SCREENING AND IDENTIFICATION

Workplace Alcohol Testing Program by Combined Use of Ethyl Glucuronide and Fatty Acid Ethyl Esters in Hair

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Abstract — Aims: The applicability of fatty acid ethyl esters (FAEEs) and ethyl glucuronide (EtG) in hair in a workplace alcohol testing program was investigated. Methods: A total of 78 hair samples from employees in jobs with a high endangering potential were tested for EtG and FAEEs. In most cases excessive drinking was suspected. For 59 of these cases additional data of the traditional alcohol markers aspartate aminotransferase, alanine aminotransferase and gamma-glutamyltransferase and of the mean corpuscular volume of the erythrocytes (58 cases) were available. Results: By application of the cut-offs of the Consensus of the Society of Hair Testing and of a gradual system for combined interpretation of FAEEs and EtG in hair no indications of alcohol abuse were obtained in 50 cases (64%), slight indications were seen in 13 cases (17%) and clear indications in 11 cases (14%). In four cases, the results were inconclusive with strongly conflicting results of both markers, the reason for which could not be cleared. The traditional markers confirmed the hair results only partly and displayed altogether a lower portion of positive results. Conclusion: EtG and FAEEs in hair, especially when interpreted in combination, are suitable for application in workplace alcohol testing programs. Nevertheless, the results obtained by hair analysis for alcohol markers can only be one part of a proper assessment aiming at the question whether an employee is addicted to alcohol or not.

INTRODUCTION

In the report on drugs and addiction 2009 published by the German federal government, it was stated that approximately 9.5 million people in Germany consumed alcohol at a risky level (Bätzing, 2009). A total of 1.3 million of them were addicted to alcohol. About 73,000 people died as a result of alcohol consumption in Germany per year. The economic and social costs of alcohol abuse are immense. As a range for social drinking an ethanol intake below 20 g for females and 40 g for males per day is accepted (World Health Organization, 2004). For risky drinking 40–120 g per day and as excessive drinking levels higher than 120 g daily ethanol intake can be assumed (Bühringer et al., 2000; Kraus and Augustin, 2001; Pragst et al., 2010).

One of the most serious acute effects of alcohol is the decrease of mental and physical performance which increases the risk of accidents by orders of magnitude. This is generally known for driving ability, but it is particularly threatening in jobs with a high endangering potential, where the life of a large number of people and goods of immense value can be destroyed by a wrong reaction. Addiction to alcohol and alcohol abuse cannot be tolerated in such jobs. Since heavy drinking is often concealed, reliable and objective methods for determination of excessive alcohol consumption are needed.

In this study, the results of a workplace testing program are presented aiming at alcohol abuse by employees in jobs with a high endangering potential. The examination was mostly performed if alcohol abuse was suspected. It was based on hair analysis for fatty acid ethyl esters (FAEEs) and ethyl glucuronide (EtG) as direct alcohol markers (Pragst and Yegles, 2006). In agreement with previous investigations, for FAEEs the sum of the concentrations of the four ethyl esters of myristic (E14), palmitic (E16), oleic (E18:1) and stearic (E18) acid was determined. In some of the cases gamma-glutamyltransferase (GGT), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and the mean corpuscular volume of the erythrocytes (MCV) were also determined.

The biochemical and physiological basics, analytical determination, advantages and limitations of FAEEs and EtG in hair for detection of excessive drinking were described in several papers (Auwärter et al., 2001; Pragst et al., 2001; Yegles et al., 2004; Politi et al., 2006; Pragst and Balikova, 2006; Appenzeller et al., 2007; Pragst and Yegles, 2008; Kronstrand et al., 2011). Because of their specificity, sensitivity and the time window of several months, they have found a large practical application in the last years. EtG in hair is now regularly used in driving ability examination in Germany and Switzerland (Schubert and Mattern, 2009), whereas determination of FAEEs is used for the detection of alcohol abuse during pregnancy (Kulaga et al., 2009) and the determination of FAEEs and EtG in combination is frequently performed in child custody cases (Suesse et al., 2010). The results of this study should also be interesting for these areas of expertise. Although in a workplace alcohol testing program other selection procedures are applied and test persons who might have a different social background are included. The employer can, according to the employment contract, ask on spec for a hair sample on short notice, whereas in driving ability examinations or child custody cases the test persons usually have several weeks or months to prepare for the hair test. As a result a different distribution, possibly with a higher rate of positive and borderline positive cases, requiring a gradual assessment can be expected.

Based on clinical studies and for uniform analytical performance and interpretation, in June 2009, the Society of Hair Testing adopted and published the ‘Consensus of the Society of Hair Testing on hair testing for chronic excessive alcohol consumption’ (Kintz, 2010) which was in essential confirmed in March 2011. Accordingly, it is recommended to use the proximal hair segment 0–3 cm for analysis, to
apply the cut-off values of 0.5 ng/mg for the sum concentration of the four FAEEs and of 30 pg/mg for EtG to discriminate between social and excessive drinking. Furthermore, it is currently assumed that EtG concentrations in hair lower than 7 pg/mg do not contradict a statement of teetotalism (Schubert and Mattern, 2009; Agius and Kinzt, 2010). However, social drinkers can fall below this level (Politi et al., 2006; Kronstrand et al., 2011).

MATERIALS AND METHODS

Hair and blood samples

Head hair samples were collected from 78 male employees between November 2008 and April 2010 by the staff at a medical facility of the place of work, where the testing was performed and were sent to the laboratory for determination of FAEEs and EtG. Mainly employees with suspected alcohol abuse were tested. The hair was cut as close as possible to the skin in the vertex posterior region. In 59 of the cases, blood samples were also taken and GGT, AST, ALT and MCV were determined in the routine investigation of the clinical laboratory of the facility.

The hair samples were stored in a paper envelope and analyzed within 2 weeks after collection. In case of bunched hair samples with a hair length ≥3 cm the proximal hair segment 0–3 cm was analyzed. Seven shorter hair samples and seven longer disordered and not bunched samples were investigated in full length.

For this study an approval of the ethics committee of the Charité—University Hospital Berlin was received.

Reference substances and reagents

Ethyl myristate, ethyl palmitate, ethyl oleate and ethyl stearate and the corresponding deuterated standards D5-ethyl myristate, D5-ethyl palmitate, D5-ethyl oleate and D5-ethyl stearate were purchased from LGC Promochem (Wesel, Germany), synthesized by Lorado (Malmö, Sweden) and cer-

Determination of EtG in hair

For the determination of FAEEs in hair, we applied a procedure based on the headspace solid-phase microextraction (HS-SPME) method previously published by Auwärter et al. (2001) and Pragst et al. (2001) which was optimized and newly validated.

The hair samples were cut into pieces of ~1 mm length and ~20 mg were weighed into a 4 ml glass vial. For decontamination, the hair pieces were washed two times for 10 min with 1 ml of n-heptane on a thermo shaker (5433, Eppendorf, Hamburg, Germany) at 25°C and 800 rpm. After removal of the washing solution 0.5 ml of dimethylsulfoxide (DMSO) and 2 ml of n-heptane as well as the deuterated internal standards (a mixture of 20 ng of each of the four FAEEs in 10 µl n-heptane) were added. The sample was shaken for 15 h at 20°C, then cooled down to 0°C to freeze the DMSO layer. The heptane layer was decanted into a 10 ml headspace vial, the solvent evaporated by a nitrogen stream, and 1 ml Sörensen phosphate buffer pH 7.6 was added to the residue. In order to enhance the extraction yields of the FAEEs, 0.5 g sodium chloride was added.

The automated HS-SPME was performed using a 65 µm polydimethylsiloxane/divinylbenzene fiber (Supelco, Deisenhofen, Germany) on a multi purpose sampler MPS 2 (Gerstel, Mülheim, Germany) under the following conditions: Preheating 5 min at 90°C, headspace adsorption for 30 min at 90°C each with the agitation at 250 rpm and desorption for 10 min in the injection port of the gas chromatograph (GC) at 260°C. A GC 6890 equipped with a mass selective detector 5973 (Hewlett Packard GmbH, Waldbronn, Germany) was used for the GC-MS measurements. Chromatographic separation was performed on a HP 5-MSi capillary column (30 m x 0.25 mm x 0.25 µm; Supelco, Bellefonte, PA, USA) with helium 5.0 as carrier gas. The injection mode was pulsed splitless with an injector temperature of 260°C. A constant flow of 1 ml/min was applied. The oven program starts with an initial temperature of 70°C, holding for 2 min, then increasing with 10°C/min up to 300°C. The temperatures of transfer line, ion source and quadrupol were 280, 230 and 150°C. For detection in selected ion monitoring mode the following m/z (target ions in bold type) were selected: D5-E14: 106, 162, 261; E14: 101, 157, 256; D5-E16: 106, 162, 289; E16: 101, 157, 284; D5-E18: 93, 106, 315; E18: 88, 101, 310; D5-E18: 106, 162, 317; E18: 101, 157, 312.

Chromatograms from the hair of a teetotaler and of a hair sample tested positive for FAEEs are shown in Figs 1 and 2.

For forensic purposes, the method was newly calibrated and validated using a children’s hair pool spiked with the four esters. All concentrations were corrected for the native content of FAEEs in this pool (E14 0.000, E16 0.040, E18:1 0.000, E18:2 0.040, E18:3 0.040).
0.033 and E18: 0.000 ng/mg). Since the calibration curve was slightly bent (Pragst et al., 2001) a low range (0.05–0.5 ng/mg) and a high range calibration (0.5–5 ng/mg) was done in order to get sufficient linearity in each range. About 20 mg of cut and washed children’s hair were spiked with the FAEEs at the following concentration levels: Low range 0.05, 0.10, 0.20, 0.30, 0.40 and 0.50 ng/mg; high range: 0.50, 1.0, 2.0 and 5.0 ng/mg. Five separately prepared samples per calibration level were measured. All acquired data were checked for outliers according to the Grubbs test on a 95 and a 99% level of significance. Homogeneity of variance was checked according to the Cochran test on a
level of significance of 95%. In order to show that the linear calibration curve can be used, the Mandel-$F$-test was performed at a level of significance of 99%. The calibration curves of all analytes showed homogeneity of variance and good linearity. The correlation coefficients were between 0.995 and 0.9998.

As lowest limit of quantification (LLOQ) the lowest calibration level (0.05 ng/mg) was applied and no extrapolation was done for results below this level. Nevertheless, the estimated possible limits of detection (LOD) and LLOQ according to DIN 32645 (German standard specification (Arbeitsausschuss, 2008)) were lower (see Table 1).

The accuracy was checked at children’s hair spiked with the FAEEs at the concentration levels 0.075, 0.35 and 2.5 ng/mg. At 5 days, two samples at each level were prepared and measured. The relative standard deviation, repeatability and bias were calculated and were all in the acceptable range given by the forensic guidelines of the German Society of Toxicological and Forensic Chemistry (GTFCh) (Peters et al., 2009).

The stability of the analytes in hair stored over a period of 7 days at room temperature and the stability of the hair extracts with phosphate buffer stored over 30 h at room temperature could be proved according to procedures described in the forensic guidelines of the GTFCh (Peters et al., 2009).

The uncertainty of measurement was estimated according to EURACHEM/CITAC Guide CG 4 (EURACHEM/CITAC Guide CG 4, 2000). Main sources of uncertainty were repeatability and bias. The estimated expanded uncertainties with a coverage factor of 2 are also given in Table 1.

### RESULTS AND DISCUSSION

The criteria used for interpretation of the results are given in Table 2. They are based on earlier studies or on current guidelines (Schubert and Mattern, 2009; Kintz, 2010; Pragst et al., 2010). Accordingly, values <7 pg/mg for EtG and <0.2 ng/mg for FAEEs are not in contradiction to alleged abstinence but do not prove abstinence. Social drinking can be assumed for concentrations between 7 and 30 pg/mg for EtG and 0.2 and 0.5 ng/mg for FAEEs. Levels ≥30 pg/mg (EtG) and ≥0.5 ng/mg (FAEEs) were interpreted as indications of chronic excessive alcohol consumption.

#### FAEE in hair

From the 78 hair samples 33 were tested negative for FAEEs (<0.2 ng/mg). In 23 samples, concentrations between 0.2 and 0.5 ng/mg were determined. Twenty-two samples were tested positive at a level typical for chronic excessive drinkers (≥0.5 ng/mg). Figure 3 shows the FAEEs concentrations of the positive cases in decreasing order. For comparison, the corresponding EtG results are given. The mean concentration ratio of the four FAEEs was calculated with 8:45:37:10. This is very close to earlier reported results (Auwärter et al., 2001; Suesse et al., 2010). For single cases a relative large deviation from this mean concentration ratio was observed.

#### EtG in hair

Forty-eight hair samples were tested negative (EtG <7 pg/mg). EtG ≥7 pg/mg was measured in 30 samples, between them 14 samples with concentrations in the typical range for chronic excessive alcohol consumption (≥30 pg/mg).
**Table 3. Combined interpretation of FAEEs and EtG concentrations in the 0–3 cm hair segment for detection of alcohol abuse according to Pragst et al. (2010), applying the recent cut-off values FAEE 0.5 ng/mg and EtG 30 pg/mg of the consensus on alcohol markers of the SoHT (Kintz, 2010).**

<table>
<thead>
<tr>
<th>$C_{\text{FAEE}}$ (ng/mg)</th>
<th>$C_{\text{EtG}}$ (pg/mg)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤0.20</td>
<td></td>
<td>Not detected</td>
</tr>
<tr>
<td>0.20 – 0.49</td>
<td></td>
<td>Moderate drinking, abstinence excluded</td>
</tr>
<tr>
<td>0.20 – 0.49</td>
<td>≤30</td>
<td>Moderate drinking, abstinence excluded</td>
</tr>
<tr>
<td>0.20 – 0.49</td>
<td>≥60</td>
<td>Repeat analysis of both parameters. If confirmed: social drinking, weak indication of abuse</td>
</tr>
<tr>
<td>0.49 – 0.99</td>
<td>≤30</td>
<td>moderate indication of alcohol abuse</td>
</tr>
<tr>
<td>0.49 – 0.99</td>
<td>30 – 60</td>
<td>Repeat analysis of both parameters. If confirmed: look for reasons in aggressive hair cosmetics. Inconclusive. Collect new sample and/or analyze body hair other than pubic hair</td>
</tr>
<tr>
<td>0.99 – 1.00</td>
<td>≥30</td>
<td>Strong indication of alcohol abuse</td>
</tr>
<tr>
<td>≥1.00</td>
<td></td>
<td>Strong indication of alcohol abuse</td>
</tr>
</tbody>
</table>

**Table 4. Interpretation of alcohol markers in 78 hair samples of the workplace testing program according to the proposed system given in Table 3 and results of classical markers in blood samples from 59 of the cases according to the reference ranges given in Table 2.**

<table>
<thead>
<tr>
<th>Assessment by FAEEs + EtG</th>
<th>Number of cases</th>
<th>Number of results above reference range (Table 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstinence or moderate drinking</td>
<td>25 (32%)</td>
<td>GGT AST ALT De-Ritis-Qt. MCV</td>
</tr>
<tr>
<td>Moderate drinking, abstinence excluded or improbable</td>
<td>25 (32%)</td>
<td>2 0 4 3 0</td>
</tr>
<tr>
<td>Social drinking, weak indication of abuse</td>
<td>13 (17%)</td>
<td>1 0 2 7 0</td>
</tr>
<tr>
<td>Indication of abuse</td>
<td>3 (4%)</td>
<td>0 0 1 2 0</td>
</tr>
<tr>
<td>Strong indication of abuse</td>
<td>8 (10%)</td>
<td>3 1 2 5 0</td>
</tr>
<tr>
<td>Inconclusive</td>
<td>4 (5%)</td>
<td>0 0 0 1 0</td>
</tr>
</tbody>
</table>

Figure 4 shows the EtG results ≥30 pg/mg in decreasing order together with the corresponding FAEEs concentrations.

**Combined interpretation of FAEEs and EtG**

A correlation between the area under the curve (AUC) of blood alcohol concentration versus time and EtG in hair was described and can also be assumed for FAEEs (Kharbouche et al., 2010). The AUC is not only affected by the dose, but also by the drinking habits. Ethanol shows a zero order kinetic. Therefore binge drinking once per week results in a much higher AUC than drinking in sum the same amount of alcohol, but consumed evenly throughout the week (Pragst et al., 2010). In addition there are many absorption, distribution and metabolic steps from the ethanol intake to the incorporation of the direct alcohol markers into hair. That is why there is only moderate proportionality between alcohol dose and the concentrations of FAEEs or EtG in hair. Therefore, the measurement and combined interpretation of both markers are recommended in order to improve the discrimination between alcohol abuse and social drinking or abstinence (Pragst and Yegles, 2006, 2008; Pragst et al., 2010). A proposal for this combined interpretation which includes also the upper levels for abstinence and the double of the cut-off values for excessive drinking of both markers was published by Pragst et al. (2010) and was updated with respect to the actual cut-off values in Table 3. It allows a more gradual assessment and takes into account the effect of biological variability and differences in hair care and cosmetics.

The application of this system to the 78 hair results is shown in Table 4. Twenty-five samples (32%) of the results were interpreted as indication of abstinence or moderate drinking. Four samples (5%) had strongly diverging results for EtG and FAEEs. The remaining 49 samples (63%) indicate a consumption of alcohol. Among them 25 samples (32%) lead to the suggestion of moderate drinking, 13 samples (17%) of social drinking with a weak indication of abuse, 3 samples (4%) of an indication of abuse and 8 samples (10%) of a strong indication of abuse.

Two of the four inconclusive samples showed very high EtG and low FAEEs concentrations and the other two samples showed highly positive FAEEs and negative EtG results. These results were confirmed by repetition of the analysis in one case, the sample amount was not sufficient for a repetition in the other three cases. Such strongly diverting findings can be due to different sources of error. EtG can be decomposed by bleaching (Morini et al., 2010) or be washed out by frequent and intensive shampooing. FAEEs can be formed when alcohol containing hair care products such as hair lotions or hair sprays are regularly applied (Hartwig et al., 2003; Gareri et al., 2011). But that could only be the reasons for the two cases with strongly positive FAEEs and negative EtG results. For the two samples with the opposite difference no simple explanation can be given. It was shown in a recent study that formation of EtG from external ethanol in hair does not occur (Martins et al., 2011). However, a false positive EtG result was described after use of hair lotion which contained EtG itself (Sporkert et al., 2011).
of scalp hair after social drinking when EtG is incorporated from urine. Since these possibilities can be excluded in the two cases, a false negative FAEEs result is more probable in view of the EtG concentrations of 88 and 219 pg/mg. It is supposable that this could be a result of the frequent use of strongly lipophilic hair care products that extract FAEEs from the hair or of alkaline products that increase the hydrolysis of the esters.

For comparison, in Table 4 also the results of the classical alcohol markers were included. From the 59 analyzed serum samples, values above the reference range (Table 2) were obtained for GGT in five cases, for AST in one case and for ALT in nine cases. MCV was in none of the 58 blood samples above the reference range. As expected, testing for GGT, ALT, AST and MCV produced fewer positive results than FAEEs or EtG, and there was no or only a low correlation to the alcohol markers in hair as it can also be seen from Table 4. In particular, these indirect alcohol markers were also not helpful in the four cases with inconclusive results from hair.

CONCLUSION

Alcohol abuse and addiction to alcohol are incompatible with jobs of high endangering potential. Since they are frequently concealed by the employees who often do not realize the seriousness of their drinking problem, reliable markers for chronic excessive alcohol consumption are very important for a corresponding workplace testing. It follows from the results of this study that EtG and FAEEs in hair are suitable for this purpose. The data show that the combined interpretation of EtG and FAEEs provides a more reliable and sophisticated interpretation of the drinking habits.

Nevertheless, consequences for the employees should not be based only on the hair result. This is particularly important in the borderline and inconclusive cases of Table 4. A positive hair result should be the reason for a deeper inspection of the drinking behavior and could lead to a corresponding therapy.

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REFERENCES


