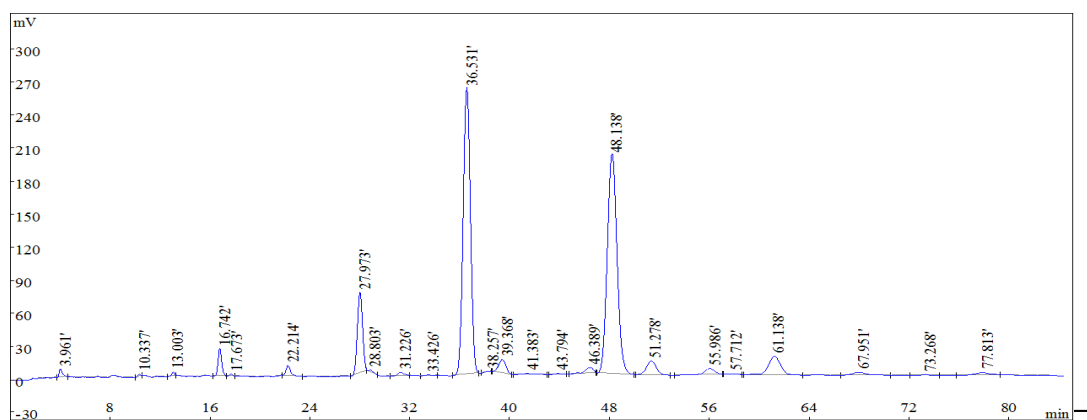


The chromatographic purification of DHA and EPA from fish oil

The chromatography is a kind of important technology for separation; especially separate the components which are difficult to separate by other technology. This document will describe some information about DHA and EPA purification from fish oil by preparative HPLC system of our company (CXTH).

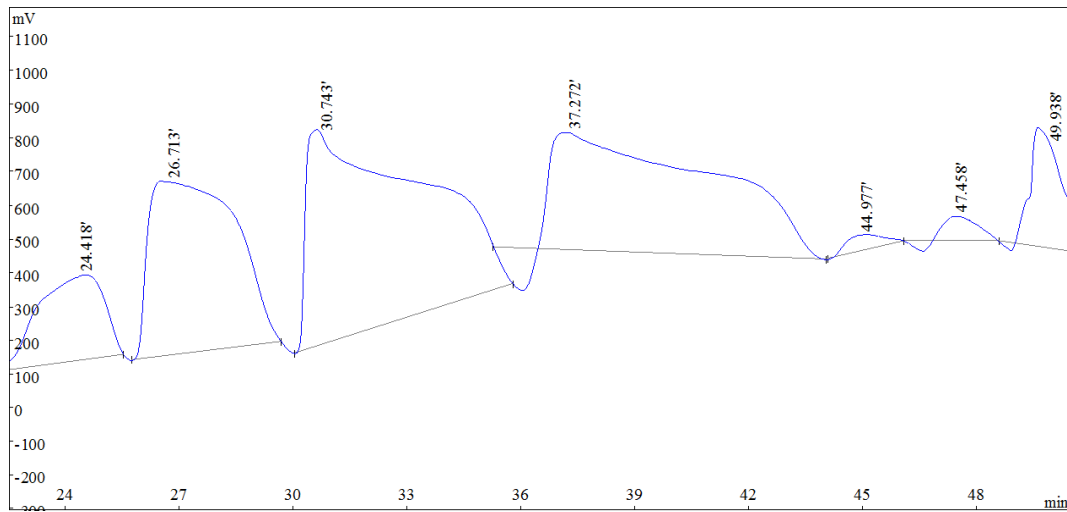
We finish the experiment on pilot preparative system with ID50mm column (RP packing material). The crude fish oil is divided into several fractions. The recovery fractions include DHA part, overlap part of DHA and EPA, EPA part. These three parts can be done the second step purification by PHPLC. After that, the collected single component of DHA or EPA can reach above 98%.

We start the purification from crude fish oil. The follow chromatogram is the result detected by HPLC.



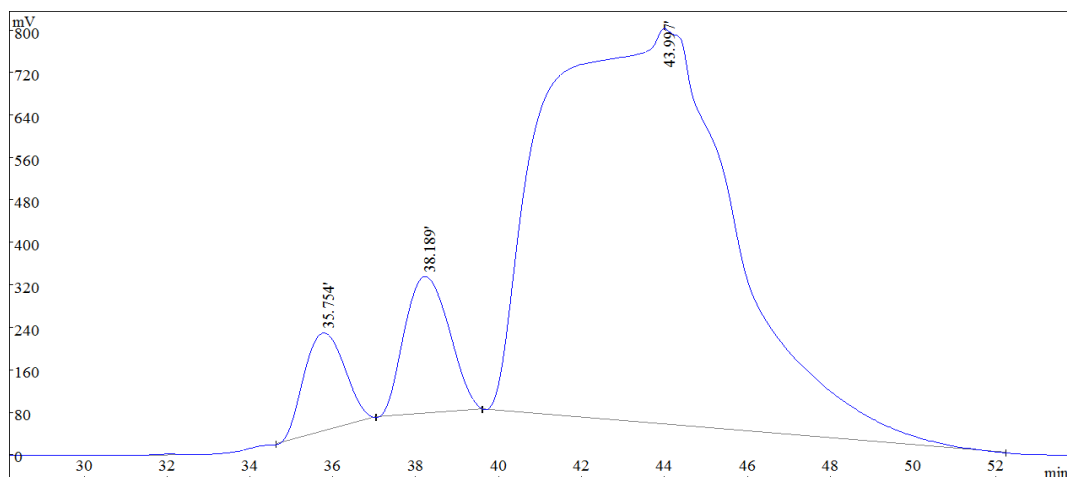
No.	Name	Ret.time	Conc	Area
11	EPA	36.531	37.25	10548573
12		38.257	0.08755	24791
13		39.368	1.792	507304
14		41.383	0.4416	125050
15		43.794	0.1662	47056
16		46.389	0.8618	244035
17	DHA	48.138	38.15	10800982
Total		100		23198972

The first step purification: divition of crude fish oil



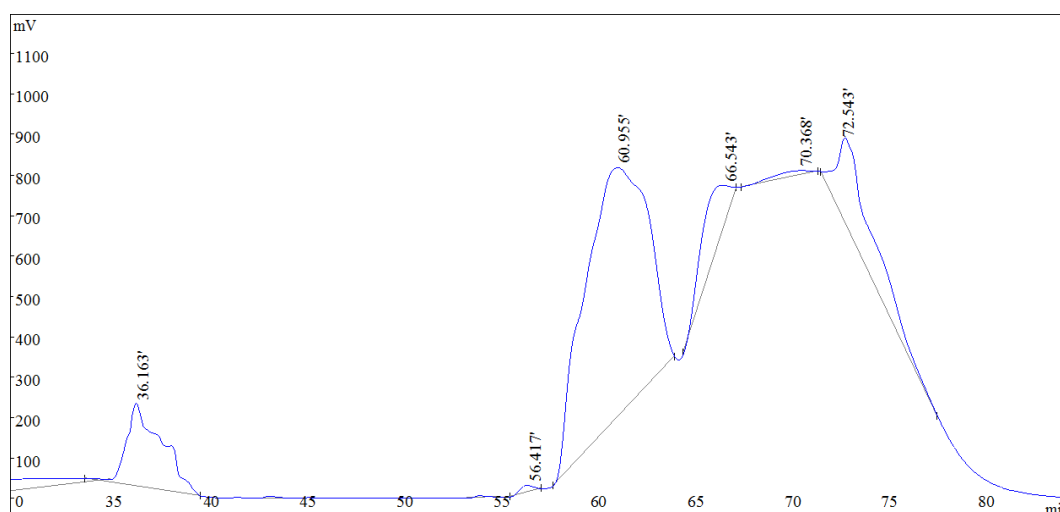
No.	Collecte number	Time (min)	volume	EPA (%)	DHA (%)
1	0225[2-1]	30.3-31.2		96.08	
2	0225[2-2]	31.2-33.7		96.86	
3	0225[2-3]	33.7-35.5		94.33	
4	0225[2-4]	35.5-37.2		18.16	49.71
5	0225[2-5]	37.2-41			91
6	0225[2-6]	41-42.8			96.51
7	0225[2-7]	42.8-44.2			92.69

The second step purification for EPA:



No.	Collecte number	Time (min)	volume	EPA (%)	DHA (%)
1	0305[2-1]	40.0-42.7		99.58	
2	0305[2-2]	42.7-46.0		99.77	
3	0305[2-3]	46.0-51.2		98.29	

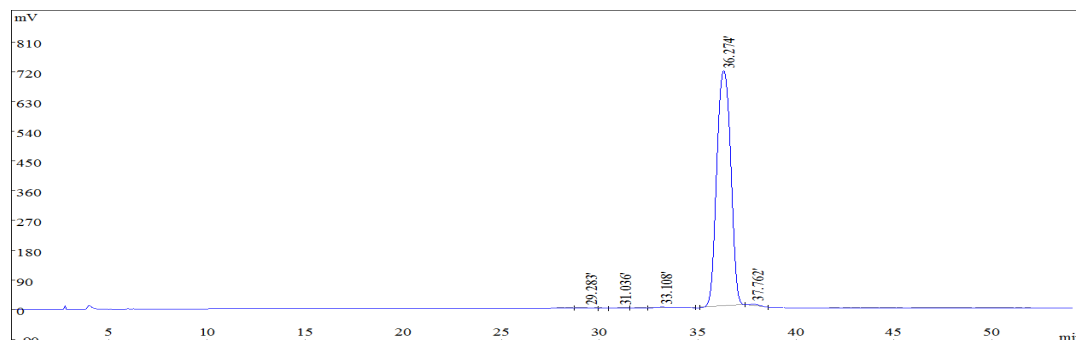
The second step purification for DHA



No.	Collecte number	Time (min)	volume	EPA (%)	DHA (%)
1	0306[2-1]	64.54-69.3			91.68
2	0306[2-2]	69.3-72.5			99.19
3	0306[2-3]	72.5-78			98.22
4	0306[2-4]	78-82.6			82.44

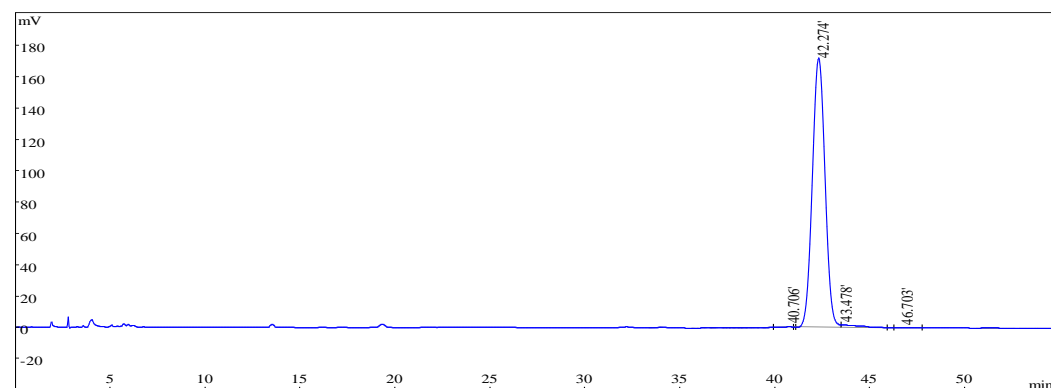
After these chromatographic separation processes, the qualified fraction can be combined. The single component of EPA or DHA can be tested by HPLC. In the combination process we can decide the final purity of the product by the fraction purity. The final purity of EPA can reach 98.95%. The DHA also can reach 98.5%.

The chromatogram of final EPA product



No.	Name	Ret.time	Conc	Area
1		29.283	0.09117	32526
2		31.036	0.04221	15058
3		33.108	0.4122	147066
4	EPA	36.274	98.95	35299290
5		37.762	0.5056	180355
Total		100		35674295

The chromatogram of final DHA product



No.	Name	Ret.time	Conc	Area
1		40.706	0.1863	15154
2	DHA	42.274	98.5	7991038
3		43.478	1.556	126618
4		46.703	0.03099	2521
Total		100		8135331

The estimated product yield by linear magnification

	Sample loading for one injection	Product yield/day	Product yield/month	Product yield/year
DAC300	0.68kg	EPA0.603kg DHA 0.603kg	EPA 18kg DHA 18kg	EPA 180kg DHA180kg
DAC500	1.9kg	EPA 1.68kg DHA 1.68kg	EPA 50.4kg DHA 50.4kg	EPA504kg DHA504kg
DAC600	2.736kg	EPA 2.41kg DHA2.41kg	EPA 72.36kg DHA 72.36kg	EPA 723.6kg DHA723.6kg
DAC800	4.864kg	EPA 4.28kg DHA 4.28kg	EPA 128kg DHA128kg	EPA 1280kg DHA1280kg
DAC1000	7.6kg	EPA 6.7kg DHA 6.7kg	EPA 200kg DHA 200kg	EPA 2000kg DHA2000kg

Note: DAC300, DAC500, DAC600, DAC800, DAC1000, these number means the column inner diameter.