How the Origin of Donors can Influence the Quantitation of a Compound in Plasma: Case Studies of Matrix Effect

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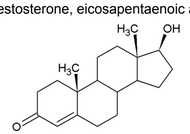
Introduction

It is recommended in the guidelines for method validation that matrix effect from special population should be studied.

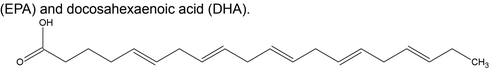
"If samples from special populations (such as renally or hepatically impaired populations) are to be analysed it is also recommended to study matrix effects using matrix from such populations."

However, when studies are done in countries with different alimentary customs, should the matrix effect on that population be evaluated? Herein two case studies are described, demonstrating the influence of the origin of donors on bioanalysis of testosterone, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).



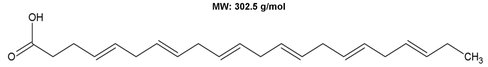
Testosterone

MW: 288.42 g/mol



Eicosapentaenoic Acid

MW: 302.5 g/mol



Docosahexaenoic Acid

MW: 328.5 g/mol

Method – EPA/DHA

Extraction Procedure

- Internal Standard: EPA-d_5 and DHA-d_5
- Sample Volume: 40 µL
- Protein precipitation , alkaline hydrolysis and dilution
- Human EDTA K_3 plasma
- 2-100 µg/mL for EPA and 10-200 µg/mL for DHA
- Concentration factor: 0.00077

LC-MS/MS Analysis

Chromatographic mode:	Reversed Phase
Analytical Column:	Zorbax Extend 50 x 4.6mm, 3.5µm
Column Temperature:	25° C
Elution mode:	Isocratic
Mobile Phase A:	Milli-Q Type Water / Methanol (24/76), Ammonium Acetate 1mM, Ammonium Hydroxyde 0.1%
Flow Rate:	1.5 mL/minute
Retention Times:	1.10 minutes for EPA 1.63 minutes for DHA
Acquisition time:	3.0 minutes
Detector:	AB Sciex API 4000
Source:	TurboIonSpray, Negative mode
Ion Monitored:	EPA: 301→257 amu DHA: 327→283 amu

Method - Testosterone

Extraction Procedure

- Internal Standard: Testosterone-d_5
- Sample Volume: 150 µL
- Automated liquid-liquid extraction with MTBE
- Human EDTA K_3 plasma
- 60-12000 pg/mL
- Concentration factor: 0.6

LC-MS/MS Analysis

Chromatographic mode:	Reversed Phase
Old Analytical Column:	X-Bridge C18, 4.6 x 50 mm, 3.5 µm
New Analytical Column:	Zorbax SB-C18, 4.6 x 30 mm, 3.5 µm
Column Temperature:	Room Temperature
Elution mode:	Isocratic with column flush
Mobile Phase A:	Milli-Q Type Water / Acetonitrile (36/64), Ammonium formate 5mM, Formic Acid 0.1%
Flow Rate:	1.0 mL/minute
Old Retention Times:	2.40 minutes
New Retention Times:	2.70 minutes
Acquisition time:	4.60 minutes
Detector:	AB Sciex API 5000
Source:	TurboIonSpray, Positive mode
Ion Monitored:	289→97 amu

Results

EPA/DHA

Table 1. EPA/DHA Endogenous Level in Human Plasma from Vegetarian Indian Donors

Donor	Concentration (µg/mL)	
	Total EPA	Total DHA
A	0.60	7.16
B	1.61	14.67
C	1.80	7.64
D	2.28	16.58
E	1.85	10.50
F	1.77	9.27
G	2.14	20.24
H	2.49	12.61
I	0.98	8.17
J	1.90	11.01
K	0.62	7.99
L	1.95	10.25
M	3.37	22.36
N	1.52	8.68
O	1.09	22.39
P	1.17	8.71
Q	1.13	8.61
R	1.53	8.86
S	3.17	14.68
T	2.72	12.70
Mean:	1.78	12.15

Bold: estimated values (< LLOQ)

When plasma was collected from vegetarian Indian donors, the mean baseline level was 1.8 µg/mL for EPA and 12.2 µg/mL for DHA. The mean baseline level in plasma used in validation was 6 µg/mL and 30 µg/mL, respectively **(Tables 1 and 2)**. Therefore, the dynamic ranges were modified for 0.5-50 µg/mL and 5-100 µg/mL for EPA and DHA, respectively, to measure the baseline level in that population.

Table 2. EPA/DHA Endogenous Level in Human Plasma from Fish Eaters

Donor	Concentration (µg/mL)	
	Total EPA	Total DHA
A	2.43	32.25
B	1.06	12.99
C	1.23	11.01
D	2.42	27.00
E	27.94	64.72
F	10.90	42.87
Mean:	7.66	31.81

Bold: estimated values (< LLOQ)

Testosterone

Study samples from Indian donors were analyzed with the validated testosterone assay. Internal standard responses in study samples were 80% lower in study samples compared to the QCs in serum used during method validation **(Table 3)**.

In order to verify if the analyte was also affected, study samples were spiked with known concentration of testosterone to verify the accuracy. A negative bias of 44% was observed on the recovered testosterone concentration **(Table 4)**.

Table 3. Internal Standard Responses of Study Samples and QCs

Sample	IS response
Sample A	37570
Sample B	49991
Sample C	35831
Sample D	45215
Sample E	36787
Sample F	41041
Sample G	46083
Sample H	40226
Sample I	44763
Sample J	43140
Sample K	40104
Sample L	42962
Sample M	57334
Sample N	51327
Sample O	50348
Sample P	50248
QC	183790

Table 4. Accuracy of Spiked Testosterone in Study Samples

	Testosterone Concentrations (pg/mL)			
Donors	Unspiked	Spiked with 4000 pg/mL	Recovered Spiked T	Accuracy (%)
A	2458.6	4649.2	2271.7	56.8
B	2924.8	5591.4	2763.1	69.1
C	1872.1	4245.2	2434.9	60.9
D	3189.8	6389.4	3304.9	82.6
E	2878.5	5342.0	2558.6	64.0
F	1768.2	4149.5	2439.6	61.0
G	1618.5	4147.2	2582.1	64.6
H	2300.3	4672.3	2447.9	61.2
I	2964.5	5183.5	2316.9	57.9
J	1201.4	3518.4	2356.6	58.9
K	3731.9	5957.9	2349.1	58.7
L	1756.1	4485.4	2787.2	69.7
M	2172.6	4784.7	2683.7	67.1
N	3212.0	5261.5	2155.5	53.9
O	5673.9	8830.6	3344.0	83.6
P	2154.5	4658.0	2574.6	64.4

It was demonstrated that ionic suppression affected the analyte at a higher extent than the IS. Phospholipids were monitored for each type of serum and different impact on the analyte was observed. The analytical column was changed for a Zorbax SB-18 to get rid of this ionic suppression. No matrix effect was observed for eight different Indian donors compared to Caucasian donors **(Table 5)**. Study samples were analyzed with the improved chromatography and the IS responses were similar to the QC samples throughout the project.

Table 5. Accuracy of Spiked Samples

Donor	Testosterone Concentration (pg/mL)		Accuracy	IS response
	Unspiked	Spiked with 9000 pg/mL		
1	1812.0	10381.2	95.4	244075
2	258.5	9015.4	97.3	265311
3	305.3	9214.5	99.0	228232
4	2416.1	10857.0	94.1	261053
5	1745.6	10464.5	97.1	245315
6	232.4	9257.2	100.3	231651
7	294.5	9063.2	97.5	245114
8	382.6	9464.0	100.9	233317
Normal QC	73.9	9128.9	100.6	244493

Results

Testosterone

Table 6. Matrix Effect at Low QC level

Untreated Standard (MFQC1)				Reference Solution (RSQC1)		Calculated Matrix Factor (Analyte)	Calculated Matrix Factor (IS)	IS-Normalized Matrix Factor
Analyte Responses	Expected Concentration	Corrected Area	Internal Standard Responses	Analyte Responses	Internal Standard Responses			
43474	293	26708	143169	29380	142911	0.8892	0.9704	0.9164
42413	279	27363	141431	29243	147039	0.9110	0.9586	0.9504
59565	438	24479	141996	29913	147271	0.8150	0.9625	0.8468
68435	498	24736	141289	30045	146665	0.8236	0.9577	0.8600
58918	412	25741	143020	30292	151328	0.8570	0.9694	0.8841
42294	284	26806	141650	31336	149993	0.8925	0.9601	0.9296
			Mean	30034.8	147534.5			0.8979
			SD(±)					0.0408
			CV(%)					4.55

Note: Analyte areas are corrected depending on the endogenous level. Matrix 3 and 4 are from Indian donors.

Table 7. Matrix Effect at High QC Level

Untreated Standard (MFULOQ)				Reference Solution (RSULOQ)		Calculated Matrix Factor (Analyte)	Calculated Matrix Factor (IS)	IS-Normalized Matrix Factor
Analyte Responses	Expected Concentration	Corrected Area	Internal Standard Responses	Analyte Responses	Internal Standard Responses			
1953024	12113	1934805	146972	1983969	151614	0.9750	0.9753	0.9997
1893102	12099	1877612	143546	1991652	149868	0.9461	0.9525	0.9933
1904373	12258	1864291	142390	1993156	149812	0.9394	0.9449	0.9942
1945581	12318	1895354	145361	1985909	151755	0.9551	0.9646	0.9902
1890491	12232	1854635	142251	1975978	149033	0.9346	0.9439	0.9901
1851324	12104	1835417	140306	1976199	152104	0.9249	0.9310	0.9934
			Mean	1984477.2	150697.7			0.9935
			SD(±)					0.0035
			CV(%)					0.35

Note: Analyte areas are corrected depending on the endogenous level. Matrix 3 and 4 are from Indian donors.

Conclusion

It was observed that donors that might have different customs, specifically related to their alimentary habits, may introduce matrix effect to specific methods. In special occasions, it is recommended that matrix effect using donors from different population must be studied during method development.

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