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A New Tool for the Automated Sample Preparation of Whole Blood Samples by LC-MS using a Commercial Autosampler

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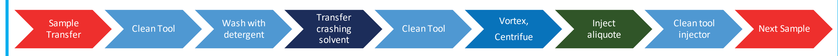
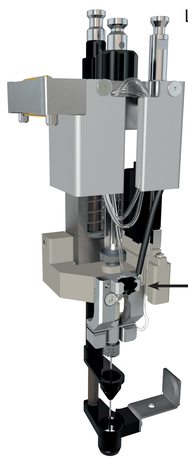
Introduction

Automated sample preparation reduces the costs per sample and minimizes sample handling errors. The use of robots is well established for therapeutic drug monitoring or diagnostics based on blood samples. Usually expensive and highly specialized pipetting robots are used. However, most of these systems are not designed with a direct interface for LC-MS applications. In addition common pipetting systems are not optimized for smaller scale sample series.

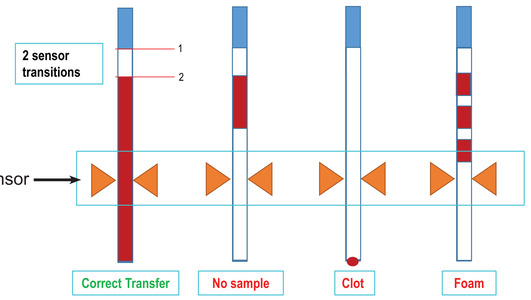
Here we present a new tool for liquid handling of whole blood samples and direct sample injection. The tool features an optical sensor that monitors all liquid handling steps. It detects presence or absence of sample, standards and reagents. The sensor is essential to ensure process safety for automated liquid handling steps.



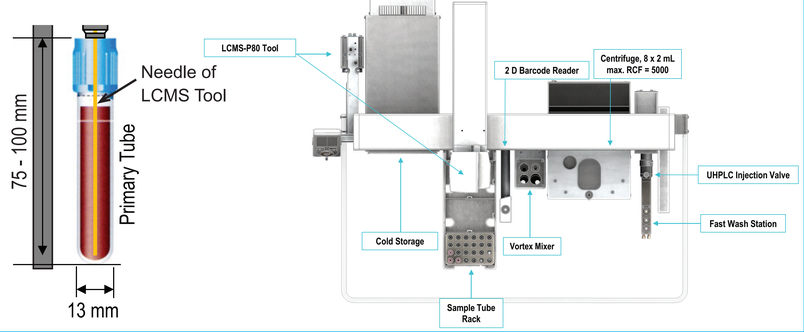
The PAL RTC (instrument view below) can perform additional sample prep steps, e.g. standard and reagent addition, centifugation, liquid / liquid extraction (LLE). The LCMS-P 80 Tool features a special needle that allows direct sampling from primary tubes. With special adapters it can transport primary tubes to and from modules on the system, e.g. for vortexing. Different adapters allow the use of different formats of primary tubes.



LCMS-P 80 Tool



The optical sensor positioned on the sample loop (orange triangles in the sketch above) of the tool moitores the aspiration of sample, air gap or bubbles. Different sampling situations (injection with air gaps, clogging, bubbles) generate distinct patterns of the sensor signal triggering defined actions of the system.



Materials and Methods

PAL RTC autosampler with a 1 mL syringe, LC MS P80 injection tool, vortex mixer, centrifuge.

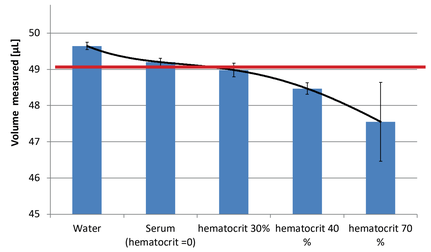
Agilent LC 1200, Agilent MS 6460 TripleQ-MS

Tested laboratory blood was supplied by blood donation in primary containers (Vacuette™, Vacutainer™, etc.). All chemicals wer purchased from Sigma-Aldrich.

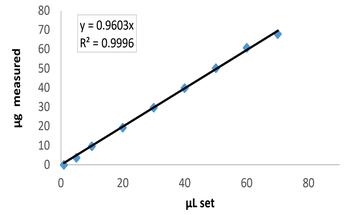
Hematocrit levels were prepared by mixing defined ratios of plasma and erythrocytes.

Results - Pipeting blood

Whole blood is a difficult liquid class of highly variable viscosity (defined by hematocrit value). To pipet blood reproducibly and accurately adjustment factors are needed. However, in the typical hematocrit range (30 - 50%) an average correction is sufficient to correct for that variability.



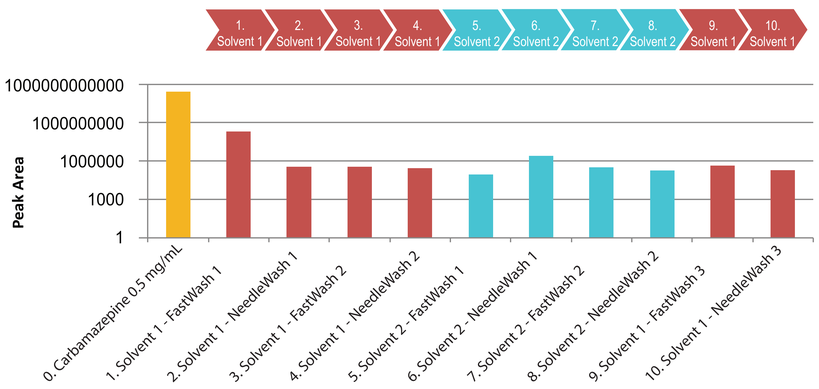
Blood of different hematocrit levels was pipetted and weighed. The volume was determined using the experimentally determined density of the used blood. The red line corresponds to the nominal volume to be pipetted (50 µL). Each bar represents n=9 measurements.



The tool pipettes 1 to 70 µL of blood with good precision (<5% CV, @ 40% hematocrit). The volume was determined gravimetrically using the experimental determined density of the used blood.

Automated Sample Prepraration Task and cleanining Procedure

Whole blood consists of water, salt, proteins, and intact erythrocytes, which tend to coagulate on surfaces or in contact with organic solvents. Therefore, it is important to wash properly to avoid precipitation in the system as well as the deposition of biofilms. Different wash steps applying two different solvents were applied to reduce potential carryover. However, an additional step using a protein removal detergent (Decon™ Contrad™ 70) was used to remove remaining proteins.



The effect of the individual wash steps was followed with carbamazepine as marker. Each wash solution was collected and analyzed separatly after a transfer of 10 µL of a carbamazepinesolution of 0.5 mg/mL. The first wash by solvent 1 (H2O, 0.95 g/L NaN3) was defined as 100% peak area. Solvent 2 (H2O/MeOH/ACN/2-Prop 1:1:1:1, step 6.) wash reduces carryover further.

Results - Examples for Therapeutic Drug Monitoring (TDM)

The LCMS-P 80 Tool was used to prepare samples for several typical drugs for TDM as well as compounds relevant in diagnostics. Spiked and blank whole blood samples were analyzed.

Carbamazepine

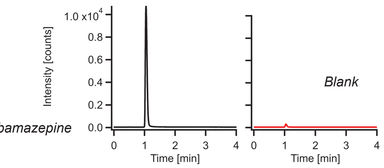
Carbamazepine is a representative of an anti-epileptic drug and very important to monitor in blood to set a proper and individual dosage. [1]

Method: Eluent A: Water 0.1% Formic acid / Eluent B: Methanol 0.1% Formic acid // *Flow*: 0.45 ml/min // *Runtime*: 6 min

Column: Waters Coretecs C18 2.1x100 mm 2.7 µm

MS Parameter: Mode: positive MRM: 237>194

Blood Volume: 10 µL / Injection volume: 2 µL



10 µg/mL carbamazepine

Diclophenac

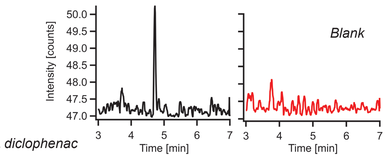
Diclophenac is a nonsteroidal anti-inflammatory drug and a representative compound for drug monitoring acording its molecular properties [2]

Method: Eluent A: Water 2 mM NaF / Eluent B: Acetonitril 2 mM NaF // *Flow*: 0.5 ml/min // *Runtime*: 7 min

Column: Zorbax SB-C8 4.6x50 mm 1.8 µm

MS Parameter: Mode: positive MRM: 296>249

Blood Volume: 10 µL / Injection volume: 2 µL



233 ng/mL diclophenac

25 Hydroxyvitamin D3

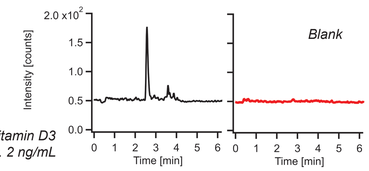
25 hydroxy vitamn D3 is the important maker for the storage depot of vitamin D [3]

Method: Eluent A: Water 0.1% Formic acid / Eluent B: Methanol 0.1% Formic acid // *Flow*: 0.45 ml/min // *Runtime*: 6 min

Column: Waters Coretecs C18 2.1x100 mm 2.7 µm

MS Parameter: Mode: positive MRM: 401>383

Blood Volume: 10 µL / Injection volume: 20 µL



80 ng/mL 25 hydroxy vitamin D3
LOD of the method approx. 2 ng/mL

Conclusions

- The new LCMS-P 80 Tool is capable of pipetting whole blood with normal hematocrit levels (30-50%) directly from primary tubes with good accuracy and precision, applying one average correction factor. For high hematocrit levels a different correction factor should be applied.
- Wash procedures were established to minimize carryover of analytes. A procedure to minimize the deposition of blood on system surfaces proofed successful.
- The system consisting of a commercial PAL RTC autosampler, connected to an Agilent LC-MS system can perform sample prep and LC-MS analysis for whole blood samples from primary tube. The performance of the system was tested with several typical compounds in therapeutically relevant concentrations.
- Future work is aimed at evaluating the capability of the sensor to reliably detect certain error conditions.

References
[1] Linder et al.; Bioanalysis (2015) 1(16) 2013-2039
[2] Kearney et al.; BMJ. (2006) 332(7553):1302-8
[3] Shah et al.; Nutrition Journal (2011) 10:4

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