Short Communication: Rapid separation of *cis*9, *trans*11- and *trans*7,*cis*9-18:2 (CLA) isomers from ruminant tissue using a 30 m SLB-IL111 ionic column

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Turner, T., Rolland, D. C., Aldai, N. and Dugan, M. E. R. 2011. **Short Communication: Rapid separation of *cis*9,*trans*11- and *trans*7,*cis*9-18:2 (CLA) isomers from ruminant tissue using a 30 m SLB-IL111 ionic column**. Can. J. Anim. Sci. **91**: 711–713. Rumenic acid (*cis*9,*trans*11-18:2) is the main natural isomer of conjugated linoleic acid (CLA). Rumenic acid has many purported health benefits, but effects of most other CLA isomers are unknown. Typically *trans*7,*cis*9-18:2 is the second most abundant CLA isomer, but it co-elutes with rumenic acid on conventional polar gas chromatography (GC) columns, requiring complimentary analysis with silver-ion high performance liquid chromatography (Ag^+-HPLC). Herein we report a rapid method for analyzing rumenic acid and *trans*7,*cis*9-18:2 using a 30 m ionic-liquid GC column. Optimal resolution of the two CLA isomers was at 145°C and analysis of backfat from barley-fed cattle compared well with GC/Ag^+-HPLC ($y = 0.978x - 0.031$, $r = 0.985$, $P < 0.001$).

Key words: Biohydrogenation, conjugated linoleic acid, gas chromatography

Turner, T., Rolland, D. C., Aldai, N. et Dugan, M. E. R. 2011. **Brève Communication: Séparation rapide des isomères *cis*9,*trans*11- et *trans*7,*cis*9-18:2 de l'ALC dans le tissu des ruminants grâce à une colonne ionique SLB-IL111 de 30 m**. Can. J. Anim. Sci. **91**: 711–713. L'acide ruménique (*cis*9,*trans*11-18:2) est le principal isomère naturel de l'acide linoléique conjugué (ALC) et est réputé bénéfique pour la santé, mais on ignore les effets de la plupart des autres isomères de l'ALC. L'acide *trans*7,*cis*9-18:2 est habituellement le deuxième isomère le plus abondant de l'ALC, cependant, dans les colonnes à chromatographie gazeuse polarisée usuelles, il se combine à l'acide ruménique, si bien qu'on doit procéder à une analyse complémentaire par chromatographie liquide à haute performance avec ions argent (Ag^+-HPLC). Les auteurs exposent une méthode rapide pour analyser l'acide ruménique et l'acide *trans*7,*cis*9-18:2 avec une colonne à chromatographie gazeuse ionique de 30 m. La résolution optimale des deux isomères de l'ALC est de 145 °C et l'analyse du gras dorsal des bovins engraissés à l'orge donne des résultats comparables pour la chromatographie gazeuse et la Ag^+-HPLC ($y = 0{,}978x - 0{,}031$, $r = 0{,}985$, $P < 0{,}001$).

Mots clés: Biohydrogénation, acide linoléique conjugué, chromatographie gazeuse

Conjugated linoleic acid (CLA) is the collective term for geometric and positional isomers of octadecadienoic acid with a conjugated double bond system. Ruminant products are natural sources of CLA and these result from partial biohydrogenation of polyunsaturated fatty acids (PUFA) in the rumen. The most prominent isomer in ruminant products is *cis*(*c*)9,*trans*(*t*)11-18:2 (rumenic acid) (Yurawecz et al. 1998). A number of health benefits have been associated with rumenic acid consumption in animal models, including anti-carcinogenic, anti-atherogenic and anti-inflammatory properties (Park and Pariza 2007). The second most abundant CLA isomer in concentrate-fed ruminant tissues is *t*7,*c*9-18:2; however, its biological effects are presently not known. Accurate analysis of individual CLA isomers is, therefore, required to enable determination of net health effects of CLA in whole foods.

When using conventional polar (cyanopropyl) gas chromatography (GC) columns, minor CLA isomers in ruminant tissues including *t*9,*c*11-, *t*10,*c*12- and *t*11,*c*13-18:2 are well resolved, but rumenic acid co-elutes with *t*7,*c*9-18:2 (Yurawecz et al. 1998; Cruz-Hernandez et al. 2004). Resolution of the major CLA isomers was enabled using silver ion–high performance liquid chromatography (Ag^+-HPLC) (Sehat et al. 1998). The presence of *t*7,*c*9-18:2 in ruminant products was established using Ag^+-HPLC and confirmed by GC-electron ionisation mass spectrometry (Yurawecz et al. 1998). Optimal resolution of most CLA isomers is now attained using three Ag^+-HPLC columns in series (Sehat et al. 1999), but analytical run times are typically 80 min and retention times can tend to drift over

Abbreviations: **c**, Cis; **CLA**, conjugated linoleic acid; **FAME**, fatty acid methyl esters; **GC**, gas chromatography; **PUFA**, polyunsaturated fatty acid: **Ag^+-HPLC**, silver-ion high-performance liquid chromatography; **t**, trans

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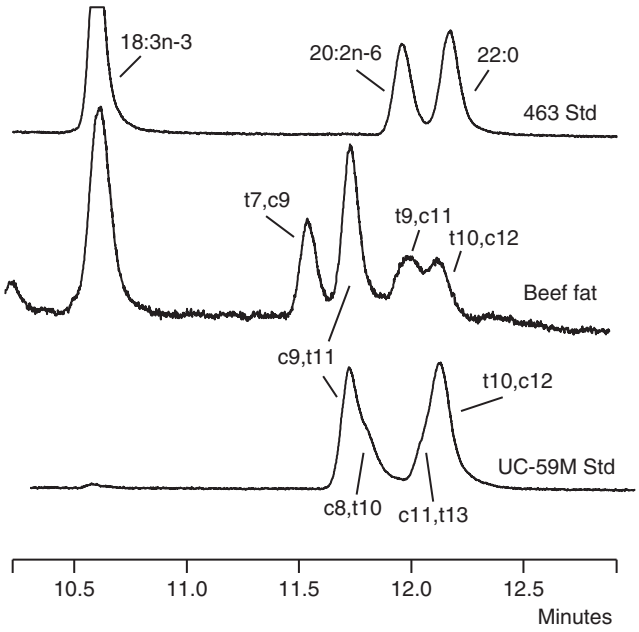

Fig. 1. Partial chromatograms of fatty acid methyl esters (FAME) using the SLB IL-GC 30 m column. (a) Nu-Chek Prep. #GLC-463 FAME standard; (b) bovine backfat; (c) Nu-Chek Prep. #UC-59 CLA standard.

successive runs (Delmonte et al. 2005). The introduction of ionic liquid GC columns offers improved resolution of 18-carbon monoenes, dienes and trienes (Ragonese et al. 2009). A new ionic liquid 100 m GC column (SLB-IL111, Supelco Inc., Bellefonte, PA) is reported to be the first to separate synthetic *t*7,*c*9- and *c*9,*t*11-18:2 (Delmonte et al. 2011). These two isomers were well resolved with the 100 m column, indicating a shorter (i.e., 30 m) column might be able to be used for their analysis with a relatively short run time.

Comprehensive analysis of ruminant tissues typically includes GC using a 100-m conventional polar column to analyze most fatty acids (Kramer et al. 2008), together with complimentary Ag^+-HPLC to complete CLA isomer analyses (Cruz-Hernandez et al. 2004). The objective of the present study was to develop a rapid method using a 30 m SLB-IL111 GC column, as a substitute for analysis of *t*7,*c*9- and *c*9,*t*11-18:2 by Ag^+-HPLC, to compliment conventional GC analyses for the comprehensive fatty acid analysis of ruminant tissues.

For method development, subcutaneous fat samples were collected from 56 crossbred steers finished (120 d) on ad libitum intake of a diet containing 73% steam rolled barley, 22% barley silage and 5% vitamin mineral supplement (as fed). Animal welfare followed guidelines outlined by the Canadian Council on Animal Care (1993). Backfat from between the 12th and 13th ribs overlying the longissimus muscle was collected at slaughter, frozen immediately and stored at −80°C

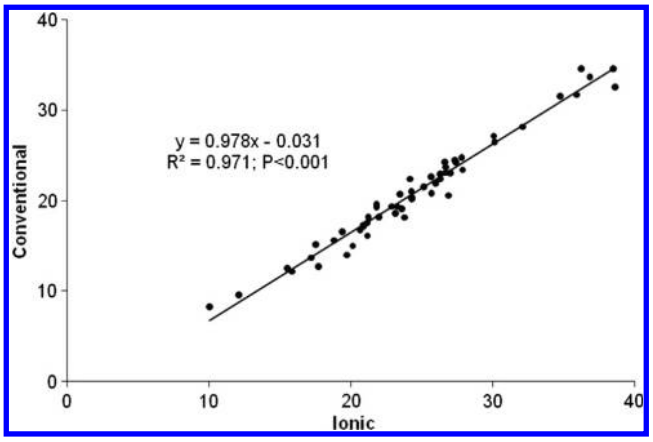

Fig. 2. Relationship between *t*7,*c*9-18:2 as a percentage of *t*7,*c*9- and *c*9,*t*11-18:2 when analyzed using an ionic GC column (30 m SLB IL 111) compared with conventional GC/Ag^+-HPLC analysis.

until analyzed. For analysis, samples of backfat (50 mg) were freeze dried and direct methylated with sodium methoxide as described by Yurawecz et al. (1999), and fatty acid methyl esters (FAME) were extracted into hexane and dried over sodium sulfate. The FAME, including CLA isomers, were then analyzed using methods described by Cruz-Hernandez et al. (2004) and using the following equipment and conditions. For GC analysis, 1 μL of FAME (1 mg mL^{-1}) were injected using a 1079 split/splitless injector (Varian Inc., Walnut Creek, CA) and a 20:1 split onto a CPSil88 column (100 m, 25 μm i.d., 0.2 μm film thickness; Varian Inc., Walnut Creek, CA) in a CP-3800 gas chromatograph (Varian Inc., Walnut Creek, CA). Hydrogen was used as the carrier gas under constant pressure (25 psi, initial flow rate of 1 mL min^{-1}) and injector and detectors temperatures were held at 250°C. The initial temperature was 45°C held for 4 min, increased to 175°C at 13°C min^{-1}, held for 27 min, increased to 215°C at 4°C min^{-1} and held for 35 min and FAME were detected using flame ionization detection. HPLC analysis of CLA isomers included the use of three 250 mm × 4.6 mm Chromspher 5 Lipids columns (Varian Inc., Walnut Creek, CA) hooked in series in a TS-43 column oven (Phenomenex Thermasphere, Torrance, CA) maintained at 25°C, a Prostar 410 autosampler, Prostar 230 HPLC and a Prostar 335 Photo-Diode-Array Detector (Varian Inc., Walnut Creek, CA). The flow rate of mobile phase (hexane with 0.1% acetonitrile, 0.5% diethyl ether in hexane) was 1 mL min^{-1}. Samples (10 μL) were injected at a concentration of 25 mg·mL^{-1} and CLA isomers were detected at 233 nm.

To determine if a 30 m SLB IL 111 GC column (0.25 mm, 0.2 μm film thickness, Supelco Inc., Bellefonte, PA) could be used to separate and aid in quantifying the two major natural isomers of CLA (i.e., *t*7,*c*9- and *c*9,*t*11-18:2) we used the same GC equipment described above. Samples (1 μL; 5 mg.mL^{-1})

1) were injected using a 20:1 split and hydrogen as a carrier gas (15 psi constant pressure, initial flow rate 2.9 mL min^{-1}). The initial temperature was 45°C, held for 2 min and then ramped by 50°C per minute and held at different temperatures (140°C, 145°C, 150°C, 155°C, 160°C, 165°C, 170°C and 175°C) to determine the temperature for optimal resolution of *t*7,*c*9- and *c*9,*t*11-18:2. For FAME identification, the Nu-Chek Prep Inc. (Elysian, MN) GLC-463 and UC-59M CLA standards were used. The CLA isomers not included in the standard, which included *t*7,*c*9-18:2, were identified by their retention times and elution orders (Cruz-Hernandez et al. 2004; Delmonte et al. 2011).

To test the new method, beef backfat results (*t*7, *c*9-18:2 area as a percentage of the combined *t*7,*c*9- and *c*9,*t*11-18:2 areas) from the SLB IL-111 GC column were regressed versus the percent area obtained during conventional GC/Ag^+-HPLC using SAS Proc Reg and Proc Corr (SAS Institute, Inc. 2001).

Previously, Delmonte et al. (2011) reported that an isothermal setting of 168°C gave optimal separation of most FAME when using a 100 m ionic-liquid column. Resolution of saturated fatty acids was noted to be a problem throughout chromatograms, but clear separation of *t*7,*c*9-18:2 from rumenic acid was obtained. All peaks in the CLA region of milk fat samples could be clearly resolved in 140 min at 130°C (Delmonte et al. 2011). Separations of FAME from beef fat using a 30 m SLB IL111 GC column are shown in Fig. 1. Optimal resolution of *t*7,*c*9-18:2 and rumenic acid was achieved at 145°C, with the two peaks eluding between 11.5 and 12 min. Lowering the temperature to 140°C increased peak breadth, whereas increasing above 155°C caused non-conjugated FA (i.e., 20:2n-6 and 22:0) to elute faster and overlap with CLA isomers of interest. Moreover, *t*9,*c*11- and *t*10,*c*12-18:2 began to co-elute at 150°C. Due to the highly polar nature of the ionic columns, a small change in temperature has been noted to cause substantial shifts in FAME retention times (Delmonte et al. 2011).

Comparing 30m SLB IL111 GC and conventional GC/Ag^+-HPLC analysis of backfat samples showed a strong linear correlation ($y = 0.978x - 0.031$, $r = 0.986$, $P < 0.001$) between the two methods over a range of CLA levels (Fig. 2). This is in agreement with Delmonte et al. (2011), who suggested complementary analysis by Ag^+-HPLC for the major CLA isomers would not be necessary if using the 100 m SLB IL-111 m column. Analysis with a 30 m SLB IL-111 GC column thus appears to provide a rapid, acceptable alternative to GC/Ag^+-HPLC analysis of the *t*7,*c*9- and *c*9,*t*11-18:2 in ruminant tissues, as a complement to separation of other FAME by 100 m polar column.

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