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LC-ESI-MS-MS determination of ethyl glucuronide and ethyl sulfate in serum

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AIMS: To develop a sensitive, selective and fully validated method for the simultaneous determination of two metabolites of ethanol, ethyl glucuronide (EtG) and ethyl sulfate (EtS), in serum.

METHODS: Serum samples (0.5 ml) were deproteinised with acetonitrile (1:1), vortex mixed for 1 min, 1: 10 diluted in deionised water, and ultracentrifuged at 13000 rpm for 10 min. 5 µl of the supernatant were injected in a 4000 QTrap (Applied Biosystems/MSD Sciex) LC-MS-MS system. Isocratic separation at 0.2 ml/min (99% formic acid (0.1%) and 1% acetonitrile) was performed on a C18 column (Chrompack Inertsil ODS-3, 100 × 3 mm × 3 µm). Electrospray negative ionisation was enhanced by post-column addition of acetonitrile (0.1 ml/min).
Two MS-MS reactions together with the surviving ions were monitored for identification: m/z 221 → 75, 221 → 85, and 221 → 221 (EtG), and m/z 125 → 97, 125 → 80, and 125 → 125 (EtS). Pentadeuterated-EtG was used as internal standard (m/z 226 → 75, 226 → 85, and 226 → 226).

RESULTS: Possible interference on EtS determination from ethyl phosphate (EtP), another reported ethanol metabolite isobaric with EtS, was studied and no overlapping in chromatograms was observed (relative retention times were 0.73 and 1.58 for EtP and EtS, respectively). The mean correlation coefficient of the calibration curve from 0.05 to 10 mg/l (6 levels) calculated on 5 different days was better than 0.9998 for both EtG and EtS. The limit of detection (LOD) and the lower limit of quantification (LLOQ) were 0.01 and 0.050 mg/l, respectively, for both compounds. Accuracy (measured as bias) and precision (relative standard deviation), studied at four different quality control levels, were always better than 9%. Matrix effects – investigated by comparing the analytes response in serum and water – were found to be negligible.
Degradation of analytes was found to be negligible in real samples for the first week of storage at -20°C; a decrement in the analytes concentration at high levels was observed starting from the second week. The method was applied to several real samples, obtained from known alcoholics and social drinkers.

CONCLUSIONS: A sensitive and specific determination of EtG and EtS in serum samples was achieved despite a simple and fast sample preparation. To our knowledge, this is the first fully validated method for the simultaneous determination of the two alcohol metabolites.

KEYWORDS: *Ethanol, Ethylglucuronide, Ethylsulfate, LC-MS*

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