Development, validation and application of a GC-MS method for quantification of glucose and lactate in vitreous humor

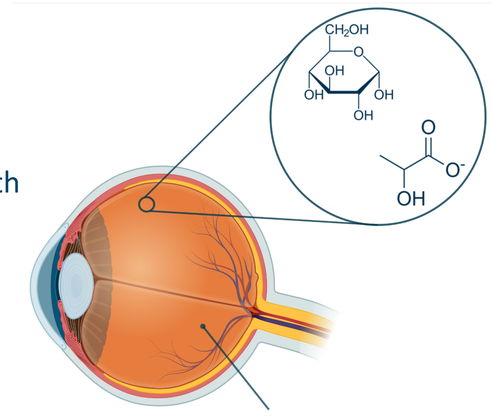
Celine Gys[1], Karolien Van Puyvelde[1], Sofie Abrahams[1], Eleanor Berry[1], Alexander van Nuijs[1], Adrian Covaci[1]

[1]Toxicological Centre, University of Antwerp, Antwerp, Belgium

Introduction

Glucose & lactate measurement

- Routine forensic toxicological screening
- Vitreous humor
  - Assessment of hypo-, normo- or hyperglycaemic status before death
- No in-house chromatographic method available
  - Diluted vitreous humor samples analysed by external laboratory
  - Spectrophotometric method not validated for vitreous humor



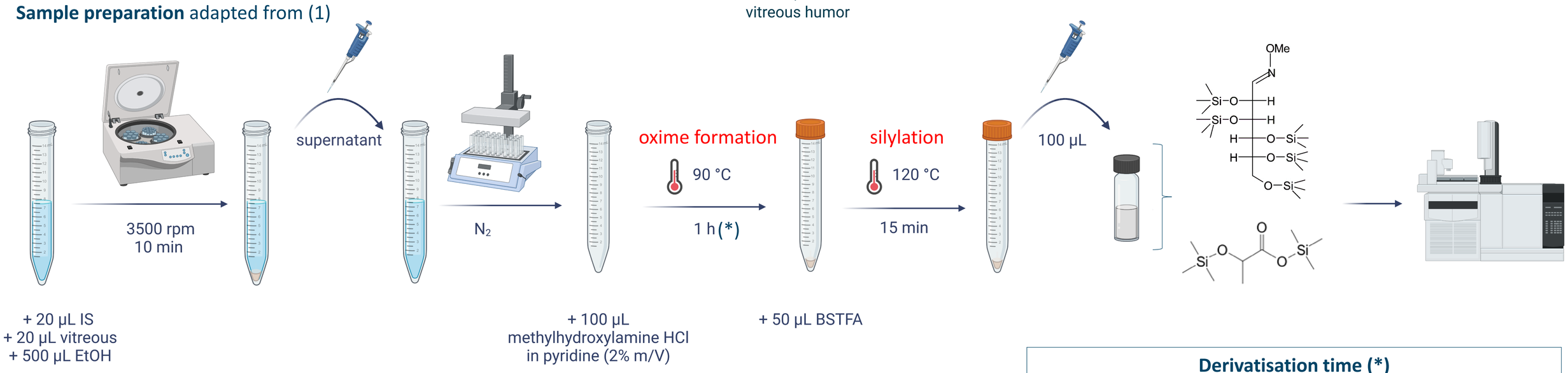
vitreous humor

Aims

- Development & validation of a straightforward method for quantifying glucose and lactate simultaneously in vitreous humor
  - Two-step derivatisation (necessary for glucose)
  - GC-MS based
- Application of the method to an archive of authentic vitreous humor samples & comparison with spectrophotometric method

Methods

Sample preparation adapted from (1)



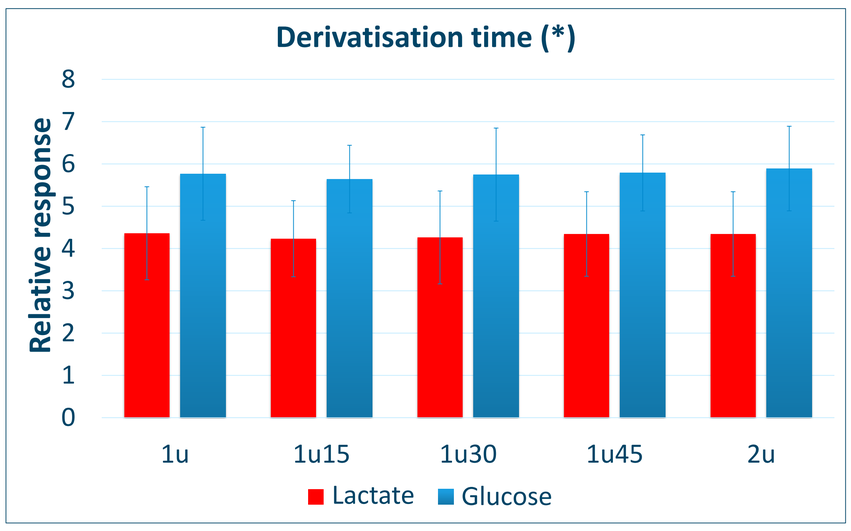
Analytical method

Compound	RT (min)	Q ion (*m/z*)	q1 ion (*m/z*)	q2 ion (*m/z*)
Lactate	5.26	117	191	
Lactate-$^{13}C_3$	5.26	119	193	
Glucose	10.87	217	205	319
Glucose-$^{13}C_6$	10.87	220	323	207

- Agilent 6890N GC – 5973N single quadrupole MS
- DB-5MS column 30 m x 0.25 mm x 0.25 µm
- Pulsed split mode

Calibration & quality control

- 6 calibration levels: 12.5 – 250 mg/dL
- Linear – no weighing
- $R^2 > 0.99$
- 3 blanks per batch
- 3 internal QC samples per batch



Validation

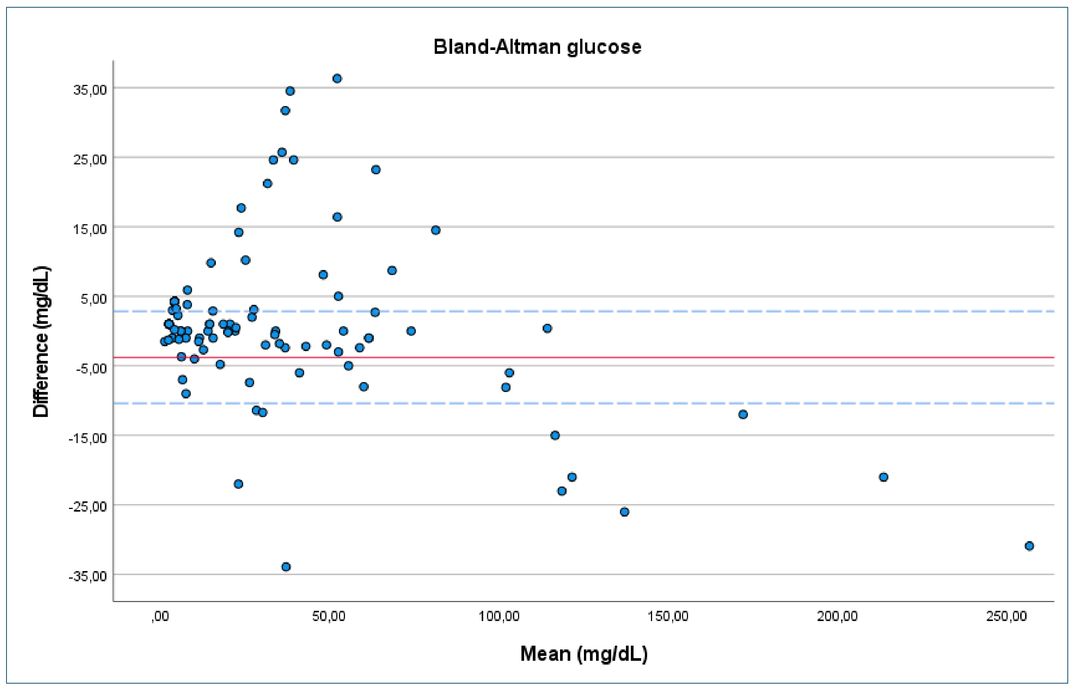
- **Guidelines European Medicines Agency**
- **LLOQ** = 12.5 mg/dL
  - Hypoglycaemia if Σ (glucose + lactate) < 160 mg/dL
- **Carry-over** < 20%
- **Calibration levels** 85-115%
- **Accuracy** 85-115%
- **Precision** RSD < 20%

Application

- **Archived authentic vitreous humor samples: n = 118**
- **Spearman correlation** between results of both methods
  - Glucose **ρ = 0.930 (p < 0.001)**
  - Lactate **ρ = 0.840 (p < 0.001)**
- **Bland-Altman plots**
  - Outliers
    - storage conditions > -20 °C
    - storage time too long?

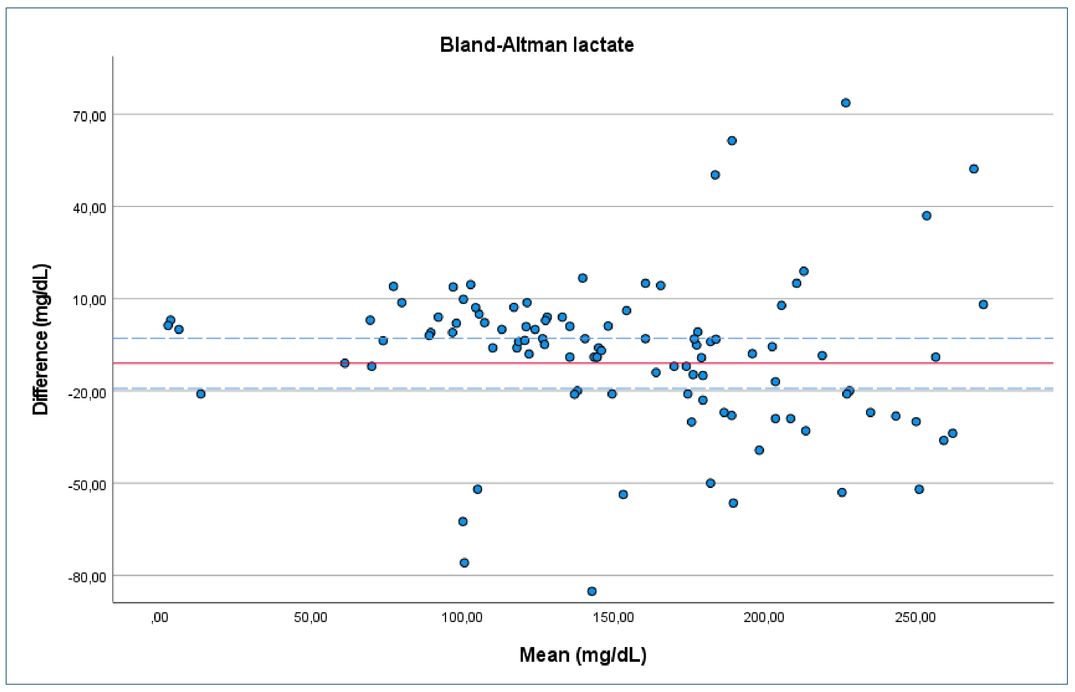
Future perspectives

- **External QCs:** artificial vitreous humor was tested = inappropriate
- **Matrix effects:** difficult matrix (small volume, large variability)
- **Stability:** storage conditions, time
- **Continued analysis of authentic vitreous humour samples**



This method median 19.4 mg/dL **Spectrophotometric method median 17.0 mg/dL**

0 ∈ 95% CI mean difference



This method median 150.4 mg/dL **Spectrophotometric method median 158.4 mg/dL**

0 ∉ 95% CI mean difference

Toxicological Centre
University of Antwerp

1. Shojaee-Moradie et al., Journal of Mass Spectrometry, 1996, 31, 961-966.

Figures created using BioRender.com