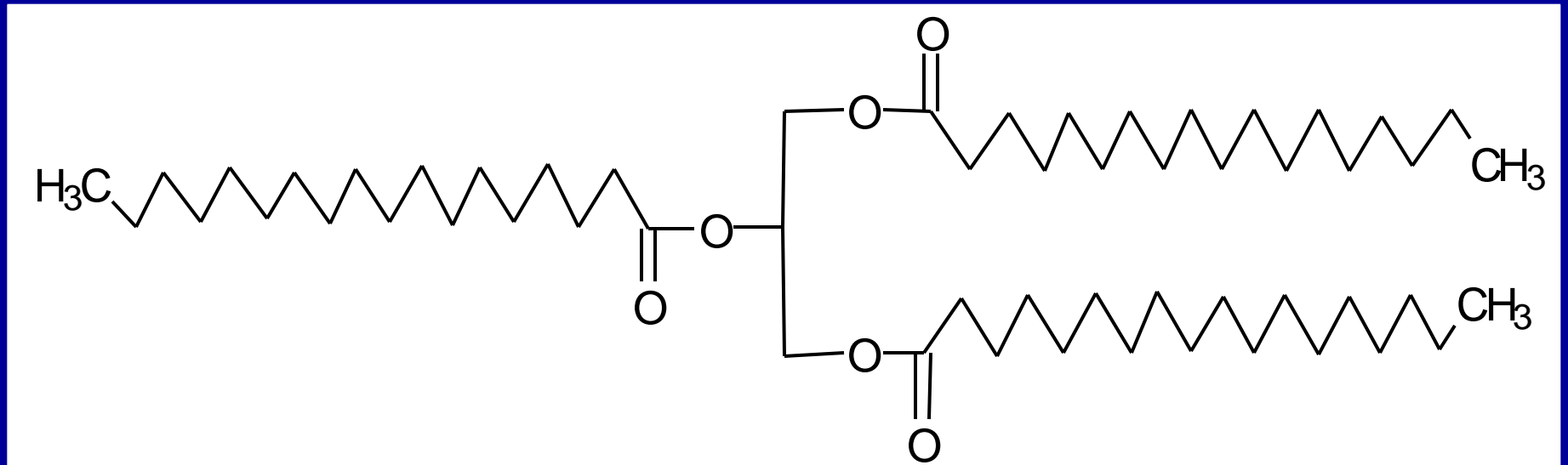


The Analysis of Marine Oil Based FAMES by Capillary Gas Chromatography



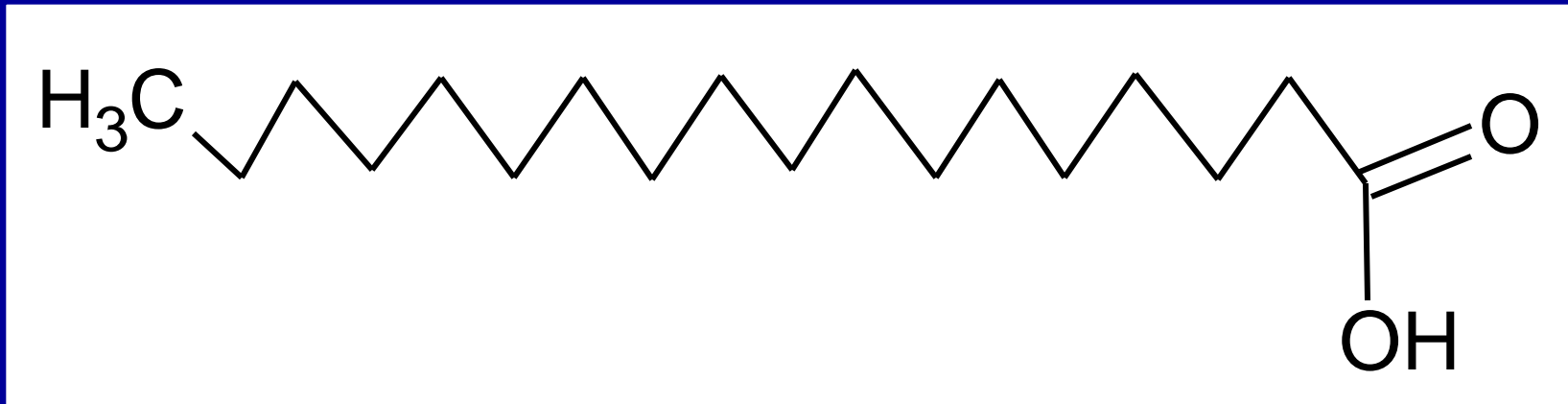
Food Lipids: Acylglycerols



Tristearin or glycerol tristearate

Neutral fats: mono-, di-, or triesters of glycerol with fatty acids

Food Lipids: Free Fatty Acids



Three fatty acids are contained in a triglyceride

- Saturated \approx no double bonds in the carbon chain
- Unsaturated \approx one or more double bonds in the chain
- *Cis* or *trans* possible around the double bond
- $\omega 3$ \approx double bond is on 3rd carbon from the methyl end

Typical Fatty Acid Compositions

Fatty Acid	Corn Oil	Soy Oil	Olive Oil	Peanut	Lard
C14:0			Trace	Trace	3
C14:1					Trace
C16:0	13	11	14	6	29
C16:1			2	Trace	3
C18:0	4	4	2	5	14
C18:1	29	25	64	61	38
C18:2	54	51	16	22	11
C18:3		9			Trace
C20:0	Trace	Trace	Trace	2	Trace
C20:1					Trace
C20:2					Trace
C22:0	Trace	Trace		3	

Marine/Fish Oil Fatty Acids

Fatty Acid	Herring	Cod	Trout
C14:0	6.4	1.4	2.7
C16:0	12.7	19.6	20.9
C16:1	8.8	3.5	3.9
C18:0	0.9	3.8	8.3
C18:1	12.7	13.8	18.4
C18:2w6	1.1	0.7	7.3
C18:3w3	0.6	0.1	1.6
C18:4w3	1.7	0.4	3.2
C20:1	14.1	3.0	ND
C20:4w6	0.3	2.5	1.7
C20:5w3	8.4	17.0	5.8
C22:1	20.8	1.0	ND
C22:5w3	0.8	1.3	Trace
C22:6w3	4.9	29.8	7.0

From: deMan, Principles of Food Chemistry

Nutraceuticals & Functional Foods

- Naturally derived, biologically active compounds
- Health benefit
- Consumed in various forms
 - ◆ Dietary supplement
 - ◆ Functional food or ingredient
- Regulation is still unclear

Nutraceutical Products

Marine/Fish Oils

- Rich in Polyunsaturated Fats
 - ◆ ω -3 fatty acids: potential health benefit
 - ◆ PUFAs can oxidize more readily
- Consumed as Fish or in Capsule Form
 - ◆ Enrichment of PUFAs possible in capsules
- Fatty Acid Composition Monitored by GC
 - ◆ Levels of DHA, EPA
- Lipid Class Separations by LC

Marine Oil FAME Analysis

Preparation of the FAMEs

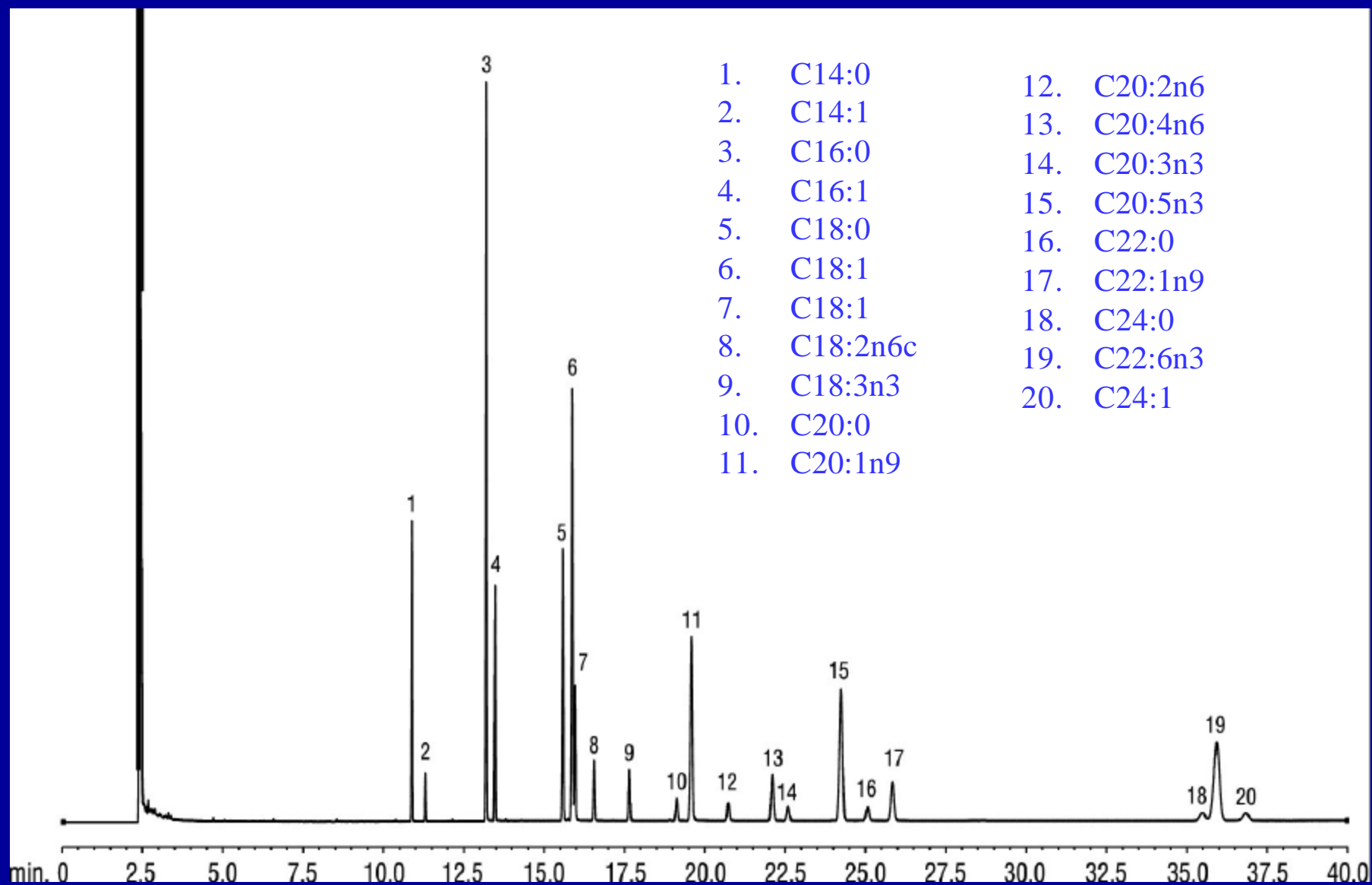
- Standard Methods
 - ◆ AOCS Ce 1b-89
 - ◆ European Pharmacopeia 2001:1352
- Saponification with NaOH or KOH
- Methylation
 - ◆ BF_3/MeOH or BCl_3/MeOH
- Extraction
 - ◆ Add saturated salt solution
 - ◆ Extract into isooctane

Marine Oil FAME Analysis

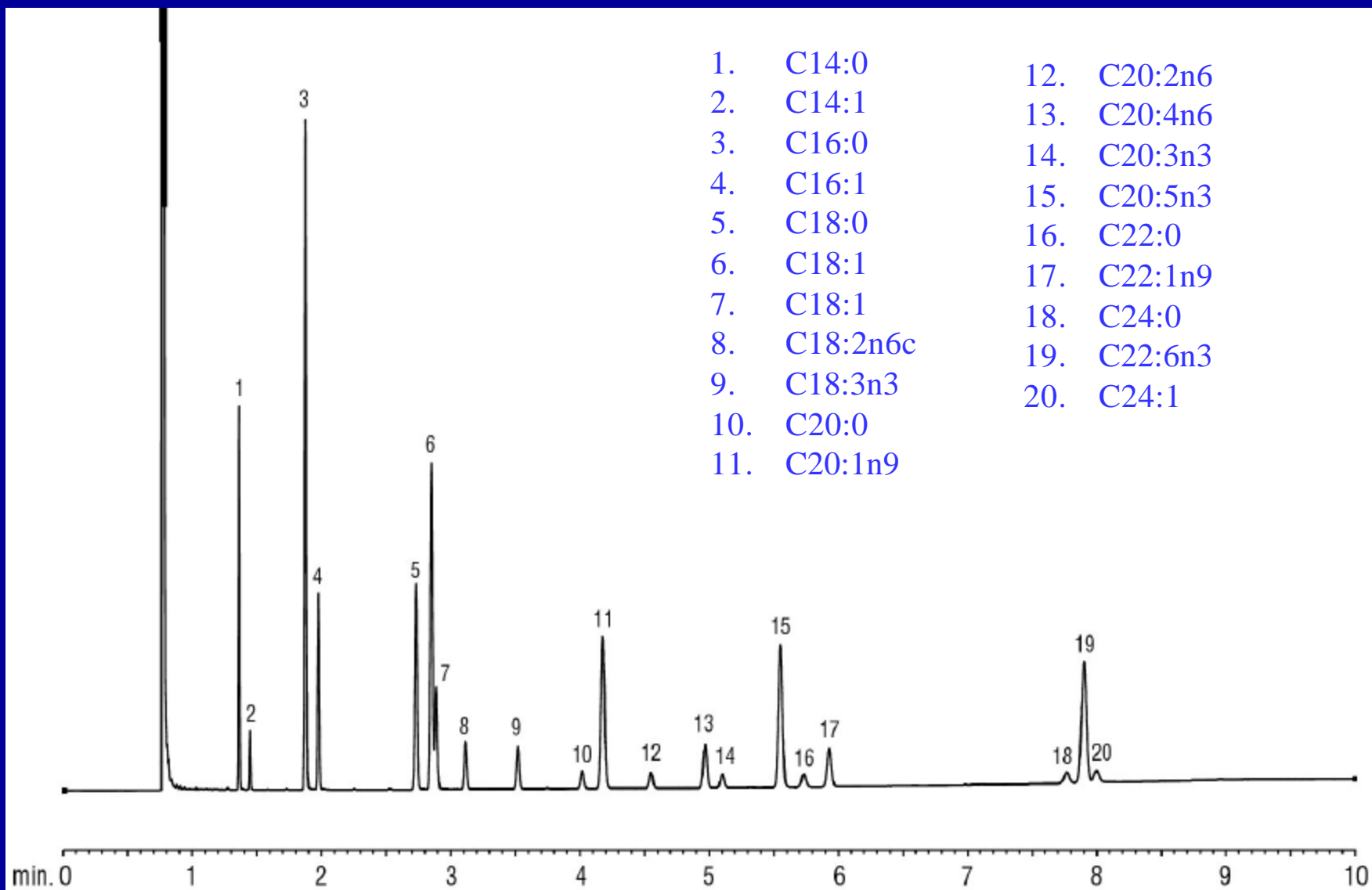
Standard GC Conditions

- Analytical Column
 - ◆ FAMEWAX™, 30m x 0.32mm x 0.25µm
- Oven Program
 - ◆ 100°C to 210°C at 8°C/min, 30 min hold
- Flow Rate
 - ◆ Hydrogen @ 1 mL/min
- Injector
 - ◆ 250°C, 100:1 split
- FID @ 250°C (or MSD)

Marine Oil FAMES - Standard



Marine Oil FAMES - Standard



Marine Oil FAME Analysis

Fast vs. Slow GC Programs

- Oven Program

SLOW: 100°C to 210°C at 8°C/min, 30 min hold

FAST: 195°C to 240°C at 5°C/min, 1 min hold

- Flow Rate

SLOW: Hydrogen @ 1 mL/min

FAST: Hydrogen @ 3 mL/min

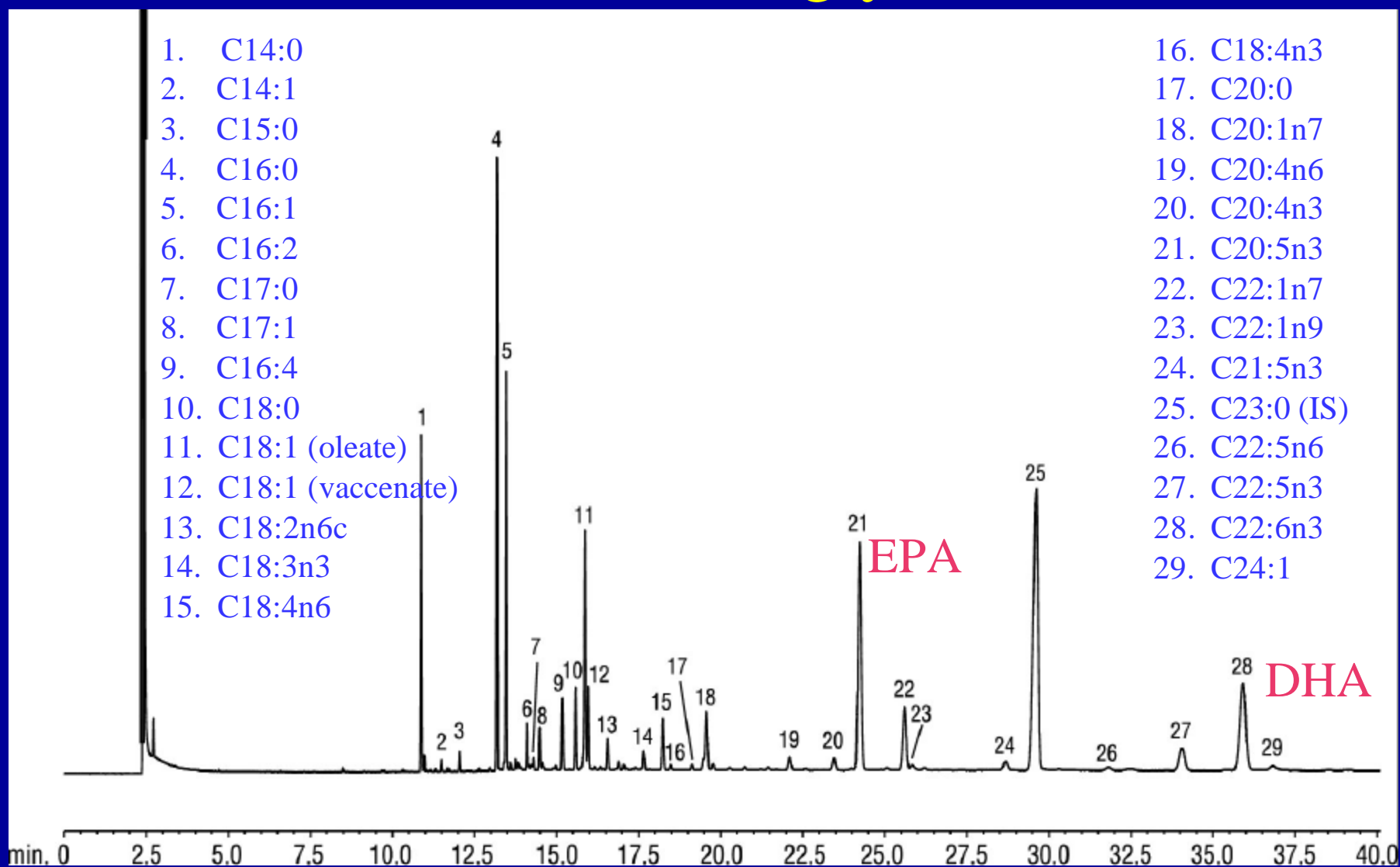
- Injector

SLOW: 100:1 split

FAST: 300:1 split

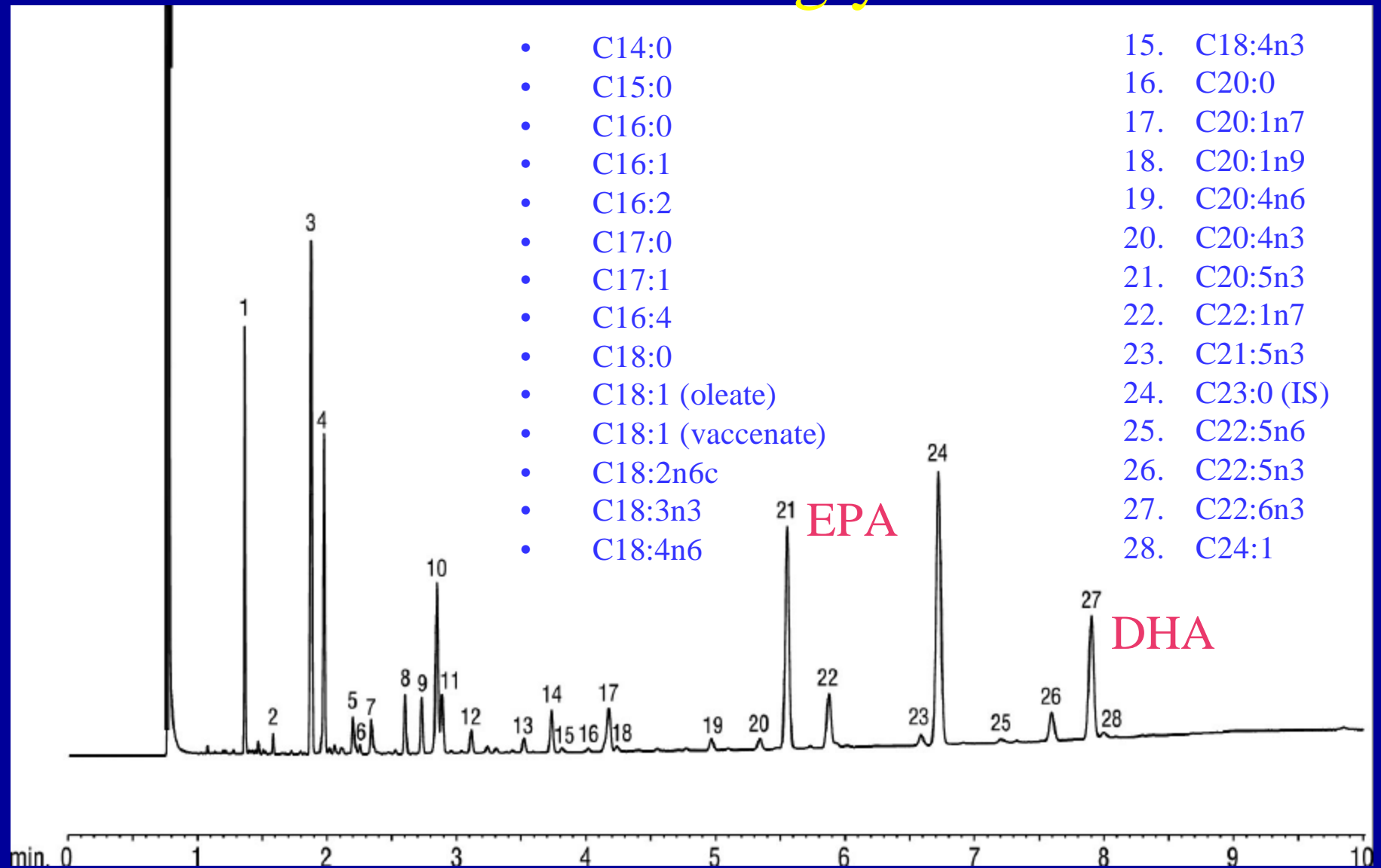
Marine Oil FAMES – Slow Program

18% EPA/12% DHA Triglycerides



Marine Oil FAMES – Fast Program

18% EPA/12% DHA Triglycerides



Validation Study

18/12 Triglyceride Capsule

SLOW GC PROGRAM		FAST GC PROGRAM			
mg of EPA	mg of DHA	mg of EPA	mg of DHA	% Difference	
				EPA	DHA
191.87	128.93	192.38	129.66	0.26	0.57
190.97	127.81	191.54	128.45	0.30	0.51
190.64	128.16	192.65	129.24	1.06	0.84
190.44	128.46	192.07	128.66	0.86	0.16
191.36	128.49	191.45	129.37	0.05	0.68
190.52	128.81	192.61	129.92	1.10	0.86
172.40	110.22	172.26	108.98	0.08	1.12
			AVERAGE-->	0.53	0.68

Data courtesy of Dave Waddell, Ocean Nutrition Canada

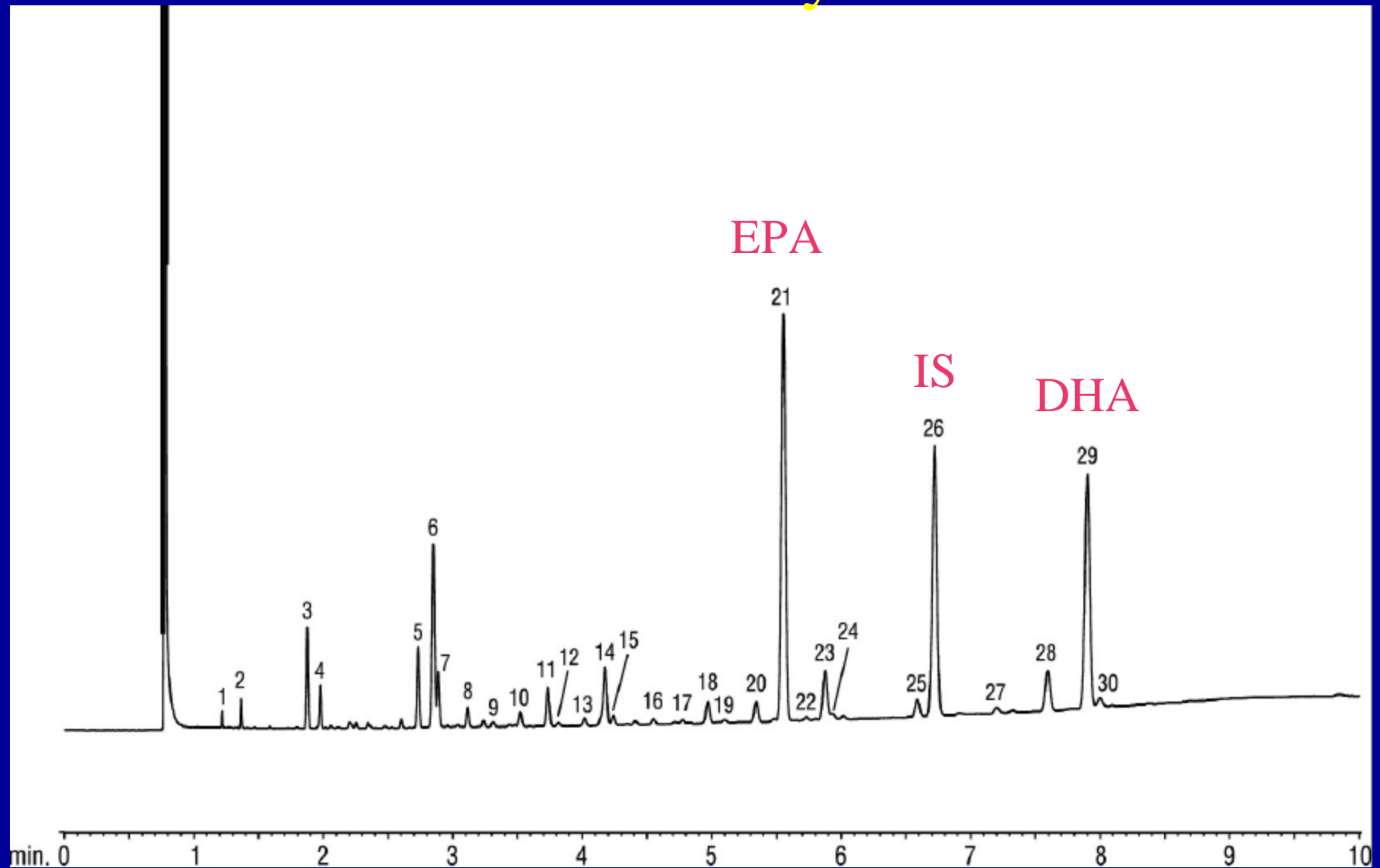
Summary of Validation Study: Slow vs. Fast GC Programs

Product	% Difference EPA	% Difference DHA
18 EPA/12 DHA Triglyceride	0.53	0.68
30 EPA/12 DHA Ethyl Ester	0.56	0.61
40 EPA/20 DHA Triglyceride	0.53	0.52
High EPA Triglyceride	0.20	0.38

Data courtesy of Dave Waddell, Ocean Nutrition Canada

Marine Oil FAMES – Sample

30% EPA/20% DHA Ethyl Esters



Summary of Marine Oil FAME Analysis

- Preparation of the Methyl Esters
 - ◆ AOCS vs. European Pharmacopeia
- Column Selection
 - ◆ PEG vs. high cyano phases
 - ◆ Column configuration
- GC Program Optimization
 - ◆ Oven program and linear velocity
 - ◆ 40+ minute run to under 10 minutes!
 - ◆ Excellent agreement between methods