# Plasma fatty acids analysis

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## Abstract

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## Protocol

Took blood samples and separated the plasma

**Step 1.**

Blood samples of 10 ml were collected in tubes containing 1 g EDTA/l after subjects had fasted for at least 12 h. Plasma was separated within 3 h by centrifugation (3000 rpm for 10 min) at room temperature.

Prepared plasma fatty acid methyl esters

**Step 2.**

Plasma fatty acid (FA)  methyl esters were prepared as previously described Moser et al.

Moser AB, Jones DS, Paymond GV, Moser HW. Plasma and red blood cell fatty acids in peroxisomal disorders*. Neurochem Res.* 1999; 24:187-97.

Briefly, 200 µl plasma and 10 µg C13:0 as an internal standard were mixed with 1 ml methanol/methylene chloride 3:1 (v/v). Subsequently, 200 µl of acetyl chloride, were added and the samples were placed in a 75ºC water bath for 1 h. After cooling down, 4 ml of 7% potassium carbonate and 2 ml hexane were added, and samples were mixed and centrifuged for 10 min at 3000 rpm at room temperature.

Analyzed the plasma fatty acids using flame ionization detection

**Step 3.**

The plasma sample in hexane layer was dried using nitrogen gas and analyzed with Agilent 7820A GC using flame ionization detection on a SP-2560 polar fused silica capillary column (100 m x 0.25 mm x 0.2 µm, Supelco Inc.) with nitrogen as carrier gas. The oven temperature program was initially set to 60ºC for 1 min, then increased by 25ºC per minute to 160ºC, then increased by 2ºC per minute to 240ºC for 10 min, and finally increased by 5ºC per minute to 245ºC for 5 min.

Identified the composition of plasma fatty acids

**Step 4.**

The Fatty acid (FA) peaks were identified by comparing retention times from our samples with retention times of a standard mixture of GLC-68A, GLC-481, GLC-532, GLC-744 (Nu-Chek Prep), 37 FAME, cis/trans 18:2n-6 and cis/trans 18:3n-3, (all from SUPELCO). The FA composition was expressed as the weight of a percentage of the total weight of carbon 12 to carbon 24 FAs (wt%).