Biomarkers of substance use disorder: A review and update

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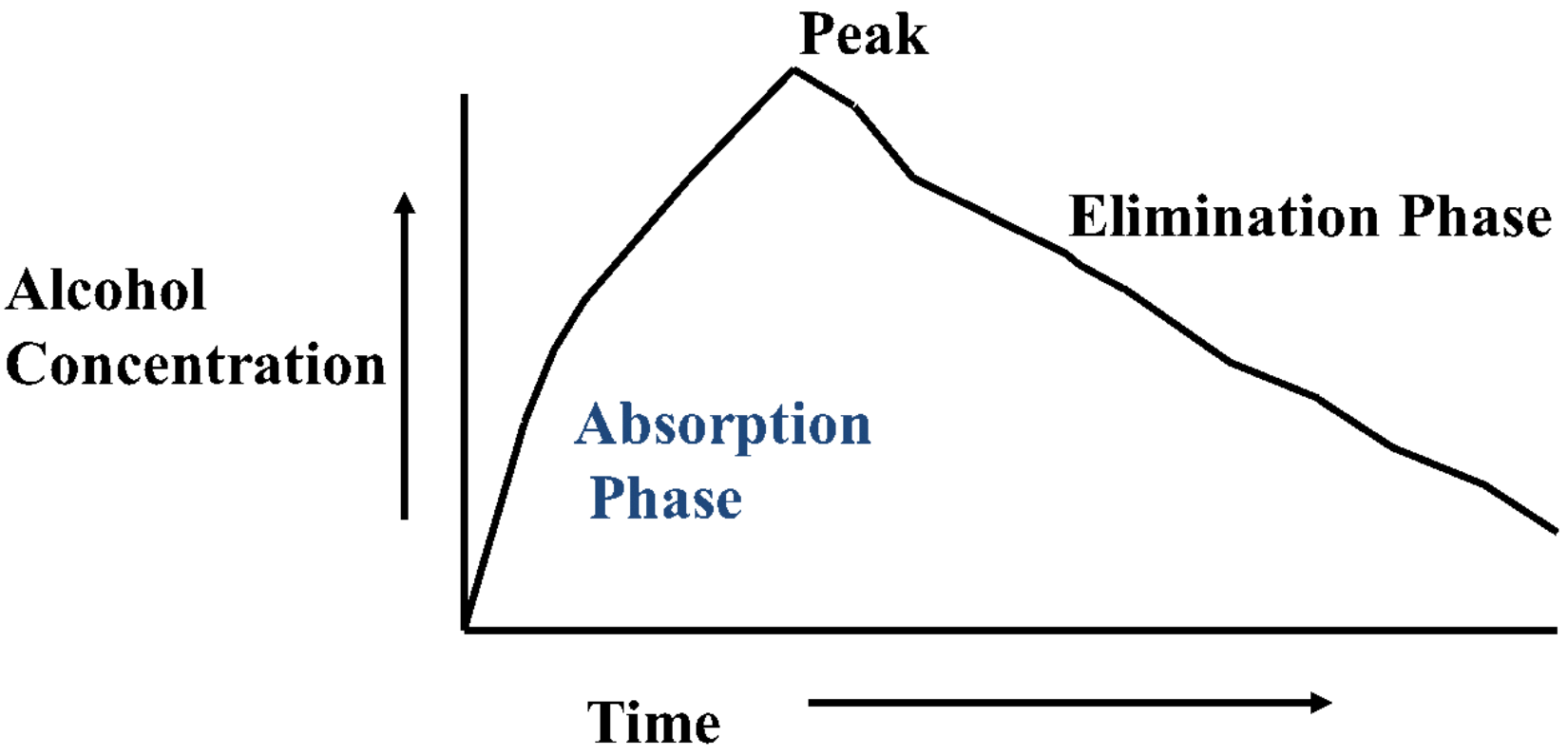
Introduction

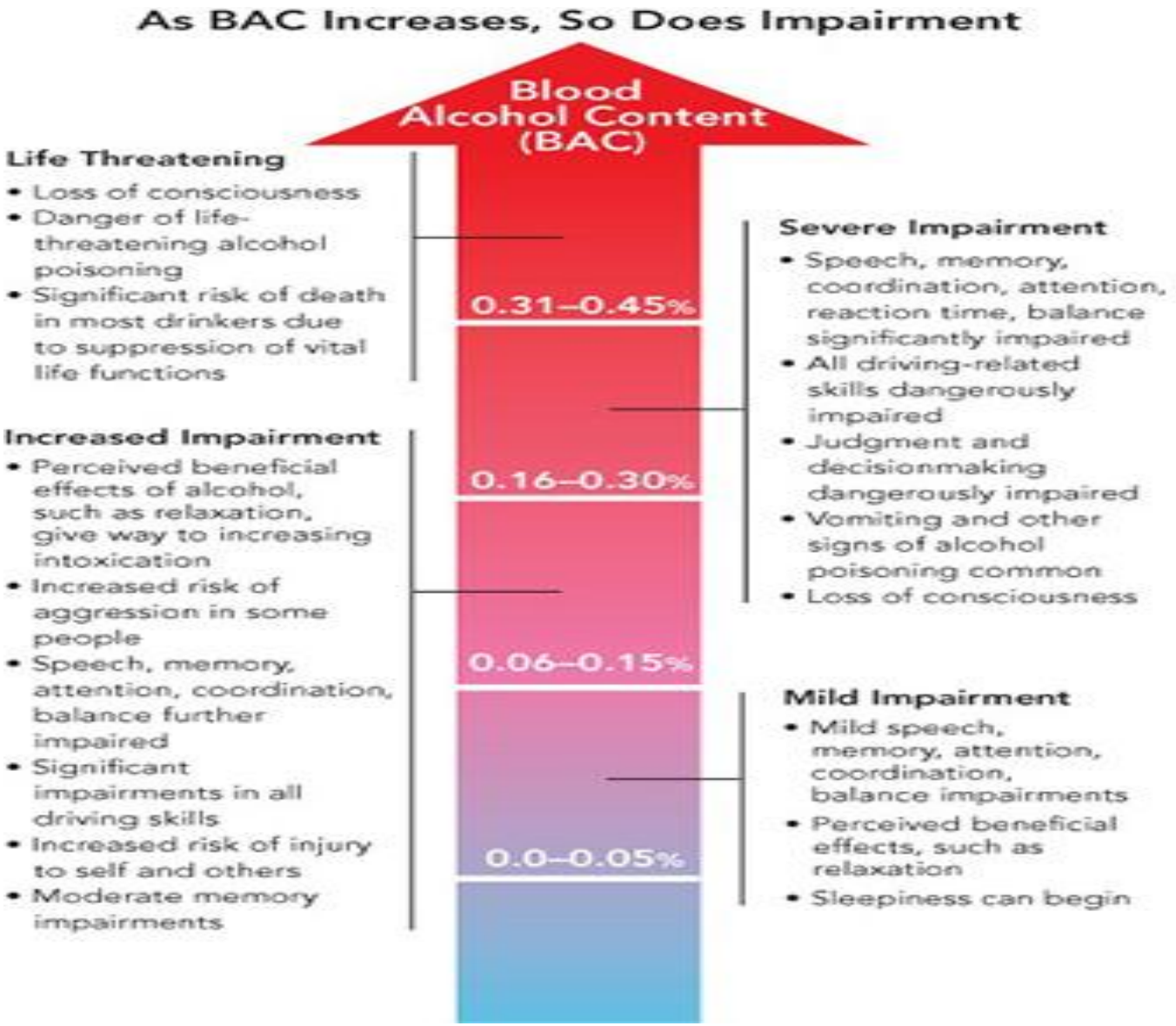
- Substance Use Disorder (SUD) refers to a range of medical condition involves heavy or frequent substance use even when it causes mental and physical problems.
- The use of sensitive and specific biomarkers to diagnose SUD is necessary due to the growing concern of this problem around the world.

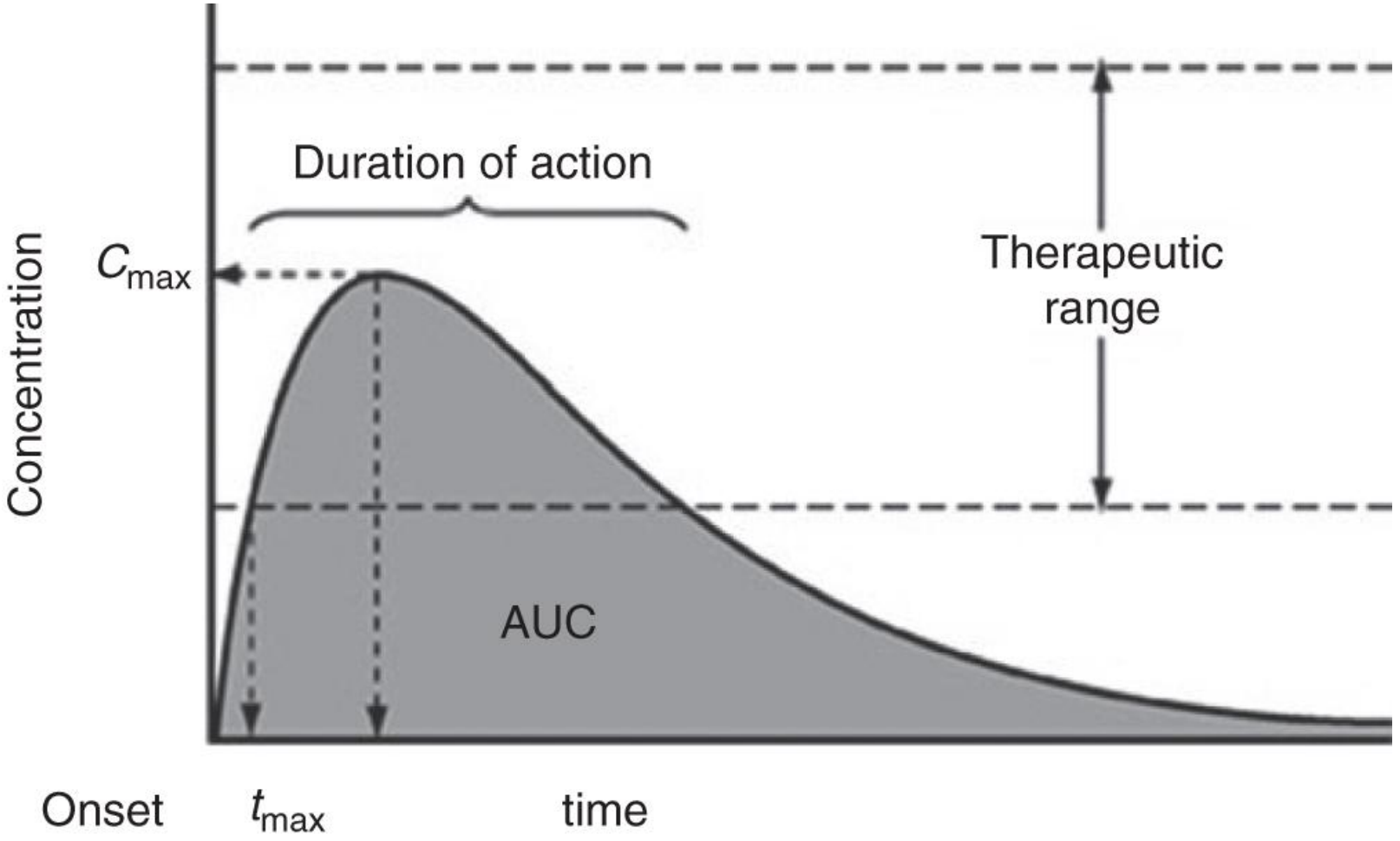
Biomarkers of Alcohol Use

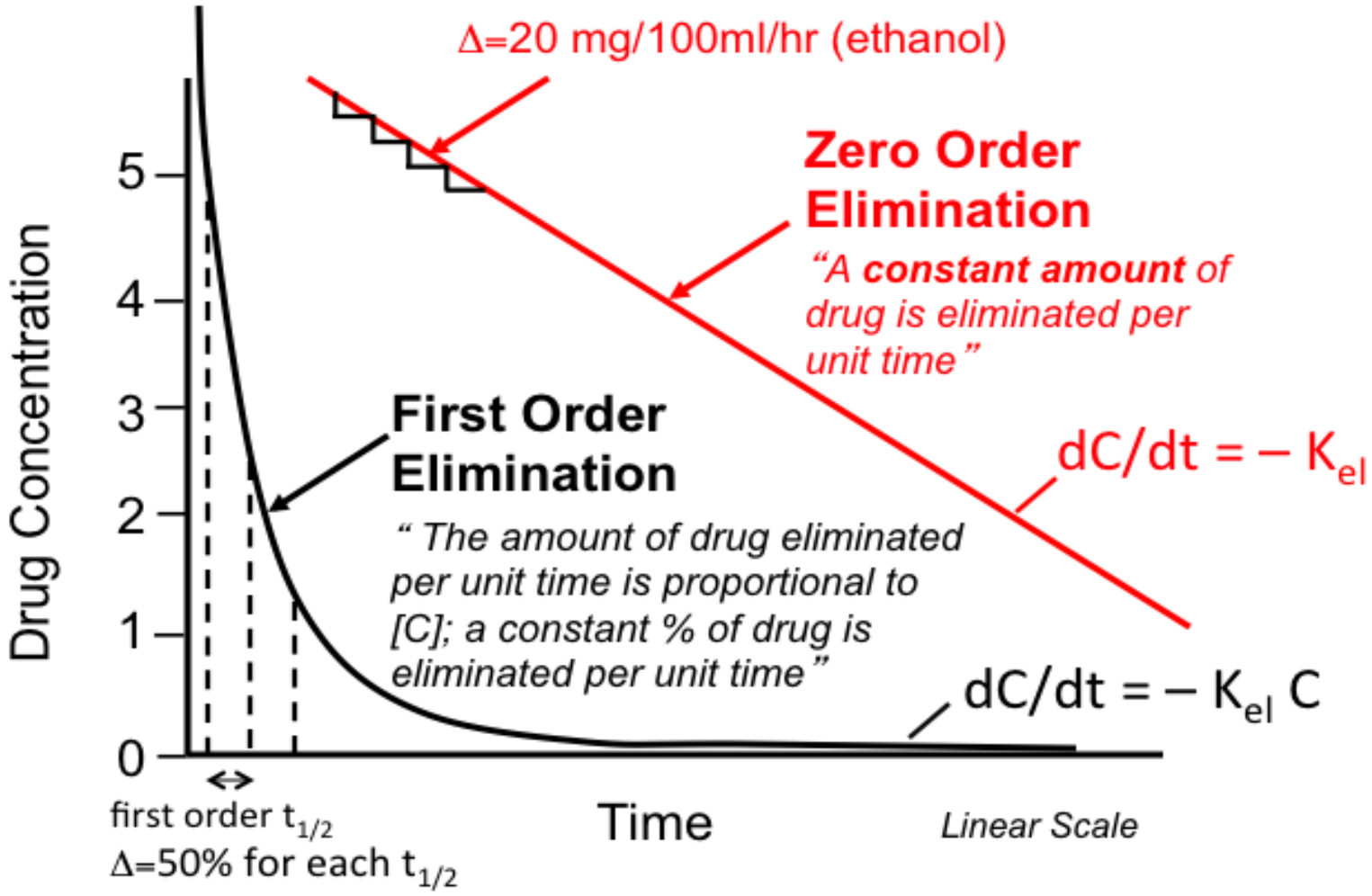
- **Direct Markers:**
- Blood ethanol level (BAC)
- Ethyl glucuronide (EtG) and ethyl sulfate levels (in blood, serum, urine, hair and nails)
- Concentration ratio of 5-hydroxytryptofol to 5-hydroxy-indole acetic acid (5 -HTOL/ 5-HIAA) (in urine)
- Phosphatidyl ethanol content (in blood)
- Concentration of fatty acid ethyl esters (FAEE)(in tissue)
- **Indirect Markers**:
- DNA methylation in peripheral blood monocytes
- Blood cortisol level
- Liver Enzymes (GGT, AST, ALT)
- Carbohydrate-deficient transferrin (CDT)
- Mean corpuscular volume (MCV)

Blood Alcohol Concentration (BAC)

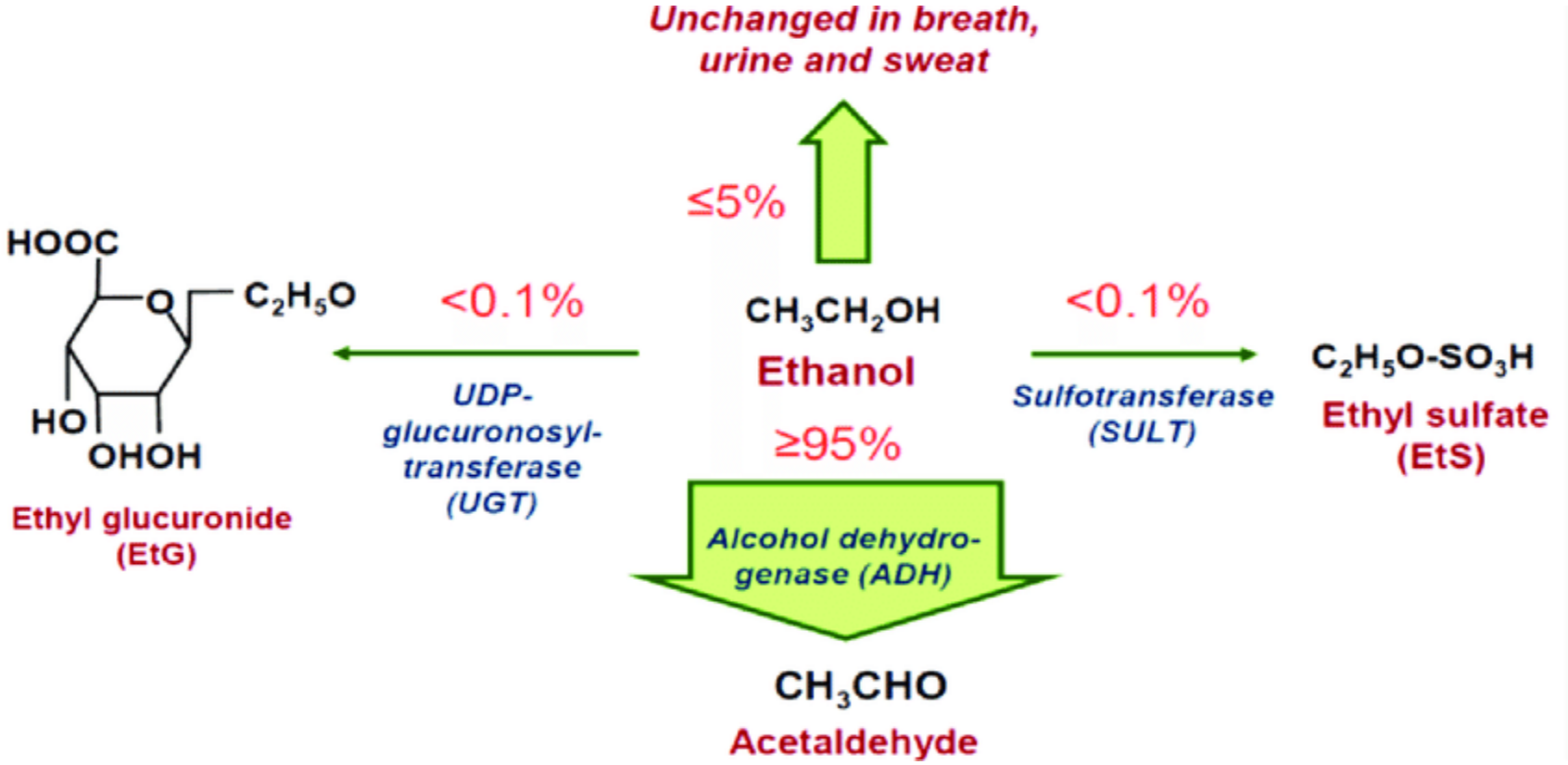


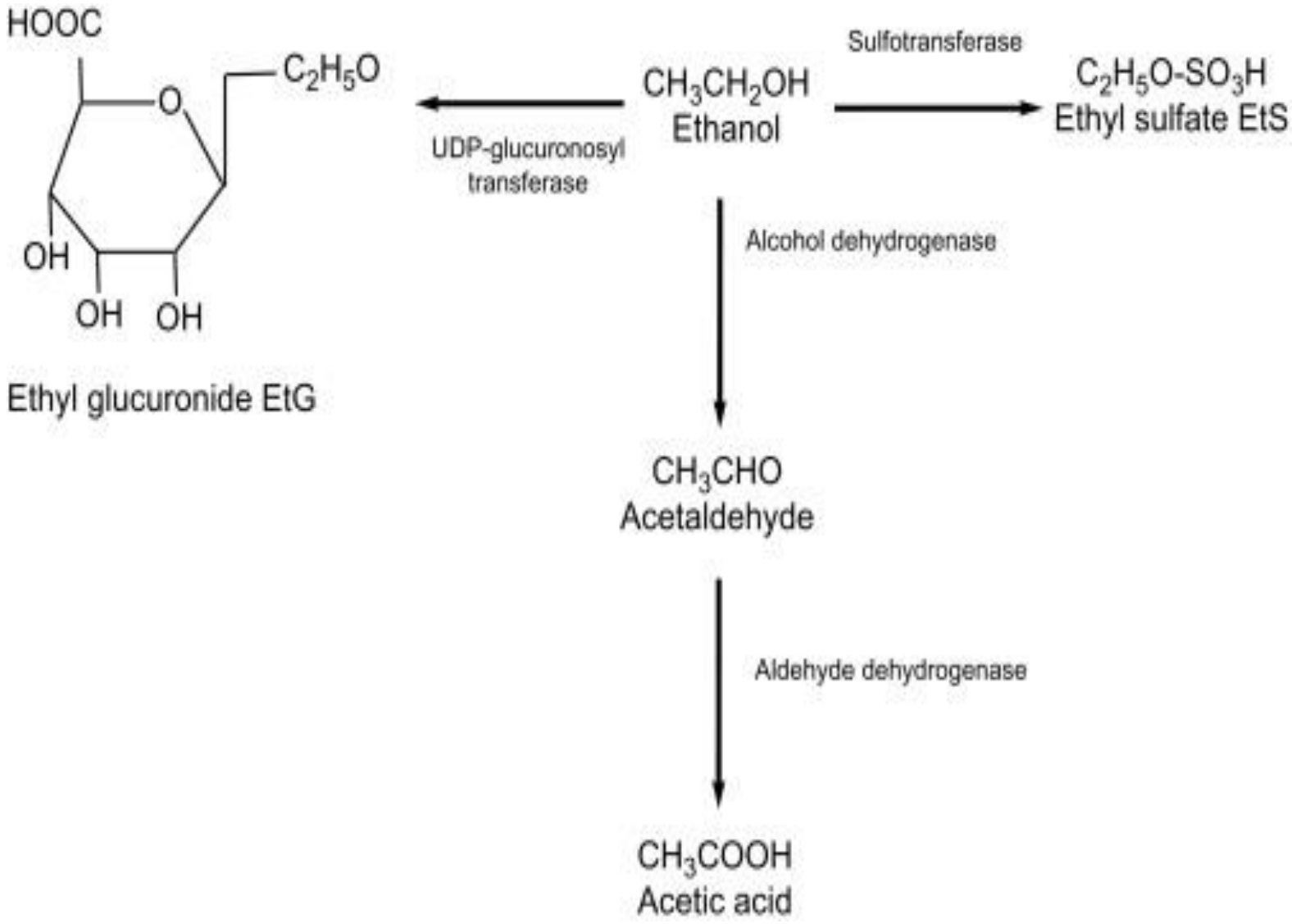

As BAC Increases, So Does Impairment
Blood Alcohol Content (BAC)
Life Threatening
• Loss of consciousness
• Danger of life-threatening alcohol poisoning
• Significant risk of death in most drinkers due to suppression of vital life functions
Increased Impairment
• Perceived beneficial effects of alcohol, such as relaxation, give way to increasing intoxication
• Increased risk of aggression in some people
• Speech, memory, attention, coordination, balance further impaired
• Significant impairments in all driving skills
• Increased risk of injury to self and others
• Moderate memory impairments
0.31–0.45%
0.16–0.30%
0.06–0.15%
0.0–0.05%
Severe Impairment
• Speech, memory, coordination, attention, reaction time, balance significantly impaired
• All driving-related skills dangerously impaired
• Judgment and decisionmaking dangerously impaired
• Vomiting and other signs of alcohol poisoning common
• Loss of consciousness
Mild Impairment
• Mild speech, memory, attention, coordination, balance impairments
• Perceived beneficial effects, such as relaxation
• Sleepiness can begin


Duration of action
Therapeutic range
Concentration
C_{max}
AUC
Onset
t_{max}
time


Δ=20 mg/100ml/hr (ethanol)
Zero Order Elimination
"A constant amount of drug is eliminated per unit time"
First Order Elimination
" The amount of drug eliminated per unit time is proportional to [C]; a constant % of drug is eliminated per unit time"
dC/dt = – K_el
dC/dt = – K_el C
Drug Concentration
5
4
3
2
1
0
first order t1/2
Δ=50% for each t1/2
Time
Linear Scale

Ethyl glucuronide (EtG)




HOOC
C_2H_5O
O
OH
OH
OH
Ethyl glucuronide EtG
UDP-glucuronosyl transferase
CH_3CH_2OH
Ethanol
Sulfotransferase
C_2H_5O-SO_3H
Ethyl sulfate EtS
Alcohol dehydrogenase
CH_3CHO
Acetaldehyde
Aldehyde dehydrogenase
CH_3COOH
Acetic acid

- Apart from oxidative metabolism, the phase II metabolite ethyl glucuronide (EtG; 0.02–0.06% of the ingested alcohol) and ethyl sulfate (EtS; 0.010–0.016%) are created from alcohol to a minor extent.
- They can be detected in blood not long after alcohol consumption (less than 45 min.).

- The time during which EtG can be detected in **serum** is by up to 8 hours longer compared to ethanol. EtS can be detected in serum about twice as long as ethanol.
- EtG can be detected in **urine** for up to about 24 hours even after consumption of small quantities; after excessive consumption, the window of detection is up to 130 hours (Average: 90 hours).
- Sensitivity is dependent on alcohol quantity, time interval between sample collection and alcohol intake as well as the cut-off level of the method applied.

- EtG accumulates in **hair** and is proposed as a stable marker for the detection of chronic and excessive alcohol consumption above a cut-off level of 30pg/mg hair. A correlation between drinking behavior and EtG hair concentrations is observed, but large variability exists.
- EtG to indicate alcohol consumption over the previous 90 days, or ~3 months as is the normal practice in **hair** analysis. The results confirm that **fingernails** can be a useful alternative matrix where hair samples are not available.

Measuring EtG in hair offers two advantages:

- The analysis of a proximal 3–6 cm long hair segment allows to retrospectively evaluate a period of several months.
- Short-term reduction in alcohol consumption has no effect on test results.

- Based on internationally adopted cut-off concentrations, abstinence from alcohol can be verified (EtG in hair 30 pg/mg).
- Concentrations between 7 and 30 pg EtG/mg hair are regarded as a strong indicator of regular alcohol consumption.

- Since the levels in urine are dependent on diuresis, the intake of larger volume of water results in a steep decrease in EtG and EtS urine levels.
- This may lead to false-negative test results. Therefore, it is important to interpret EtS and EtG levels based on the urine creatinine levels or to state at least a minimum requirement—usually >20 mg/dL

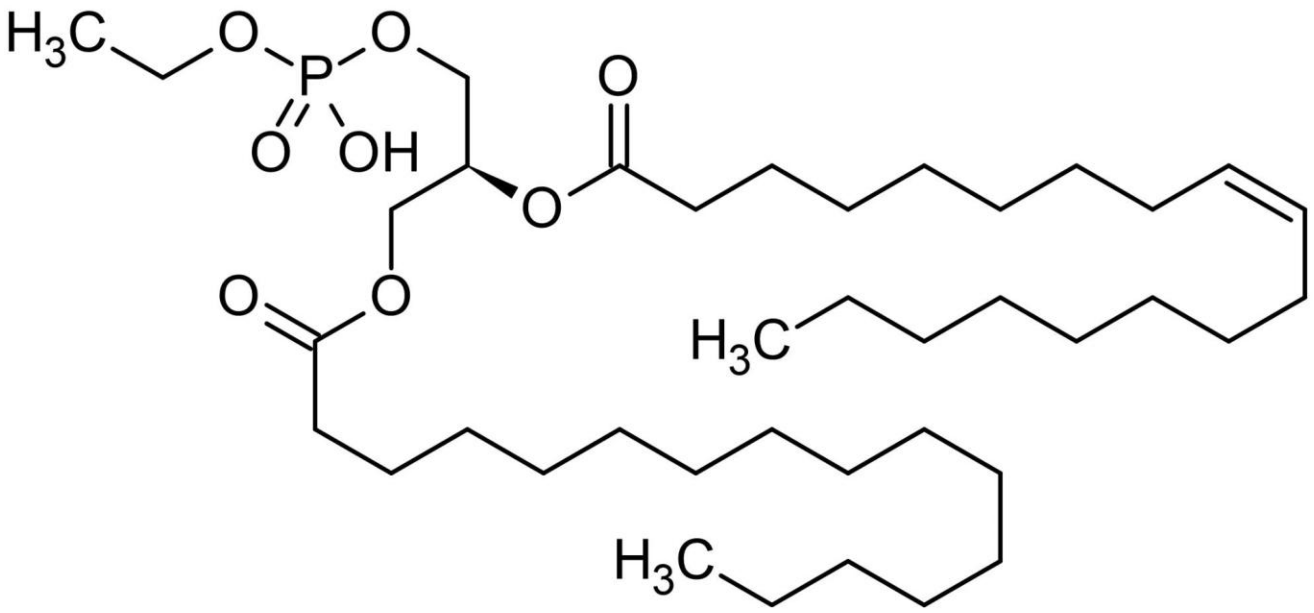
Disadvantage

- However, the disadvantage of the very high sensitivity of this method is that the EtG/EtS levels in urine do not allow to distinguish between a binge drinking event several days ago and a (potentially unintended) minor alcohol intake a few hours before the sample was taken.

Analytical Methods for EtG assay

- GC-MS
- LC-MS-MS

Phosphatidylethanol (PEth)



- PEth is an abnormal phospholipid which is produced after alcohol exposure in cell membranes of, for example, human erythrocytes.
- By measuring PEth levels, daily alcohol consumption of more than 60 g ethanol can clearly be distinguished from lower alcohol consumption.
- Thus, PEth can be used to identify individuals with chronic excessive drinking behavior. Currently, no cut-offs have been officially set, but several pertinent recommendations exist.

- PEth is a recently introduced biomarker with high specificity, high sensitivity, and response correlating with alcohol consumption.
- The PEth results indicate that the participants had, on average, under-reported their alcohol consumption several-fold. The findings suggest that PEth in blood has a sufficient long-term stability for use as an alcohol biomarker in prospective case-control studies.

- A study showed that the PEth concentration correlated well with past weeks alcohol intake, albeit with a large inter-individual scatter. This indicates that it is possible to make only approximate estimates of drinking based on a single PEth value, implying risk for misclassification between moderate and heavy drinking.

- Helander et al (2019). Dose-Response Characteristics of the Alcohol Biomarker Phosphatidylethanol (PEth)-A Study of Outpatients in Treatment for Reduced Drinking. Alcohol Alcohol. 54(6):567-573.

- Even though the preanalysis and analysis of PEth is complex, this biomarker can now be determined in routine analysis, thanks to the use of dried blood samples, the availability of deuterated standards and of modern analytic technologies. Since PEth can detect chronic and one-time alcohol consumption, this biomarker is well suited to monitor abstinence and drinking behavior and to identify relapse.

- PEth is not a single molecule but a group of glycero - phospholipids with fatty acid groups of various lengths with various degrees of saturation.
- So far, 48 PEth species have been identified in human blood samples.
- The PEth homolog 16:0/18:1 is currently the species most commonly determined in analyses. It accounts for the largest proportion of the PEth homologs produced after alcohol intake and is often simply referred to as PEth.
- In drinking experiments, PEth was detected in blood already after 30 minutes; PEth levels peaked after 90 to 120 minutes. With frequent alcohol consumption, PEth accumulates in whole blood.

- Since PEth can already be detected in blood after about 1 to 2 hours and for up to 12 days after one-time alcohol intake, this biomarker can be used to determine both current consumption and abstinence.

Phosphatidylethanol analysis

- For the analysis of PEth, whole blood samples are generally used; tissue homogenates are almost exclusively used in research.
- Whole-blood samples should preferentially be stored in tubes with added EDTA, heparin or (less common) fluoride/oxalate and should not be centrifuged. Blood samples can be stored at room temperature for up to 24 hours and at 4 °C for up to 3 weeks; for longer periods, a storage temperature of –80 °C is required.
- Alternatively, dried blood spots (DBS) can be prepared. Over a very broad range of concentrations, DBS analysis yielded results matching those obtained with native blood samples of corresponding concentrations.

Extraction of PEth

- PEth is typically isolated from blood samples by a multi-step extraction process involving the use of 2-propanol, followed by n-hexane or n-heptane after the addition of an internal standard.
- As the internal standard, phosphatidylpropanol or phosphatidylbutanol was initially used.
- Recently, deuterated compounds (PEth-d5, PEth-d31) have become commercially available.

Analytical Methods

- GC-MS
- Enzyme-linked immunosorbent assay (ELISA) test kit, using serum
- Capillary electrophoretic (CE) separation with ultraviolet (UV) detection
- LC-MS(MS) technologies can ionize and detect all PEth homologs ("PEth species"), using electrospray ionization in negative ion mode.

Fatty acid ethyl esters

- Fatty acid ethyl esters (FAEEs) are produced in the presence of ethanol from, for example, triglycerides or free fatty acids by specific FAEE synthases and other enzymes.
- These products of nonoxidative ethanol metabolism can be detected in blood, tissue and also in hair.
- Besides EtG, FAEE concentrations can be measured in hair as a plausibility control, but alone they are not suitable to verify abstinence.

FAEEs Chemical Structure

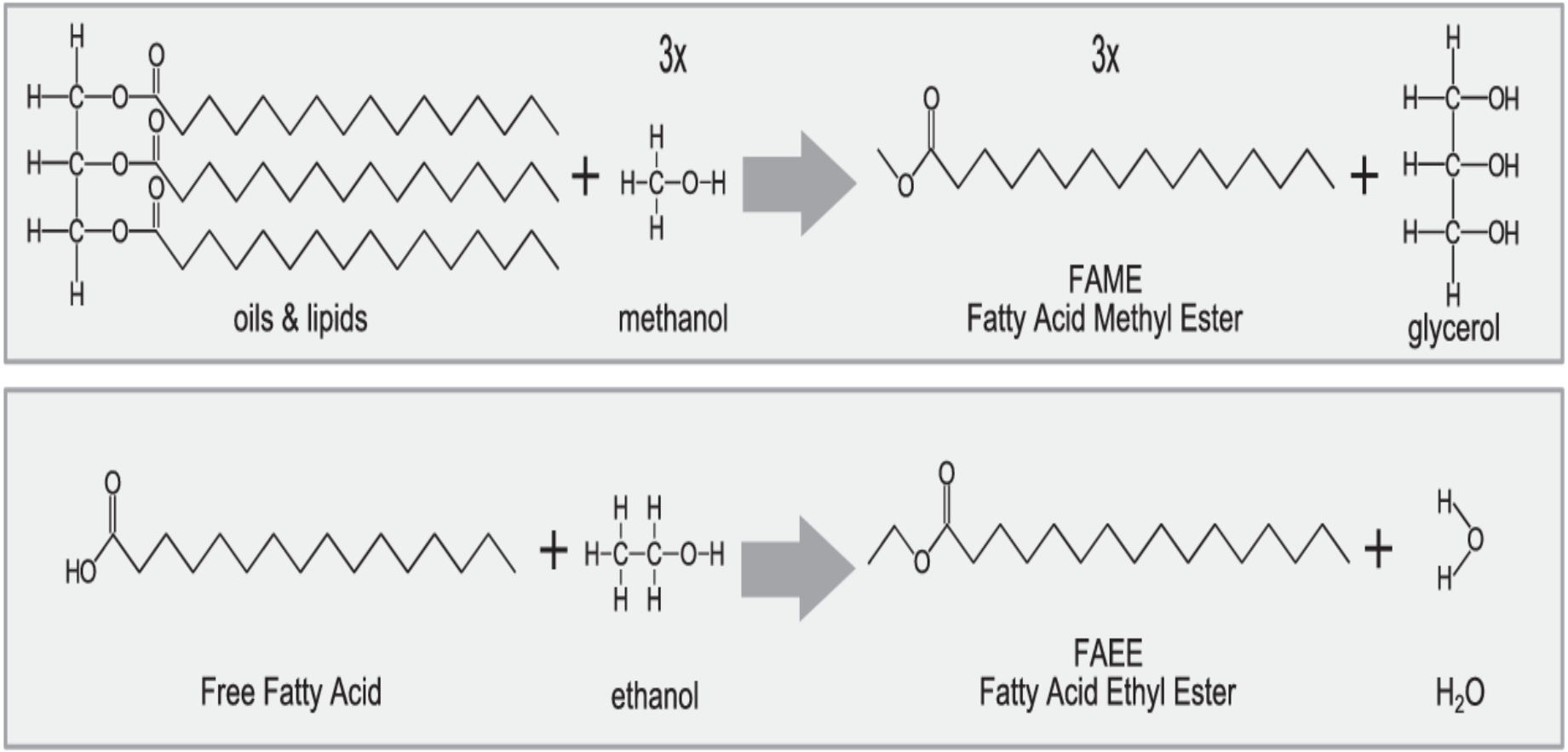
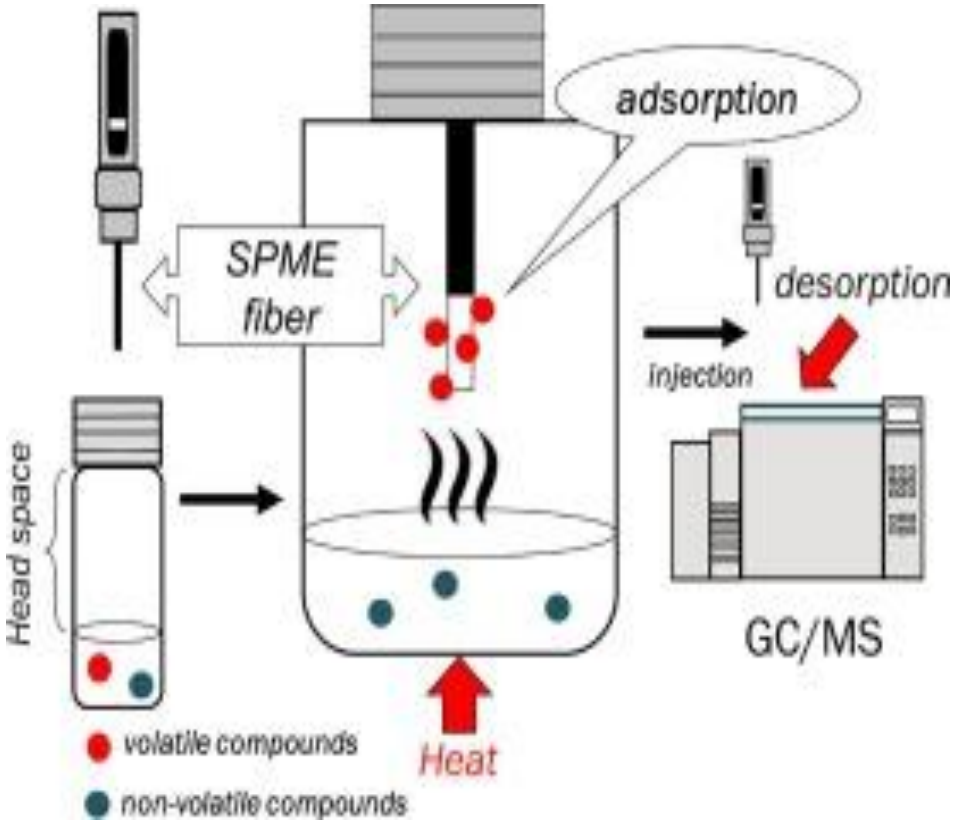


Figure 1.2: Chemical reaction of FAMEs from oils and lipids and biochemical conversion of FAEEs from free

- For both EtG and FAEEs in hair it applies that occasional alcohol consumption is not ruled out by a negative result. Consequently, alleged abstinence cannot be verified, but at best disproved.
- It should be noted that 3 to maximal 6 cm long proximal hair segments are recommended as samples. If this is not the case, quantitative results should be interpreted with great caution.
- Cosmetic hair treatment (tinting, coloring, bleaching, perming, straightening) can significantly reduce the concentrations of the analyte. Ethanol-containing hair care products have no effect on EtG, but may lead to false-positive FAEE results

Analytical Method for FAEEs in hair

- HS-SPME-GC-MS



Novel Molecular Biomarkers for Alcohol Consumption/Use

- DNA methylation/ DNA adduct may provide novel biomarkers of alcohol use.
- Methylation of cytosine-phosphate-guanine dinucleotide (CpG) sites in relation to alcohol intake from monocyte-derived DNA samples for current heavy alcohol intake (⩾42 g per day in men and ⩾28 g per day in women).
- In conclusion, a robust alcohol-related DNA methylation signature and shown the potential utility of DNA methylation as a clinically useful diagnostic test to detect current heavy alcohol consumption.
- Lin et al. (2018). A DNA methylation biomarker of alcohol consumption. Mol Psychiatry. 23(2):422-433.

Specificity and sensitivity of alcohol biomarkers in relation to the reported amount drunk

Parameter	Sensitivity	Specificity	Amount drunk	References
MeOH	70%	98%	> 0.5 ‰ for several hours	(8)
CDT	46–90%	70–100%	Chronic excessive drinking	(15) (e2) (14)
GGT	37–95%	18–93%	Chronic excessive drinking	(15)
AST	25–60%	47–68%	Chronic excessive drinking	(15)
ALT	15–40%	50–57%	Chronic excessive drinking	(15)
MCV	40–50%	80–90%	Chronic excessive drinking	(15)
CDT, MCV and GGT in combination	88%	95%	Chronic excessive drinking	(9)
EtG in urine	100%	NS	1.2 g/L BAK after 24 h (Cut-off 100 ng/mL)	(20)
	50 and. 100%, resp.	NS	0.2 g/L BAK after 24 h / 12 h (cut-off 100 ng/mL)	(20)
	89%	99%	Abstinence monitoring	(9)
EtG in hair	75%	96%	Chronic excessive drinking (cut-off 30 pg/mg)	(36)
FAEE in hair	90–97%	75–90%	Chronic excessive drinking (dependent on cut-off)	(33) (34)
PEth	88–100%	48–89%	see *eTable*	see *eTable*

Substance Use/Abuse/ Addiction Biomarkers

- **Metabolomics studies:**
- ***Metabolomics*** defined as "The comprehensive quantitative analysis of all the metabolites of an organism or specified biological sample".
- Metabolomics has evolved highly over the last years and is now described as the study of metabolites using advanced high throughput analytical approaches and bioinformatics.
- Metabolomics monitor changes in small molecules (<1500 Da) with modifications appearing in organisms in response to a specific stimulus

- In contrast to the other “omics sciences”, metabolomics can link both gene and environmental interactions.
- The metabolites present in biological systems, defined as the metabolome, include endogenously derived biochemicals like amino acids, lipids, fatty acids, steroids, carbohydrates, or vitamins.

Applications of Metabolomics in Forensic Toxicology

- In 2011, Shima et al. used a combination of GC-TOF- MS and CE-MS-MS for global and targeted analyses and proposed the endogenous compounds to be considered as potential markers of methamphetamine (MA) intoxication.
- Potential biomarkers related to MA-induced poisonings included 5-oxoproline, saccharic acid, uracil, 3-hydroxybutyrate, adipic acid, glucose, glucose 6-phosphate, fructose 1,6-bisphosphate, and fumarate.
- Saliva samples of healthy volunteers were collected at pre-dose and after smoking herbal components SCs and analyzed by high-resolution mass spectrometry.

- The potential positives based on the analysis of two markers (scopoletin and 2-hydroxyethyl dodecylamine) identified in the herbal blends, whose ratio permitted distinguishing the herbal smokers from non-smokers

- Zeng et al. used GC-MS-based metabolomics to study the effect of 10-days of heroin exposure, followed by a withdrawal of four days in a rat animal model.
- Analysis of the metabolites revealed that heroin administration decreased tryptophan and 5-hydroxytryptamine levels in peripheral serum, but increased tryptophan and 5- hydroxyindoleacetate in urine.
- Withdrawal of heroin for 4 days efficiently restored all metabolites to baseline, except serum myo-inositol-1-phosphate, threonate, and hydroxyproline in the urine.

- **Genomics Studies:**
- In a study, dynorphin and kappa-opioid receptor level in the human blood lymphocytes demonstrated as a possible role as a biomarker in severe opioid use disorder.

Conclusion

- The advantage of the clinica/ forensic use of substance use biomarkers is that the actual substance consumption of a patient can be assessed.
- Finally, it is important to emphasize that substance use biomarkers test results should never be interpreted in isolation, but always in the context of medical history, clinical findings and the patient's mental and physical state of health.