Drugs of abuse and heavy alcohol use markers in hair samples: analytical methodologies and data interpretation in forensic toxicology

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Xenobiotics incorporation into hair
• From blood stream during hair growth
• From sebum
• From sweat
• From environment (smoke)

Xenobiotics incorporation into hair
Basic drugs bind to melanin
• Concentration dependent on melanin content (Kronstrand et al., 1999)
• Bias on hair color
• Positive linear relationship between total melanin content of hair and $C_{max}$ of codeine, cocaine, and metabolites following controlled dosing (Scheidweiler et al., 2005)

Hair growth
• Head hair:
  – Anagen: 4-8 years, 0.6-1.4 cm/month
  – Catagen: 2 weeks
  – Telogen: 10 weeks
• Average growth rate in head hair: 1 cm/month
Surveillance window

Hair analysis

- Environmental Toxicology (trace elements/pollutants)
- Nutrition (lack of elements)
- Disease diagnosis (Down syndrome, schizophrenia, ...)
- Forensic Toxicology

Hair analysis in Forensic Toxicology

- Napoleon (Smith et al. 1962)
- John Keats (Baumgartner et al. 1989)

Drugs of abuse:
- Drivers license issue or re-issue
- Monitoring during addiction treatment
- Workplace drug testing
- Divorce / children custody
- Cause of death
- Unwilling sedation

Season of mists and mellow fruitfulness
Close bosom—friend of the maturing sun
Conspiring with him how to load and bless
With fruit the vines that round the thatch—eaves run...
Hair analysis: steps

- Segmentation
- Decontamination
- Cutting or pulverization
- Incubation
- Extraction of analytes from incubation solvent
- Instrumental analysis
  - Gas chromatography – mass spectrometry (GC-MS)
  - Liquid chromatography – tandem mass spectrometry (LC-MS-MS)

Recommended Lower Limits of Quantification

<table>
<thead>
<tr>
<th>Substance</th>
<th>LLOQs</th>
</tr>
</thead>
<tbody>
<tr>
<td>MORPHINE</td>
<td>0.2 ng/mg</td>
</tr>
<tr>
<td>6-ACETYL MORPHINE</td>
<td></td>
</tr>
<tr>
<td>COCAINE</td>
<td>0.5 ng/mg</td>
</tr>
<tr>
<td>COCAINE METABOLITES</td>
<td>0.05 ng/mg</td>
</tr>
<tr>
<td>AMPHETAMINES</td>
<td>0.2 ng/mg</td>
</tr>
<tr>
<td>THC</td>
<td>0.1 ng/mg</td>
</tr>
<tr>
<td>THC-COOH</td>
<td>0.2 pg/mg</td>
</tr>
</tbody>
</table>

Recommendations for hair testing in forcing cases

1. Sampling, shipping, storage
2. Decontamination
3. Hair disintegration and extraction
4. Screening tests
5. Criteria for MS analysis
6. Specific drug classes
7. Internal quality control
8. External quality control

VALIDATION
IDENTIFICATION
RECOMMENDED
LLOQs

Hair analysis for drivers license regranting

- Depending on National (or local) Law
- As an example, in Florence, Italy:
  - Opiates
  - Buprenorphine
  - Cocaine
  - Cannabinoids
  - Amphetamines
  - Methadone
Evidence of surreptitious administration

- A 9 year old girl was assaulted
- Seven weeks later a hair strand was collected
- Segmented in 1 or 2 cm
- Each segment (about 20 mg) was analysed for diphenhydramine (LC-MS-MS, 2 transitions, validated method)

Kintz et al. 2007

Evidence of surreptitious administration

- Diphenhydramine: OTC drug, antihistaminic, sedative, antiemetic

<table>
<thead>
<tr>
<th>Segment</th>
<th>Concentration (pg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1 cm</td>
<td>37 pg/mg</td>
</tr>
<tr>
<td>1-3 cm</td>
<td>39 pg/mg</td>
</tr>
<tr>
<td>3-5 cm</td>
<td>33 pg/mg</td>
</tr>
</tbody>
</table>

- Use of diphenhydramine as a drug-facilitated crime and subsequent impairment of a 9-year-old female victim

Kintz et al. 2007

Evidence of surreptitious administration

- 51 years old male admitted 9 times to hospital for drowsiness, ataxia, sedation, muscular weakness, and marked somnolence
- Hair strand of 7 cm, analysed in segments by LC-MS-MS (2 transitions)
- Alprazolam in 4 segments

<table>
<thead>
<tr>
<th>Segment</th>
<th>Concentration (pg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1 cm</td>
<td>&gt; LOD (2 pg/mg)</td>
</tr>
<tr>
<td>1-2 cm</td>
<td>71 pg/mg</td>
</tr>
<tr>
<td>2-3 cm</td>
<td>8 pg/mg</td>
</tr>
<tr>
<td>3-4 cm</td>
<td>15 pg/mg</td>
</tr>
<tr>
<td>5-6 cm</td>
<td>&lt; LOD (2 pg/mg)</td>
</tr>
<tr>
<td>6-7 cm</td>
<td>&lt; LOD</td>
</tr>
</tbody>
</table>

Kintz et al. 2007

Evidence of neonatal exposure

- Two twin female infants born at 34 weeks of gestation by caesarean section performed because of maternal preeclampsia.
- During preceding week pregnancy had been complicated by maternal hypertension. The mother reported use of venlafaxine during pregnancy.
- Both twins presented neonatal abstinence syndrome

Favretto et al. 2010
Evidence of neonatal exposure

- 2.5 mg hair washed twice with CH₂Cl₂
- Automatic pulverization with 145 µl of water, 20 µl of acetonitrile, 20 µl of 1 M trifluoroacetic acid, and 15 µl of IS working solution, 5 minutes.
- Double centrifugation
- 10 µl of supernatant injected directly into analytical column

Evidence of neonatal exposure

- LTQ-Orbitrap MS in positive ESI mode
- AtlantisT3 analytical column by gradient at 100 µl/min
- Accurate mass measurements of MH⁺ ions: m/z 278.21146 for venlafaxine

Evidence of neonatal exposure

- HRMS permitted the detection of:
  - Venlafaxine (VEN)
  - O-desmethylvenlafaxine (ODV)
  - N,O-didesmethylvenlafaxine (N,O-DDV)
  - N,N-didesmethylvenlafaxine (N,N-DDV)
  - N-desmethylvenlafaxine (NDV)

- 10 ng/mg and 12 ng/mg of VEN in infants hair
Drug facilitated sexual assault

- 61-year-old woman hospitalised for a minor surgical operation
- Local analgesia (mepivacain, ropivacain), general anaesthesia (propofol)
- On completion of operation fully awakened, was transferred to her ward
- The patient reached her ward with considerable delay and still unconscious
- Head and pubic hair collected 6 weeks later

Frison et al. 2003

Drug facilitated sexual assault

- SPME and GC-MS-MS (ion trap MS), monitoring 3 product ions per compound
- Validated method

<table>
<thead>
<tr>
<th></th>
<th>Pentobarbital (ng/mg)</th>
<th>Thiopental (ng/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 A Proximal</td>
<td>0.40</td>
<td>0.30</td>
</tr>
<tr>
<td>1 A Distal</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>1B Proximal</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>1B Distal</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>2 Pubic</td>
<td>0.40</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Frison et al. 2003

Drug facilitated sexual assault

- Lack of collection and toxicological analysis of traditional biological fluids in the immediacy of a DFSA in a healthcare setting
- Quali-quantitative results obtained from SPME and GC–MS-MS analysis of the victim head and pubic hair
- Document the use of the anaesthetic agent thiopental to sedate her quickly and deeply and commit sexual assault.

Frison et al. 2003

Pre-Columbian Mummies

- Chilean mummies, dated 2000 BC: benzoylecgonine (Cartmel et al. 1991)
- Eight pre-columbian mummies
  - Woman from Perú or Argentina
  - Child from Peruvian Chancay culture
  - Female skull with braided hair
- Positive to nicotine (57.5, 14.1, 11.4 ng/mg, Musshoff et al. 2009)
Egyptian Mummies

- Nine mummies dated from 1000 BC to 400 AD, included a priestess, Henut Taui
- Hair positive to:
  - THC
  - Cocaine
  - Nicotine

Balabanova et al. 1992

β-D-ethyl glucuronide

- Non-volatile
  - water-soluble, slightly acidic
direct metabolite of ethanol
- Indicative of ethanol intake for:
  - hours (serum)
  - days (urine)
  - months (hair)

Question:

Is there a relationship between the pattern of alcohol use and β-D-ethyl glucuronide (EtG) levels in hair?
Ethyl glucuronide in hair - HEtG

During 2000:
• Skopp et al. Alcohol and Alcoholism
• Alt et al. Alcohol and Alcoholism

Since 2004:
• LLOQs down to 2 - 3 pg/mg
• Fully validated methods
• Wider populations examined

Sample preparation

• Hair (50-100 mg, 3-5 cm, proximal segment)
• Washing: CH₂Cl₂, MeOH
• 1-2 mm scissor cut
• Overnight incubation in 700 μl H₂O (D₂-EtG)
• 2-hour ultrasonication
• 13000 rpm centrifugation
• Injection (8 μl)

Politi et al. 2006

Sample preparation

• Hair (50-100 mg, 3-5 cm, proximal segment)
• Washing: CH₂Cl₂, MeOH
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• Overnight incubation in 700 μl H₂O (D₂-EtG)
• 2-hour ultrasonication
• 13000 rpm centrifugation
• Injection (8 μl)

Politi et al. 2006

Sample preparation

LC-ESI-MS-MS method

• C₁₈ (100 × 3 mm, 3 μm id)
• 0.1% formic acid/ACN 99:1
• 200 μl/min
• 100 μl/min ACN post-column
• Negative electrospray ionization
• MRM transitions:
  - EtG: m/z 221 → 75, 221 → 85, 221 → 221
  - D₂-EtG: 226 → 75, 226 → 85, 226 → 226

Politi et al. 2006

LC-ESI-MS-MS method

• Full validation (selectivity, linearity, accuracy, precision, matrix effect, stability, reproducibility)
• LLOQ: 3 pg/mg
• LOD: 2 pg/mg
• 2 MRM
• Surviving Ion

Politi et al. 2006
Correlation EtG – ethanol daily intake

• Correlation between hair EtG and ethanol daily intake normalized by the individual body weight

Politi et al. 2006

Ethanol Daily intake - EDI

• Politi et al. 2006:
  – HEtG correlated with:
    • EDI
    • Body Mass Index

• Appenzeller et al. 2007:
  – Linear proportional relationship between HEtG and EDI
  – HEtG > 23 pg/mg corresponds to an EDI > 60 pg/mg

HETG cut-offs

Suggested cut-offs:

Appenzeller et al. (2007) 23 pg/mg
Pragst and Yegles (2008) 25 pg/mg
Bendroth et al. (2008) 30 pg/mg
Kintz et al. (2008) 50 pg/mg

Factors possibly influencing EtG formation or incorporation: sex, age, gender, body mass index, cosmetic treatments, hygienic habits, etc.

Questions

Definition of a cut-off level for HEtG able to effectively discriminate a heavy drinking behaviour.

Evaluation of the influence of factors known to affect ethanol metabolism and/or the diagnostic power of other markers of ethanol use, and of issues possibly affecting substance incorporation into hair.
Study protocol

98 subjects:
– teetotallers, social drinkers, and heavy drinkers at the beginning of a withdrawal treatment

• EDI 2-week and 3-month ← Questionnaire, anonymous, self-administered

• HEtG, 3-cm prox ← LC-MS-MS
– LLOQ: 3 pg/mg, full validation
– 2 transitions per analyte

EDI and basic characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>3-month EDI &lt;60 g/day (n=23)</th>
<th>3-month EDI ≥60 g/day (n=75)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>gender (% females)</td>
<td>26 (n=11)</td>
<td>21 (n=12)</td>
<td>0.5839</td>
</tr>
<tr>
<td>age (years)</td>
<td>41 (31-52)</td>
<td>45 (40-54)</td>
<td>0.0543</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23 (21-26)</td>
<td>23 (21-26)</td>
<td>0.5473</td>
</tr>
<tr>
<td>smokers (%)</td>
<td>55 (n=12)</td>
<td>83 (n=49)</td>
<td>0.0085</td>
</tr>
<tr>
<td>brown or black hair colour (%)</td>
<td>56 (n=13)</td>
<td>79 (n=50)</td>
<td>0.2203</td>
</tr>
<tr>
<td>hair treatment (%)</td>
<td>44 (n=7)</td>
<td>41 (n=16)</td>
<td>0.8537</td>
</tr>
<tr>
<td>n. shampoos/week</td>
<td>3 (2-6)</td>
<td>3 (2-4)</td>
<td>0.6461</td>
</tr>
<tr>
<td>wine as prevalent beverage (%)</td>
<td>83 (n=15)</td>
<td>67 (n=35)</td>
<td>0.2332</td>
</tr>
</tbody>
</table>

Receiver Operated Characteristic (ROC) curves

Sensitivity and Specificity (1)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n</th>
<th>SE</th>
<th>SP</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-week EDI (≥60 g/day)</td>
<td>98</td>
<td>0.92</td>
<td>0.85</td>
<td>0.88</td>
</tr>
<tr>
<td>3-month EDI (≥60 g/day)</td>
<td>98</td>
<td>0.92</td>
<td>0.96</td>
<td>0.94</td>
</tr>
<tr>
<td>Females</td>
<td>42</td>
<td>0.90</td>
<td>0.91</td>
<td>0.91</td>
</tr>
<tr>
<td>Males</td>
<td>56</td>
<td>0.93</td>
<td>1.00</td>
<td>0.95</td>
</tr>
<tr>
<td>Age 20-34</td>
<td>11</td>
<td>0.75</td>
<td>1.00</td>
<td>0.75</td>
</tr>
<tr>
<td>Age 35-49</td>
<td>42</td>
<td>0.88</td>
<td>1.00</td>
<td>0.88</td>
</tr>
<tr>
<td>Age ≥ 50</td>
<td>29</td>
<td>0.95</td>
<td>0.86</td>
<td>0.95</td>
</tr>
</tbody>
</table>
Characteristics  | n  | SE | SP  | AUC  
---|---|---|---|---
BMI<25       | 52 | 0.91| 1.00 | 0.96  
BMI≥25       | 30 | 0.88| 0.86 | 0.83  
Non-smokers  | 20 | 0.90| 1.00 | 0.98  
Smokers      | 61 | 0.90| 0.92 | 0.89  
Hair colour blonde/grey | 31 | 1.00| 0.90 | 0.96  
Hair colour brown/black | 63 | 0.90| 1.00 | 0.95  
Hair treatments | 32 | 0.88| 1.00 | 0.87  
No hair treatments | 23 | 0.91| 0.89 | 0.96  
1-2 shampoos/week | 35 | 0.88| 1.00 | 0.94  
3 or more shampoos/week | 41 | 0.90| 0.92 | 0.90  
Prevalent beverage beer | 19 | 0.91| 0.93 | 0.92  
Prevalent beverage wine | 50 | 0.88| 1.00 | 0.92  

**Correlation EDI- HEtG**

**EtG in Hair and Racial Bias**
No correlation found between EtG in hair and melanin content

(Appenzeller et al. 2007. Ethyl glucuronide concentration in hair is not influenced by pigmentation)

**Correlation EDI- HEtG: conclusions**
When a fully validated analytical method providing adequate sensitivity and selectivity of detection is used:

- HEtG is able to ascertain chronic heavy drinking (EDI of 60 g/day or higher within the last 3 months) with high sensitivity (0.92) and specificity (0.96)
- A 27 pg/mg cut off presented the best test performance in accordance with the results of previous studies.
Correlation EDI- HEtG: conclusions

• None of the examined factors was found to significantly affect HEtG performance.

• Further studies on larger groups of individuals are required in order to establish if the differences observed for some factors (e.g. BMI, smoke, and hair treatments), as well as associations between factors, may reach statistical significance.

Carbohydrate Deficient Transferrin

CDT

Monosialo Transferrin

Tetrasialo Transferrin

Trisialo Transferrin

Disialo Transferrin

Asialo Transferrin

CDT: sensitivity and specificity

WHO/ISBRA Collaborative Study:

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (SE)</td>
<td>60%</td>
<td>29%</td>
</tr>
<tr>
<td>Specificity (SP)</td>
<td>92%</td>
<td>92%</td>
</tr>
</tbody>
</table>

as compared to patient’s interview

• Low CDT serum levels when < 20 years

• High serum CDT with low BMI (< 20 kg/m²)

Conigrave et al. 2002

Questions

Is hair EtG a better indicator than CDT?

What is sensitivity (SE) and specificity (SP) for EtG in hair and CDT in serum as markers of chronic heavy alcohol use?
**Methods**

Healthy volunteers (teetotalers n=5, social drinkers n= 16) and alcoholics at the beginning of an in-patient or an out-patient treatment (n= 65)

Hair samples (n=86, 3-cm proximal segment) for EtG determination

Serum (n=86) for CDT determination by immunonephelometry (n=30) or by HPLC (n=56)

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**Hair EtG and CDT**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Cut-off (EDI 60 g/die)</th>
<th>EDI 2-weeks SE</th>
<th>EDI 2-weeks SP</th>
<th>EDI 3-months SE</th>
<th>EDI 3-months SP</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEtG</td>
<td>27 pg/mg</td>
<td>0.96</td>
<td>0.70</td>
<td>0.98</td>
<td>0.89</td>
</tr>
<tr>
<td>CDT*</td>
<td>2.5%</td>
<td>0.50</td>
<td>0.70</td>
<td>0.51</td>
<td>0.78</td>
</tr>
<tr>
<td>CDT*</td>
<td>2.2%</td>
<td>0.63</td>
<td>0.60</td>
<td>0.62</td>
<td>0.56</td>
</tr>
</tbody>
</table>

* CDT by HPLC.

Morini et al. 2009

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**Hair EtG and CDT: conclusions**

• At detecting an ethanol daily intake of 60 g/day or higher Hair EtG provides equal selectivity of CDT (by both immunonephelometry and HPLC)
  • within the last 2 weeks
  • within the last 3-months

• Hair EtG proved to be about 2 times more sensitive than either of the two CDT methods

Morini et al. 2009
**Consensus of the Society of Hair Testing on hair testing for chronic excessive alcohol consumption**

1. Alcohol is a legal compound in many countries and is consumed in much higher amounts in comparison to other drugs of abuse and by a much larger portion of the population compared to other substances, and detection of chronic excessive alcohol consumption by hair analysis has some specific characteristics.

2. Consistent with the World Health Organization and a broader survey, chronic excessive alcohol drinking corresponds to a consumption higher than 60 g per week on a daily basis during the previous month.

3. For clinical and forensic purposes, there is a need to establish chronic excessive alcohol consumption.

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**Fatty acid ethyl esters FAEE**


Gas chromatography tandem mass spectrometry for biomarkers of alcohol abuse in human hair

Analysis of fatty acid ethyl esters in hair as possible markers of chronically elevated alcohol consumption by headspace solid phase microextraction (HS-SPME) and gas chromatography mass spectrometry (GC-MS).

P. Pfeifer, V. Immervoll, E. Spodnick, K. Spiegel

Analytical Chemistry Branch, National Institute on Alcohol Abuse, Alcoholism & Drug Abuse, National Institute of Mental Health, Bethesda, MD, USA.
Is it possible to determine hair FAEE by gas chromatography – single quadrupole mass spectrometry with respect to analytical selectivity and sensitivity?

Study design

- 60 volunteers (teetotallers, social, and heavy drinkers)
- Hair strand
- Questionnaire:
  - Alcohol daily consumption habits
  - Pathological conditions and pharmaceuticals continuously used
  - Hair treatments

Politi et al. 2011
Sample preparation

- Double washing in MeOH of hair, 3-cm proximal segment
- Finely scissor cut
- Addition of internal standard (alpha-colestan, 50 ng)
- Overnight incubation with 0.5 ml DMSO + 4 ml n-hexane

Politi et al. 2011

Sample preparation

Incubation medium on aminopropyl SPE cartridges:

- cartridges conditioned with 3 ml CH₂Cl₂ + 3 ml n-hexane
- n-hexane load
- elution of FAEE with 3 ml n-hexane + 3 ml CH₂Cl₂
- n-hexane and CH₂Cl₂ evaporated to dryness
- re-constitution in n-hexane (50 µl)

Politi et al. 2011

GC-MS

- Agilent 7890A/5975C Inert MSD with autosampler 7683B
- Capillary fused silica column HP-5MS (30 m x 0.25 mm x 0.25 µm methyl silicone 5% diphenyl silicone)
- Splitless mode injection - Helium 1 ml/min
- Injector: 300°C
- T at 100°C for 2 min; 12°C/min up to 200°C; 8°C/min up to 300°C, final isotherm for 3 min
- Interface: 300°C

Politi et al. 2011

GC-MS

- Ethyl Linolenate
- Ethyl Linoelate
- Ethyl Arachidonate
- Methyl Stearate
- ...
Validation

• Specificity (non significant FAEE)
• Linearity: 0.01; 0.05; 0.1; 0.2; 0.5 ng/mg
• LLOQ: 0.01 ng/mg
• Accuracy and precision
• Recovery

Ethanol daily consumption and FAEE

• Questionnaire
  • Ethanol daily consumption: 0 – 246 g/day
  • median: 13 g/day

• SPE-GC-MS analysis
  • FAEE tot: 0.02 – 10.78 ng/mg
  • median: 0.66 ng/mg

Ethanol intake and FAEE: Results

<table>
<thead>
<tr>
<th></th>
<th>FAEE &lt; 0.5 ng/mg</th>
<th>FAEE &gt; 0.5 ng/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ETOH &lt; 60g/day</td>
<td>ETOH &gt; 60g/day</td>
</tr>
<tr>
<td>All samples (n=60)</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>Excluding lotions (n=49)</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>Excluding lotions, hormonal treatments (n=40)</td>
<td>19</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>SE</th>
<th>SP</th>
</tr>
</thead>
<tbody>
<tr>
<td>All samples</td>
<td>100%</td>
<td>40%</td>
</tr>
<tr>
<td>Excluding lotsions</td>
<td>100%</td>
<td>52%</td>
</tr>
<tr>
<td>Excluding lotions, hormonal treatments</td>
<td>100%</td>
<td>70%</td>
</tr>
</tbody>
</table>

- False negatives: 0 → Sensitivity 100%
- True negatives: 19
- False positives: 28
- True positives: 13

11 EtOH-containing trichological lotions
8 hormonal based contraceptives
Ethanol intake and FAEE: Results

• False positives: 9 → Specificity 70%

• Tentative reasons:
  • Individual genetic variability in FAEE formation?
  • Alcohol use not reported in questionnaire?
  • Not reported hair lotion use?
  • ...?

Method – Alcohology Unit

• 53 volunteers upon admission to an Alcohology Unit treatment
• Hair strand for FAEE analysis, 3-cm prox
• Alcohol daily consumption habits estimated by the Alcohology Unit operator during anamnesis

Results – Alcohology Unit

Hair FAEE: conclusions

• GC-MS is a valid technique for hair FAEE analysis
• Factor to be taken into account:
  • Use of trichological lotions
  • Use of hormonal contraceptives ?
  • Binge drinking ?
FINAL CONSIDERATIONS

• When hair analysis is aimed at forensic purposes, always:
  • VALIDATION
  • IDENTIFICATION
  • INTERPRETATION

FINAL CONSIDERATIONS

• Refer to specific Guidelines:

• Proficiency Tests

Thank you!