

## SEPARATION OF CRITICAL PAIRS OF C18:1 ISOMERS AND CONJUGATED LINOLEIC ACIDS IN MILK FAT

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The analysis of fatty acids (as methyl esters, FAME) from milk fat is usually performed in high polarity 100 m capillary columns. Due to sample complexity, however, sometimes proper resolution between C18:1 *cis/trans* isomers, as well as conjugated linoleic acid (CLA) isomers, is not achieved. As part of an ongoing project on CLA, herein we report a study on a set of chromatographic conditions towards the separation of these compounds in a single run, using a CP-Sil 88 column (88% cyanopropyl-aryl-polysiloxane, 100 m x 0.25 mm x 0.2 μm). All tests were performed in an Agilent 6890N gas chromatograph fitted with a flame ionization detector (kept at 280°C). Separation of critical pairs of C18:1 and of CLA were carried out at constant temperature (160°C, 170°C and 175°C), as well as at temperature gradient from 160°C to either 175°C, 210°C or 215°C. Carrier gas (hydrogen) flows ranging from 0.5 to 2.5 mL/min were tested. FAME and CLA standard solutions were injected (1.0 μL) at 250°C and split 1:50. For a given constant carrier gas flow, best separation was obtained with the 160°C constant temperature analysis. With temperature gradient analysis, flows between 1.0 and 2.0 mL/min led to better resolution. Best overall separation conditions were at 160°C and variable carrier gas flow: 2.0 mL/min (32 min), then 1.5 mL/min (32 min) and 2.5 mL/min (28 min), leading to a total analysis time of 92 min. Complete separation of the critical pair C18:1 *cis/trans* and CLA isomers was observed. It was also important to note that the signal corresponding to C21:0, which usually lies among CLA peaks, leading to miscalculations of this group, was observed in a chromatographic region well separated from CLA. The optimized method was applied to milk fat samples, six from bovines and six from ovines, fed with a diet rich in unsaturated fatty acids. The CLA yield varied from 0.5 to 2.0% in the milk from bovines, and from 0.6 to 0.9% in the milk from ovines.

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