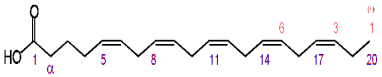
LC/MS/MS quantitation of eicosapentaenoic acid in rat plasma

Rachel Sun, Jordan Nally, Tim Shoaf
***BASi,* 2701 West Lafayette, IN 47906**

Introduction and Purpose

Eicosapentaenoic acid (EPA) is an omega-3 fatty acid, which has been documented to be important on biochemical and physiological processes including inflammatory response, blood clotting, and the immune system. Eicosapentaenoic acid (EPA) is different forms in the blood circulation, such as triglyceride, ethyl ester, or free acid. In this work, we presented an LC/MS/MS method to quantitate the total eicosapentaenoic acid in rat plasma

Structure of EPA



Extraction procedure

Transfer sample/Cal/QCs/blanks to 96 well plate
Add internal standard working solution
Add 200 µL of hydrolyzation solvent ACN/6N HCl (90:10)
Hydrolyze the samples at 100 °C for 1 hour and 15 min
Cool down the plate to room temperature. Centrifuge the plate briefly.
Add 650 µL of hexane for liquid-liquid extraction
Transfer the supernatant to the clean plate with 10 µL DMSO in each well.
Evaporate to dry ness and add reconstitution solutions
Vortex and centrifuge.
Seal the plate and inject samples.

LC/MS Conditions

Column: Kinetex, XB-C18, 50*2.1, 2.6 µ)
Mobile Phase A: 10% ACN/90% water,
Mobile Phase B: 90% ACN/10% water, 10 mM Ammonium acetate.
Run time: 3.5minutes

TSQ Quantum mass spectrometer

Source:	HESI
Ion mode:	Negative
Resolution (Q1/Q3):	Unit/Unit
MRM masses(*):	301/257 (EPA)
	306/262 (EPA-d5)

Results

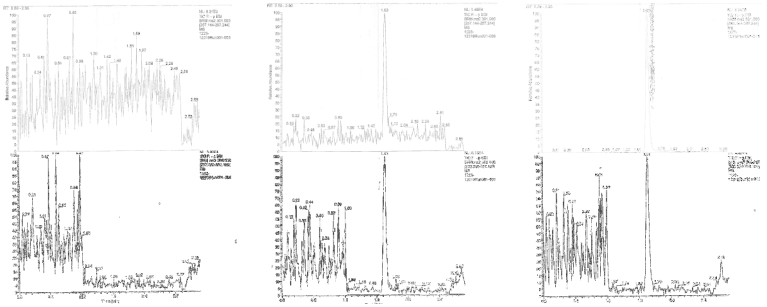
Calibration statistics

Assay Date	Analytical Run Number	STD 0.500 0.500 µg/mL	STD 1.00 1.00 µg/mL	STD 2.00 2.00 µg/mL	STD 5.00 5.00 µg/mL	STD 20.0 20.0 µg/mL	STD 50.0 50.0 µg/mL	STD 100 100 µg/mL	STD 250 250 µg/mL
18-Mar-2013	1	0.526	1.08	2.18	5.21	21	53.4	104	277
		0.481	0.888	1.82	4.9	18.4	49.3	92.7	224
18-Mar-2013	2	0.46	0.942	1.87	5	19.4	48.5	99.3	250
		0.524	1.11	2.13	5.17	19.7	50.2	101	253
19-Mar-2013	3	0.485	1.04	2.03	5.35	20.5	53.2	104	263
		0.519	0.985	1.77	4.98	18.8	47.8	99.9	233
19-Mar-2013	4	0.412	1.01	1.93	5.04	19.4	51.4	100	248
		0.598	0.971	1.98	5.09	19.5	51.2	105	243
20-Mar-2013	5	0.479	1.07	1.94	5.04	19.9	50.6	101	244
		0.522	0.949	1.89	5.27	19.7	50.4	98.8	256
20-Mar-2013	6	0.51	0.899	1.74	5.01	20.1	51.2	105	237
		0.526	1.02	1.98	5.12	20.3	50.6	108	246
Mean		0.504	0.997	1.94	5.1	19.7	50.7	102	248
S.D.		0.0454	0.0708	0.133	0.131	0.714	1.65	3.98	13.9
%CV		9	7.1	6.9	2.6	3.6	3.3	3.9	5.6
%Bias		0.8	-0.3	-3	2	-1.5	1.4	2	-0.8
n		12	12	12	12	12	12	12	12

QC statistics

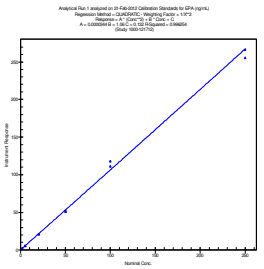
Run Date	Curve Number	QC 2.00 2.00 ng/mL	QC 80.0 80.0 ng/mL	QC 200 200 ng/mL
18-Mar-2013	1	2.02	83.4	221
		1.78	77.2	200
18-Mar-2013	2	2.07	80.9	206
		2.08	78.9	208
19-Mar-2013	3	2.11	77.2	206
		1.93	76.1	199
19-Mar-2013	4	2.02	77.4	199
		1.93	80.1	263
20-Mar-2013	5	2.17	82	201
		1.91	79.6	218
20-Mar-2013	6	1.87	81.4	194
		2.16	82.2	185
Mean		2	79.7	208
S.D.		0.121	2.36	19.8
%CV		6.1	3	9.5
%Theoretical		100	99.6	104
%Bias		0	-0.4	4
n		12	12	12

Chromatograms



Proxy Blank- **LLOQ** **ULOQ**

Typical calibration curve



Discussion

proxy matrix for endogenous compound: parallelism test

Calibrators in proxy matrix

Assay Date	Analytical Run Number	STD 0.500 0.500 µg/mL	STD 1.00 1.00 µg/mL	STD 2.00 2.00 µg/mL	STD 5.00 5.00 µg/mL	STD 20.0 20.0 µg/mL	STD 50.0 50.0 µg/mL	STD 100 100 µg/mL	STD 250 250 µg/mL
21-Feb-2012	1	0.547	1.02	1.95	5.21	19.7	49.3	111	239
		0.46	0.963	1.96	4.96	19.5	47.4	105	249
Mean		0.504	0.992	1.96	5.09	19.6	48.4	108	244
S.D.									
%CV									
%Bias		0.8	-0.8	-2	1.8	-2	-3.2	8	-2.4
n		2	2	2	2	2	2	2	2

QCs in plasma and proxy matrix

Run Date	Curve Number	Proxy matrix QC 2.00 2.00 µg/mL	Proxy matrix QC 80.0 80.0 µg/mL	Proxy matrix QC 200 200 µg/mL	Plasma Matrix QC 2.00 PL 8.00 µg/mL	Plasma Matrix QC 80.0 PL 86.0 µg/mL	Plasma Matrix QC 200 PL 206 µg/mL	Plasma Matrix QC 250 PL 256 µg/mL
21-Feb-2012	1	2.08	76.4	193	9.25	85.8	199	239
		2.5	78.9	189	8.26	87	184	243
		2.09	75.2	190	8.24	85.7	195	254
		1.95	78.1	190	8.53	86.5	202	247
Intrarun Mean		2.16	77.2	191	8.57	86.3	195	246
Intrarun SD		0.239	1.67	1.73	0.472	0.614	7.87	6.4
Intrarun %CV		11.1	2.2	0.9	5.5	0.7	4	2.6
Intrarun %Bias		8	-3.5	-4.5	7.1	0.3	-5.3	-3.9
n		4	4	4	4	4	4	4

Hydrolysis of EPA conjugates

Majority of EPA is found in plasma phospholipids and lipoprotein complexes. Plasma samples are hydrolyzed at acidic condition to release EPA from conjugates. The hydrolysis was carried out in a 96 well plate with an in house made clamp to prevent acetonitrile evaporation.

Conclusions

A fast, simple, specific, and robust method was developed with excellent linearity, precision, and accuracy for the quantification of EPA in rat plasma. The method has been used successfully to assay samples from non GLP studies.

Contact Information

BASi
2701 Kent Ave
West Lafayette, IN 47906
www.basinc.com

Contact:
Rachel Sun
rsun@basinc.com