

GC-MS or GC-MS/MS for the determination of ethyl glucuronide in hair?

D. Cappelle¹, C. L. Crunelle¹, A. L. N. Van Nuijs¹, M. Yegles², A. Covaci¹, H. Neels^{1,3}

¹ Toxicological Centre, University of Antwerp, Wilrijk, Belgium;

² Laboratoire National de Santé, Service de Toxicologie, Dudelange, Luxembourg;

³ Toxicology Laboratory, ZNA Stuivenberg, Antwerp, Belgium.



Introduction & aim

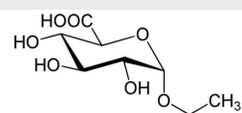


Fig. 1: Structure of ethyl glucuronide (EtG)



Ethyl glucuronide (EtG) in hair: used as a long-term alcohol marker

Methods with low LOQs: required to differentiate teetotalers from moderate drinkers according to the cut-offs (7 pg/mg hair; SoHT)

Determination of EtG: performed by LC-MS/MS or GC-(MS/MS)^[1]

Use of negative ion chemical ionization (NICI) mode^[1]:

- Improves the analytical sensitivity of GC-MS
- Is expected to improve the analytical sensitivity of GC-MS/MS
- Direct comparison of GC-MS and GC-MS/MS in NICI has never been investigated

Aim: Compare **GC-MS** and **GC-MS/MS** in **NICI** for EtG determination in 64 real hair samples

→ Assess the additional qualitative and quantitative advantages of GC-MS/MS

Materials & methods

Sample preparation

Hair segments^[2]

- 64 volunteers: teetotalers, moderate and excessive drinkers
- Wash in: H₂O, acetone
- Pulverization in a ball mill



30 mg pulverized sample

- + 2 mL H₂O
- Ultrasonication (1h 30min)
- + 2 ng EtG-d₅ (internal standard)



Solid phase extraction

- Wash: 1 mL 5% NH₄ in H₂O, 2 mL MeOH
- Elute: 2 mL 2% FA in MeOH
- Evaporation under N₂



Derivatization

- + 100 µL HFBA (30min @ 60°C)
- Evaporation under N₂
- Reconstitution in 50 µL ethyl acetate

GC-(MS/MS) method

Gas Chromatography

Agilent 7890A Gas Chromatograph
Automatic injector Agilent AS 7693



Injection: volume: 3 µL

temperature: 250°C

Column: HP-5 MS (5% phenyl methyl siloxan)

(length 30 m × diameter 0.25 mm × film thickness 0.25 µm)

Carrier gas: helium @1 mL/min

Oven: 100°C for 2min, to 170°C @ 10°C/min, to 300°C @ 40°C/min

Mass Spectrometry

Agilent 7000C Triple Quadrupole Mass Spectrometer

Detector: NICI mode

temperature: 280°C

Compound	GC-MS	GC-MS/MS
	Selected ion-monitoring mode	Multiple reaction-monitoring mode
	Monitored ions (m/z)	Monitored ion transitions (m/z)
EtG	596	596 → 213
	397	397 → 213
EtG-d ₅	601	601 → 213

Results

Sensitivity

	GC-MS	GC-MS/MS
LOD	0.022 pg/mg	0.005 pg/mg
LOQ	0.075 pg/mg	0.017 pg/mg

Calibration curve

R² of calibration curves of both methods > 0.998.

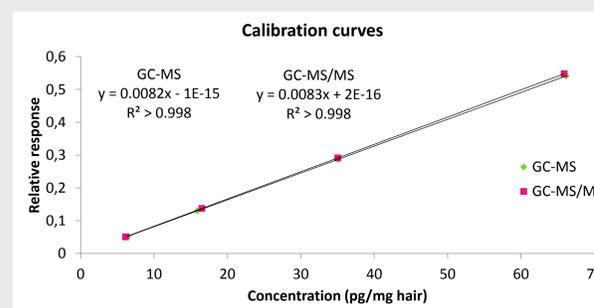


Fig. 2: Calibration curves obtained for GC-MS and GC-MS/MS

Background noise

Lower background noise observed using GC-MS/MS

Method comparison

GC-MS compared to GC-MS/MS for all concentrations:

No significant difference between GC-MS and GC-MS/MS with paired t-test ($p > 0.05$)

High correlation between both methods ($r^2 = 0.9993$)

Similar EtG concentrations obtained with both methods (mean CV = 1.01%)

GC-MS compared to GC-MS/MS for lower and higher concentrations:

Differences between both methods: larger for lower EtG concentrations (teetotalers and moderate drinkers; range 0.6-20 pg/mg hair; $r^2 = 0.9974$; mean CV = 1.67%) than for higher EtG concentrations (excessive drinkers; range 8-674 pg/mg hair; $r^2 = 0.999$; mean CV = 0.64%)

→ Suggests a higher selectivity of GC-MS/MS at lower concentrations

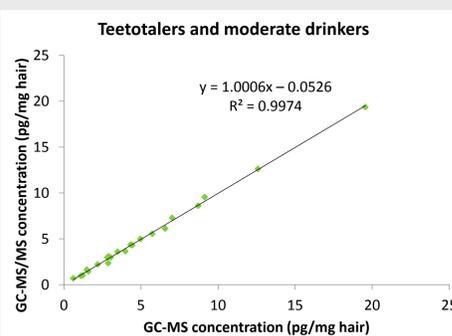


Fig. 3: Method comparison for teetotalers and moderate drinkers

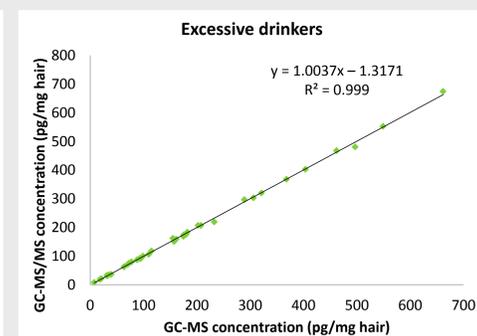


Fig. 4: Method comparison for excessive drinkers

Conclusion & future perspectives

Using GC-MS/MS instead of GC-MS:

- Higher analytical selectivity
- Improved signal to noise ratio

→ GC-MS/MS method of choice to differentiate between teetotalers and moderate drinkers

References

- 1 CRUNELLE, C.L., YEGLES, M., VAN NUIJS, A.L., COVACI, A., DE DONCKER, M., MAUDENS, K.E., SABBE, B., DOM, G., LAMBERT, W.E., MICHIENSEN, P. & NEELS, H. 2014. Hair ethyl glucuronide levels as a marker for alcohol use and abuse: a review of the current state of the art. *Drug Alcohol Depend*, 134, 1-11.
- 2 KERKES, I., YEGLES, M., GRIMM, U. & WENNIG, R. 2009. Ethyl glucuronide determination: head hair versus non-head hair. *Alcohol Alcohol.*, 44, 62-66.

Contact information:

Delphine Cappelle
Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk (Antwerp), Belgium
E-mail: delphine.cappelle@uantwerpen.be