

# QUANTITATIVE AND QUALITATIVE ANALYSIS OF LIPIDS AND CHOLESTEROL DERIVATIVES IN SMOFlipid® 20% INTRAVENOUS LIPID EMULSION

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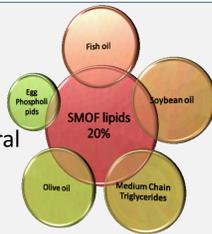
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## PURPOSE

SMOFlipid® 20% is an intravenous lipid emulsion (ILEs) used for parenteral nutrition to provide essential fatty acids for patients who have difficulty absorbing nutrition through digestive tract. These emulsions are made from natural sources like fish oil, medium chain triglycerides, soybean oil and olive oil. The composition of these lipids can vary based on sources and manufacturing processes and may also contain various degradation products, that can potentially affect patients. Accurate quantification of lipid constituents and identification of possible lipid degradation products, as well as cholesterol oxidation products, are necessary for a comprehensive evaluation of the formulation in post-market studies. In this study we analyzed the lipid composition of SMOFlipid® 20% using UHPLC-QTOF-ESI/APCI.



## METHODS

A series of UHPLC-HRMS (QTOF) methods was developed to detect and quantify free fatty acids, triglycerides, cholesterol derivatives, phospholipids, and lyso-phospholipids present in SMOFlipid® 20%. The study was furthered to compare the amounts of all these components with their USP-grade sources of fish oil, soybean oil, olive oil, and medium-chain triglyceride (MCT) from different vendors.

The lipid and other components were separated using reversed-phase UHPLC methods and detected using ESI/APCIQTOF (electron spray ionization/atmospheric pressure chemical ionization- Quadrupole time-of-flight) mass spectrometry techniques. The Premier Biosoft SimLipid® software was used to identify the separated lipids using their MS/MS fragmentation patterns from the software lipid database. The composition of identified lipids was quantitatively or semi-quantitatively determined with commercially available lipid standards.

For the method validation, calibration curves were built using six concentrations and spiked with an internal standard. Similarly, quality control (QC) samples were independently prepared at five concentration levels for each standard. Inter-day and intra-day method validation as well as precision calculation were performed on different days for all standards.

## RESULTS

### Free fatty acids (FFA)

- Ionized by ESI source in the negative mode.
- 14 free fatty acids were identified in SMOFlipid® 20%
- Most abundant FFA: Oleic acid (C18:1) - 54.80 mg
- Other abundant FFA: linoleic acid (C18:2) & caprylic acid (C8:0)

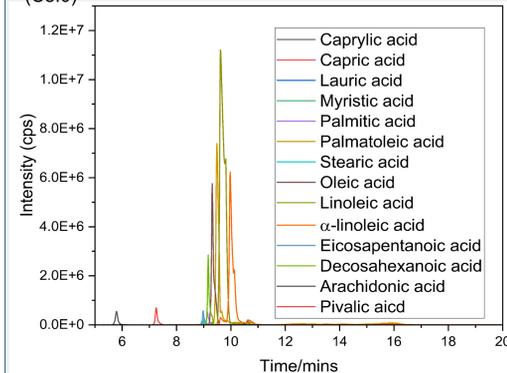


Figure 1. Individual Ion Chromatogram of FFA in SMOFlipid® 20%

### Cholesterol

- Ionized by APCI source in the positive mode.
- 11 cholesterol derivatives identified in SMOFlipids® 20%
- Cholesterol derived from fish oil present in avg 54.62 mg
- Phytosterols such as campesterol (126.47 mg) and beta-sitosterol (46.70 mg) present in considerable amounts

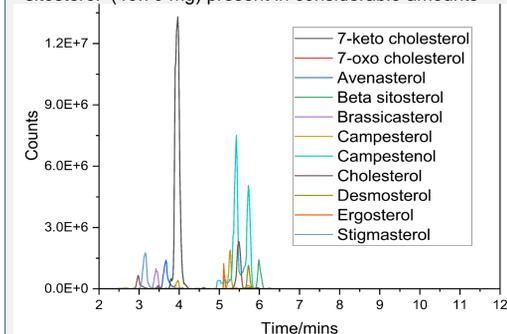


Figure 2. Individual Ion Chromatogram of Cholesterol derivatives present in SMOFlipid® 20%

## ADDITIONAL INFORMATION

- Identified compounds in SMOFlipid® 20% included 14 free fatty acids, 11 cholesterol and cholesterol derivatives, 23 triglycerides (TG, comprising 7 medium-chain triglycerides (MCTs) and 17 long-chain triglycerides), 21 lyso-phospholipids, and 51 phospholipids
- SMOFlipid® 20% and its component (olive oil, fish oil, soybean oil and MCT) contain more than 95% of triglycerides by weight
- Majority of the long chain triglycerides in SMOFlipid® 20% are made up of C18 carbon chains, and majority of medium chain triglycerides are made of C8 carbon chains, and the sole contributor of medium chain triglyceride was MCT
- The main cholesterol contributor to the SMOFlipid® 20% emulsion is fish oil, but the most abundant sterol derivatives are phytosterols derived from plant-based lipid sources such as olive oil and MCT
- Phosphocholines were the most abundant phospholipids class present in SMOFlipid® 20% (more than 80% of total phospholipids by mass)

Table 1. Quantification of Phospholipids (mg/dL ± (SD)) present in SMOFlipid® 20% (blue) and in purified egg phospholipids from different vendors (violet, green)

Phospholipids	Vendor 1 (1.2%)					Vendor 2 (1.2%)					Vendor 1 (1.2%)					Vendor 2 (1.2%)						
	SMOF (mg)	Avg (mg)	SD	Avg (mg)	SD	SMOF (mg)	Avg (mg)	SD	Avg (mg)	SD	SMOF (mg)	Avg (mg)	SD	Avg (mg)	SD	SMOF (mg)	Avg (mg)	SD	Avg (mg)	SD		
LPC 14:0	0.4	0.4	0	ND	0	LPE 18:0	9.4	8.6	0.9	7.6	0.7											
LPC 16:0	14	14	1.7	1.8	1.6	PE 16:0/18:2	10	9.9	0.6	13	1.1											
LPC 16:1	2.4	2.6	0.2	1.6	0.1	PE 16:0/18:0	13	8	0.5	6	0.4											
LPC 18:0	9.2	4.9	0.3	6.9	0.5	PE 18:1/18:2	3.6	2.3	0.2	2.1	0.1											
LPC 18:1	55	17	1.6	17	1.9	PE 18:0/18:2	6.8	8.4	0.8	8.5	0.8											
PC 16:0/16:1	31	36	2.8	30	2.4	PE 18:0/18:1	28	27	1.8	17	1.3											
PC 16:1/18:2	15	18	1.7	17	1.3	PE 18:1/20:1	27	29	2.2	32	1.8											
PC 16:0/18:2	223	145	9.5	145	10	PE 18:0/20:1	35	55	3.1	57	2.2											
PC 16:0/18:1	247	201	12	208	15	PE 18:0/22:6	89	104	4.7	113	4.2											
PC 16:0/20:4	64	44	3.1	36	2.2	LPI 18:0	2.1	5	0.4	ND	0											
PC 18:1/18:2	49	53	4.7	57	4.2	PI 16:0/16:1	2.9	3.4	0.2	2.8	0.2											
PC 18:0/18:2	190	145	8.3	161	8.1	PI 16:0/18:2	6.6	11	1.1	6.1	0.7											
PC 18:0/18:1	168	144	6.5	146	8.4	PI 16:0/18:1	9.9	9.7	0.8	12	1.1											
PC 16:0/22:6	45	37	2.5	40	2.8	PI 16:0/20:4	13	8.5	0.5	12	1.2											
PC 16:0/22:5	20	27	2.2	26	1.9	PI 18:0/18:2	2.6	2.6	0.2	3.5	0.3											
LPE 14:0	0.5	0.8	0	ND	0	PI 18:0/18:1	2.6	2.8	0.2	3.2	0.3											
LPE 16:0	5	4.3	0.3	8.3	0.5																	

Average was taken from 6 samples for all the lipids; N=6

Table 2. Quantification of Lyso-phospholipids (mg/dL ± (SD)) present in SMOFlipid® 20% and in olive oil, soybean oil, fish oil, and MCT from different vendors

Lyso-Phospholipids	Olive oil 5%					Soybean oil 6%					MCT 6%					Fish oil 3%							
	SMOF (mg)	Avg (mg)	SD	Avg (mg)	SD	SMOF (mg)	Avg (mg)	SD	Avg (mg)	SD	SMOF (mg)	Avg (mg)	SD	Avg (mg)	SD	SMOF (mg)	Avg (mg)	SD	Avg (mg)	SD			
LPC 14:0	0.44	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.013	0.001	0.016	0.001				
LPC 16:0	13.98	ND	ND	ND	0.181	0.012	0.118	0.011	0.062	0.005	0.492	0.015	0.379	0.012	0.291	0.008	ND	0.000	0.000				
LPC 16:1	2.37	ND	ND	ND	0.095	0.006	0.159	0.008	0.069	0.006	0.224	0.007	0.143	0.005	0.145	0.005	ND	ND					
LPC 18:0	9.16	2.844	0.186	2.596	0.090	0.861	0.055	3.750	0.263	3.298	0.174	2.040	0.108	3.644	0.148	2.587	0.138	1.358	0.074	0.829	0.039	1.311	0.071
LPC 18:1	54.55	10.629	0.277	5.311	0.175	8.283	0.215	1.911	0.125	2.416	0.146	1.212	0.097	24.684	0.585	16.409	0.322	11.565	0.282	0.491	0.034	0.712	0.034
LPG 16:0	0.14	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.021	0.001	ND	0.014	0.001	0.083	0.005	0.028	0.002				
LPG 18:0	0.081	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.039	0.003	ND	0.029	0.001	0.019	0.001	ND					
LPE 14:0	0.54	ND	ND	ND	0.014	0.001	ND	ND	ND	ND	ND	0.008	0.001	ND	ND	ND	ND	ND					
LPE 16:0	4.98	0.044	0.003	0.064	0.005	0.031	0.002	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND					
LPE 18:0	9.37	0.042	0.003	ND	0.014	0.002	0.012	0.001	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND					
LPA 16:0	0.77	0.163	0.010	0.099	0.008	0.088	0.009	0.238	0.006	0.185	0.005	0.175	0.008	0.484	0.026	0.257	0.014	0.327	0.013	ND	ND		
LPA 18:1	0.53	0.107	0.008	0.111	0.010	0.069	0.005	0.216	0.010	0.061	0.002	0.101	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.000	ND	

Average was taken from 6 samples for all the lipids; N=6

Table 3. Quantification of Phosphocholines (mg/dL ± (SD)) present in SMOFlipid® 20% and in olive oil, soybean oil, fish oil, and MCT from different vendors

Phospholipids	Olive oil 5%					Soybean oil 6%					MCT 6%					Fish oil 3%							
	SMOF (mg)	Avg (mg)	SD	Avg (mg)	SD	SMOF (mg)	Avg (mg)	SD	Avg (mg)	SD	SMOF (mg)	Avg (mg)	SD	Avg (mg)	SD	SMOF (mg)	Avg (mg)	SD	Avg (mg)	SD			
PC 16:0/22:4	9.48	ND	ND	ND	ND	2.242	0.119	3.814	0.203	1.340	0.083	ND	ND	ND	7.744	0.423	4.759	0.284					
PC 16:0/18:2	223.38	2.124	0.116	ND	0.697	0.048	ND	ND	ND	2.622	0.086	1.235	0.110	0.993	0.075	1.188	0.077	0.328	0.027				
PC 18:0/18:2	190.03	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	30.742	1.267	26.989	0.912					
PC 16:0/22:6	44.82	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	6.178	0.393	9.857	0.623					
PC 18:3/18:3	12.22	1.684	0.100	2.615	0.173	1.377	0.143	ND	ND	2.749	0.170	3.694	0.161	1.190	0.078	0.811	0.046	1.235	0.082				
PC 14:0/14:0	34.77	7.081	0.372	5.655	0.236	3.953	0.124	6.267	0.276	11.894	0.412	7.756	0.325	7.800	0.203	5.905	0.232	3.972	0.137	12.866	0.473	13.384	0.570
PC 16:1/16:1	10.78	ND	ND	ND	ND	2.702	0.168	4.668	0.224	1.800	0.113	3.595	0.162	2.446	0.131	1.562	0.078	4.359	0.271	1.634	0.080		
PC 18:2/18:2	7.55	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	7.355	0.366	6.503	0.313					
PC 16:0/18:1	246.55	1.802	0.113	0.971	0.085	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.897	0.051	0.271	0.022					
PC 20:1/20:1	16.73	ND	ND	ND	ND	ND	ND	ND	ND	6.911	0.227	3.735	0.139	2.265	0.112	13.571	0.587	8.348	0.488				
PC 22:1/22:1	10.90	1.406	0.097	0.993	0.024	1.425	0.092	5.030	0.167	6.083	0.245	4.468	0.413	1.615	0.133	2.825	0.073	3.267	0.196	3.467	0.143	2.821	0.167
PC 16:0/20:4	64.13	1.260	0.138	2.310	0.133	2.070	0.144	1.841	0.136	1.655	0.140	1.320	0.123	0.708	0.063	0.658	0.069	0.426	0.041	1.103	0.105	1.884	0.132
PC 18:0/22:6	8.53	1.535	0.120	2.045	0.074	1.876	0.100	1.186	0.069	1.516	0.166	1.833	0.115	0.885	0.056	0.539	0.048	0.532	0.040	3.506	0.182	5.302	0.138
PC 18:0/18:1	168.39	3.054	0.210	2.829	0.174	2.732	0.164	1.340	0.100	0.970	0.080	1											