After alcohol ingestion, the bulk of ethanol ingested (≥95%) is rapidly eliminated in the liver in a two-stage oxidation process: first to acetaldehyde by alcohol dehydrogenase and then to acetic acid by aldehyde dehydrogenase. The remainder is excreted mainly unchanged in urine and expired air. However, another small fraction of the ingested ethanol dose (<0.1%) (1) undergoes a phase II conjugation reaction catalyzed by UDP-glucuronosyltransferase (UGT) to produce ethyl glucuronide (EtG), which is eventually excreted in the urine (2–4). Because EtG has a longer period of elimination than the parent compound, the interest in EtG has largely focused on its use as a sensitive and specific biomarker of recent alcohol intake with clinical and forensic applications (5, 6).

Animal studies have indicated that ethanol may also undergo sulfate conjugation through the action of sulfotransferase to produce ethyl sulfate (EtS) (7–9). After an oral dose of ethanol and injection of 35S-labeled sulfate in rats, EtS was apparently excreted in urine mainly during the first 24 h (10). However, a general limitation in these studies was the lack of reliable methods for unequivocal identification of EtS and precise quantification.

In the present study on humans, we used a sensitive and specific liquid chromatographic-mass spectrometric (LC-MS) method to determine whether EtS is formed after intake of alcohol and is excreted in the urine. Urine samples were collected from a healthy male individual at timed intervals after ingestion of a single dose of ethanol. Urine samples were also selected randomly from those sent to the laboratory for routine detection of recent drinking by measurement of the ratio of 5-hydroxytryptophol to 5-hydroxyindoleacetic acid, a biomarker of recent alcohol intake (11). The urine specimens were stored at −20 °C until analysis. The procedures followed were approved by the ethics committee at the Karolinska University Hospital.

A direct electrospray LC-MS method for urinary EtS was developed from an existing method used for quantitative analysis of EtG (12) by extending the analysis time to ~15 min and monitoring the ion for EtS. Analysis was performed in the negative-ion mode, with selected-ion monitoring of the pseudomolecular ions at m/z 125 for EtS (M, 126.1) and m/z 226 for EtG-d5 (used as internal standard). The EtS concentration of unknown samples was determined from the peak-area ratio of EtS to EtG-d5 by reference to a calibration curve (ethyl sulfaric acid sodium salt was purchased from TCI). The calibration curve was linear (R² = 0.9999; P < 0.0001) up to at least 800 μmol/L (~100 mg/L) EtS, and the limit of detection was ~0.5 μmol/L (signal-to-noise ratio of 3). In four clinical samples containing high EtS concentrations, the identification of EtS in urine was further confirmed by the correct relative abundance of the 34S isotope at m/z 127 (5.3–5.4%; standard, 5.2%). Urinary ethanol was determined by headspace gas chromatography and creatinine by the Jaffe reaction.

The urinary excretion profiles for ethanol, EtS, and EtG in a healthy male (age, 42 years; weight, 75 kg; height, 185 cm) who had ingested a single dose of 0.5 g/kg ethanol over 30 min in a fasting state are shown in Fig. 1. According to the self-report, he had abstained from alcohol for at least 48 h before starting the experiment. EtS and EtG are expressed in relation to creatinine to compensate for variations in urine dilution (1). The ethanol concentration peaked at 2 h and had returned to below the detection limit at 8 h. EtS was not detected in the first urine sample (0 h) but was detected in the second sample, collected at 1 h after intake. EtS showed a time course similar to that for EtG, but with slightly higher concent-

Fig. 1. Concentration–time profiles for urinary ethanol, EtS, and EtG after alcohol ingestion (A), and LC-MS chromatograms for EtS (B and C).

(A), urine samples were collected from a healthy male after ingestion of 0.5 g/kg ethanol over 30 min in a fasting state. EtS and EtG are expressed in relation to creatinine (crea) to compensate for urine dilution. (B), chromatograms showing the peaks for EtS (m/z 125; solid line) and the internal standard EtG-d5 (m/z 226; dashed line) for a clinical urine sample containing ~16 μmol/L EtS. (C), chromatograms showing the peak for EtG-d5 but no EtS for a clinical urine sample collected after abstinence from alcohol for several days.
trations for EtG. The peak values for both compounds were obtained in the 4-h collection, and both compounds were still measurable in the sample collected at 29 h, but not at 32 h.

Among 54 clinical urine samples selected at random from those sent to the laboratory for routine testing of recent alcohol consumption, all 31 samples with a detectable EtG (mean, 427 μmol/L; range, 1.7–3162 μmol/L) were also positive for EtS (mean, 257 μmol/L; range, 1.1–2095 μmol/L), and 2 others were positive only for EtS (0.6 and 2.4 μmol/L, respectively). There was a good correlation between EtS and EtG \((r^2 = 0.839; \ P < 0.0001)\) with somewhat higher mean concentrations for EtG (mean, 427 μmol/L) compared to overall ethanol metabolism in agreement with previous data on the relative importance of EtG with somewhat higher mean concentrations for EtG (mean, 427 μmol/L).

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1 Department of Clinical Chemistry, BovenJ Hospital, Staterijachstraat 1, 1034 CS Amsterdam, The Netherlands; 2 Department of Clinical Chemistry, Medical Center Alkmaar, Alkmaar, The Netherlands; 3 Department of Clinical Chemistry, Elkerliek Hospital, Helmond, The Netherlands; 4 Department of Clinical Chemistry, Gemini Hospital, Den Helder, The Netherlands; 5 Beckman Coulter Netherlands B.V., Mijdrecht, The Netherlands; a author for correspondence: fax 31206346529, e-mail h.huijgen@bovenj.nl

Homocysteine (Hcy) is a sulfur amino acid and a metabolite of the amino acid methionine. Hcy is very reactive because it contains a free sulfhydryl (thiol) group, and it readily oxidizes to form various disulfides. The fraction of free Hcy in plasma is therefore <2% of total plasma Hcy (tHcy) (17).

Increased Hcy has been associated with cardiovascular, cerebrovascular, and peripheral vascular disease (2–4) and is recognized as an independent risk factor. The need for a simple automated assay is therefore increasing, and various analytical methods have become available. Currently the two most widely used techniques are HPLC and immunochemistry.

We implemented a new automated enzymatic method for the measurement of tHcy on two different routine clinical chemistry analyzers and compared these assays with HPLC and the AxSYM immunoassay.

Reagents and calibrators for the enzymatic method