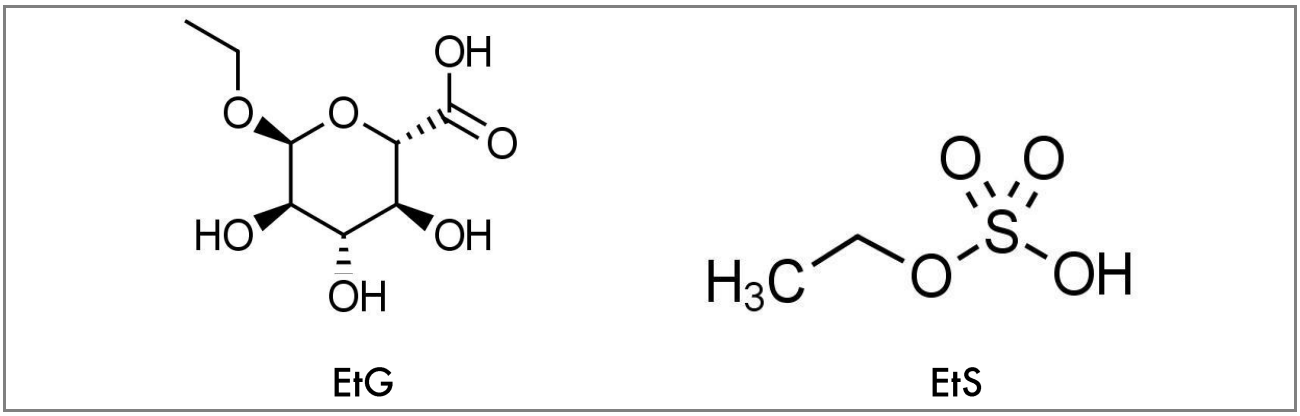
MassChrom® Ethyl Glucuronide and Ethyl Sulfate

Reliable Testing in Urine

What are EtG and EtS?

Ethyl glucuronide (EtG) and etyhl sulfate (EtS) are alcohol metabolites in the human body present after alcohol consumption. More than 90 % of the alcohol is metabolised in an oxidative degradation process to acetaldehyde and acetate and finally into CO_2 and water. However, a small fraction of ethanol (EtOH) undergoes a non-oxidative degradation into glucurono- and sulfo-conjugated EtG and EtS.

As serum EtOH reveals only recent alcohol intake within hours - in saliva, breath or blood < 12 hours and in urine a few hours longer[1] - attention has focused on EtG and EtS as biomarkers that are detectable for longer time frames. This means that the analysis of EtG offers a sensitive test of recent drinking, which is why it is being applied to medical-psychological assessments for example in Germany to check abstinence compliance of past drink drivers. The usefulness has also been demonstrated in routine post-mortem toxicology when ante-mortem drinking and alcohol-related deaths are investigated. Because EtOH is metabolised in the liver until the time of death, the autopsy urine alcohol concentrations could be negative, however, EtG-positive testing can reveal that the deceased had consumed alcohol in the immediate ante-mortem period[2].



EtG EtS

Why not CDT or direct alcohol analysis?

The EtOH metabolites EtG and EtS have a longer detection time frame than the parent compound. Depending on the amount of alcohol consumed, EtG is detectable up to 70 h after the elimination of EtOH itself from blood[3].

In contrast, carbohydrate-deficient transferrin (CDT) is only sensitive for alcohol consumption with at least 1000 g of alcohol within the last 14 days. It therefore provides an indication of the cumulative amounts of alcohol consumed only, thus short-term relapses into drinking may not be detectable.

EtG is therefore a specific marker for alcohol consumption that can be detected for an extended time period after the complete elimination of EtOH from the body. Thus, EtG and EtS close the gap between short-term markers (i.e. EtOH itself) and long-term markers (CDT).

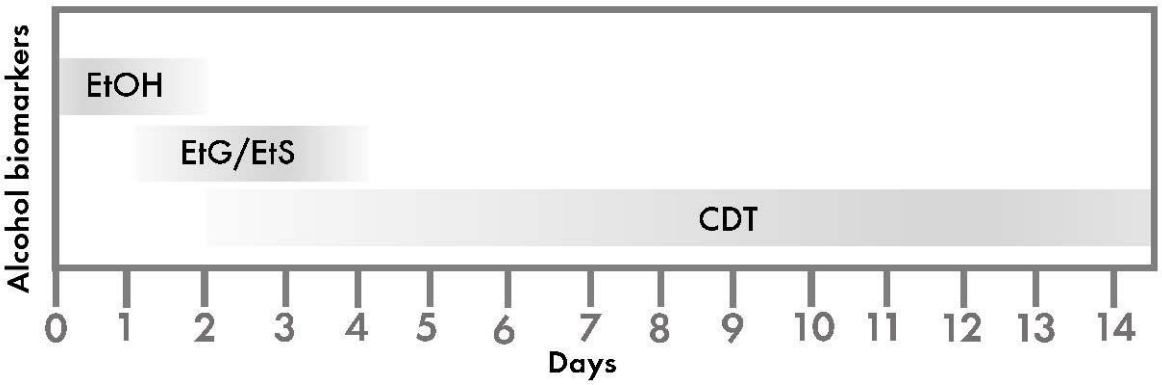


Figure 1. Schematic illustration of the timeframe for several alcohol biomarkers (0 to 14 days).
EtG and EtS are closing the diagnostic gap between the determination of alcohol (EtOH) itself and the long-term marker CDT (about 2 - 3 weeks; sensitivity < 60 g alcohol/day is limited). In Germany, the determination of EtG in urine is already standard for monitoring abstinence to specify driver fitness.

Reliability of EtG and EtS in Urine

Studies have shown a significantly improved verification of abstinence through urinary EtG in patients participating in long-term alcohol dependence treatment programmes[4]. In addition to EtG, recent scientific studies have identified EtS as a second specific metabolite or biomarker of EtOH. It is known, that EtG and EtS are also detectable in hairs, but this matrix is described as inhomogeneous which could result in large deviations[5]. The specificity of EtG and EtS analysis exceeds that of all other known ethanol markers[6]. The detection of both, EtG and EtS, offers greater sensitivity and accuracy for determination of recent alcohol consumption than by measuring either biomarkers alone[7].

Why using mass spectrometry?

Available methods for EtG and EtS include enzyme immunoassays, GC-MS and LC-MS/MS. Many assays show an LOD of 0.1 mg/l or higher, which can be a problem for assays with higher dilutions of urine samples for example. Major differences exist with regard to the extraction procedure applied. In contrast to other methods, LC-MS/MS combines its high sensitivity, similar to GC, with a fast and simple sample preparation procedure. LC-MS/MS can therefore be considered as the most efficient, sensitive and accurate method available that can be applied to any laboratory with an MS instrument with sufficient sensitivity.

Why using *MassChrom*® EtG/EtS kit?

- Complete kit with a highly reliable and robust method in the clinical diagnostics routine - from the sample preparation to the final result
- Comprehensive validation of the assay that has been performed in accordance with the guidelines of the German Society of Toxicology and Forensic Chemistry (GTFCh)[8].
- Easy and efficient sample prep procedure
- One single run for both analytes with a fast run time of 6 minutes
- Support supplied as needed for installation and troubleshooting

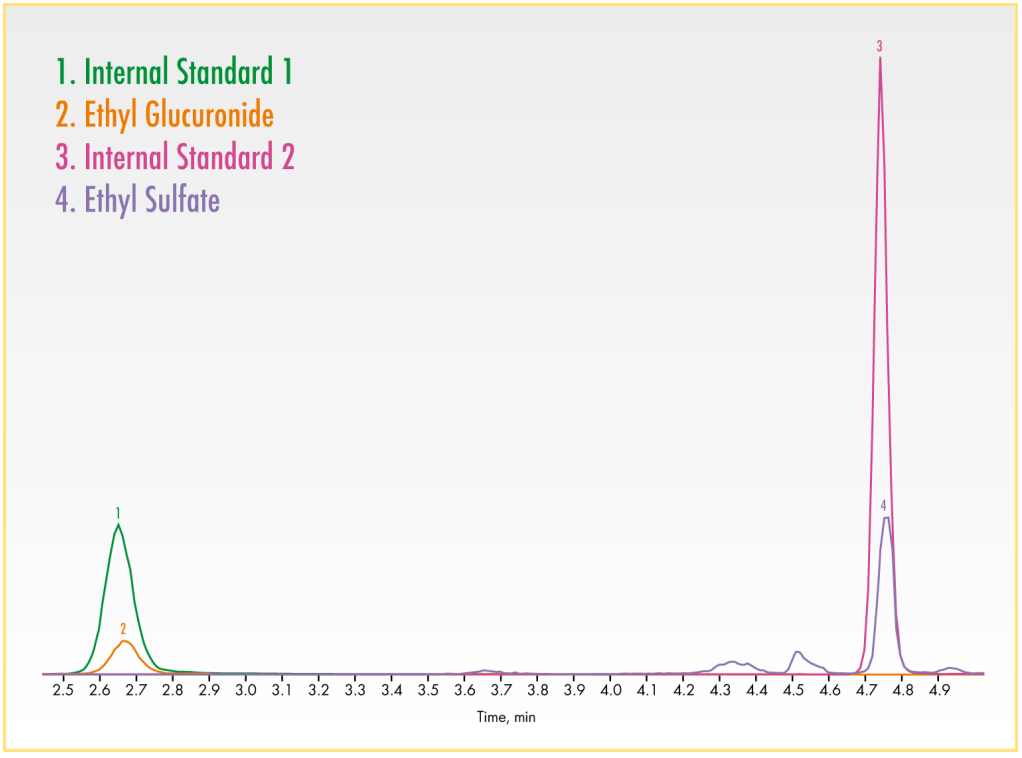


Figure 2. Chromatogram for the determination of EtG and EtS.
The determination of EtG and EtS takes places within 6 min. The chromatogram shows a positive patient sample after alcohol intake.

References

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[6]Wurst FM, Kempter C, Seidl S, Alt A. (1999) Ethyl glucuronide–a marker of alcohol consumption and a relapse marker with clinical and forensic implications. *Alcohol Alcohol* **34**(1): 71-7.

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[8]Peters FT, Hartung M, Herbold M, Schmitt G, Daldrup T, Mußhoff F. (2009) Anhang B zur Richtlinie der GTFCh zur Qualitätssicherung bei forensisch-toxikologischen Untersuchungen: Anforderungen an die Validierung von Analysenmethoden. *Toxichem Krimtech* **76**(3):185–208.

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