APPLICATION OF AUTOMATED 96-THIN FILM SPME FOR DETERMINATION OF TRANEXAMIC ACID IN CARDIAC SURGICAL PATIENT OPERATED WITH USE OF CARDIOPULMONARY BYPASS

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Abstract

Tranexamic acid (TA) is a lysine analog used as antifibrinolytic agent in cardiac surgery to minimize bleeding and reduce the need for transfusion. It was confirmed that concentration of TA significantly changes in patients placed onto cardiopulmonary bypass secondary to an increased central volume of distribution and reduced elimination from the central compartment. The objectives of the studies were i) to assess the accuracy of measuring blood tranexamic acid concentrations with thin film microextraction (TFME) versus standard sample preparation and ii) to measure in vivo blood concentrations of tranexamic acid when using dosage protocol previously developed and to verify whether a therapeutic concentration is achieved and sustained. Ten patients have been selected to do the study. Drug concentration was monitored in the specified time intervals before, during and after the surgery. Liquid chromatography (hilic method) coupled with tandem mass spectrometry was used for analysis. Sample preparation step was performed with the use of automated TFME system using 96-well plate format and thin film device. The main advantage of automated TFME system over traditional methods used in biomedical analysis is that TFME offers very good clean-up and high throughput. TFME data was compared with the results obtained with the use of standard methods – protein precipitation and ultrafiltration. Bland-Altman statistical analysis showed a good correlation between the used methods. Our TFME results also confirmed the previously calculated mathematical model of TA dosing.

Introduction

Up to 1.25 million patients undergo cardiac surgery every year worldwide. Ten to twenty percent of patients who have undergone surgery using cardio-pulmonary bypass (CPB) exhibit excessive post-operative bleeding with 5% requiring immediate re-exploration in order to achieve haemostasis. Tranexamic acid (TA) has become the forefront antifibrinolytic agent. However, controversy still surrounds the optimal dosing regime to achieve and sustain a therapeutic blood concentration. In the current studies we determined TA concentration in plasma samples of patients undergoing cardiac surgery with the use of CPB. Tranexamic acid was administered according to previously proposed scheme.

Therapeutic drug monitoring is very challenging due to the complexity of samples and low concentration of the analytes. The most commonly technique to measure drug concentration in blood because of its excellent selectivity and sensitivity is liquid chromatography – tandem mass spectrometry (LC-MS/MS). However, this technique requires extensive sample preparation prior to analysis to remove potential interferences when dealing with complex biological fluids such as blood or plasma. Standard sample preparation methods i.e. plasma protein precipitation and solid phase extraction may provide poor sample clean-up which leads to matrix effects which affect assay accuracy and precision as well as decrease the analytical column lifetime.

Solid Phase Microextraction (SPME) available in different formats, among others thin film geometry (TFME), is a simple, fast and sensitive technique where the amount of analyte extracted is proportional to free fraction of ligand. SPME combines sampling and sample preparation step. Coated fibre format and non-exhaustive nature of SPME allow the use of small sample volume for analysis, and minimizes solvent consumption. Due to the non-exhaustive nature of SPME only small percentage of the analyte and interferences is extracted, so SPME provides very good sample clean-up and eliminates or significantly reduces matrix effects. SPME does not require sample pretreatment and is applicable to complex biofluids even whole blood, in contrast to methods such solid-phase extraction.

For clinical laboratories, hundreds of samples must be analysed with the use of the simplest, cheapest and fastest methods, thus high-throughput automation is required. One of the reasons for limited use of SPME in clinical practice to date, was the lack of suitable automation, but this was recently addresses through the commercial availability of a fully automated robotic CONCEPT 96 system. This software-operated platform consists of four stations for preconditioning, extraction, wash and desorption steps and automates all steps of SPME according to application requirements. The system was further refined to employ thin-film geometry applied in 96-blade system (thin film microextraction, TFME) which increases the extraction efficiency due to the larger coating surface. This is an advantage when trace analytes or compounds at low affinity are of interest.

In this study, tranexamic acid plasma concentration of patients undergoing cardiopulmonary bypass surgery was measured by SPME/TFME coupled with HILIC LC-MS/MS platform. The usefulness of both, manual SPME with the use of commercial fibres as well as automated 96-thin film system for drug monitoring in clinical practice was reported. The results were further compared with data obtained by conventional methods: protein precipitation and ultrafiltration. This is the first large scale clinical study of the suitability of SPME in combination with LC-MS/MS for the analysis of clinical samples.

Sample collection procedure

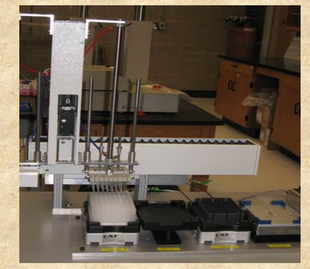
After induction of anesthesia, TA were administered intravenously by a 30 mg/kg bolus infused over 15 minutes using an infusion pump followed by an infusion of 16 mg/kg/hr until closure of the sternotomy with a 2 mg/kg load within the pump prime.

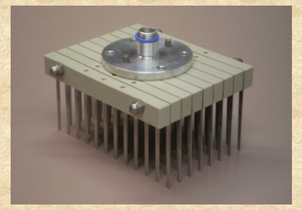
Blood samples were taken at baseline and then 5 mins after the bolus. Once starting the infusion, samples were taken immediately before and after commencing bypass followed thereafter at 30 minute intervals whilst on cardiopulmonary bypass. On discontinuation of the infusion, samples were taken at 5, 60 and 120 minutes.

Blood samples were collected into standard citrate collecting tubes and tubes were inverted a minimum of 5 times to ensure proper mixing with anti-coagulant. Samples were randomly assigned a number and thus blinded to the analysing laboratory. The standard citrate tubes were stored on ice and then centrifuged at 2000g for 15 mins at 4 deg.C with the subsequent supernatant frozen and stored at -70 deg.C until analysed.

The plasma samples were divided into two portions: (1) one portion were subjected to SPME/TFME (2) second portion were subjected to standard sample preparation technique.

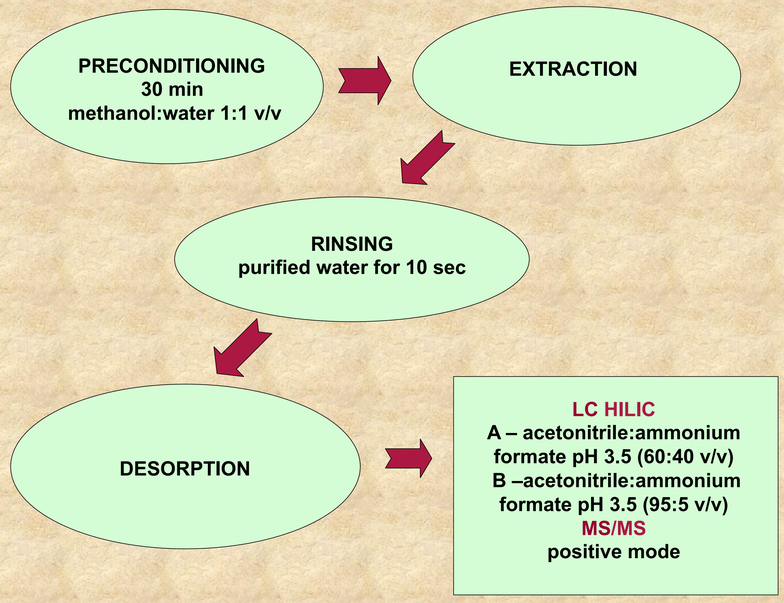
TFME method development



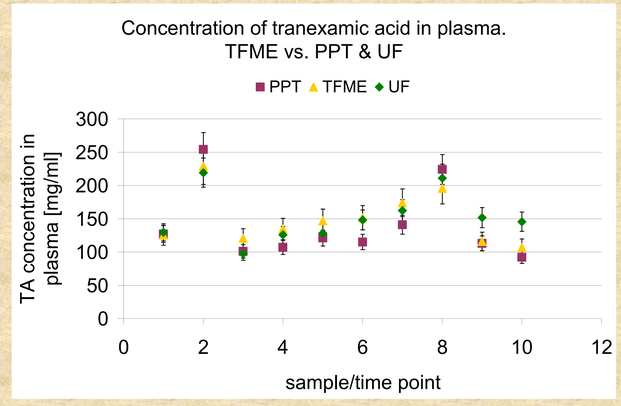


Coating	PAN-C18
Sample volume	1 ml
Plasma dilution with PBS pH7.4	1:3
Extraction time	120 min at 1000 rpm (equilibrium)
Desorption time	120 min at 1200 rpm
Desorption solvent	4:1 v/v acetonitrile/water with 0.1% v/v formic acid
Desorption solvent dilution	1:10
Desorption solution volume	1.5 ml
Evaporation/reconstitution	No

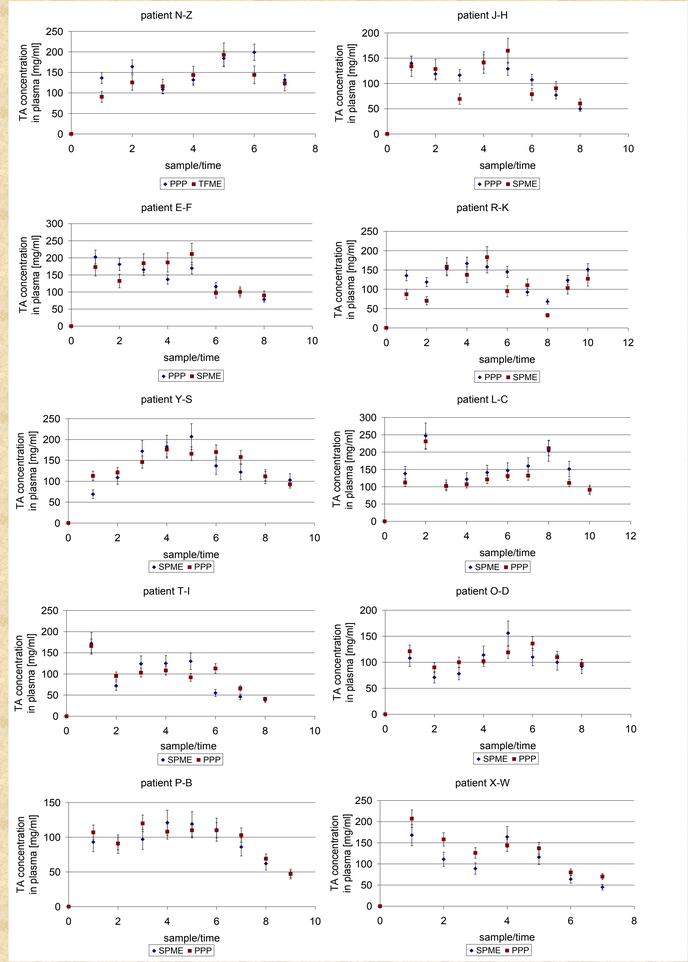
Experimental workflow



Validation of SPME Method with protein precipitaion and ultrafiltration



Excellent agreement between TFME, protein precipitation and ultrafiltration



Bland-Altman analysis performed on the basis of all clinical data showed that the mean difference (bias) ± standard deviation between TFME and PPP methods was 7.02 ± 25.86 and limits of agreement at the 95% rate of 57.7 to -43.68. Linear regression identified the relationship between TFME and PPT with a correlation coefficient of 0.82.

CONCLUSIONS

TFME is a good alternative for standard sample preparation techniques in clinical practice. The application of CONCEPT 96 robotic system allows to save researcher's time, especially when a great number of samples needs to be analysed. Moreover, reusability of the coating allows to decrease total cost of analysis. The biocompatibility of the extraction phase allows for direct extraction from complex biological matrices with no sample pretreatment.