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VOLUMETRIC ABSORPTIVE MICROSAMPLING AS AN ALTERNATIVE SAMPLING STRATEGY FOR CEREBROSPINAL FLUID

Lisa Delahaye / 11th EBF Open Symposium / November 2018

Based on: Delahaye et al. *Volumetric absorptive microsampling as an alternative sampling strategy for the determination of paracetamol in blood and cerebrospinal fluid*. Anal Bioanal Chem (2018).

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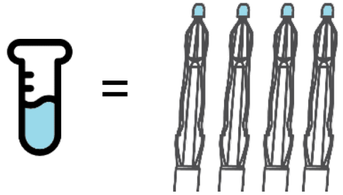
CSF MICROSAMPLING WITH VAMS

- Low sampling volume

  ! Especially interesting in pediatric patient population

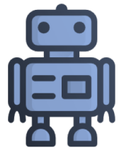


- No need for micropipette to obtain volumetric samples

  → Allows minimal sample handling, even in hospital setting

= fixed volume

- Easy sample storage and transportation
- Possibly automated workflow in the future



- Same workflow for blood and CSF samples

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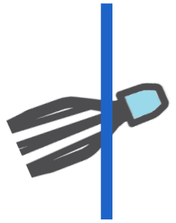
QUANTIFICATION OF PARACETAMOL

Preparing the VAMS
+
Dry for 2 hours



Remove VAMS tip

Extraction solvent:
300 µL 80/20 MeOH/H_2O
+ IS* + 0.01% FA

* IS = Paracetamol -D4

Thermomixer:
10 min; 22 °C; 1400 rpm
Centrifugation:
10 min; 10 000 g

250 µL supernatant
+ 750 µL H_2O (0.01% FA)

LC-MS/MS

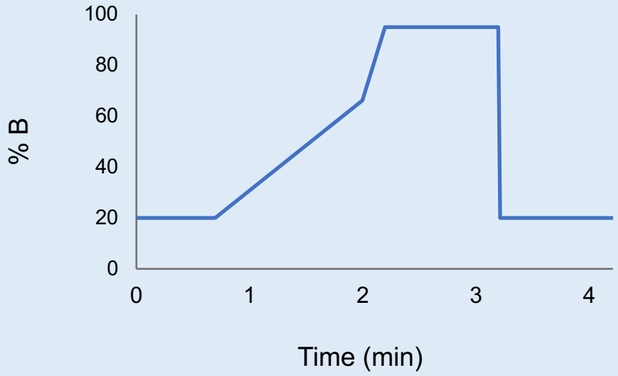
Instrument set-up and parameters:

LC: Waters UPLC®

Column: ACQUITY UPLC® HSS T3 1.8 µm (2.1 x 100 mm) – 45 °C

MP A: 95/5 H_2O/MeOH + 0.01% FA

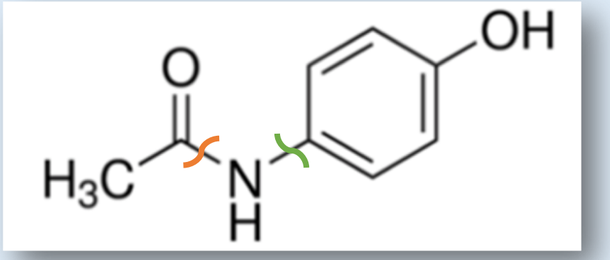
MP B: 95/5 MeOH/H_2O + 0.01% FA



MS: SCIEX API 4000

+ MRM

- Quantifier ion: 152.04 → 110.00 m/z
- Qualifier ion: 152.04 → 93.00 m/z



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VALIDATION

- No blank CSF available for generation of calibrators and QCs
  → Alternative matrix needed: can *water* be used?

- Calibration line with spiked water (= alternative matrix) → generate VAMS samples
- Real-life liquid CSF patient samples (6) → generate VAMS samples
- Dilute same CSF patient samples (6) 1/10 with water (6) → generate VAMS samples

Concentration undiluted samples ✓ = Concentration diluted samples (after recalculation)

Average difference = 3.33 %
%RSD of difference = 7.71%

Demonstrates equality of process efficiency between two matrices

VALIDATION

- No blank CSF available for generation of calibrators and QCs
  → Evaluation of selectivity? Evaluation of matrix effect?

– Preparation of artificial CSF
– Comparison of transition ratio's of calibrators/QCs (*n*=16) vs. patient samples (*n*=55)
– Evaluation of IS area in calibrators/QCs vs. patient samples

No interfering peaks detected in artificial CSF
Transition ratios samples within ±4% of the ratio in calibrators/QCs
→ No issues with interferences for this assay

IS had an overall an %RSD of 7.3% in calibrators, QCs and patients samples
→ No significant ion suppression/enhancement in patient samples

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VALIDATION

Calibration:

Range: 100 ng/mL to 40 µg/mL

Calibration model: linear model with $1/x^2$ weighting

Selectivity:

No interferences detected

No carry over detected

Meets acceptance criteria ✓

Accuracy and precision:

QC Level	Accuracy (%bias)	Intra-day precision (%RSD)	Total precision (%RSD)
LLOQ	16.8	6.9	6.9
Low	2.5	5.3	5.3
Medium	6.0	4.5	4.5
High	-0.43	3.9	7.6

Meets acceptance criteria ✓

Dilution integrity:

Dilution factor	Accuracy (% bias)	Precision (%RSD)
2	1.0	5.4
5	7.5	5.9

Meets acceptance criteria ✓

Recovery:

QC level	Recovery*	%RSD
Low	70 %	9
High	78 %	3

* ANOVA did not reveal any statistically significant difference between the groups

Meets acceptance criteria ✓

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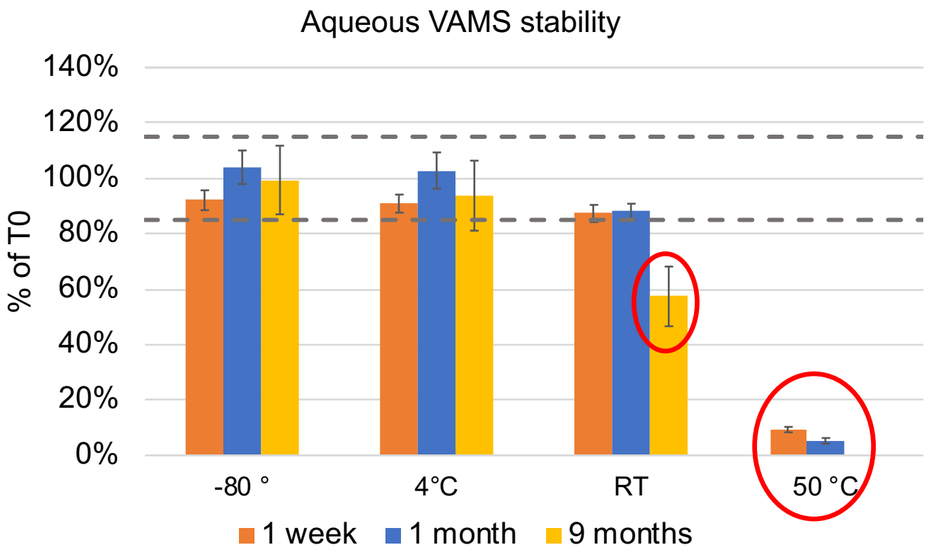
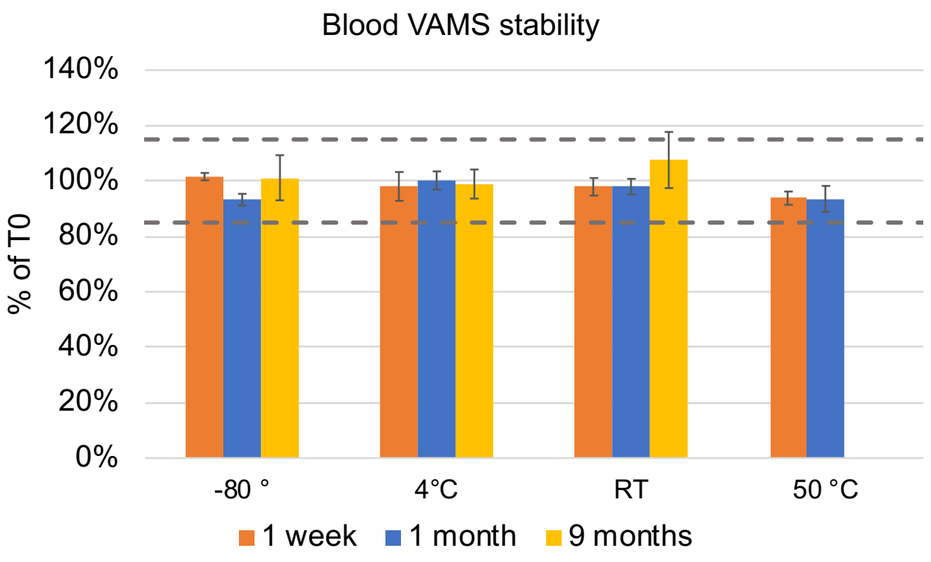
VALIDATION - STABILITY





- Aqueous VAMS samples are stable for at least **9 months** when stored at **-80°C and 4°C** ✓
- Aqueous VAMS samples are stable for at least **1 month** when stored at **RT** ✓
- Aqueous VAMS samples stored at 50°C and for more than 1 month at RT show *decreased concentrations* ✗

? Stability data for **blood** VAMS samples extracted following the same protocol are **within the acceptance** criteria for all tested conditions
→ no problem with chemical stability of paracetamol

Apparent stability issue is most likely **recovery related**
→ Cause of decreased extractability?

Extractability-mediated recovery bias can be matrix dependent!

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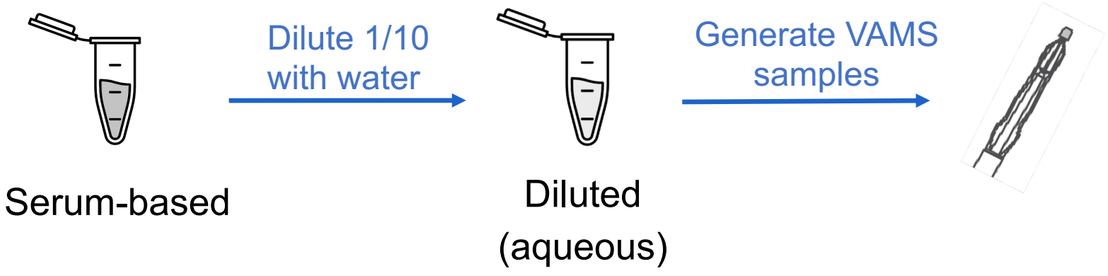
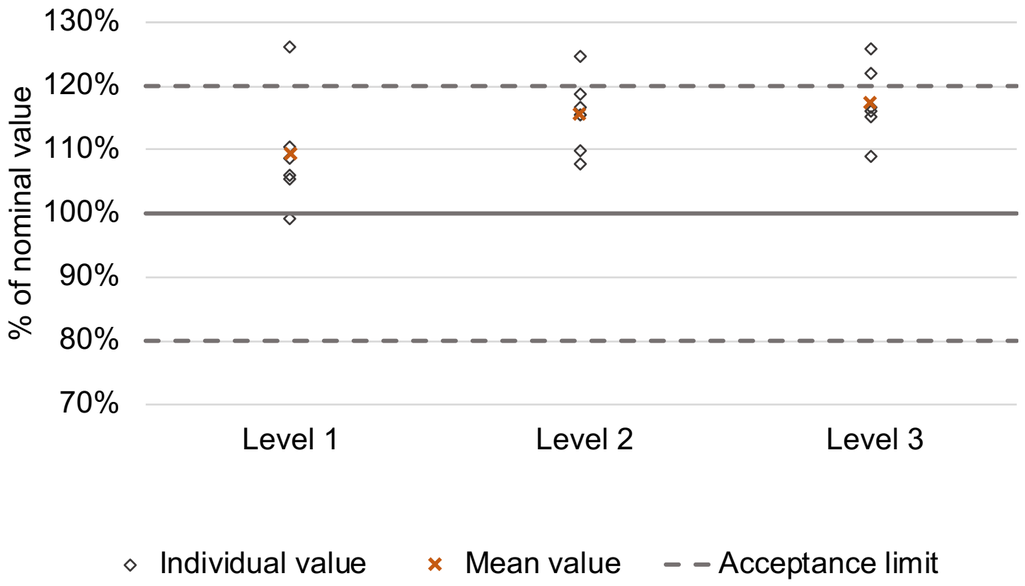
ANALYSIS OF EXTERNAL QUALITY CONTROL MATERIAL

No CSF-based QC material available

→ Start from serum-based QC material





- 14/18 individual measurements are within the acceptance limits
- Mean values per QC level are within the acceptance limits
- Concentrations of the QCs are within the therapeutically relevant concentrations

  → Supports validity of the method

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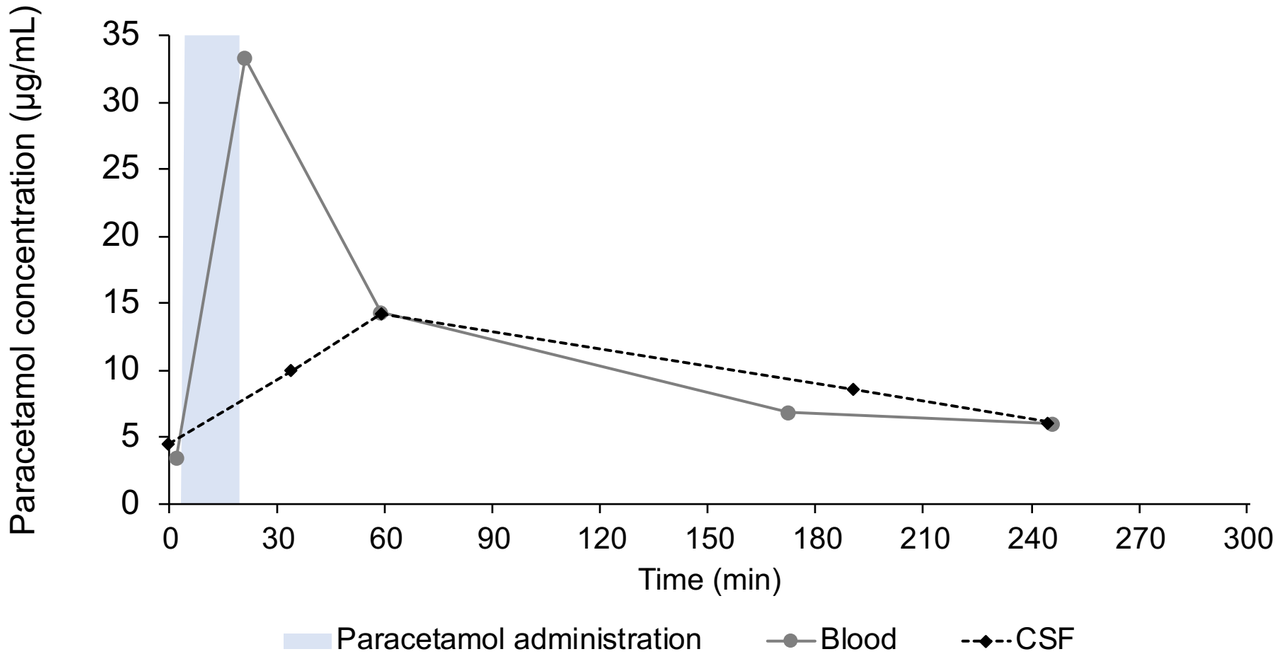
ANALYSIS OF PATIENT SAMPLES

– Venous blood and CSF VAMS samples collected from pediatric patients treated with IV paracetamol



- Concentrations of patient samples were within the calibration range of the method
- No unforeseen issues encountered with the analysis of patient samples

! Visual inspection of CSF VAMS samples = difficult → Need for good sample quality!

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CONCLUSION

- Development of methods for the quantitative determination of **paracetamol** in **blood and CSF using VAMS**
- **Successful validation** of these methods based on international guidelines
- The methods were successfully applied on **external QC material** and **authentic patient samples**
- An extractability-mediated recovery bias can be **matrix dependent**

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