, such as HR-Patient 20 and HR-Patient 21, although TXA concentrations remain high (greater than 100 µg/mL), there is a more obvious decreasing trend of TXA plasma concentrations over the course of surgery, unlike that seen with HR-Patient 24. All three patients of this risk group belong to stage 4 chronic renal dysfunction. Despite belonging to the same stage and receiving the same dosing schedule, there are clear differences in the profiles between these patients. LR-Patient 26 and LR-Patient 28 experienced a sharp drop in concentration around CPB-30, however the concentration levels essentially plateaued at these lower concentrations with a slight spike at pre-closure and postop 1 hour from LR-Patient 26 until concentrations finally decreased during the post op period around 12 hours and 24 hours for LR-Patient 26 and LR-Patient 28 respectively. However, LR-Patient 6 experienced a much sharper decreasing trend than the other patient counterparts in the same risk group and especially in comparison to those patients in the high-risk group. The concentration plateau seen in LR-Patient 26 and LR-Patient 28 are indicative of increased residence time of TXA in plasma due to poor renal filtration as both patients belong to stage 4 renal dysfunction whereas LR-Patient 6 belongs to stage 2 renal dysfunction. These results comparing MOI-MS/MS to LC-MS/MS are astounding, especially considering the large difference in time of analysis that exists between the two methods. For instance, approximately 19 continuous hours would be needed to process 96 samples via LC-MS/MS as opposed to the less than 2 hours needed for the manually operated MOI-MS/MS workflow to produce comparable results. Moreover, the proposed method proves robustness, as the samples were run on a blinded basis and completely randomized.

Statistical validation of the MOI methodology

Figure 3 and Figure 4 demonstrate the results of the statistical validation performed on the data acquired from both methods. The Passing-Bablok regression, shown in Figure 3, was constructed in order to statistically cross-validate the SPME-MOI-MS/MS method for monitoring TXA in plasma with the previously validated SPME-LC-MS/MS method. The Passing-Bablok regression uses an orthogonal regression algorithm which assumes that measurement imprecision is present in both methods under comparison. Six patients, were analysed initially by SPME-LC-MS/MS for the purposes of the high throughput study.[4] Both methods exhibited consistency in signal response over concentration. From the 86 sample pairs that were obtained from measurements with LC vs MOI from 6 patients, 25 pairs exhibited statistically different results ($p < 0.05$) and were therefore excluded from further analysis. As such, n = 63 pairs were used for further statistical cross validation of the two methods. The results from the regression, which included 63 sampling pairs suggest equal suitability for both methods, with a slope of 0.984 (0.909 to 1.06), and an intercept of 11.049 (1.91 to 16.3). A statistical comparison of the two methods via the Spearman correlation coefficient yielded a value of 0.958 (0.931 to 0.974), further indicating that the methods have a linear relationship and are highly correlated ($p < 0.01$). Furthermore, to supplement the results of the Passing-Bablok regression, a Bland-Altman plot was constructed. The Bland-Altman plot shown in Figure 4 shows that the mean absolute difference in TXA quantification was observed to be 9.0. A value of ±1.96 standard deviation (SD) was used for the limit of agreement values (LOA), thus obtaining an interval from −39.6 (lower agreement limit) to 57.6 (upper agreement limit). The number of data pairs observed to be beyond the LOA value was 5 out of 63, which confirmed a Bland–Altman index of 7.9%. Thus, 92.1 % of the compared pairs were distributed within the acceptable range (within the ±1.96 SD limit). The comparative results presented low discrepancy between both methods, with the majority of the values distributed around the mean (9.0) and completely random, indicating that no tendency is present in the analysis. Thus, confirming the suitability of Bio-SPME-MOI-MS/MS for the monitoring of TXA in plasma samples.

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MS-based POC technologies

The MOI has already demonstrated to have about one order of magnitude more sensitivity than the open port probe interface for Bio-SPME applications for several targeted compounds.[9,14] The main differences were attributed to the small desorption volume employed as well as the static desorption allowing for a very sharp injection plug. Additionally, this technology is complementary to other ambient technologies such as DART and DESI which have been implemented in a number of clinical applications and are largely employed in a wide range of bioanalytical applications including screening of small molecules in biological fluids, 2D imaging of tissues and high throughput screening and drug discovery, respectively.[25–28] However, most of these applications are directed towards the analysis of compounds present in high concentrations where strong matrix effects are not compromising the detectability. When sample preparation for direct-to-MS approaches is taken into account, the most widespread is SPE.[29,30] Rapidfire™ especially, has been demonstrated to be useful for therapeutic drug monitoring of immunosuppressive drugs in whole blood with sample turnaround times of less than 15 s.[30] However, for total analysis time, the number of steps is not fully contemplated including protein precipitation, incubation and centrifugation. Noteworthy, Unlike SPME, in SPE this protocol cannot be skipped in clinical samples such as whole blood or plasma due to the chances of cartridge clogging. Regarding SPME-MS interfaces, Coated blade spray (CBS)[7] is another concept, that has also shown potential for high throughput therapeutic drug monitoring affording sample analysis times of less than 55 s.[11] As a substrate spray method, the entire blade can be exposed to a fluid sample for a predetermined amount of time, thereby conducting an SPME based extraction.[10,11] Alternatively, similar to Paper spray (PS), a spot analysis can be performed wherein a small sample volume can be directly applied to the blade coating surface prior to desorption/ ionization. There are fewer methodologies still, like PS, that are used for quantitative therapeutic drug monitoring and POC analysis.[31,32] Given that SPME fibers can be easily interfaced with the MOI, this technology offers a unique advantage not only as an alternative approach to the bulk of these strategies but also a complementary technique as it can be used for both quantitative *in vivo* and *ex vivo* analysis.[9,13,14,16] Owing to the SPME device's small diameter (Ø < 250 µm), MOI presents a breakthrough for rapid quantitative and qualitative practices for assessing more complex biological materials such as various tissues/organs that have been subjected to therapy.

Conclusions

The work herein presented demonstrates that employment of liquid chromatography instrumentation for quantitation of TXA in plasma can be circumvented by directly coupling SPME to mass spectrometry via the developed MOI, thereby dramatically improving sample throughput. In fact, to the best of our knowledge, it is the first time that a SPME-MS approach is validated for a large number of clinical samples demonstrating the feasibility for rapid therapeutic drug monitoring. Since SPME integrates sample preparation and sample clean-up in one step, while being amenable for automation with the 96-concept, a large number of plasma samples from patients can be prepared and subsequently submitted to instrumental analysis within a short period of time, affording total analysis time of less than 30 seconds per sample. The currently presented method achieved a linear dynamic range between 25 µg/mL – 1000 µg/mL, which is comparable to that produced by TFME-LC-MS/MS. This LOQ of 25 µg/mL result is suitable for the range of TXA concentrations expected from such clinical applications; and undoubtedly reproduced the profiles of 6 patients from two different risk groups, with an average accuracy and precision of 95% and 20 %, respectively. It must also be noted that despite Bio-SPME-MOI-MS/MS did not achieve similar precision as found by TFME-LC-MS/MS method, the attained values are nonetheless sufficient and acceptable for a POC or screening methodology. Presumably, one of the main contributing factors behind the observed difference in performance may be attributed to the absence of an autosampler for conducting the analysis since the fibers were introduced manually to the MOI. Uncertainties in the final quantitative data may arise because of variations in the reaction times of placing or removing the fibers into or from the MOI, as well as variations in the amount of time that elapsed for desorption and switching the flow to allow aspiration. Additionally, the fibers used were prepared in laboratory using a relatively novel dipping strategy developed in house.[12] The use of commercially available fibers can be a source of potential improvement. Furthermore, although use of an internal standard is meant to correct for any possible variations, the internal standard used was not an isotopically labelled standard which may not completely address the system variations. Nevertheless, the Passing-Bablok regression was performed to cross-validate the developed MOI methodology against the LC-MS/MS gold standard. The correlation was slightly lower in the lower concentration range (intercept) due to the differences in LOQ achieved by LC-MS/MS vs MOI-MS/MS respectively. However, the assays agreed reasonably well in the expected TXA concentration range for both methods (slope of the regression line 0.931 to 0.974). Bio-SPME-MOI offers a breakthrough for rapid sample analysis and high sample throughput — requirements for on-site clinical applications such as therapeutic drug monitoring and POC testing. With the necessity for rapid sample analysis afoot, based on the work presented herein and the possibility of mass spectrometers being introduced into clinical settings, this technology can be implemented for real-time analysis of biological samples in hospitals and clinics, providing and easy to use tool for POC-testing, screening, and onsite therapeutic drug monitoring in biofluids and/or tissues either *in vivo* or *ex vivo*.

Conflicts of interest

The authors do not have conflicts of interest to declare

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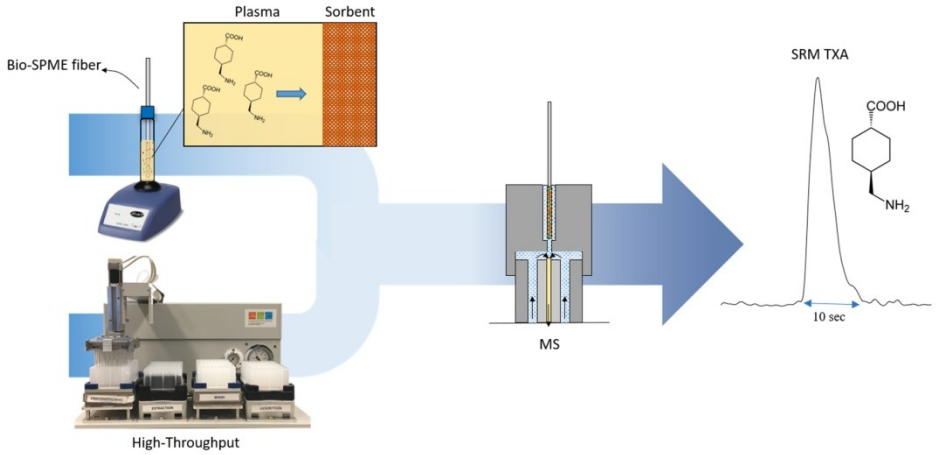

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