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A new internal standard for HPLC assay of conjugated linoleic acid in animal tissues and milk

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A new method for the quantification of underivatized conjugated linoleic acid (CLA) isomers and CLA-metabolites by silver ion liquid chromatography (Ag^+-HPLC) with photodiode array detection (DAD) is described. Conjugated fatty acids (CFA) and sorbic acid as the internal standard (IS) were separated on two 5 µm Chrompac ChromSpher Lipids columns (250 × 4.6 mm). Biological samples were hydrolyzed with 1M KOH in methanol and 2M KOH in water at room temperature for 12 h. Hydrolyzates were acidified and the free fatty acids were extracted with dichloromethane. The organic solvent was removed and then the residue was re-dissolved in hexane and centrifuged. The supernatant was injected onto the columns. The mobile phase of 1.6% acetic acid and 0.0125% acetonitrile in hexane was chosen as the optimum mobile phase for fractionation of IS, CLA isomers and CLA-metabolites in all assayed biological samples. The use of two silver ion-exchange columns with direct UV detection (Ag^+-HPLC-DAD) offers satisfactory precision of the IS quantification and low limits of detection of IS and CLA isomers (0.60 and 0.21–0.35 ng, respectively). The presented simple Ag^+-HPLC-DAD method with sorbic acid as the IS can be used for direct determination of underivatized CLA isomers in specimens of animal origin.

Keywords:

sorbic acid; internal standard; CLA isomers; HPLC; photodiode array detection; biological samples

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