## **ANALYSIS OF FREE FATTY ACIDS IN FOOD BY UPC<sup>2</sup>-MS**

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### **INTRODUCTION**

The development of a fast and simple analytical method for the routine simultaneous identification and quantification of a variety of free fatty acid (FFA) is desirable for use in various fields <sup>(1)</sup>. FFA content in crude edible oil is used to characterize both high quality pressed oils and fish oils. FFA content is also a parameter that may be used to monitor oil degradation that arises from storage under different conditions and to follow the thermal degradation of oils that are used to cook or fry. The determination of fatty acid profile has mainly been carried out by gas chromatography (GC) after the acids are converted to esters <sup>(2)</sup>. However, the nonvolatility of longer-chain acid esters and the thermally labile property of unsaturated acids can complicate the GC analysis. Liquid chromatography (LC), including silver-ion chromatography and reversed-Phase chromatography (RPLC), have been applied to the fatty acid analysis <sup>(1)</sup>. Silver-ion chromatography is the method of choice for separation and isolation of *cis* and *trans* fatty acids, but it needs to be coupled with other techniques (such as GC) for complex samples' fatty acid peak identification. RPLC has been widely studied for the fatty acid determination, either with or without derivatization. However, the separation efficiency in RPLC is not as great as that in GC.

UltraPerformance Convergence Chromatography<sup>TM</sup> (UPC<sup>2®</sup>) is a newgeneration supercritical fluid chromatography (SFC). It has been demonstrated that it has excellent separation efficiency and speed in a wide range of application areas <sup>(3)</sup>, including the edible oils, acylglycerols, and short-chain fatty acids <sup>(4-6)</sup>. This poster demonstrates the separation and quantitation of FFAs in food samples, including the separation of positional and geometrical isomers by UPC<sup>2</sup>-MS.

## **EXPERIMENTAL**

Samples:

Free fatty acid standard mix (GLC-463 fatty acids) from Nu-Check Prep (Elysian, MN) Details of the individual compounds see Table 1. Food samples were fat extracted from food with petroleum ether and dried in water bath. The samples were dissolved in chloroform at 1 to 4 wt%.

<u>UPC<sup>2</sup> conditions</u> ACOUITY<sup>®</sup> UPC<sup>2</sup> with ACOUITY UPC<sup>2</sup> PDA and Xevo<sup>®</sup> TO System: S MS Software: MassLynx<sup>®</sup> V4.1 Column: 2 pieces of ACQUITY UPC<sup>2</sup> HSS C18 SB 3.0 x 150 mm, 1.8µm (186006685) connected in series. Col. Temp.: 10°C Co-solvent: MeOH/AcN(50/50) with 1% Formic acid Ini Vol.: 0.5 µL Flow Rate: 0.70 mL/min Run time: 27 min Col. Equil.: 7 min. initially at 1500 psi, at 20 min change to 2500 psi, and ABPR: back to 1500 psi at 29 min. Elution Gradient. Time (Min) Flow Rate %A Curve (mL/Min) 0.700 Initial



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## **RESULTS AND DISCUSSION**

#### 1) Chromatography method development

The effects of different columns, the co-solvents (mobile phase) and the gradient, the sample diluents, and the ABPR pressure were investigated. Two ACQUITY UPC<sup>2</sup> HSS C18 SB (3.0 x 150 mm), 1.8 µm columns gave the best separation of the isomers. These two columns were installed in the ACQUITY UPC<sup>2</sup> Column Manager. The connection tubing in the Column Manger was altered so the two columns were connected in series. The column position II ports were not used. Figure 1 shows the examples of Extracted Ion Chromatograms (XIC) for the isomers in the GLC-462 reference mix (fatty acids). Figure 2 shows the overlay of the 51 fatty acid chromatograms.

#### 2) Separation of the fatty acids and their isomers

From Fig. 1 and Fig. 2, one can see that under this UPC<sup>2</sup> conditions, the fatty acids were separated based on their chain length, the degree of saturation, and the geometrical configuration. The retention time increased with increasing chain length and decreasing number of double bonds. The *trans* isomers were eluted after the corresponding *cis* isomers. In addition, the closer the double bond to the carboxyl group, the more reduction in the RT (see Fig 1. B, D and E).

The separation of the fatty acid isomers is impressive. Under this 28 minute gradient, the *trans/cis* isomers, the C16:1(9E) and C16:1(9Z), and the C18:2 (9Z,12Z) and C18:2(9E,12E), as well as the positional isomers, the C20:3 (8Z,11Z,

#### ,14Z) and C20:3(11Z,14Z,17Z) were well separated (Rs>1.5). The five challenging isomers of C18:1 were partially separated. However, the C20:1(5) co-elute with the C20:1(8). The elution order of these isomers were confirmed with the RTs of the individual standards.

#### 3) Calibration results

The calibration curves for each compounds were obtained using a serial dilution of a stock solution of the GLC-463 fatty acids standards mix. The weighted least squares (1/x) 2nd order polynomial fitting was used for all compounds. The retention times (RT), the calibration equations, the  $R^2$  values, the estimated limit of quantitation (LOQ) at signal to noise ratio 10 (S/N=10), and the calibration concentration range are in Table 2. Majority of the compounds' LOQs were lower than the lowest concentration in the calibration range.

#### 4) Analysis of food samples

Compound 50: C22:6 1.6 0.0112 Total free fatty acids (%): 0.4294 0.1896 Six food samples were analyzed using this UPC<sup>2</sup>-MS method. The samples were 37.46 35.77 13.99 33.86 32.43 otal sample conc. (mg/mL) 14.23 fat that were extracted from food products using petroleum ether and dried in water bath. There was no saponification or derivatization. So, the free fatty acids were determined in the presence of the fat matrix (Triacylglycerols). Figure 3 is the XIC of fatty acids found in sample A. Table 2 shows the analysis results for the six samples. In Figure 3, there were lots of unknown peaks in the late elution stage on many of the XICs. These peaks were believed from the fat matrix.

Compound 47: C22:4

Compound 48: C24:0

Compound 49: C22:5

Sample

Compound 10: C12:0

Compound 14: C14:0

Compound 16: C15:0

Compound 18: C16:0

Compound 19: C16:1(9Z)

Compound 25: C18:1(9Z)

ompound 26: C18:1(11Z)

ompound 29: C18:2(9Z,12Z)

Compound 31: C18:3(6Z,9Z,12Z)

Compound 39: C20:3(8Z,11Z,14Z)

Compound 41: C20:4(5Z,8Z,11Z,14Z)

Compound 38: C20:2(11Z,14Z)

Compound 23: C18:0

**Table 1.** Details of the fatty acid standards in the GLC-463 reference mix and their retention times, square of the correlation coefficients, limit of quantitations, and calibration curve concentration ranges.

|       | Fatty Acids in standard mix   |                                   |                      |              |                    |               | Calibration Results |       |           |                |
|-------|---|-----------------------------------|----------------------|--------------|--------------------|---------------|---------------------|-------|-----------|----------------|
| No    | Name  | common Name                       | Description          | Mol. Formula | [M-H] <sup>-</sup> | wt% in<br>mix | RT (min)            | R^2   | LOQ (ppm) | Range<br>(ppm) |
| 1     | Butanoic acid   | Butyric a cid                     | C4:0                 | C4H8O2       | 87.1               | 1             | 11.09               | 0.998 | 4         | 4-162          |
| 2     | Pentanoic acid  | Valeric acid                      | C5:0                 | C5H10O2      | 101.1              | 1             | 11.35               | 0.998 | 4         | 4-162          |
| 3     | Hexanoic acid   | Caprioc acid                      | C6:0                 | C6H12O2      | 115.2              | 1             | 11.68               | 0.997 | 3         | 3-162          |
| 4     | Heptanoi c acid   |                                   | C7:0                 | C7H14O2      | 129.2              | 1             | 12.06               | 0.999 | 2         | 2-162          |
| 5     | Octanoic acid   | Caprylic acid                     | C8:0                 | C8H16O2      | 143.2              | 2             | 12.52               | 0.999 | 3         | 3-324          |
| 6     | Nonanoic acid   |                                   | C9:0                 | C9H18O2      | 157.2              | 1             | 13.04               | 0.999 | 3         | 3-162          |
| 7     | Decanoic acid   | Capric acid                       | C10:0                | C10H20O2     | 171.3              | 2             | 13.61               | 0.997 | 12        | 12-324         |
| 8     | Undecanoic acid   | Undecylic acid                    | C11:0                | C11H22O2     | 185.3              | 1             | 14.24               | 0.999 | -         | 2-162          |
| 9     | 10Z-Undecenoic acid   | Undecylenic acid                  | C11:1(10Z)           | C11H20O2     | 183.3              | 1             | 13.62               | 0.999 | -         | 2-162          |
| 10    | Dodecanoic acid   | Lauric acid                       | C12:0                | C12H24O2     | 199.3              | 4             | 14.94               | 0.998 | -         | 6-648          |
| 11    | Dodecenoic acid   |                                   | C12:1                | C12H22O2     | 197.3              | 2             | 14.23               | 0.999 | -         | 3-324          |
| 12    | Tridecanoic acid  | Tridecylic acid                   | C13:0                | C13H26O2     | 213.3              | 1             | 15.68               | 0.999 | -         | 2-162          |
| 13    | Tridecenoic acid  |                                   | C13:1                | C13H24O2     | 211.3              | 1             | 14.90               | 0.999 | -         | 2-162          |
| 14    | Tetradecanoic a cid   | Myristic acid                     | C14:0                | C14H28O2     | 227.4              | 4             | 16.47               | 0.996 | -         | 6-324          |
| 15    | 9Z-Tetradecenoic acid   | Myristoleic acid                  | C14:1(9Z)            | C14H26O2     | 225.4              | 2             | 15.32               | 0.997 | -         | 3-324          |
| 16    | Pentadecanoic acid  | Pentadecylic acid                 | C15:0                | C15H30O2     | 241.4              | 1             | 17.30               | 0.998 | -         | 2-162          |
| 17    | 10Z-Pentadecenoic acid  |                                   | C15:1(10Z)           | C15H28O2     | 239.4              | 1             | 16.04               | 0.998 | -         | 2-162          |
| 18    | Hexadecanoic acid   | Palmitic acid                     | C16:0                | C16H32O2     | 255.4              | 4             | 18.16               | 0.992 | -         | 6-154          |
| 19    | 9Z-Hexa decenoi cacid   | Palmitoleic acid                  | C16:1(9Z)            | C16H30O2     | 253.4              | 4             | 16.58               | 0.998 | -         | 6-154          |
| 20    | 9E-Hexa decenoi c acid  | Palmitelaidicacid                 | C16:1(9E)            | C16H30O2     | 253.4              | 1             | 16.87               | 0.985 | -         | 2-81           |
| 21    | Heptadecanoic acid  | Margaric acid                     | C17:0                | C17H34O2     | 269.4              | 2             | 19.09               | 0.992 | 3         | 3-77           |
| 22    | 102-Heptadecenoic acid  |                                   | C1/:1(102)           | C1/H32O2     | 267.4              | 2             | 17.39               | 0.996 | 3         | 3-88           |
| 23    | Octade canoic acid  | Stearic acid                      | C18:0                | C18H36O2     | 283.5              | 4             | 20.04               | 0.990 | -         | 6-62           |
| 24    | 62-Octadecenoic a cid   | Petroselinic acid                 | C18:1(6Z)            | C18H34O2     | 281.5              | 1             | 17.84               | 0.985 | -         | 2-10           |
| 25    | 92-Octadecenoic acid  | Cleic acid                        | C18:1(92)            | C18H34O2     | 281.5              | 4             | 18.02               | 0.997 | -         | 0-02           |
| 20    | 9E-Octadecenoic a cid   | Elaidic acid                      | C18:1(9E)            | C18H34U2     | 281.5              | 1             | 10.10               | 0.999 | 2         | 2-10           |
| 27    | 11E-Octadecenoic acid   | Vaccenic acid                     | C18.1(112)           | C18H34O2     | 201.5              | 1             | 19.10               | 0.001 | -         | 6-62           |
| 28    | 97 127-Octadecerioic acid   |                                   | (18-2/97 127)        | C18H34O2     | 281.5              | 4             | 17.18               | 0.901 | -         | 2-21           |
| 30    | 9E 12E-Octadecadienoic acid   | Linolelaidic acid                 | C18-2(9E 12E)        | C18H32O2     | 279.4              | 2             | 17.10               | 0.995 | -         | 2-39           |
| 31    | 67 97 127-Gamma-Octadecatrienioc acid   | Gamma-linolenic acid              | (18-3/67 97 127)     | C18H30O2     | 277.4              | 1             | 16.14               | 0.996 | -         | 2-81           |
| 22(1) | 97 127 157-Alpha-Octa docatrionoic acid   | Alpha-linglonic acid ALA (n-2)    | (18-2/07 127 157)    | C18H20O2     | 277.4              | 4             | 10.14               | 0.000 |           | 2.01           |
| 22    | Nonadocanois acid   | Nonadogylic acid                  | (10.0)               | C10U2802     | 2075               | 1             | 21.04               | 0.002 | 2         | 2-20           |
| 33    | 107-Nonadecenoic acid   | Nonadecynic acid                  | (19-1(107)           | C19H36O2     | 297.5              | 1             | 18.62               | 0.992 | 2         | 2-35           |
| 25    | Eicosanoic acid   | Arachidic acid                    | (102)                | C19H30O2     | 233.5              | 4             | 22.06               | 0.000 | 2         | 6-62           |
| 26(2) | 5-Eicesensis Asid   |                                   | (20.1(5)             | C20H29O2     | 200.5              | 2             | 10.57               | 0.000 |           | 0.02           |
| 30    | 8-Eicosenoic Acid   |                                   | (20.1(5)             | C20H38O2     | 200.5              | 2             | 19.57               | 0.001 |           | 2.21           |
| 37    | 11-Eicosonoic Acid  |                                   | C20:1(8)             | C20H38O2     | 209.5              | 2             | 19.57               | 0.991 | 2         | 2-21           |
| 30    | 117 147-Eicosadienoic acid  |                                   | (20.2(117 147)       | C20H36O2     | 303.5              | 2             | 19.82               | 0.330 | -         | 2-21           |
| 40    | 87 117 147-Ficosatrienoic acid  | Gamma Homo Linglenic acid         | (20-3/87 117 147)    | C20H34O2     | 305.5              | 1             | 17.61               | 0.992 | -         | 2-39           |
| 40    | 117 147 177-Ficosatrienoic acid   | Gamma Homo Emolenic acid          | C20-3(117 147 177)   | C20H34O2     | 305.5              | 2             | 18.26               | 0.985 | -         | 3-31           |
| 42    | 57 87 117 147-Eicosatetraenoic acid   | Arachidonic acid                  | C20-4(57 87 117 147) | C20H32O2     | 303.5              | 1             | 16.65               | 0.997 | -         | 2-39           |
| 43    | Eicosa penta noic Acid  | EPA                               | C:20:5               | C20H30O2     | 301.4              | 2             | 16.23               | 0.992 | -         | 3-31           |
| 44    | Docosanoic acid   | Behenic acid                      | C22:0                | C22H44O2     | 339.6              | 2             | 24.27               | 0.992 | -         | 6-29           |
| 45    | 13Z-Docosenoic acid   | Erucic acid                       | C22:1(13Z)           | C22H42O2     | 337.6              | 4             | 21.91               | 1.000 | -         | 3-77           |
| 46    | Docosadienoic Acid  |                                   | C:22:2               | C22H40O2     | 335.5              | 1             | 20.80               | 0.985 | -         | 2-39           |
| 47    | Docosatrienoic acid   |                                   | C22:3                | C22H38O2     | 333.5              | 2             | 20.07               | 0.991 | -         | 3-31           |
| 48    | Docosatetraenoic acid   |                                   | C22:4                | C22H36O2     | 331.5              | 1             | 18.04               | 0.988 | -         | 2-39           |
| 49    | Docos apentaenoi cacid  | DPA                               | C22:5                | C22H34O2     | 329.5              | 2             | 17.57               | 0.995 | -         | 3-31           |
| 50    | Docosahexaenoic acid  | DHA                               | C22:6                | C22H32O2     | 327.5              | 2             | 16.63               | 0.993 | -         | 3-77           |
| 51    | Lignoceric acid   |                                   | C24:0                | C24H48O2     | 367.6              | 2             | 26.04               | 0.984 | -         | 3-77           |
| 52    | Nervonic acid   |                                   | C24:1                | C24H46O2     | 365.6              | 1             | 24.21               | 0.994 | -         | 2-39           |
| Note: | 1) The C18:3(9Z,12Z,15Z) peak was not foun<br>2) No calibration curve was created for C20 | d in the chromatogram of the refe | erence mix.          |              |                    |               |                     |       |           |                |



**Figure 2**. Overlay of chromatograms of 51 fatty acid compounds in a standard mix (GLC-463 fatty acid). The chromatograms of fatty acids from C4 to C15 were SIR chromatograms, and the chromatograms for C16 to C24 were XIC from MS scan spectrum. Peak labels were shown in the chromatograms. The UPC<sup>2</sup>-MS conditions are in the Experimental Section.



Figure 3. Selected XIC of fatty acids in sample (A). The peaks were identified by their m/z and the reference RTs of the corresponding fatty acid standards. The shaded area are the unknowns, which are believed from the sample matrix. The identified peaks were quantified with the corresponding calibration curves, the results for the samples are in Table 2.

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## CONCLUSION

Conc in

(ug/mL) sample (%) (ug/mL) sample (%) (ug/mL) sample (%) (ug/mL) sample (%)

2.1 0.0062 2.3 0.0064

Conc in

0.0120

0.0262

0.0136

0.0207

0.0718

0.0089

0.0237

0.006

0.0062

3.9

8.5

4.4

6.7

23.3

2.9 7.7

2.1

The determination of the FFA composition in food samples has been demonstrated using Waters ACQUITY UPC<sup>2</sup> coupled with Xevo TQ-S MS. The benefits of this UPC<sup>2</sup>-MS method include:

No derivatization;

**Table 2**. Free fatty acids compositions in food samples by UPC<sup>2</sup>-MS

Conc in

ppm

Conc in

3.7 0.0099

14.8 0.0395

10.6 0.0283

1.9 0.0051

1.3 0.003

0.0163

0.0027

16.6 0.0443

6.1

1

opm Conc in ppm

2.5 0.0176

3.8 0.0267

0.6 0.0042

7.9 0.0555

5.6 0.0394

4.8 0.0337

11.8 0.0829

1.6 0.011

6.5 0.0457

1.2 0.0084

2.2 0.0155

1.8 0.0126

1.9 0.0134

7.3 0.0513

(mL) sample (%) (ug/mL) sample (%)

- Suitable for samples that contain long chain fatty acids and thermal liable fatty acids;
- Simplified sample preparation procedure;
- Reduced chemical waste;
- Fast analysis run time (35 min);

This UPC<sup>2</sup>-MS method provides an alternative approach for the analysis of the fatty acid composition in food.

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Products, Infant Formula, and Adult/Pediatric Nutritional Formul Capillary Gas Chromatography, AOAC International 2016; J. AOAC Int. 99, 210 (2016).

3) Please see website: http://upc2.waters.com for a list of UPC<sup>2</sup>

applications

4) Jinchuan Yang and Giorgis Isaac, Characterization of Triacylglycerols in Edible Oils Using the ACQUITY UPC2 System and Mass Spectrometry, Waters Technology Brief 720004809en, 2013

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