ANALYSIS OF FREE FATTY ACIDS IN FOOD BY UPC²-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 [1]. 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 [2]. However, the non-volatility 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 [1]. 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™ (UPC²®) is a new-generation supercritical fluid chromatography (SFC). It has been demonstrated that it has excellent separation efficiency and speed in a wide range of application areas [3], including the edible oils, acylglycerols, and short-chain fatty acids [4-6]. This poster demonstrates the separation and quantitation of FFAs in food samples, including the separation of positional and geometrical isomers by UPC²-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%.

UPC² conditions:

System:	ACQUITY® UPC² with ACQUITY UPC² PDA and Xevo® TQ-S MS
Software:	MassLynx® V4.1
Column:	2 pieces of ACQUITY UPC² 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
Inj Vol.:	0.5 µL
Flow Rate:	0.70 mL/min
Run time:	27 min
Col. Equil.:	7 min.
ABPR:	initially at 1500 psi, at 20 min change to 2500 psi, and back to 1500 psi at 29 min.

Elution Gradient:

	Time (Min)	Flow Rate (mL/Min)	%A	Curve
1	Initial	0.700	99	6
2	3	0.700	99	6
3	20	0.700	94	6
4	22	0.700	94	6
5	23	0.700	80	6
6	28	0.700	80	6
7	28.1	0.700	99	6

MS Conditions:

MS Modes:	ES- (SIR and Full Scan)
Capillary (kV):	2.00
Cone (V):	30.00
Source Temperature (°C):	150
Desolvation Temperature (°C):	500
Cone Gas Flow (L/Hr):	150
Desolvation Gas Flow (L/Hr):	1000
Make-up solvent:	MeOH with 1% NH_4OH aqueous solution.
Make-up solvent flow:	0.4 mL/min
Make-up solvent pump:	ISM (Isocratic solvent module)
UPC²-MS interface:	UPC² MS splitter

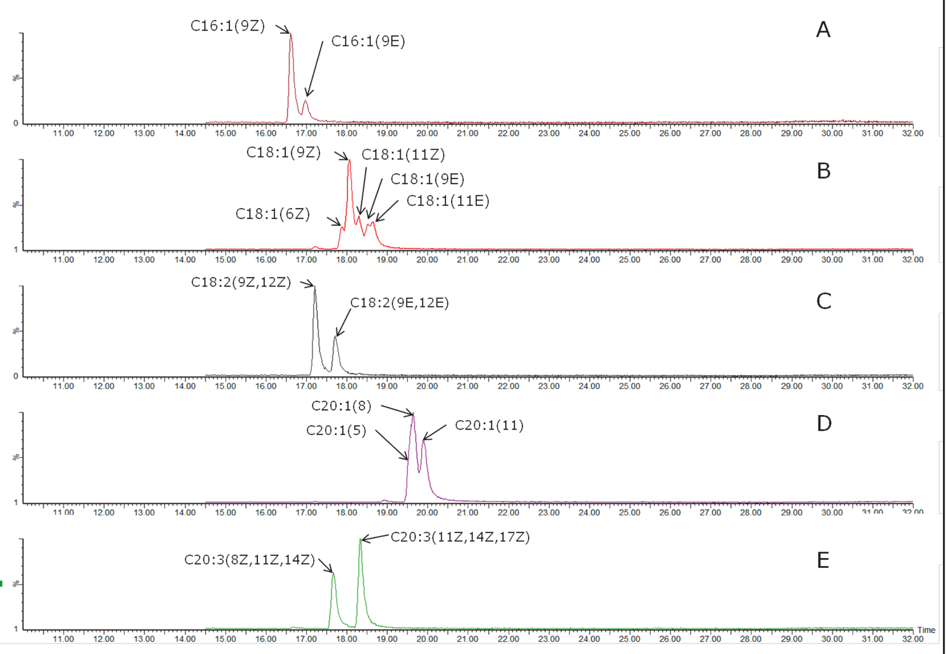

***Figure 1**. Examples of XIC showing the separation of isomers: A) C16:1 9Z, and 9E isomers; B) C18:1 6Z, 9Z, 11Z, 9E, 11E isomers; C) C18:2(9Z,12Z) and C18:2(9E,12E); D) C20:1 Δ5, Δ8, Δ11 isomers. E) C20:3(8Z,11Z,14Z) and C20:3 (11Z,14Z,17Z). The UPC²-MS conditions are in the Experimental Section. The peak IDs were verified with the individual standards.*

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 UPC² 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 UPC² 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 UPC² 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
Six food samples were analyzed using this UPC²-MS method. The samples were 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.

***Table 2**. Free fatty acids compositions in food samples by UPC²-MS*

Sample:	A		B		C		D		E		F	
Compounds	ppm (ug/mL)	Conc in sample (%)	ppm (ug/mL)	Conc in sample (%)	ppm (ug/mL)	Conc in sample (%)	ppm (ug/mL)	Conc in sample (%)	ppm (ug/mL)	Conc in sample (%)	ppm (ug/mL)	Conc in sample (%)
Compound 10: C12:0	2.5	0.0176										
Compound 14: C14:0	3.8	0.0267			3.7	0.0099					3.9	0.0120
Compound 16: C15:0	0.6	0.0042										
Compound 18: C16:0	7.9	0.0555			14.8	0.0395					8.5	0.0262
Compound 19: C16:1(9Z)	5.6	0.0394									4.4	0.0136
Compound 23: C18:0	4.8	0.0337			10.6	0.0283					6.7	0.0207
Compound 25: C18:1(9Z)	11.8	0.0829			16.6	0.0443					23.3	0.0718
Compound 26: C18:1(11Z)	1.9	0.0134			1.9	0.0051					2.9	0.0089
Compound 29: C18:2(9Z,12Z)	7.3	0.0513	3.4	0.0243	6.1	0.0163					7.7	0.0237
Compound 31: C18:3(6Z,9Z,12Z)					1	0.0027						
Compound 38: C20:2(11Z,14Z)											2.1	0.0065
Compound 39: C20:3(8Z,11Z,14Z)	1.6	0.0112										
Compound 41: C20:4(5Z,8Z,11Z,14Z)	6.5	0.0457			1.3	0.0035					2	0.0062
Compound 47: C22:4	1.2	0.0084										
Compound 48: C24:0	2.2	0.0155					2.1	0.0062	2.3	0.0064		
Compound 49: C22:5	1.8	0.0126										
Compound 50: C22:6	1.6	0.0112										
Total free fatty acids (%):		0.4294		0.0243		0.1495		0.0062		0.0064		0.1896
Total sample conc. (mg/mL):	14.23		13.99		37.46		33.86		35.77		32.43	

***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.*

No	Fatty Acids in standard mix						Calibration Results			
	Name	common Name	Description	Mol. Formula	[M-H]⁻	wt% in mix	RT (min)	R^2	LOQ (ppm)	Range (ppm)
1	Butanoic acid	Butyric acid	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	**Caproic acid**	C6:0	C6H12O2	115.2	1	11.68	0.997	3	3-162
4	Heptanoic 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 acid	**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-Hexadecenoic acid	Palmitoleic acid	C16:1(9Z)	C16H30O2	253.4	4	16.58	0.998	-	6-154
20	9E-Hexadecenoic acid	Palmitelaidic acid	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	10Z-Heptadecenoic acid		C17:1(10Z)	C17H32O2	267.4	2	17.39	0.996	3	3-88
23	Octadecanoic acid	**Stearic acid**	C18:0	C18H36O2	283.5	4	20.04	0.996	-	6-62
24	6Z-Octadecenoic acid	Petroselinic acid	C18:1(6Z)	C18H34O2	281.5	1	17.84	0.985	-	2-16
25	9Z-Octadecenoic acid	**Oleic acid**	C18:1(9Z)	C18H34O2	281.5	4	18.02	0.997	-	6-62
26	9E-Octadecenoic acid	Elaidic acid	C18:1(9E)	C18H34O2	281.5	1	18.49	0.999	2	2-16
27	11Z-Octadecenoic acid	Vaccenic acid	C18:1(11Z)	C18H34O2	281.5	1	18.18	0.977	-	2-16
28	11E-Octadecenoic acid	Vaccenic acid	C18:1(11E)	C18H34O2	281.5	1	18.59	0.981	-	6-62
29	9Z,12Z-Octadecadienoic acid	**Linoleic acid**	C18:2(9Z,12Z)	C18H32O2	279.4	4	17.18	0.993	-	3-31
30	9E,12E-Octadecadienoic acid	Linolelaidic acid	C18:2(9E,12E)	C18H32O2	279.4	2	17.66	0.995	-	2-39
31	6Z,9Z,12Z-Gamma-Octadecatrienioc acid	Gamma-linolenic acid	C18:3(6Z,9Z,12Z)	C18H30O2	277.4	1	16.14	0.996	-	2-81
32(1)	9Z,12Z,15Z-Alpha-Octadecatrienoic acid	Alpha-linolenic acid ALA (n-3)	C18:3(9Z,12Z,15Z)	C18H30O2	277.4	4				
33	Nonadecanoic acid	Nonadecylic acid	C19:0	C19H38O2	297.5	1	21.04	0.992	2	2-39
34	10Z-Nonadecenoic acid		C19:1(10Z)	C19H36O2	295.5	1	18.62	0.999	2	2-39
35	Eicosanoic acid	Arachidic acid	C20:0	C20H40O2	311.5	4	22.06	0.990	-	6-62
36(2)	5-Eicosenoic Acid		C20:1(5)	C20H38O2	309.5	2	19.57			
37	8-Eicosenoic Acid		C20:1(8)	C20H38O2	309.5	2	19.57	0.991	-	3-31
38	11-Eicosenoic Acid		C20:1(11)	C20H38O2	309.5	2	19.82	0.996	3	3-31
39	11Z,14Z-Eicosadienoic acid		C20:2(11Z,14Z)	C20H36O2	307.5	2	18.87	0.999	-	3-31
40	8Z,11Z,14Z-Eicosatrienoic acid	Gamma Homo Linolenic acid	C20:3(8Z,11Z,14Z)	C20H34O2	305.5	1	17.61	0.992	-	2-39
41	11Z,14Z,17Z-Eicosatrienoic acid		C20:3(11Z,14Z,17Z)	C20H34O2	305.5	2	18.26	0.985	-	3-31
42	5Z,8Z,11Z,14Z-Eicosatetraenoic acid	Arachidonic acid	C20:4(5Z,8Z,11Z,14Z)	C20H32O2	303.5	1	16.65	0.997	-	2-39
43	Eicosapentanoic 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	Docosapentaenoic acid	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 found in the chromatogram of the reference mix.
2) No calibration curve was created for C20:1(5).

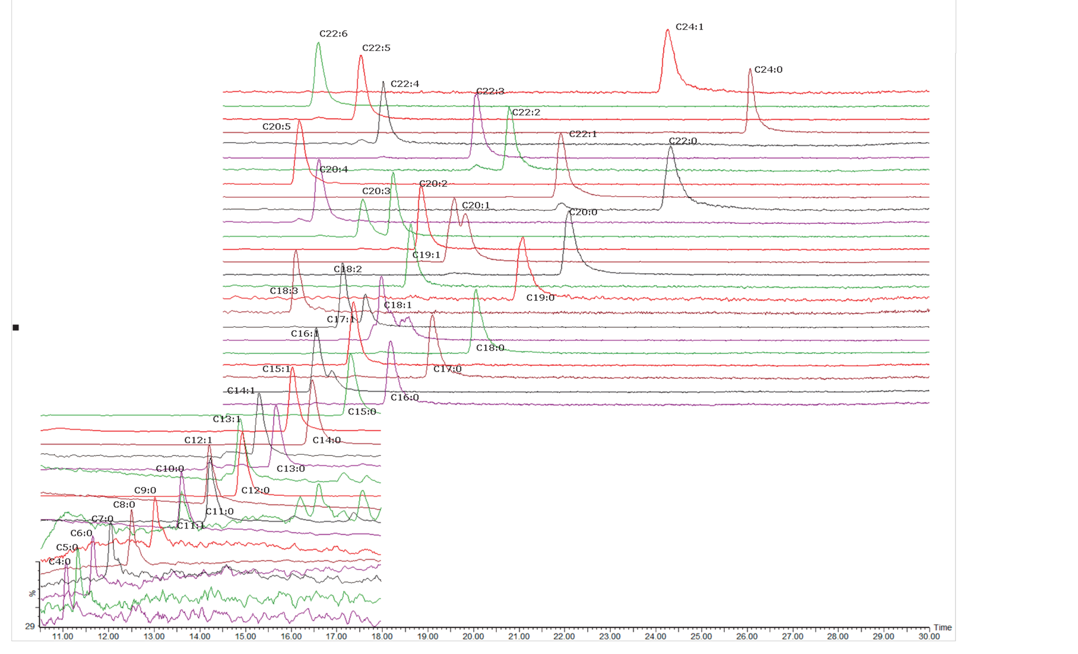

***Figure 2**. Overlay of chromatograms of 51 fatty acid compounds in a standard mix (GLC-463 fatty acid). The chromatograms of fatty acids from C4 to C15 were SIR chromatograms, and the chromatograms for C16 to C24 were XIC from MS scan spectrum. Peak labels were shown in the chromatograms. The UPC²-MS conditions are in the Experimental Section.*

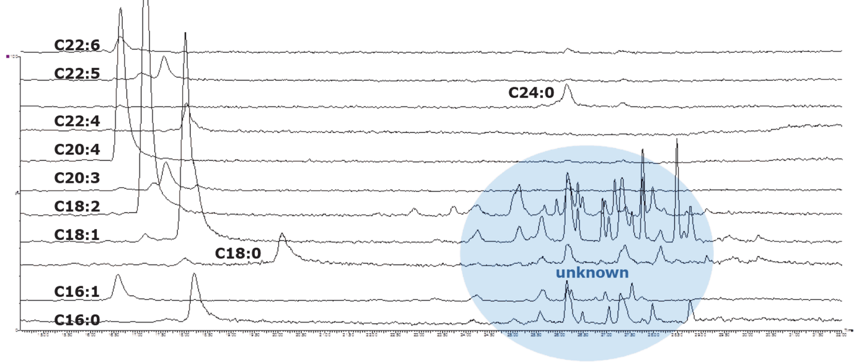

***Figure 3**. Selected XIC of fatty acids in sample (A). The peaks were identified by their m/z and the reference RTs of the corresponding fatty acid standards. The shaded area are the unknowns, which are believed from the sample matrix. The identified peaks were quantified with the corresponding calibration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

CONCLUSION

The determination of the FFA composition in food samples has been demonstrated using Waters ACQUITY UPC² coupled with Xevo TQ-S MS. The benefits of this UPC²-MS method include:

- No derivatization;
- 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 UPC²-MS method provides an alternative approach for the analysis of the fatty acid composition in food.

References

1) Domenico Marini. HPLC of Lipids. In: Leo M.L. Nolllet, ed. Food Analysis by HPLC. Second Edition, New York: Marcel Dekker, 2000, pp 175-209
2) AOAC Official Method 2012.13 Labeled Fatty Acids Content in Milk 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 UPC² 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
5) Mehdi Ashraf-Khorassani, Larry T. Taylor, Jinchuan Yang, Giorgis Isaac, Fast Separation of Triacylglycerols in Oils using UltraPerformance Convergence Chromatography (UPC2), Waters Application Note 720004871en, 2014
6) Jinchuan Yang, Carrie Snyder, Jessica Lance, B. J. Bench, Jayant Shringarpure, Fast analysis of short-chain fatty acids in feeds by UPC²-MS, Poster presented at 129th AOAC Annual Meeting, September 27-30, 2015, Los Angeles, California, USA

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