Forensic Science International 218 (2012) 10–14

Contents lists available at SciVerse ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Ethyl glucuronide in hair – A highly effective test for the monitoring of alcohol consumption☆

Ronald Agius *, Thomas Nadulski, Hans-Gerhard Kahl, Bertin Dufaux

Labor Krone, Siemensstr. 40, 32105, Bad Salzuflen, Germany

ARTICLE INFO

Article history:
Received 15 April 2011
Accepted 3 May 2011
Available online 22 October 2011

Keywords:
Ethyl glucuronide
Hair
Urine
Alcohol marker
Uncertainty of measurement
Driving license

ABSTRACT

In Germany drink driving offenders lose their license and must prove abstinence for one year in order to regain it. In this paper we assess the newly introduced ethyl glucuronide (EtG) tests in urine and hair in this alcohol abstinence monitoring. 20% (80 out of 386) of the 3 cm long hair samples were tested positive for EtG in hair, compared to only 2% (92 out of 4248 samples) in urine in the same time period. Additionally 50% of the samples positive for EtG in hair had EtG values greater than 30 pg/mg hair, indicating chronic alcohol consumption in the last three months. This study shows that four EtG tests in 3 cm hair lengths reveal a significantly higher percentage of drink driving offenders who fail to be sober in the rehabilitation period, than do six random EtG tests in urine. Presumably, the hair test is more adequate to monitor long term alcohol abstinence than the urine test as defined by the new driving license re-granting medical and psychological assessment (MPA) in Germany.

1. Introduction

Following their wide acceptance as alcohol biomarkers in various fields, ethyl glucuronide in urine (EtGU) and in hair (EtGH) have been included in recently revised German driving re-granting guidelines (medical and psychological assessment, MPA [1]). Drunken drivers have to prove complete abstinence for at least one year either by six "random" (24 hours' notice) urine tests in twelve months or EtG in four separate 3 cm long hair strands corresponding roughly to one calendar year. The aim of this paper is to compare the rate of confirmed positive EtGU to EtGH samples using statistically significant populations and hence to assess their efficacy in detecting candidates who fail to be sober during this rehabilitation period.

2. Materials and methods

2.1. Specimens

Blank urine and hair samples (brown) were obtained from volunteer teetotalers and were checked to be negative with respect to EtGU and EtGH using the same methods used for the measurement of the real samples.

2.2. Chemicals and reagents

Methanolic solutions of EtG and EtG-D5 (I.S.) and Medidrug EtG 2/09 B urine quality controls were purchased from Medichem (Steinenbronn, Germany). All chemicals were of the highest analytical grade. Methanol, ammonium hydroxide solution (25%), ethyl acetate, formic acid, pyridine and acetone were supplied by Diagonal (Münster, Germany). N,O-bis(trimethylsilyl)trifluoroacetamide, (BSTFA), heptafluorobutyric acid anhydride (HFBA) and the SPE Cleanscreen® EtG (3 mL, 200 mg) cartridges were purchased from Amchro (Hattersheim, Germany). Methane 5.5, Argon 6.0 and Helium 5.0 were supplied by Linde AG (Unterschleißheim, Germany). Deionized water was prepared with a cartridge-deionizer using a Barnstead Nanopure Diamond Lab Water System from Werner GmbH (Leverkusen, Germany).

2.3. EtG screening in urine

Urine samples were screened with an enzyme immunoassay (DRI-EtG EIA; Microgenics Corp.) run on an Olympus AU5400 instrument using the supplied calibrators at 0, 0.1, 0.5, 1.0 and 2.0 mg/L, controls at 0.375, 0.625, 0.750 and 1.250 mg/L and protocols.

2.4. EtG confirmation in urine

1 mL deionized water and 1 ng internal standard (50 μL of 20 ng/mL EtG-D_5 in methanol) were added to 600 μL blank urine and vortexed for one minute. After centrifugation at 13,000 U/min for five minutes, 1 mL supernatant was extracted using SPE Cleanscreen® EtG cartridges as follows: conditioning with 1 mL of 1% formic acid, addition of the supernatant, washing with 1 mL water and elution of EtG with 2 mL methanol/1% formic acid mixture. A RapidTrace® SPE Worksation (Caliper Life Science GmbH, Rüsselsheim, Germany) was used for automated solid phase extractions. After evaporation of the solvent under a under a stream of nitrogen, the residue was derivatized with 45 μL BSTFA/Pyridine (9:1) mixture for 20 min at 80 °C. 2 μL sample were injected into the GC–MS operated in selective ion monitoring (SIM) mode using the fragments *m/z* 261, 160, 405 for EtG and *m/z* 266, 165, 410 for EtG-D_5.

☆ This paper is part of the special issue entitled: Selected papers from the Chamonix 2011 Society of Hair Testing Meeting, Guest-edited by Pascal Kintz.

* Corresponding author. Tel.: +49 5222 8076 178; fax: +49 5222 8076 170.
E-mail address: ragius@laborkrone.de (R. Agius).

0379-0738/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.forsciint.2011.10.007

A Shimadzu GC–MS model QP-2010 was used. The injector temperature was 250 °C; transfer line 300 °C. Chromatographic separation was performed using a column ZB-5ms 20 m × 0.18 mm × 0.18 μm (Zebron), at a helium flow rate of 0.6 mL/min. The temperature program was 125 °C held for 1 min, increased to 225 °C at a rate of 15 °C/min.

2.5. Sample preparation and measurement of EtG in hair

The hair samples were prepared and measured as described by a previously fully validated method [2] as follows. 10–50 mg of hair were washed with water and acetone, dried and pulverised. After addition of water and internal standard (EtG-D_5), the sample was ultrasonicated at 40 °C for 2 h. After centrifugation, the aqueous solid phase extraction was performed with SPE Cleanscreen® EtG cartridges with the following steps: conditioning with 1 mL of 1% formic acid, addition of the sample, washing with 1 mL water, EtG is eluted with 2 mL methanol/1% formic acid mixture. After evaporation of the solvent in nitrogen stream the residue is derivatised with 40 μL heptafluorobutyric acid anhydride (HFBA) for 15 min at 80 °C. Subsequently, the remaining reagent is evaporated and the dry residue is submitted to headspace solid phase micro extraction (HS-SPME) using a 75 μm Carboxen/Polydimethylsiloxane SPME fiber for 10 min at 90 °C. The SPME fiber is injected into the GC–MS/MS operated in selected reaction monitoring (SRM) mode using the transitions m/z 596 → 427 and m/z 596 → 288 for EtG, and m/z 601 → 432 and 601 → 288 for EtG-D_5.

2.6. Validation procedures

Since no protocols were available by the supplier for EtG screening in urine at the cut-off corresponding to 0.1 mg/L EtG, as required by the new German driving license re-granting guidelines, we fully validated the DRI-EtG EIA kits for the urine matrix at the new MPA guidelines' cut-offs by confirming at 78 real samples by GC–MS following EIA screening and choosing the screening cut-off at the point where the number of false negatives was lower than 1%.

3. Results and discussion

3.1. EtG in urine screening method validation

The number of true positives (PP), false negatives (NP), true negatives (NN) and false positives (PN) as a function of arbitrary ELISA cut-offs for EtG in urine are shown in Fig. 1.

The EIA screening cut-off at 0.1 arbitrary units was chosen as the cut-off corresponding to the confirmation cut-off of 0.1 mg/L EtG. At this point, the sensitivity against GC–MS was calculated at 100% (95% specificity). Already at the EIA screening cut-off 0.11 arbitrary units, resulted in 2 false negatives and a sensitivity of 94%.

3.2. EtG in urine confirmation method validation

The calibration curve for the confirmation of EtG in urine was linear and homogenous in the range from 0.08 to 250 mg/L urine.

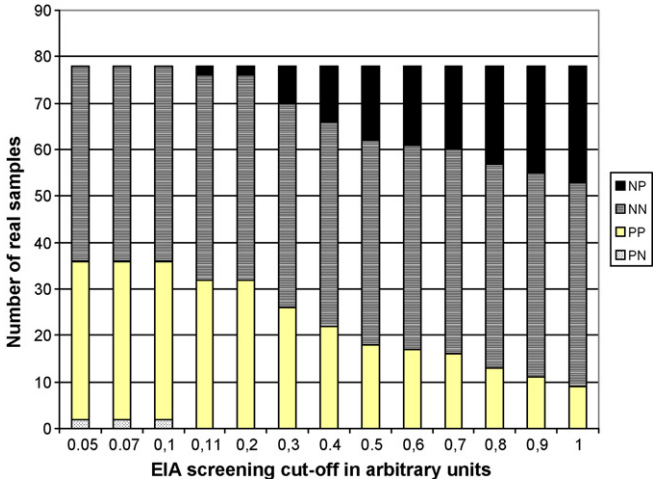

Fig. 1. The number of true positives (PP), false negatives (NP), true negatives (NN) and false positives (PN) as a function of arbitrary EIA cut-offs for EtG in urine for 78 real samples.

Linearity was verified with the Mandel-*F*-test at the 99% significance level, after ensuring homogeneity of the variances across the calibration range and the absence of straggler and outliers by means of the Grubbs-test at the 95% and 99% significance level respectively as given in DIN 32645 [3] and DIN 38402. The LLOD was calculated at 0.05 mg/L (with α-error 1% for the quantifier) and the LLOQ was0.08 mg/L (with α-error 10% for the qualifier) using linear regression at 99% significance level using the computer program VALISTAT [4]. Using Medidrug EtG 2/09 B urine quality controls at 2.70 mg/L and 4.46 mg/L, the bias was calculated at 4.2% and −3.3% respectively, intra-assay imprecision was calculated at 3.2% and 1.9% respectively and inter-assay imprecision was calculated at 9.9% and 9.0% respectively. The recoveries of samples spiked at 1.0 mg/L and 10 mg/L EtG in urine were 92.2% and 76.8% respectively.

3.3. EtG in hair method validation

The validation results were presented in detail in previously published HS-SPME-GC–MS/MS method [2]. The lower limit of quantification (LLOQ) was 2.8 pg/mg and the lower limit of detection (LLOD) detection was 0.6 pg/mg using linear regression at 99% significance level.

3.4. Quality assurance

We participated successfully in all external proficiency testing programs organized by the Society of Toxicological and Forensic Chemistry (GTFCh) and the Society of hair testing (SOHT) for both EtGU and EtGH. All samples were analyzed as real routine samples and the results are shown in Tables 1 and 2.

3.5. Uncertainty of measurement

The uncertainty of measurement at the LLOQ for both methods is ±33% at 99% confidence intervals as defined by DIN 32645 [4]. Furthermore we calculated the uncertainty of measurement using a top-down approach, as suggested by the GTFCh guidelines [5], based on EURACHEM Guide [6] and Nordtest report [7] by adding:

1) the uncertainty components due to the imprecision of internal quality control results, u(Rw),
2) the uncertainty components due to the determination of the proficiency testing target value, u(Cref) and
3) the uncertainty components due to the uncertainty component associated with the bias of the proficiency testing result, RMS(bias).

The proficiency testing results shown in Tables 1 and 2 for EtGU and EtGH respectively were used. The results are summarized in Table 3.

However, the uncertainty of measurement is expected to be different at different EtG concentrations. In order to obtain an EtG

Table 1
Proficiency testing results for EtGU from mid-2009 up to 2010.

GTFCh specimen	Our result in mg/L	Accepted range in mg/L	Target value in mg/L
ETG 2/09 A	1.000	0.560–1.120	0.840
ETG 2/09 B	3.200	2.200–3.840	3.020
ETG 1/10 A	1.280	0.760–1.440	1.100
ETG 1/10 B	0.940	0.592–1.164	0.878
ETG 2/10 A	2.000	1.140–2.100	1.620
ETG 2/10 B	2.200	1.400–2.560	1.980