Improved Sensitivity and Reduced Sample Size in Serum Fatty Acid Ethyl Ester Analysis, Walter C. Zybko, Joanne E. Cluette-Brown, and Michael Laposata * (Division of Laboratory Medicine, Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114; * address correspondence to this author at: Room 235, Gray Building, Massachusetts General Hospital, Boston, MA 02114; fax 617-726-3256, e-mail mlaposata@partners.org)

Fatty acid ethyl esters (FAEEs) are nonoxidative esterification products of ethanol metabolism found after ingestion of ethanol (1). FAEEs were proposed as toxic mediators of ethanol-induced cell injury in a study of postmortem analysis of tissues and organs (2). Subsequently, direct damage to human hepatoblastoma cells by FAEEs was reported (3), as well as in vivo pancreatic injury in rats induced by FAEE infusion (4). FAEEs in serum are both short- and long-term markers of ethanol intake (5, 6). Because serum FAEE concentrations are useful indicators of ethanol intake, test sensitivity and sample volume have become increasingly important targets for improvement.

The first use of gas chromatograph–mass spectrophotometry (GC-MS) detection systems for serum FAEE concentrations identified ethyl 16:0, ethyl 16:1, ethyl 18:0, ethyl 18:1, ethyl 18:2, and ethyl 20:4 (1). In these experiments, the FAEEs were isolated from an organic phase by thin-layer chromatography; individual species of FAEEs were then identified and quantified by GC-MS. The current procedure for isolation and quantification of FAEE using solid-phase extraction and GC-MS is based on a method developed by Bernhardt et al. (7). Improvements have increased the number of identifiable FAEEs in the current technique to include ethyl 14:0, ethyl 18:3n-3, ethyl 20:3n-6, ethyl 20:5n-3, and ethyl 22:6n-3. Preanalytical variables that affect FAEE quantification have been described in a recent report by Soderberg et al. (8).

The standard procedure for FAEE analysis uses 1 mL of serum, with the addition of 2 mL of acetone and 50 µL of internal standard. A chromatogram of the analysis of FAEEs from a subject with a blood alcohol concentration of 3210 mg/L (69.8 mmol/L) is depicted in Fig. 1. The upper tracing was obtained using a 1-mL serum sample and extraction with 2 mL of acetone. The lower tracing was obtained using a 1-mL serum sample and extraction with 4 mL of acetone. After correction for recovery using the internal standard, the 1 mL/2 mL ratio for total FAEEs was 29.36 nmol/mL, and the 1 mL/4 mL ratio was 22.66 nmol/mL. The calculation of total FAEEs is based on the area of abundance of the internal standard relative to the area of abundance of the individual FAEEs. The total amount of FAEEs is somewhat lower for the higher acetone volumes, most likely because more internal standard was recovered (7.27-fold; see FAEE ethyl 17:0 in Table 1) relative to the individual FAEEs in the serum (3.35- to 5.63-fold, except for FAEE ethyl 18:0, for which the recovery was 8.57-fold higher) as shown in Ratio 1 of Table 1.

Rather than using the same amount of serum and more acetone, to try to gain the benefit of a smaller sample size we reduced the amount of serum, but held the acetone amount of acetone, 3.35- to 8.57-fold more FAEEs were extracted into the acetone phase. The correlation between the amount obtained using 1 mL of serum and 2 mL of acetone vs 1 mL of serum and 4 mL of acetone was 0.980. After correction for recovery using the internal standard, the 1 mL/2 mL ratio for total FAEEs was 29.36 nmol/mL, and the 1 mL/4 mL ratio was 22.66 nmol/mL. The calculation of total FAEEs is based on the area of abundance of the internal standard relative to the area of abundance of the individual FAEEs. The total amount of FAEEs is somewhat lower for the higher acetone volumes, most likely because more internal standard was recovered (7.27-fold; see FAEE ethyl 17:0 in Table 1) relative to the individual FAEEs in the serum (3.35- to 5.63-fold, except for FAEE ethyl 18:0, for which the recovery was 8.57-fold higher) as shown in Ratio 1 of Table 1.

Fig. 1. Chromatograph obtained from GC-MS analysis of FAEEs from a subject with a blood alcohol concentration of 3210 mg/L (69.8 mmol/L).

The upper tracing was obtained using a 1-mL serum sample and extraction with 2 mL of acetone. The lower tracing was obtained using a 1-mL serum sample and extraction with 4 mL of acetone.
Thus the ideal sample volume to maximize FAEE detection at the lower legal limit for intoxication is also not recommended for analysis. The total FAEE amount as in Ratio 4, showing that a 0.10-mL sample size was 799.740 for FAEE ethyl 16:0. Although the FAEEs were in less abundance in the 0.1-mL sample, they were still clearly detectable in a sample with a blood ethanol concentration at the lower legal limit for intoxication. Thus the ideal sample volume to maximize FAEE detection appears to be 0.5 mL. However, even at 850 mg/L (18.5 mmol/L) blood ethanol, a sample size of 0.10 mL or possibly lower could be used for FAEE analysis.

In summary, the sensitivity for FAEE detection is improved by use of a smaller sample volume of 0.5 mL and a serum-to-acetone ratio of 1:8.

References

Relationships among Plasma Homocysteine, Cysteine, and Albumin Concentrations: Potential Utility of Assessing the Cysteine/Homocysteine Ratio, Glen L. Hortin, Patricia Sullivan, and Gyorgy Csako (Department of Laboratory Medicine, Warren Magnuson Clinical Center, NIH, Bethesda, MD 20892; * address correspondence to this author at: Department of Laboratory Medicine, NIH, Building 10, Room 2C-407, Bethesda, MD 20892-1508; fax 301-402-1885, e-mail ghortin@mail.cc.nih.gov)

McCully (1) initially observed that patients with extremely increased plasma concentrations of homocysteine (Hcy) attributable to homocystinuria have accelerated...