SUPPLEMENTARY INFORMATION

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

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Supplemental Data

This file is comprised of the 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.

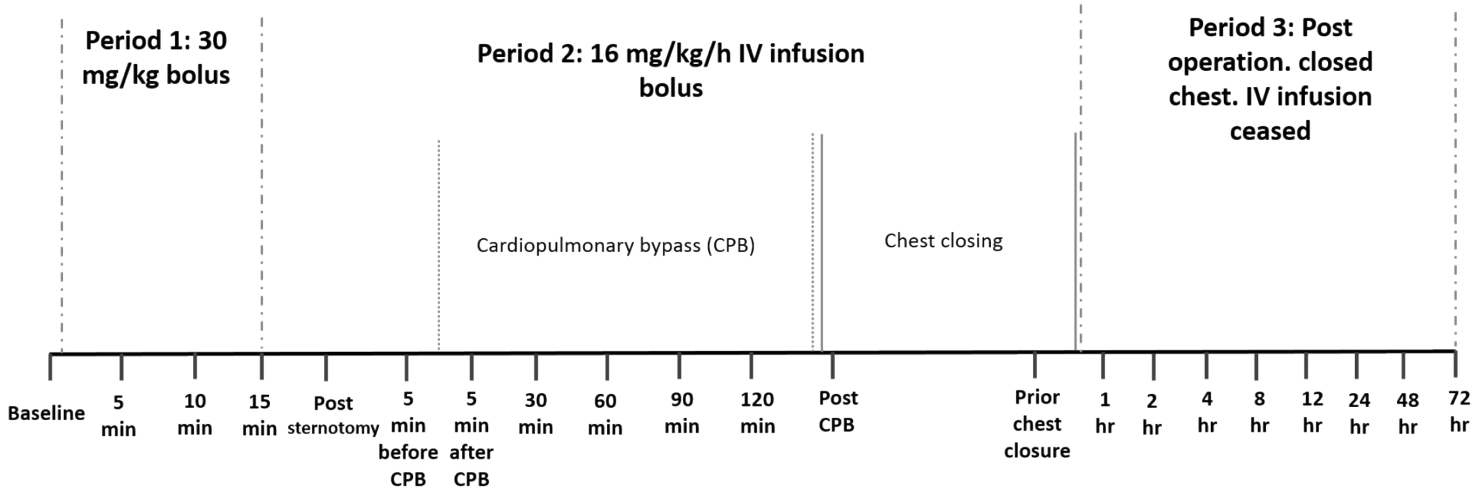

Figure S1: Patient blood samples were collected during surgery - which is segmented into 3 periods. First, baseline samples were collected prior to Period 1. Samples were then collected at 5 min and 10 min during Period 1 whereby a 30 mg/kg bolus dose of tranexamic acid is administered over 15 minutes. During period 2 of intravenous infusion of 16 mg/kg/hr, samples were collected post-sternotomy (after chest opening), 5 min before and after the start of cardiopulmonary bypass (CPB), at 30 minutes intervals during CPB for up to 4 sampling points, post CPB and prior to chest closure. During period 3, the post operation period where intravenous infusion was ceased, samples were finally collected at 1 hr, 2 hr, 4 hr, 8 hr, 12 hr, 24 hr, 48 hr and 72 hr.

1. Schedule of blood sample collection

2. Concept-96 for high throughput SPME workflow

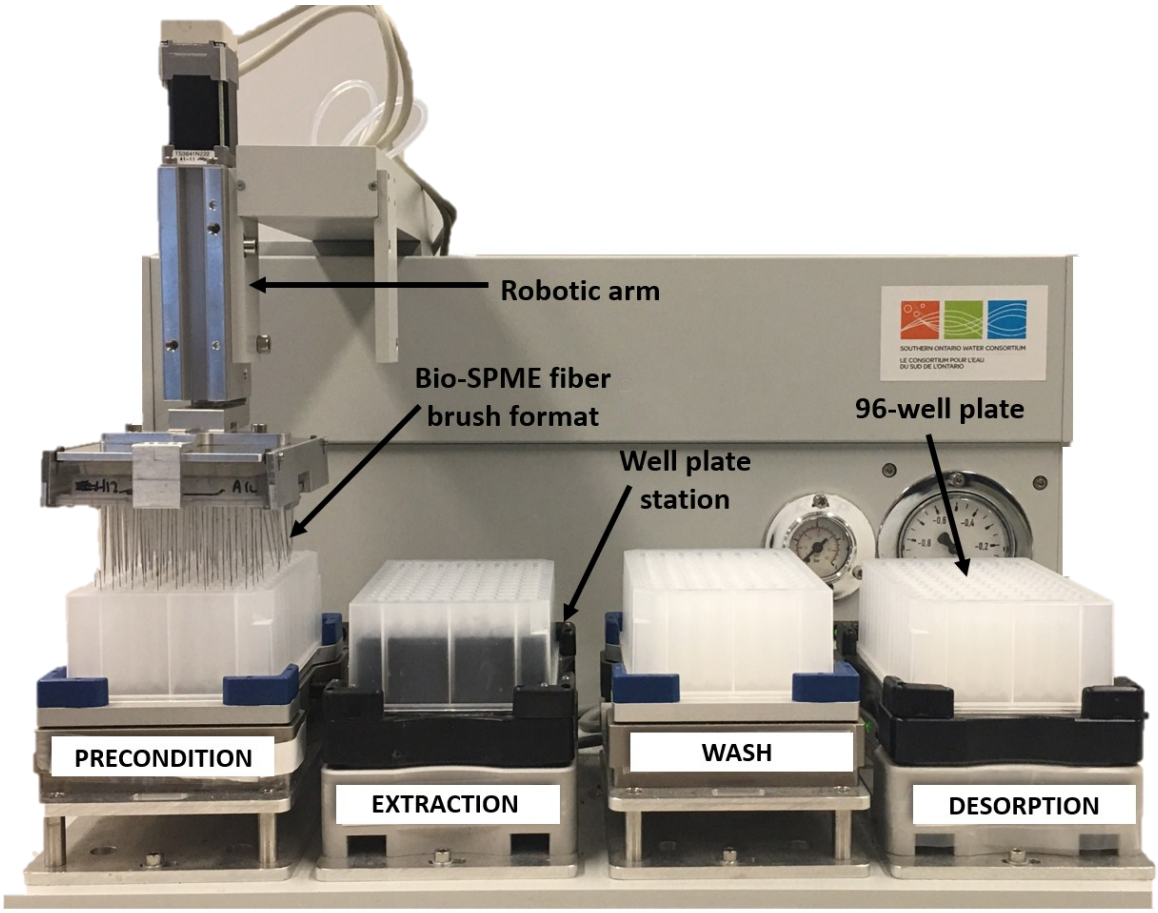

Figure S2: The Concept-96 is a software operated system that permits high throughput solid phase microextration (SPME) sample preparation of 96 samples simultaneously. It automatically performs each step of the SPME protocol – preconditioning, extraction, wash and desorption – with the help of a robotic arm that houses the brush format of the SPME configuration to be used (Bio-SPME fibers). This brush format is compatible with 96 well plates which are placed on their respective stations.

3. Calibration and quality control samples:

Stock solutions of tranexamic acid were prepared by dilution of 100 mg/mL initial stock in pure LC-MS grade water. The solutions ranged from 500 µg/mL to 100 000 µg/mL, and were stored at 4 ^{0}C in amber vials. A matrix-matched external calibration curve was prepared by adding 10 µL of the respective concentration of stock to 1 mL of human plasma. Samples were then submitted to low agitation for 45 minutes at room temperature so as to allow for equilibration to occur prior to the sample preparation step. The standard curve was validated at a working concentration of 100 µg/mL, which was prepared using the same dilution ratio of a separate stock solution of 10 000 µg/mL of TXA. Phosphate buffered saline was prepared by dissolving 1.44 g sodium phosphate, 0.2 g potassium phosphate, 8.0 g sodium chloride, and 0.2 g potassium chloride in 1 L LC-MS grade water, and adjusted to pH 7.4. The PBS was then spiked with 2 400 µg/mL of the internal standard cis-4-aminocyclohexanecarboxylic acid.

4. SPME-LC-MS/MS

High performance liquid chromatography was performed with an Accela autosampler and pump (San Jose, CA, USA). Mass spectrometric detection was carried out with a triple quadrupole mass spectrometer TSQ Vantage (Thermo Scientific, San Jose, USA). Chromatographic separation was performed on a Discovery HS F5-3 column (10 cm x 2.1 mm, 3 µm) with a corresponding PFP guard column (Supelco, Bellefonte, PA, USA). A previously developed method detailing the conditions of the chromatographic separation and detection of TXA via LC-MS/MS was described elsewhere in detail.(33) Mobile phase A was 100 % water, while mobile phase B was 100 % acetonitrile, each with 0.1% formic acid. The 8 minute gradient chromatographic method had a final flow rate of 300 µL/min, and was carried out as follows: 90 % A from 0-2 minutes, 40 % A from 2-5.5 minutes with a hold at 40% A to 6 minutes, ending with a 2 minute re-equilibration at 90% A. The ESI parameters were: positive ion mode; nitrogen gas set at GS1 = 25, GS2 = 25; collision gas (CAD) =10; curtain gas = 25; heated nebulizer temperature = 450 °C; and electrospray voltage = 5000 V.

Table S1. SRM transitions and optimized parameters such as collision energy (CE), start and stop time, and S-Lens for SPME-LC-MS/MS experiments conducted on the TSQ Vantage.

Compound	**Parent (m/z)**	**Product (m/z)**	**Collision energy (CE)**	**Start time**	**Stop time**	**S-Lens**
TXA	158.111	67.140	29	0	8.00	51
		95.150	15			
		123.100	9			
IS	144.098	79.120	30	0	8.00	52
		81.190	18			
		109.220	13			

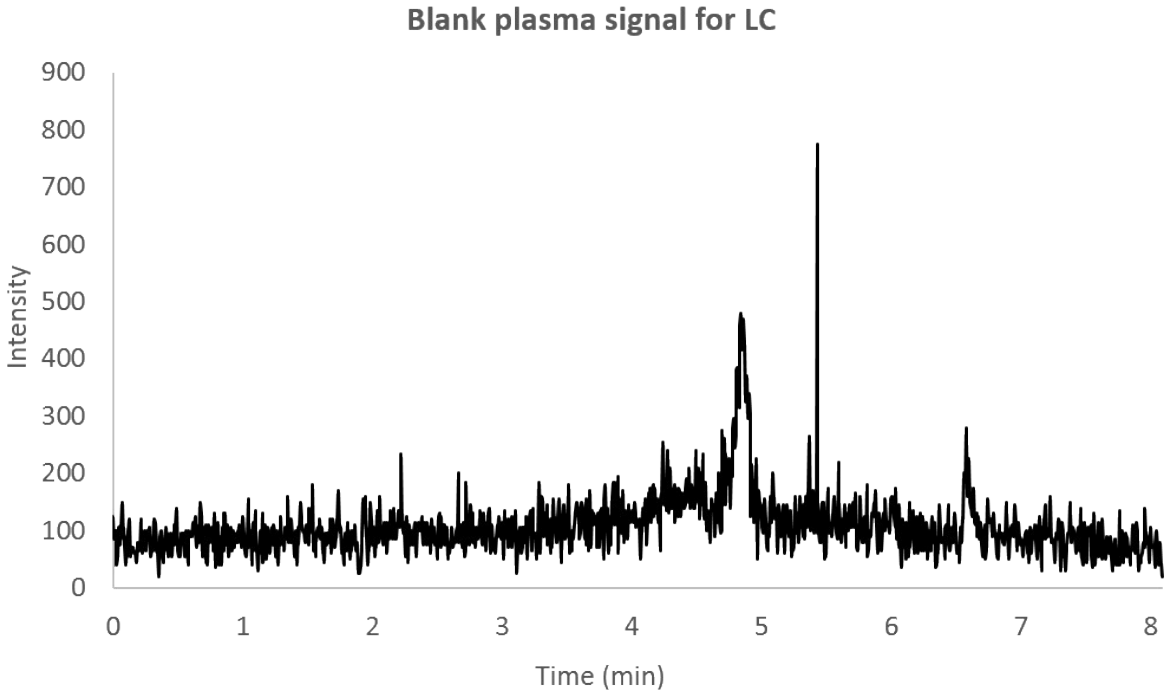

Figure S3: The signal for a blank plasma sample prepared with thin-film microextraction (TFME) and analyzed via liquid chromatography tandem mass spectrometry (LC-MS/MS).

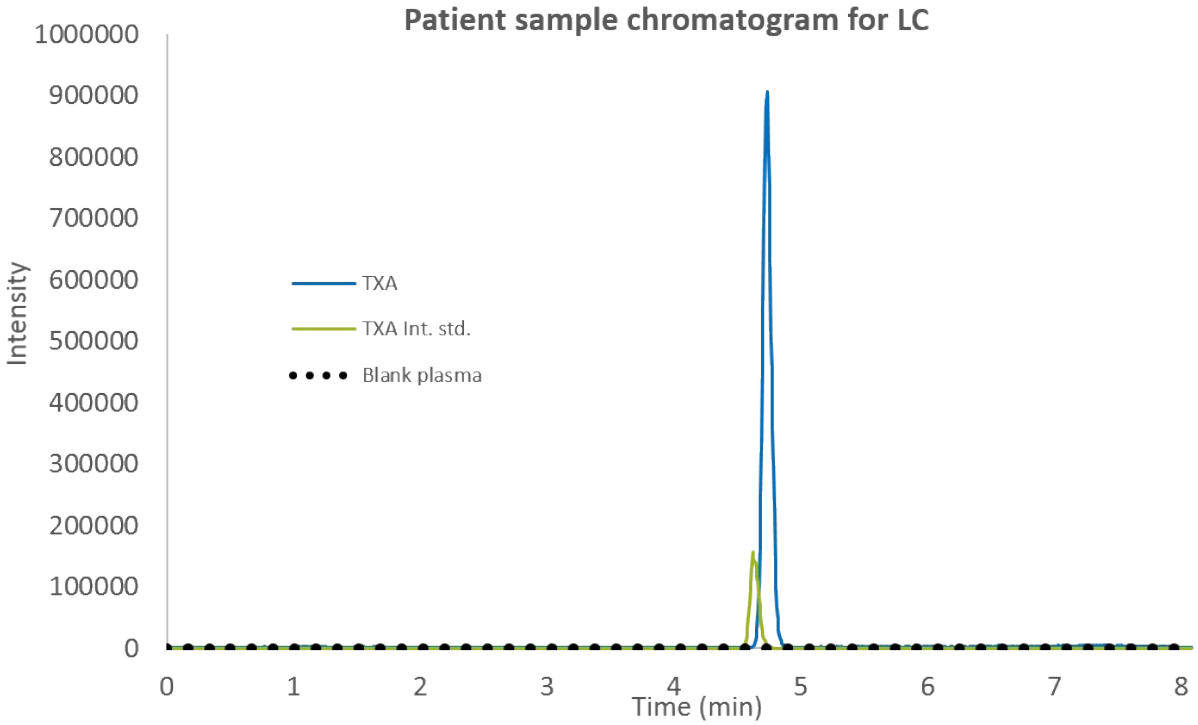

Figure S4: The signal from blank plasma superimposed with signal for a patient sample prepared with thin-film microextraction (TFME) and analyzed via liquid chromatography tandem mass spectrometry (LC-MS/MS).

5. SPME-MOI-MS/MS

Essentially, the MOI device consists of two sections. 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. 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. 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.(37) 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. The solvent employed in the MOI-MS/MS system was methanol with 0.1% v/v formic acid. The mass spectrometer employed for analyses was an API 4000 triple (Applied Biosystems, California, USA).

Table S2. SRM transitions and optimized parameters such as declustering potential (DP), entrance potential (EP), collision energy (CE) and exit potential (CXP) for MOI-MS/MS experiments conducted on the API 4000.

Compound	**Parent (m/z)**	**Fragment (m/z)**	**DP (V)**	**EP (V)**	**CE (V)**	**CXP (V)**
TXA	158	95	60.7	6.8	20.6	6.2
IS	144	81	53.4	7	28.2	4.9

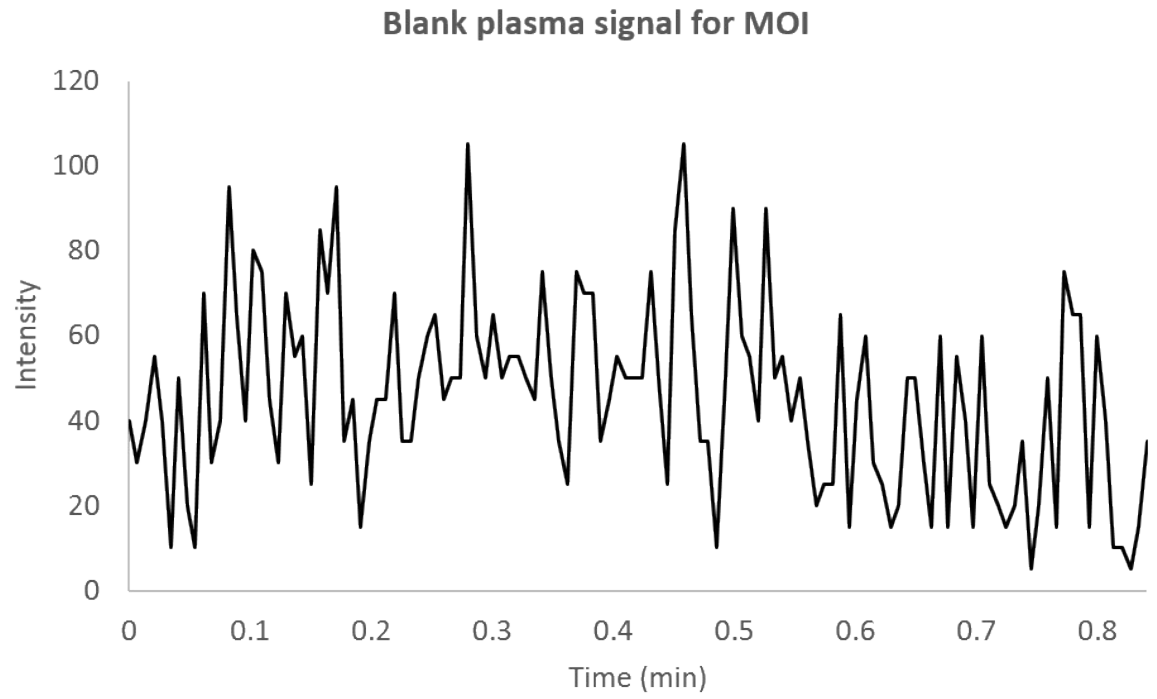

Figure S5: The signal for a blank plasma sample prepared with biocompatible solid phase microextraction (Bio-SPME) fibers and analyzed via microfluidic open interface tandem mass spectrometry (MOI-MS/MS)

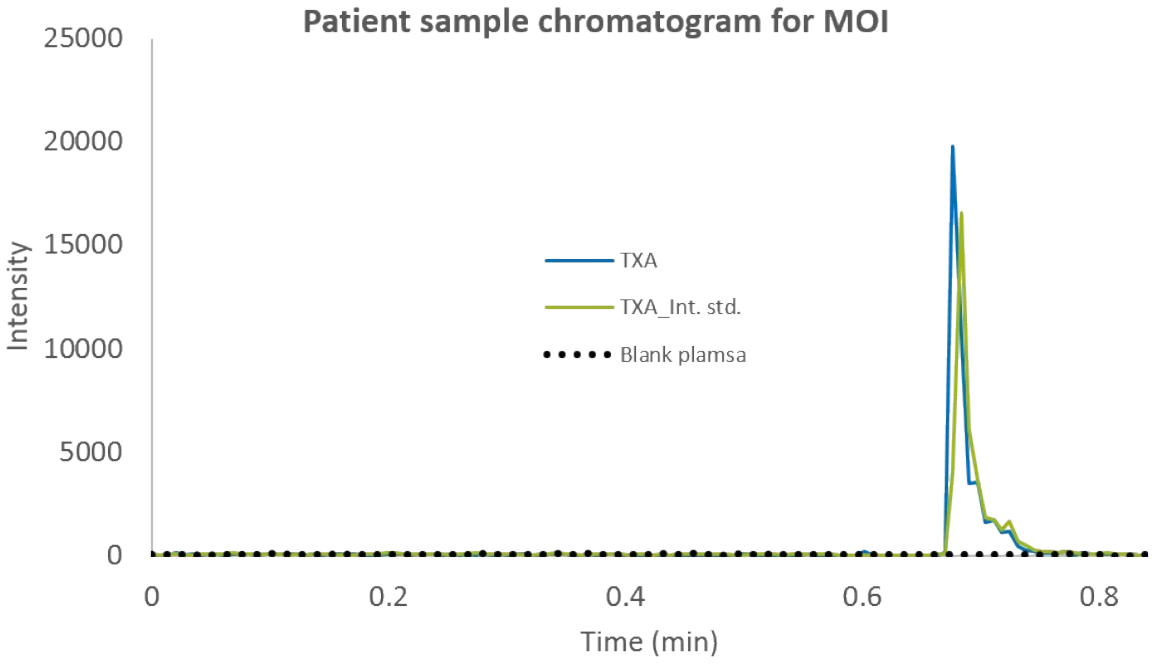

Figure S6: The signal for a blank plasma sample superimposed with a signal from patient sample prepared using biocompatible solid phase microextraction (Bio-SPME) fibers and analyzed via microfluidic open interface tandem mass spectrometry (MOI-MS/MS)

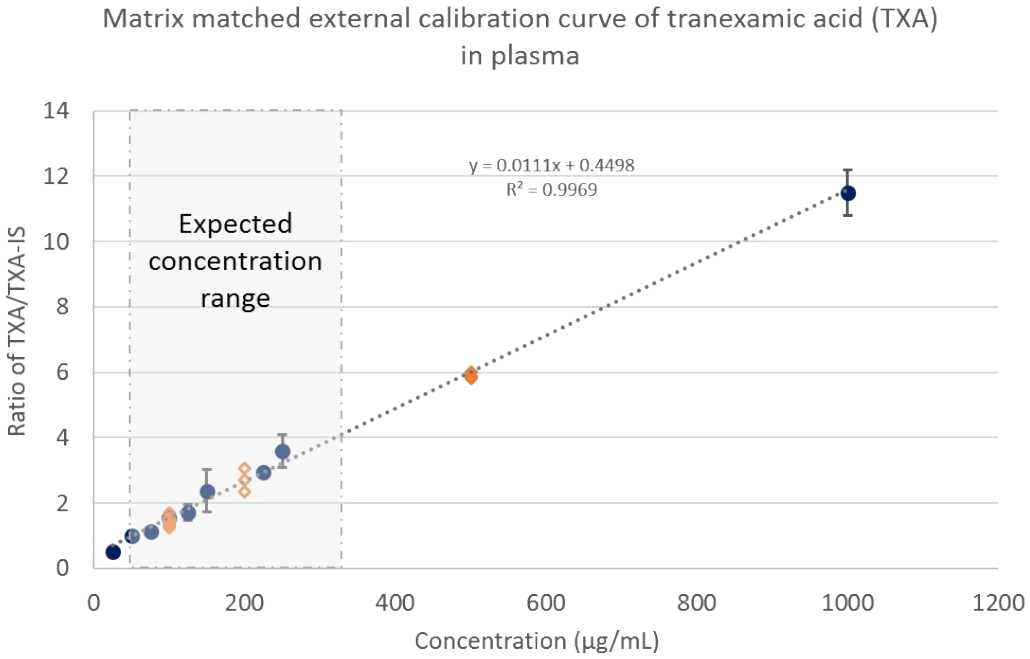

Figure S7: The matrix matched external calibration curve of tranexamic acid in plasma produced a coefficient of determination of 0.997 and a linear dynamic range from 25 µg/mL – 1000 µg/mL and an LOQ of 25 µg/mL. The expected concentration range of TXA in the plasma samples is represented by the transparent grey box that covers a concentration range from 50 µg/mL – 350 µg/mL. Quality control samples represented by orange diamonds on the calibration curve were tested at a concentration of 100 µg/mL, 200 µg/mL and 500 µg/mL found with an intra-day accuracy of 91% (n=5), 98% (n=3) and 97% (n=3) respectively and a precision of 20%, 16% and 3% respectively.

6. Bio-SPME-MOI-MS/MS as a tool for rapid quantitative analysis