



## Use of a novel technique, solid phase microextraction, to measure tranexamic acid in patients undergoing cardiac surgery

## Utilisation d'une nouvelle technique, la microextraction en phase solide, pour doser l'acide tranexamique chez les patients subissant une chirurgie cardiaque

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### Abstract

**Purpose** Solid phase microextraction (SPME) is a technique widely used and accepted in the field of food technology and in environmental and biological analyses. Despite its numerous advantages over older analytical methods, it has not been studied extensively in the medical sciences. Tranexamic acid (TXA) is currently the sole antifibrinolytic agent used during cardiac surgery involving the use of cardiopulmonary bypass (CPB). The current standard method of measuring TXA in plasma is based on plasma protein precipitation (PPP), but this analytical approach is time-consuming and not practical for routine use. The aim of the current study was to compare plasma TXA levels measured with the PPP method vs those acquired with the novel, highly efficient SPME technique. We also investigated the use of automated SPME with the

aim of improving the technique so it could be used efficiently for measuring plasma TXA levels.

**Methods** With Research Ethics Board approval, we undertook a prospective, investigator-blinded study in ten patients undergoing cardiac surgery with CPB. An initial TXA bolus of  $30 \text{ mg}\cdot\text{kg}^{-1}$  was infused over 15 min followed by a  $16 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{hr}^{-1}$  infusion until chest closure with a  $2 \text{ mg}\cdot\text{kg}^{-1}$  load in the pump prime. Each blood sample was divided into two portions and assigned a random number to blind the analyzing laboratory. The blood TXA concentration was measured using both PPP and SPME. Agreement between the two tests was analyzed using the Bland-Altman plot.

**Results** Comparisons of plasma TXA concentrations measured with the two methods (PPP and SPME) showed good agreement. Absolute recovery of TXA for PPP was 64.9–78.2%; its precision, as a percentage of the relative standard deviation (RSD) was  $< 10\%$  [with the exception of the lower limit of quantification (LLOQ), where the RSD was 18%]; and its accuracy, as the bias against the nominal concentration, was  $< 7\%$  (for LLOQ it was 15%). Thus, extraction with SPME compared favourably with the PPP technique.

**Conclusions** Solid phase microextraction is a relatively simple, rapid extraction technique that can facilitate future pharmacokinetic studies analyzing TXA drug concentrations and drug dosing in various clinical settings.

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### Résumé

**Objectif** La microextraction en phase solide (SPME) est une technique largement utilisée et acceptée dans les domaines de la technologie alimentaire et des analyses environnementales et biologiques. En dépit de ses nombreux

avantages par rapport aux méthodes d'analyse plus anciennes, elle n'a pas été étudiée de façon approfondie dans le cadre des sciences médicales. L'acide tranexamique (TXA) est actuellement le seul antifibrinolytique utilisé au cours de la chirurgie cardiaque impliquant l'utilisation d'une circulation extracorporelle (CEC). La méthode de référence actuelle du dosage de la TXA plasmatique repose sur la précipitation des protéines plasmatiques (PPP), mais cette approche analytique prend du temps et n'est pas adaptée à une utilisation en routine. Le but de la présente étude était de comparer les niveaux plasmatiques de TXA dosés par la méthode PPP à ceux obtenus avec la nouvelle technique, très efficace, de SPME. Nous nous sommes également intéressés à l'utilisation de la SPME automatisée dans le but d'améliorer la technique afin de pouvoir l'utiliser efficacement pour le dosage de la TXA plasmatique.

**Méthode** Nous avons entrepris, avec l'accord du Comité d'éthique de la recherche, une étude prospective à l'insu de l'investigateur chez dix patients subissant une chirurgie cardiaque avec CEC. Un bolus initial de  $30 \text{ mg}\cdot\text{kg}^{-1}$  a été perfusé en 15 min suivi d'une perfusion de  $16 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$  jusqu'à la fermeture de la paroi thoracique avec une dose de charge de  $2 \text{ mg}\cdot\text{kg}^{-1}$  dans l'amorçage de la pompe. Chaque échantillon de sang a été divisé en deux spécimens auxquels ont été attribués un numéro aléatoire pour assurer l'insu du laboratoire d'analyse. La concentration sanguine de TXA a été dosée par les deux méthodes de PPP et de SPME. La correspondance entre les deux tests a été analysée par la méthode de Bland-Altman.

**Résultats** La comparaison des concentrations plasmatiques de TXA dosées par les deux méthodes (PPP et SPME) a fait apparaître une concordance satisfaisante. Le taux de récupération absolue de la TXA par la méthode PPP a été compris entre 64,9 % et 78,2 %; sa précision, exprimée en pourcentage de l'écart-type relatif (ETR), était  $< 10$  % (à l'exception du seuil de détection [LLOQ] pour lequel l'ETR était de 18 %); et son exactitude, exprimée par l'écart par rapport à la concentration nominale, était  $< 7$  % (15 % pour le LLOQ). Ainsi, la technique d'extraction par SPME se comparait favorablement à la technique PPP.

**Conclusions** La microextraction en phase solide est une technique d'extraction relativement simple et rapide susceptible de faciliter les études pharmacocinétiques futures analysant les concentrations de TXA et sa posologie dans différents contextes cliniques.

Solid phase microextraction (SPME) is widely used and accepted in the field of food technology and in environmental and biological analyses. The technique is based on use of a biocompatible sorbent absorbing the substance(s)

of interest. It offers several advantages over other analytical extraction techniques, making it potentially attractive for use in clinical medicine.<sup>1,2</sup> Even though the technique has been around for two decades, its application in the medical sciences has been poorly explored. Solid phase microextraction theoretically poses several advantages over other methods used to measure drug and substance concentrations,<sup>3</sup> including its ease of use, the requirement of only small volumes of plasma or other body fluids, and the potential to measure total and free concentrations of multiple drugs, their metabolites, and substances they may influence from a single body fluid or tissue sample.<sup>1-3</sup> In addition, this method uses biocompatible sorbents immobilized on small fibres housed in a needle assembly system that can potentially be used in *in vivo* systems.<sup>1</sup>

Because of its antifibrinolytic effects, tranexamic acid (TXA) is routinely used during cardiac surgical procedures to minimize blood loss.<sup>4,5</sup> Recently, its use during trauma surgery has been shown to reduce mortality.<sup>6</sup> Optimal dosing of TXA is still unknown and several theoretical models have been developed and applied. One of the most popular dosing regimens is based on the pharmacokinetic model developed by Dowd *et al.*<sup>7</sup> It has been used in a randomized controlled trial comparing the use of three antifibrinolytic agents in high-risk cardiac surgical patients [Blood Conservation Using Antifibrinolytics in a Randomized Trial (BART)].<sup>5</sup> The plasma concentrations of TXA in patients undergoing cardiac surgery with these antifibrinolytic doses have not been studied.

Therefore, we decided to conduct a prospective study to assess whether SPME measurements of the plasma TXA concentrations in cardiac surgical patients were comparable to those measured with the traditional sample preparation technique of plasma protein precipitation (PPP). The primary objective of our study was to compare directly the TXA levels measured with the use of those two extraction techniques (PPP and manual SPME). The secondary objective of our study was to investigate whether automated SPME improved the technique used to measure the plasma TXA concentration.

## Methods

The Research Ethical Boards at University Health Network and University of Waterloo approved the study. We recruited and obtained signed informed consent from a convenience sample of ten patients undergoing cardiac surgery with cardiopulmonary bypass (CPB). All patients underwent standard anesthesia and surgical care as described elsewhere.<sup>8</sup> We prospectively collected detailed perioperative data, including demographic data, surgical information, timing and amount of blood product transfusions, perioperative medications

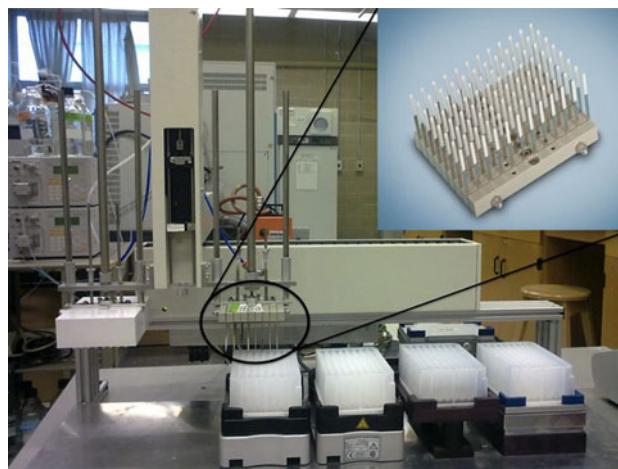
including drugs affecting coagulation, laboratory tests and postoperative outcomes.

After induction of anesthesia, intravenous TXA was administered using a protocol described previously and being used in several institutions.<sup>5,7</sup> An initial TXA bolus of  $30 \text{ mg}\cdot\text{kg}^{-1}$  was infused over 15 min followed by a  $16 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{hr}^{-1}$  infusion until chest closure with a  $2 \text{ mg}\cdot\text{kg}^{-1}$  load in the pump prime. Blood samples were obtained at baseline, five minutes after the bolus, five minutes before and after commencing CPB, and at 30-min intervals whilst on CPB. Upon discontinuation of the infusion, blood samples were obtained at 5, 60, and 120 min. Each blood sample was collected into a standard citrate tube (Vacutainer; Becton Dickson, Franklin Lakes, NJ, USA) and centrifuged; the supernatant (plasma) was stored at  $-70^\circ\text{C}$  prior to analysis. Each plasma sample was divided into two portions and was randomly assigned a number, thereby keeping them blinded to the analyzing laboratory.

Tranexamic acid was extracted from plasma samples with use of PPP or SPME, and concentrations were measured using tandem liquid chromatography-mass spectrometry.<sup>9,10</sup> Before performing measurements, standard calibration curves were obtained for TXA concentrations ranging from  $1.56$  to  $300 \mu\text{g}\cdot\text{mL}^{-1}$ . Plasma protein precipitation and subsequent steps of the measurement were performed as described previously.<sup>9,10</sup> In brief, PPP uses a mixture of acetonitrile and methanol (mixed at an 8:2 ratio) as a precipitating solvent. During the precipitation the ratio of analyzed plasma to precipitant volume was 1:10, and precipitation was performed over 60 min while the mixture was spun in a vortex working at 1,000 rpm. The samples were then centrifuged for 20 min at temp of  $4^\circ\text{C}$  at 12,000 rpm.

Manual SPME was performed using biocompatible C18 fibres (Supelco, Bellefonte, PA, USA). Tranexamic acid was extracted from plasma samples by placing a sample fibre in a small sample volume ( $300 \mu\text{L}$ ) for 90 min and spinning it in a vortex at 1,200 rpm. Following extraction, the samples were washed for 10 sec (washing solution water). Subsequently, fibres underwent the process of desorption for 120 min at 1,000 rpm. As a desorption solvent ( $300 \mu\text{L}$ ) we used a mixture of acetonitrile with water (8:2 ratio) with addition of 0.1% formic acid.

For the second stage of the study, the SPME method was reoptimized and automated. This automated technique used polyacrylonitrile octadecyl-silica (PAN-C18) thin film geometry coating immobilized on a system of 96 blades supported on a holder operated by a robotic arm of the CONCEPT 96 automated sample preparation station. The 96-blade device is fully compatible with a commercial 96-well plate (Fig. 1). Use of the CONCEPT 96 system enabled the extraction and desorption of 96 samples simultaneously.<sup>11,12</sup> This system has been described in detail elsewhere.<sup>11,12</sup> The optimized equilibrium time was 120 min



**Fig. 1** Automated robotic system for automated solid phase microextraction (SPME) analysis (CONCEPT 96). Inset. Removable brush consists of 96 blades coated with biocompatible sorbent used for direct extraction from biological fluids

at 1,000 rpm, and desorption time was 120 min at 1,200 rpm. Prior to the extraction, plasma samples were diluted fourfold with phosphate-buffered saline (PBS) at pH 7.4. Because of the high concentration of TXA in plasma samples, the extracts were further diluted with the desorption solution 1:10. No internal standard was used in the studies.

#### Statistical analysis

Continuous response variables are reported as means and standard deviations, and categorical variables are described using frequencies and percentages.

The agreement between the two tests was analyzed using the Bland-Altman plot.<sup>13,14</sup> This method offers a graphic representation of how the difference between the two measurements varies across the values of the quantity that it is measured. The latter is unknown and thus estimated by the average value of the two measurements. In the present study, the data were correlated as measurements from the same patient and as taken over time. The traditional Bland-Altman method requires independent data points and therefore is unsuitable for this study. Instead, we employed a modified version of the Bland-Altman method that takes into account the correlation structure of the data; this method generated more accurate estimates of the standard errors of the difference between the two measurements.<sup>15</sup> We estimated the bias and the confidence interval of the difference to assess the agreement. We also fit a linear and a nonlinear (curve) model through the difference points, investigating the relation between the difference and the mean value. Any systematic variation of the difference over the range of measurements is undesirable. Bland-Altman analysis was performed using R statistical software, the research methods package (NM5), and the R code developed by Nutter.<sup>16</sup>

## Results

All ten patients who were recruited into the trial completed the study protocol. The recruitment started in June 2009 and was complete in July 2009. Demographic variables are presented in the Table. The calibration curves obtained for the SPME and PPP methods are shown in Fig. 2. Absolute recovery of TXA by the PPP technique was 64.9–78.2%. The precision, as a percentage of the relative standard deviation (RSD), was < 10% [with the exception of the lower limit of quantification (LLOQ), where the RSD was 18%]. The accuracy, as bias against the nominal concentration, was < 7% (for LLOQ it was 15%). For manual (fibre) SPME, the absolute recovery, precision, and accuracy were 0.06%, < 9%, and < 6%, respectively. For the LLOQ, precision and accuracy were 11.0% and 8.9%, respectively. The automated SPME technique (Fig. 1) achieved a recovery of 2.5%, the precision was < 8%, the accuracy < 9% and the precision and accuracy at the LLOQ level were 10.0% and 8.8%, respectively.

Regarding the cross-validation of PPP and SPME, the profile of the mean concentrations of TXA obtained with the two methods are shown in Fig. 3. Comparisons of measurements obtained by PPP and by automated SPME, analyzed with the Bland-Altman approach, are shown in Fig. 4. They indicate good agreement. The bias, or average difference, between the two analytical techniques was  $3.281 \mu\text{g}\cdot\text{mL}^{-1}$  with a 95% confidence interval between  $-49.94 \mu\text{g}\cdot\text{mL}^{-1}$  and  $56.5 \mu\text{g}\cdot\text{mL}^{-1}$ . The bias, or average difference, between the two analytical techniques was  $3.28 \mu\text{g}\cdot\text{mL}^{-1}$ , and the 95% confidence interval was between  $-49.17 \mu\text{g}\cdot\text{mL}^{-1}$  and  $55.73 \mu\text{g}\cdot\text{mL}^{-1}$ .

## Discussion

Our results show that measuring plasma TXA concentrations with SPME extraction compares favorably with the traditional extraction technique using protein precipitation. Protein precipitation is a time-consuming, multistep process that requires sample agitation, centrifugation, separation of the supernatant, and dilution. By simplifying the extraction of TXA from plasma, we can more readily explore the pharmacokinetics of this important antifibrinolytic agent.

To validate an analytical technique, it must be reproducible, accurate, and precise.<sup>A</sup> SPME gives highly reproducible results with a correlation coefficient of 0.959 as reported previously.<sup>17</sup> The accuracy of SPME describes how

**Table** Patients' characteristics

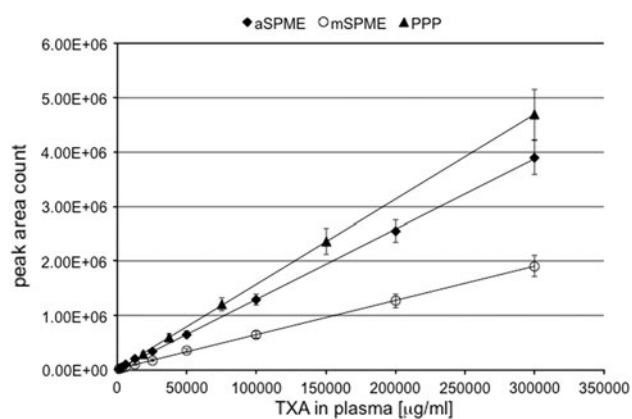
Demographics	
Age (yr)	64 (13)
Male sex	6 (60%)
Body mass index ( $\text{kg}\cdot\text{m}^{-2}$ )	28.1 (3.4)
Grade 1 LV	8 (80%)
Myocardial infarction < 30 days	0
Hypertension	3 (30%)
COPD	2 (20%)
Stroke/TIA	1 (10%)
Aspirin (within 5 days)	2 (20%)
Heparin/LMWH (within 5 days)	0
Clopidogrel (within 5 days)	0
Hemoglobin ( $\text{g}\cdot\text{L}^{-1}$ )	134.9 (13.7)
Platelets $\times 10^{-9}\cdot\text{L}^{-1}$	248.6 (45.1)
Creatinine ( $\mu\text{mol}\cdot\text{L}^{-1}$ )	74.2 (5.3)
Intraoperative variables	
ACB	3 (30%)
Single valve	6 (60%)
Complex/combination	1 (10%)
Redo	2 (20%)
CPB duration (min)	104.4 (41.5)
pRBC (units)	0.9 (1)
Platelets (units)	0.3 (0.6)
FFP (units)	0.6 (1.3)
Postoperative variables	
Hemoglobin (POD 1) ( $\text{g}\cdot\text{L}^{-1}$ )	101.2 (8.6)
Platelets (POD 1) $\times 10^{-9}\cdot\text{L}^{-1}$	157.9 (21.9)
Creatinine (POD 1) ( $\mu\text{mol}\cdot\text{L}^{-1}$ )	78.4 (15.5)
24-hr Chest tube losses (mL)	461.5 (84.1)
Hospital length of stay (days)	6.8 (2.4)

Results are the number of patients unless otherwise stated

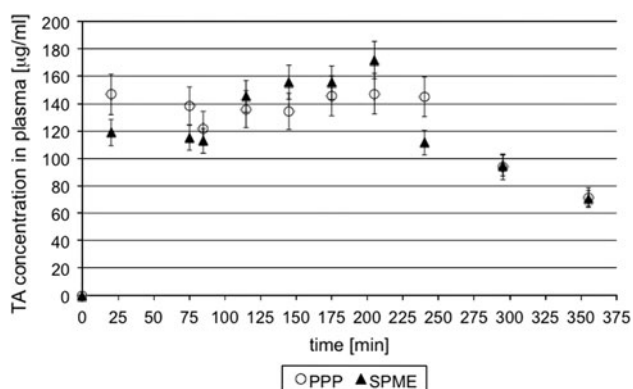
LV = left ventricle; COPD = chronic obstructive pulmonary disease; TIA = transient ischemic attack; LMWH = low molecular weight heparin; ACB = aortocoronary bypass; pRBC = packed red blood cells; FFP = fresh frozen plasma; POD = postoperative day

close the mean test results are to the known concentration. The mean test results should be within 15% of the actual concentration within the range of clinically relevant concentrations of the drug. The Food and Drug Administration (FDA) requires that the mean test result should be within 20% of the known concentration near the LLOQ. The SPME is well within these boundaries. Finally, the precision of SPME is determined by the similarity of the test results at a given concentration. To validate an analytical technique, the results need to be within 15% of each other within the range of clinically relevant concentrations of TXA and within 20% of each other near the LLOQ.<sup>A</sup> Once again, SPME is well within the FDA requirements for validation of an analytical technique. This validation of the SPME extraction technique for TXA results in adding another medication to the long list

<sup>A</sup> <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070107.pdf> (accessed 09/21/11).



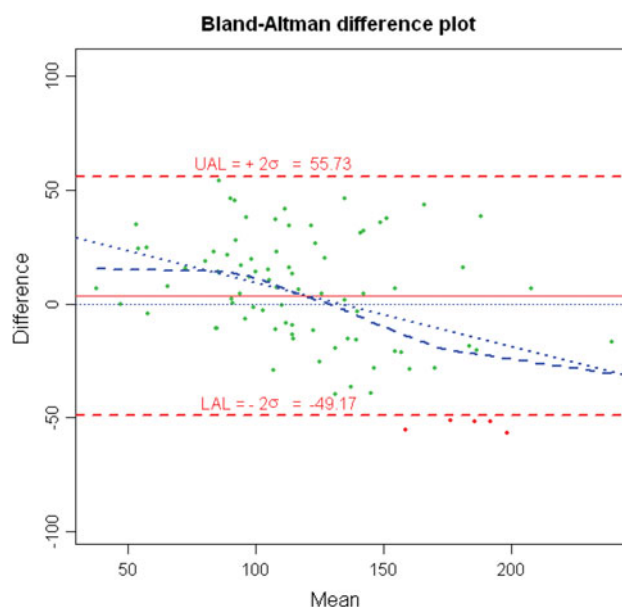
**Fig. 2** Calibration curves for tranexamic acid (TXA) in plasma obtained by the SPME and plasma protein precipitation (PPP) methods. mSPME = manual SPME with the use of fibres; aSPME = automated SPME 96-blade system



**Fig. 3** Pharmacokinetic profile of plasma TXA in patients undergoing cardiac surgery with cardiopulmonary bypass. These average values obtained from the data of ten patients were obtained with the automated SPME and PPP methods

of analytes that have been investigated previously.<sup>2,18–21</sup> It demonstrates potential of this analytical method to be applied for measurements of other agents of interest in clinical medicine.

We have taken the validation of SPME a step further by cross-validation with the traditional extraction technique of protein precipitation using a medication routinely used in cardiac surgery (TXA). The extraction efficiency of TXA from serum samples using SPME was much lower than that of PPP because SPME is a nonexhaustive extraction technique in which the fibre coating approaches equilibrium with the free concentration of the analyte in the sample. The SPME fibre extracts molecules with weights in the range of 70–3,000 Daltons.<sup>3,12</sup> This leaves behind many of the contaminating salts and large-molecular-weight substances in the original serum sample.<sup>22</sup> In contrast, PPP retains many of these contaminants in the final extract, which can interfere with the precision and accuracy of the



**Fig. 4** Tranexamic acid data were analyzed, and the two measurement methods (SPME and PPP) were compared using the Bland-Altman (BA) method. The difference between the two measurements (PPP – SPME) is plotted against their average. Three horizontal lines = the bias (mean of the difference) and the confidence interval

final measurement of the analyte. Also, the analyte is concentrated on the coating of the fibre and can be desorbed from the fibre with very small volumes of elutant.<sup>2,22</sup> The results of the TXA assays showed that the precision and accuracy of SPME are similar to those of the PPP technique within the studied range of concentrations.

The Bland-Altman analysis is used to examine the level of agreement between two measurement techniques.<sup>13,14</sup> In the case of TXA, this analysis assumes that the mean of the SPME and the PPP values represent the true concentration of TXA in the plasma. The Bland-Altman analysis illustrates the difference between the SPME and PPP values for all concentrations studied. With this analysis, if measured values are not identical or in close agreement, the difference is demonstrated on the plot. For TXA, the bias, or agreement, between the two methods was positive at low concentrations and negative at higher concentrations, but the bias remained small throughout the concentrations studied.

The use of the automated system presented in this study decreases the time of analysis to < 3 minute/sample when a bath of 96 samples was analyzed simultaneously. Therefore, we stress that the SPME fibre/device (in this case, blades) can also be used effectively in the automated mode.<sup>11,12,22</sup> For this purpose, the same CONCEPT system can be used, and fibres must be supported on a multifibre holder compatible with commercially available 96-deep-well plates. A special multifibre desorption device was also developed to improve storage conditions and increase the throughput of desorption for *in vivo* analysis.<sup>11,12,22</sup> This

simpler, faster technique can be used to facilitate further measurements and pharmacokinetic research studies in various patient populations prescribed TXA.

It is important to recognize that SPME measurements can be performed not only from plasma but from any body fluid (e.g., whole blood, urine, tissue). Moller *et al.* used SPME to extract samples from human hair.<sup>20</sup> The authors of that study detected and measured concentrations of codeine and morphine and their metabolites in patients at risk of substance abuse. This method has proven to be more sensitive than others previously reported in the literature. The authors concluded that use of SPME for opioid detection in human hair offers higher accuracy and fulfils the sensitivity and specificity required in forensic and toxicology studies.

Other investigators have used SPME to measure drug concentrations as well, demonstrating the potential application of this technique in the field of clinical medicine. Schubert *et al.* developed a model of an artificial venous system with constant flow and measured the concentration of linezolid, an oxazolidinone antibiotic.<sup>18</sup> Analysis of the drug concentration showed excellent linearity, and the amount of drug extracted from the blood did not change significantly with different flows in the system. Moreover, equilibrium was reached after five minutes, which indicates that performing SPME *in vivo* may be significantly faster than *ex vivo* because normal, pulsatile blood flow provides effective agitation, decreasing the time needed to reach equilibrium. On the other hand, the SPME fibres were prepared manually, and the authors observed large inter-fibre variability. In our study, we used commercially available C18 fibres and did not encounter the aforementioned problem.

Musteata *et al.* used an animal model of the metabolism of diazepam and its metabolites (nordiazepam, oxazepam). They introduced SPME fibres directly into a catheter in the carotid artery.<sup>21</sup> Their results correlated well with those of a standard analytical method using protein precipitation of blood samples obtained at preselected time points.

In the current study, we did not demonstrate the other advantages of SPME, which include simultaneous determination of total and free concentrations of the drug. This was because our analyte of interest, TXA, is characterized by negligible plasma binding (3%).<sup>4</sup> However, previous studies have shown that SPME can be used effectively for highly protein-bound drugs (e.g., benzodiazepines) with satisfactory accuracy and precision.<sup>12,18,19</sup> The SPME technique appears to have great potential for use in clinical medicine,<sup>22</sup> and there are numerous analytes to consider. Among the most important future applications of SPME are its ability to measure the concentration of multiple analytes with the use of a single fibre, the ability to absorb substances with short and ultra-short half-lives, and the

potential for *in vivo* use when SPME fibres can be inserted into an intravascular cannula.<sup>3,18,19</sup>

In summary, our results show that SPME can be used for relatively fast, precise measurements of plasma TXA concentrations. The SPME technique has several clinical advantages when compared to the currently used, traditional extraction techniques. Further improvements of the technique (e.g., shortening the extraction time) should allow it to be used for “bed-side” analyses. This study is a first step toward investigating potential applications of SPME to measure the pharmacokinetics of TXA. Studies are currently being conducted to investigate the pharmacokinetics of TXA during cardiac surgery, during liver transplantation, and in patients with renal dysfunction.

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**Competing interests** None declared.

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