2022.10.28, Taipei, Taiwan

喬璞科技 JOPE TECHNOLOGY

Industrial Application of Continuous Chromatography by Using Supercritical Fluid as Eluent for the Separation of EPA Ethyl Ester from Fish Oil

A Conflict of Interest

Ming-Tsai Liang

General Manager, Dynes BioTech. Co., Ltd., Pingtung County, Taiwan (ROC)

mtliang@jope-smb.com

(M) +886-912-707954

喬璞科技
JOPE TECHNOLOGY

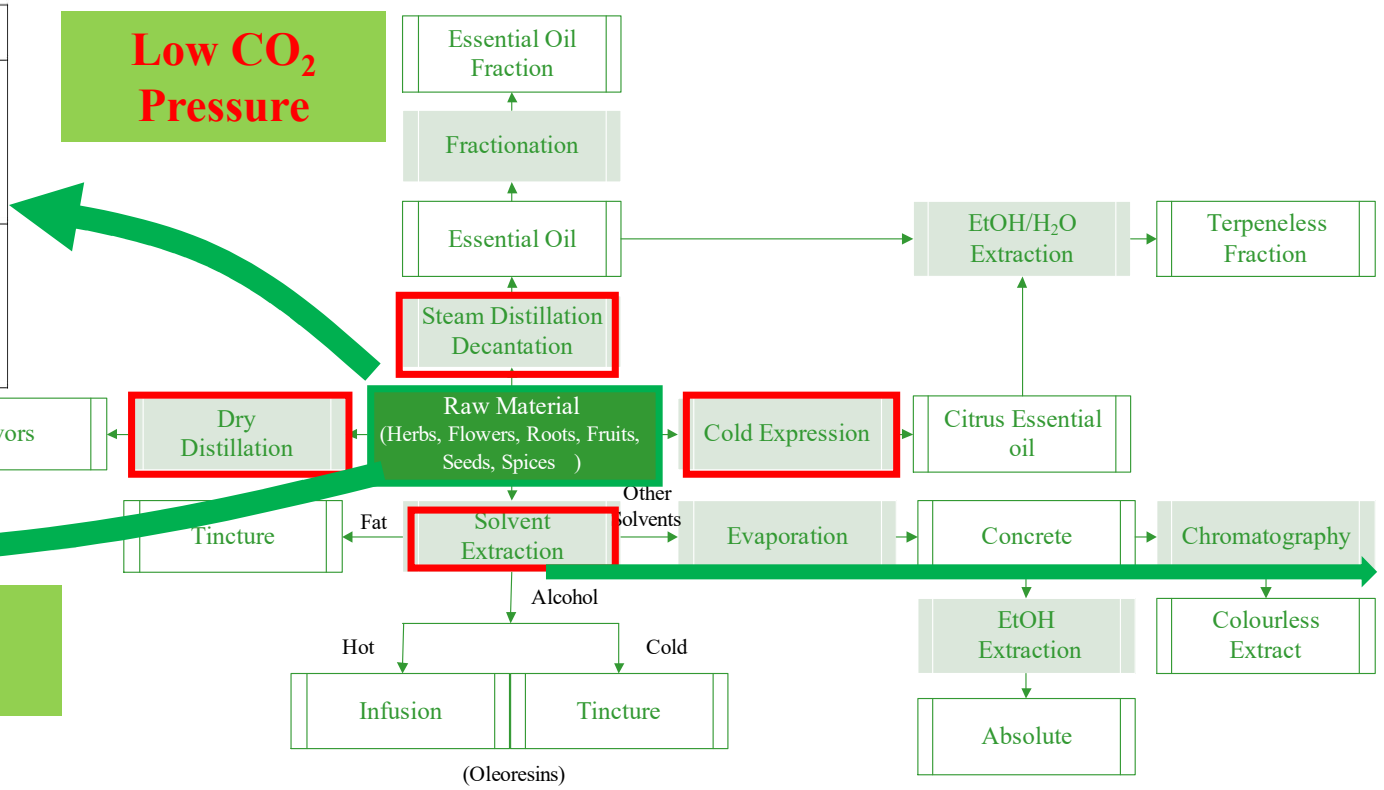
OUTLINES

- Industrial Application of SFE
- Introduction of SMB and SFC
- Isolation of EPA from Fish Oil
- Conclusion

❑ Natural Product Processing

喬璞科技 JOPE TECHNOLOGY

	100 bar	130 bar	150 bar
35℃	1.52%	1.59%	1.88%
50℃	0.96%	1.30%	1.76%



Overview of process concepts for the extraction of nature products

Bart, H.J., Pilz, S., Industrial Scale Natural Products Extraction, 2011, Wiley-VCH

❑ Process Development for Natural Products

喬璞科技 JOPE TECHNOLOGY

Raw Materials

↓

Pretreatment

↓

Solid-Liquid Extraction

(1) SFE
(2) Solvent

Parameters:
T、P、S/F、pH、Flow

↓

Purification

(1) SFF
(2) LL extraction
(3) Adsorption
(4) Membranes
(5) Precipitation
(6) Distillation

Parameters:
T、Solvent、pH、Flow

Chromatography:
(a) Prep-HPLC
(b) SMB
(c) SF-SMB

↓

Formulation

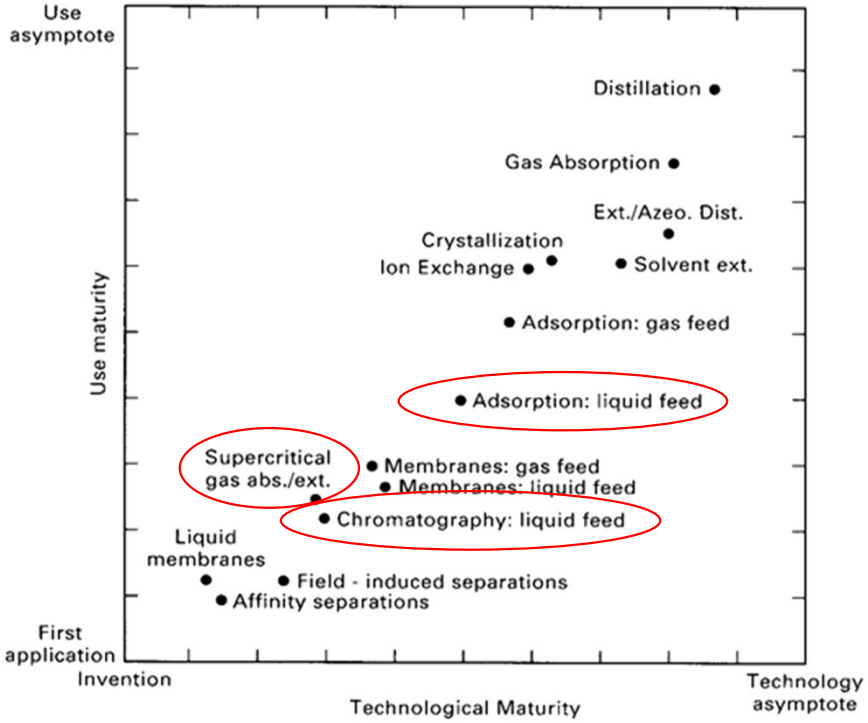


Figure 1.11 Technological and use maturities of separation processes [8].

Bart, H.J., Pilz, S., Industrial Scale Natural Products Extraction, 2011, Wiley-VCH

❑ SFE Used in Western and Eastern World

喬璞科技 JOPE TECHNOLOGY

The Applications of SFE in West

The Applications of SFE in East

❑ Application Diversity of SF

SF can be applied for cleaning

Rice-Cleaning Plant

Five-King Cereal Company

The first large application of SF in cleaning rather than in extraction.

If the dissolved solute is the desired products, it is called extraction.

If the impurities are removed by the SF, we call it cleaning.

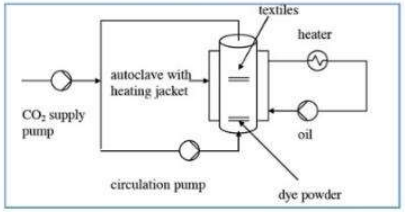
Spain – Cork Treatment

(1) The tannin in Cork is removed to enhance the tastettexture of redwine.
(2) A second plant was immediately contracted to construct right after the start-up of the first plant.

Extractor volume

3 x 8,3 m^3

Textiles Dying



The production of CBD oil

The production of high purity of fish oil

❑ Bioactive Compounds?

SFC
SF-SMB

喬璞科技 JOPE TECHNOLOGY

I: SFE | **II: MD** | **Gingerols**



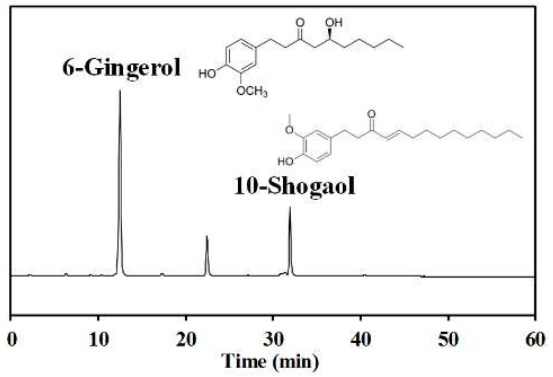

中華民國/110/發明專利/ I716829

申请号或专利号：**201910390376.0**

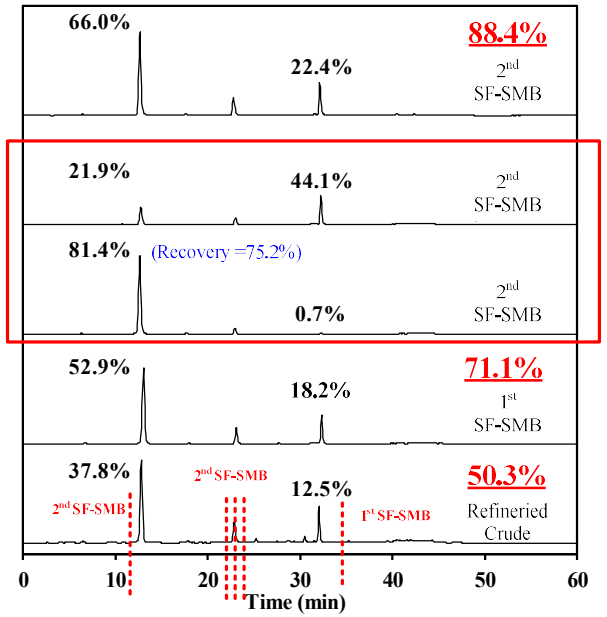
- 1st step: separate the strong retention impurities
- 2nd step: separate the weak retention impurities


Dark orange concrete

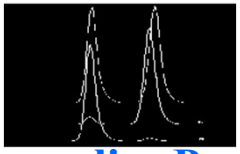
Ginger oil



YMC Pack Pro C18 (250 mm x 4.6 mm, S-5 mm, 12 nm); UV: 282 nm

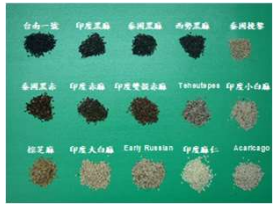


❑ Processes Development



喬璞科技 JOPE TECHNOLOGY

Sesame **Sesame oil** **Crude Lignans** **Pure Sesamolin** **Pure Sesamin**



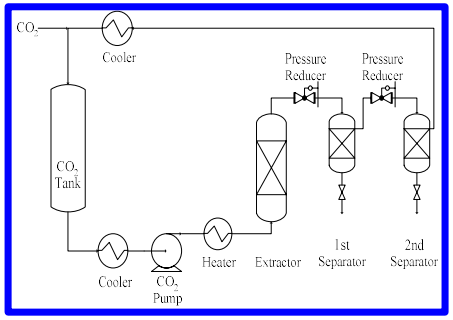


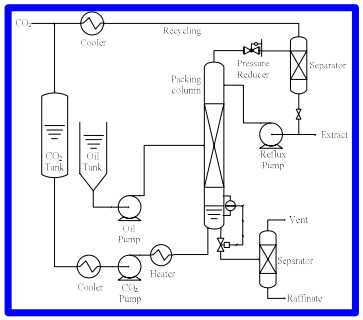
STEP I: SFE
(supercritical Fluid Extraction)

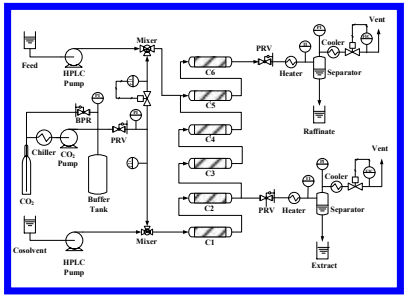
STEP II: SFF
(Supercritical Fluid Fractionation)

STEP III: SF-SMB
(Simulated Moving Bed)





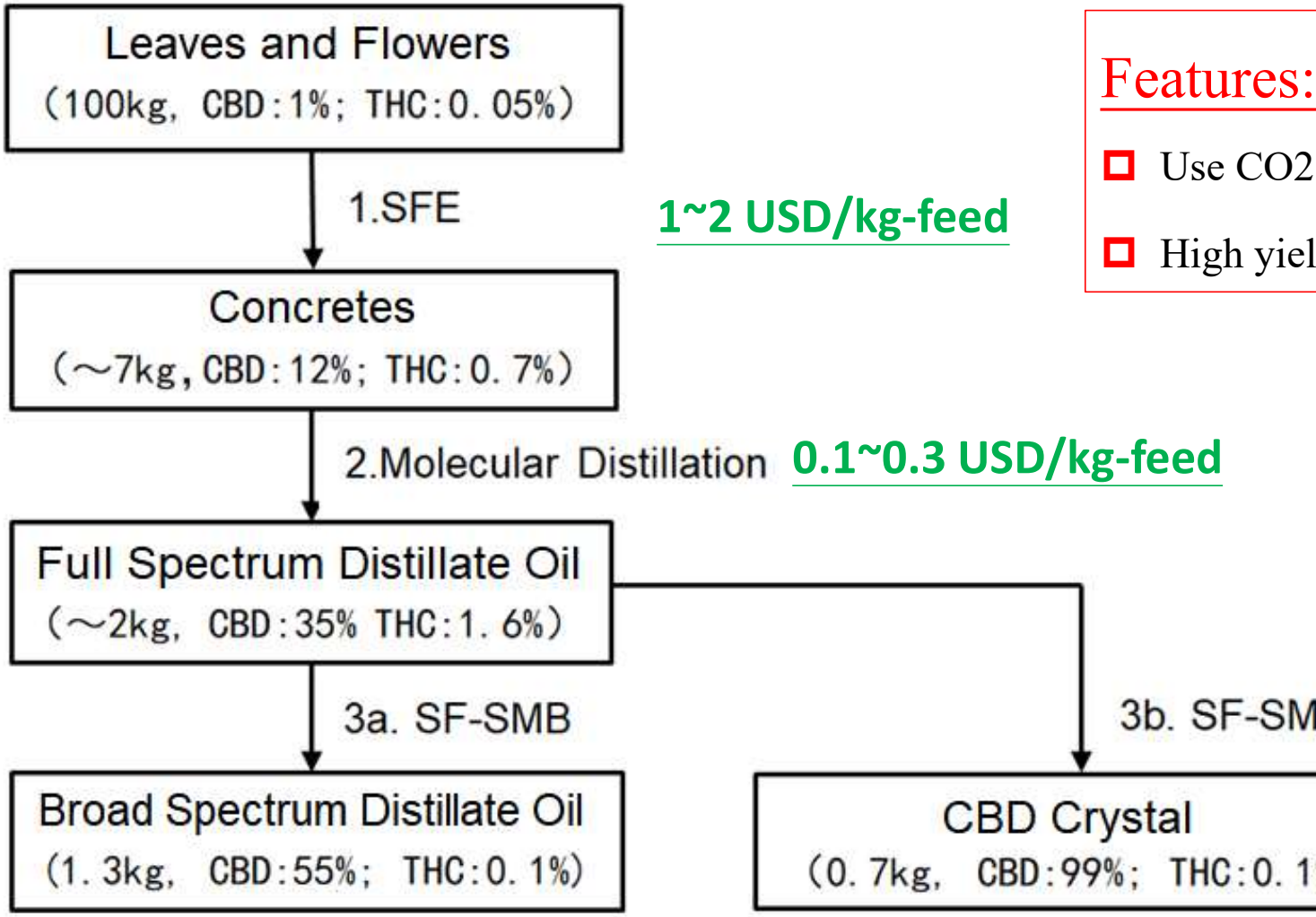




Journal of Chemistry and Chemical Engineering, vol. 5, pp. 479-786, 2011.06

Dry Processes for CBD oil Extraction and Purification

喬璞科技 JOPE TECHNOLOGY



1~2 USD/kg-feed

0.1~0.3 USD/kg-feed

Features:

- Use CO2 and ethanol.
- High yield and low energy consumption.

Operation Cost:
30~60 USD/kg-feed

喬璞科技
JOPE TECHNOLOGY

OUTLINES

- Industrial Application of SFE
- Introduction of SMB and SFC
- Isolation of EPA from Fish Oil
- Conclusion

❑ The Chromatography and Its Maturity in Use

喬璞科技 JOPE TECHNOLOGY

Chromatography: Chrom to Graph

Chromatography is 100 years old !

It was the russian botanist Mikhail Semenovich **Tswett** (1872-1919) who in 1906 first used the term chromatography: from the Greek **chroma** for color, **graphein** for writing.

Tswett (Цвет) is russian for color.

Pros: Selectivity
Cons: Dilution

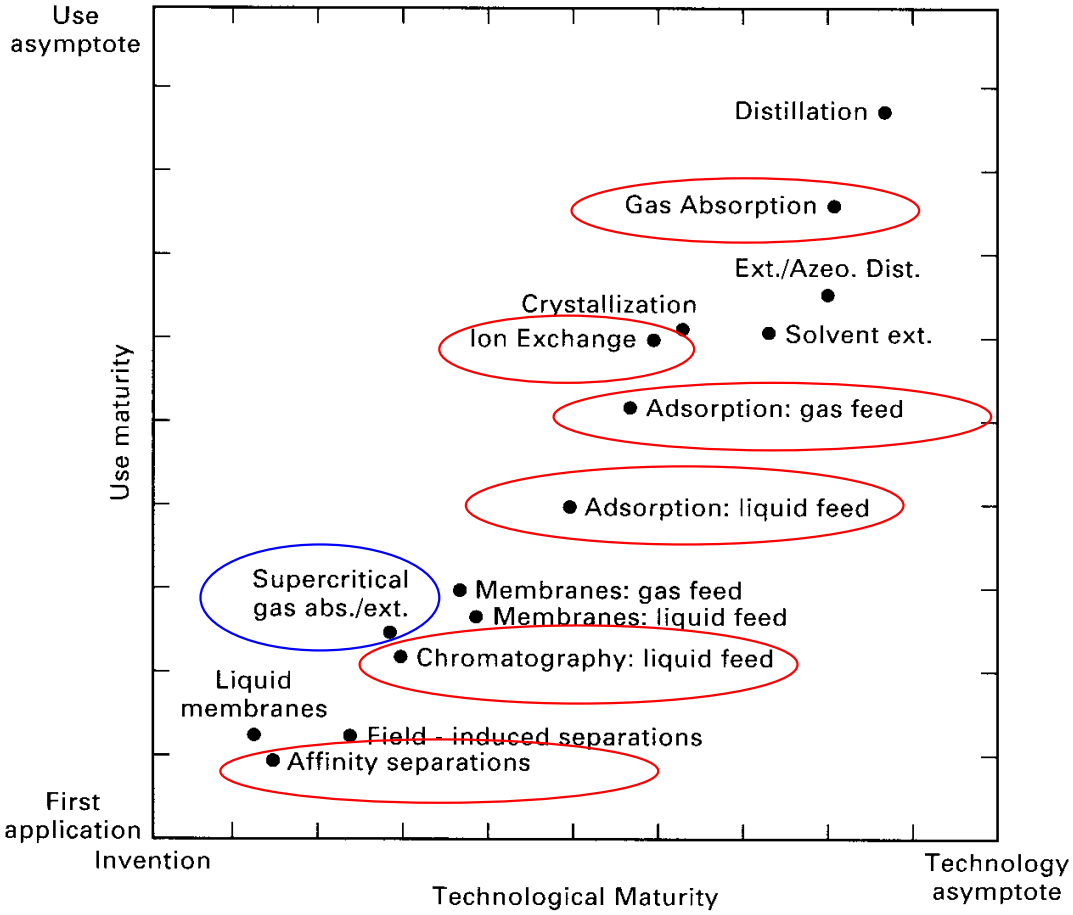
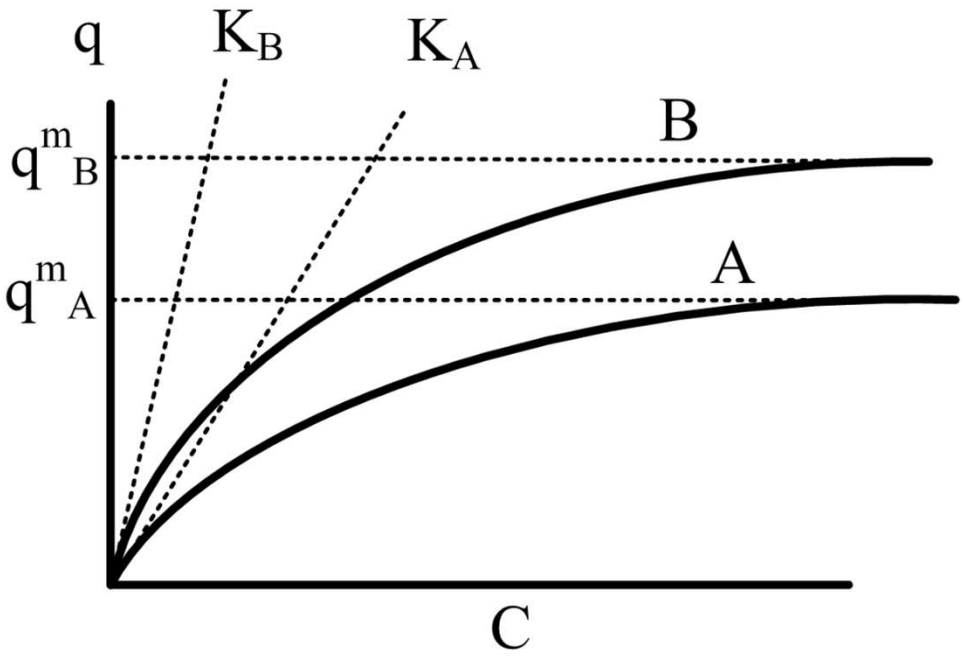


Figure 1.11 Technological and use maturities of separation processes [8].

❑ Fundamental of Chromatography

喬璞科技 JOPE TECHNOLOGY

Adsorption isotherms: An equilibrium between solid and liquid phase



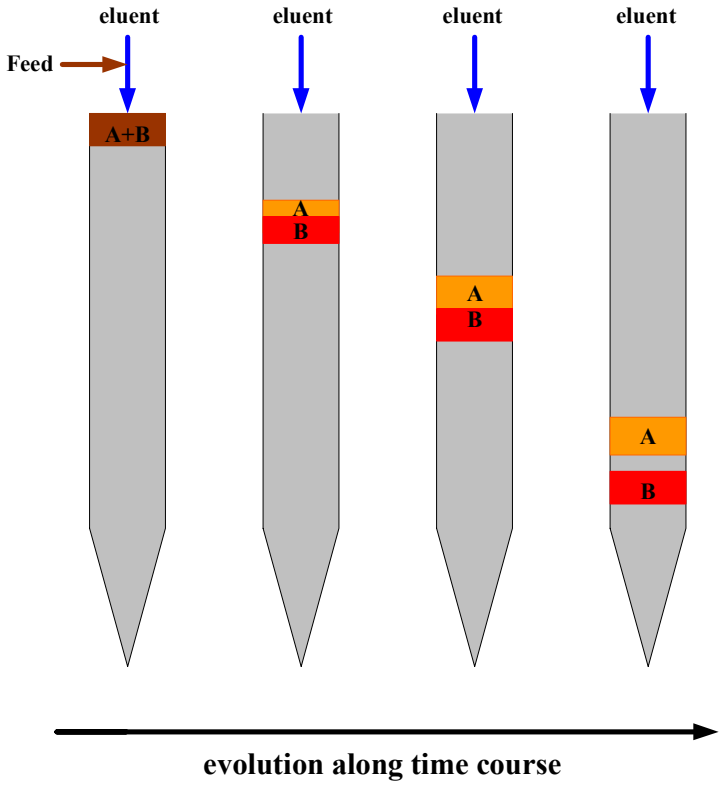
SMB(Simulated Moving Bed)：

A technology can be used for chromatography and adsorption to increase the productivity and reduce the solvent consumption.

$$\text{Selectivity} = \frac{q_B^m}{q_A^m}, \quad \frac{K_B}{K_A}$$

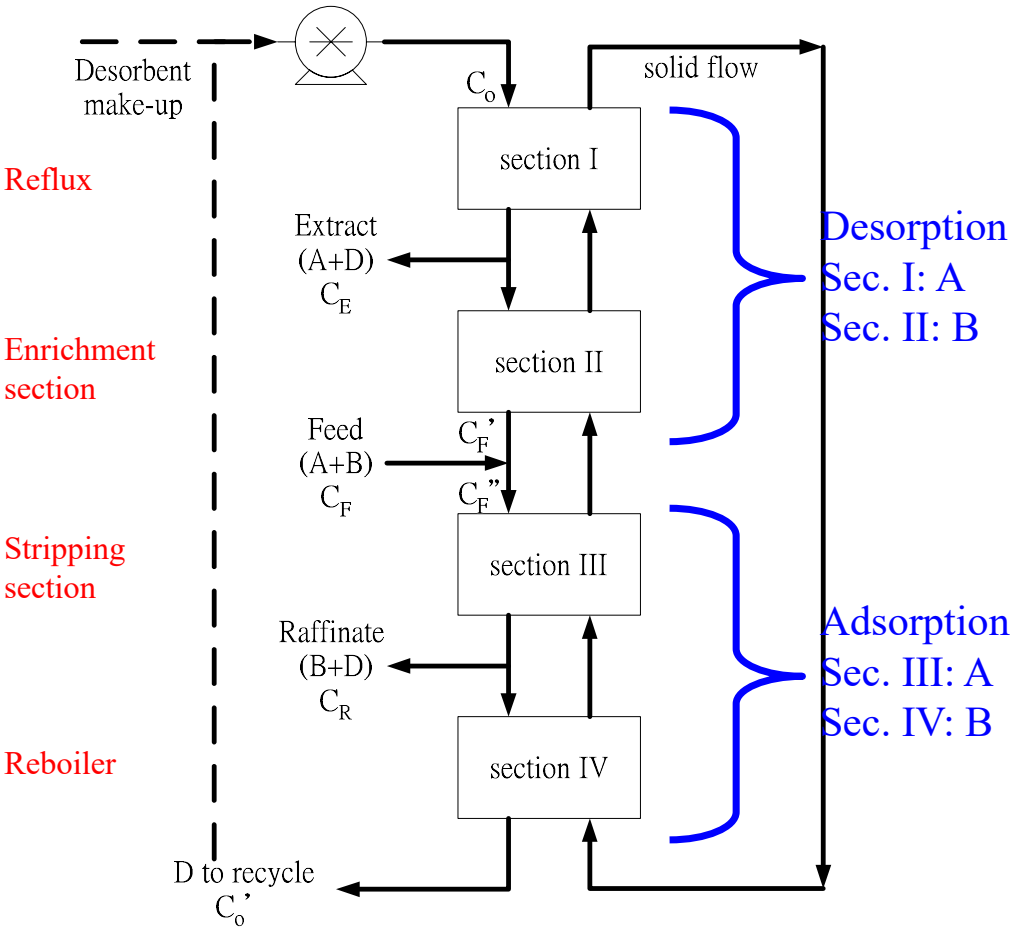
❑ Traditional Chromatography(Batch Operation)

喬璞科技 JOPE TECHNOLOGY

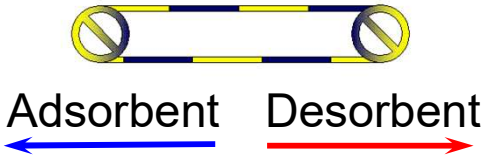


Production SFC

❑ Continuous Chromatography(TMB)



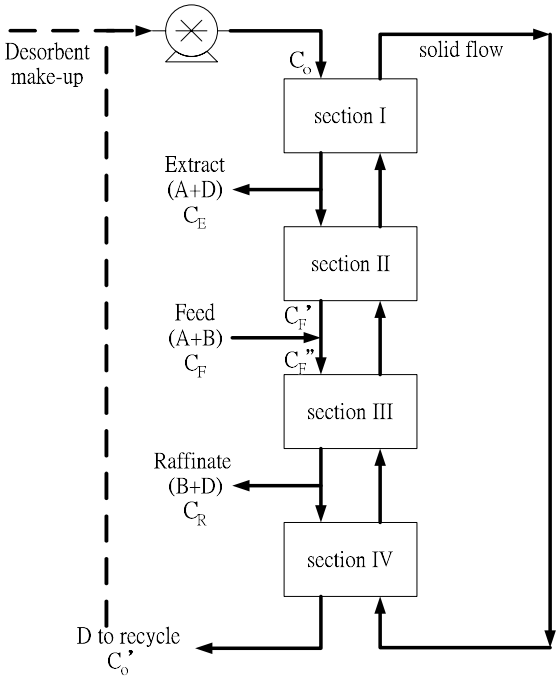
A: Strong Retention Component
B: Weak Retention Component

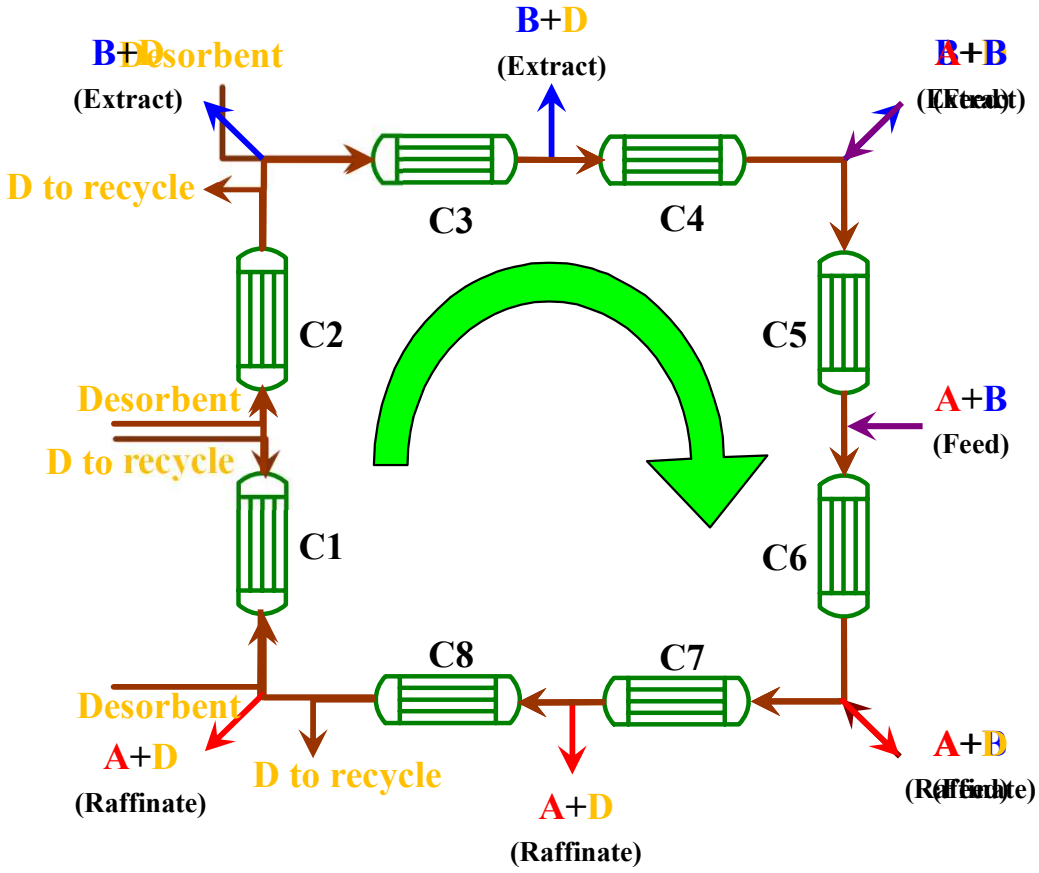


❑ Continuous Chromatography(SMB)

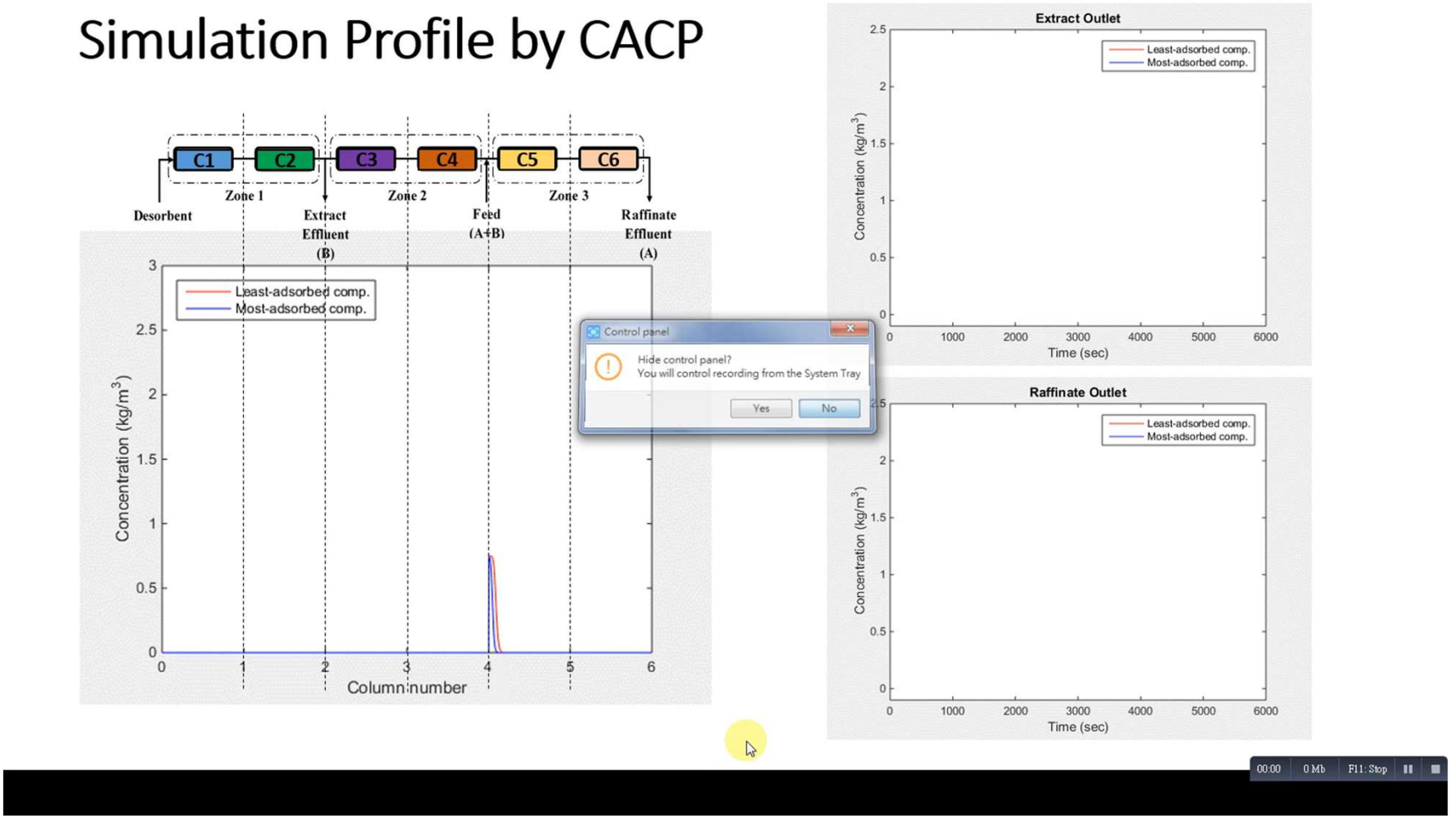
喬璞科技 JOPE TECHNOLOGY

By periodically switching the ports of inlet and outlet to simulate the flow of solid in packing bed





Periodical Change of Concentration



❑ Comparison of HPLC and SMB

喬璞科技 JOPE TECHNOLOGY

	Batch HPLC	Continuous SMB
Pros	● High degree of freedom ● multicomponent ● Easy to operate ● Easy to scale up	● Concentrated products ● High efficient of adsorbent ● Low solvent consumption ● Easy to scale up
Cons	● Diluted products ● High solvent consumption ● High operating cost	● Complicated operation ● High fixed cost ● Generally for binary system
Purities	> 85%	> 99%
Recovery	> 70%	> 99%
Productivity of adsorbent(kg/kg-day)	**0.01 ~ 0.1**	**1.0 ~ 10**

Application of SMB in Industries

- **1960s: C8 Separation in Petroleum Industry**
  Broughton and Gerhold (UOP), 1961, US patent 2985589
- **1970s: Glucose/Fructose Separation in Sugar Refinery Industry**
  Bieser and deRosset(UOP), 1977, Detmold Germany (SAREX)
- **1990s: Chiral Separation**
  Daicel Co. (1992) and Separex Co. (1993)

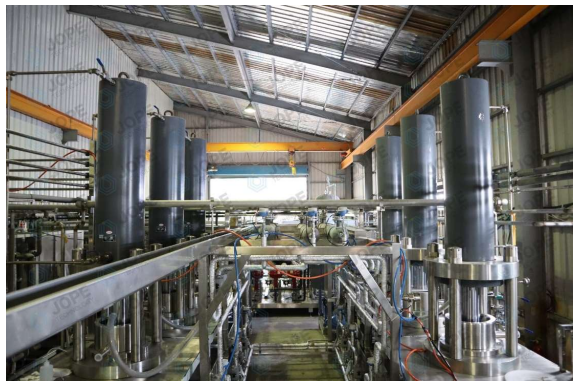
SMB 1960s

- **1996: the first publication of SF-SMB**
  Clavier, J.Y., Nicoud, R.M., Perrut
- **2015: the first pilot scale SF-SMB in Taiwan**
  Ming-Tsai Liang
- **2019~2020: Full Scale of SF-SMB in Taiwan for Fish Oil**
  Ming-Tsai Liang

SF-SMB 2015

Trends in 50 years:

- ✓ SP becomes smaller
- ✓ Operating pressure becomes higher
- ✓ **Process becomes greener**



SF-SMB 2019

❑ Application of SMB in Industries

喬璞科技 JOPE TECHNOLOGY

Chromatography

SMB

SF-SMB

Applications:

Pharmaceutical **Bio-Medical**
Biotechnology **Fats and Oils**
Nutriceuticals **Fine Chemicals**

Pharmaceutical:

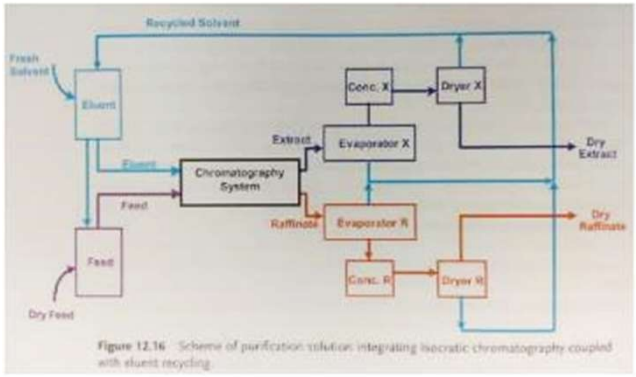
- Fractional purification of natural Tocopherols, fatty acids and phospholipids,
- Hydrophobicity parameters of drugs,
- Separation of metal ions,
- Purification and separation of vitamins A,D and E.

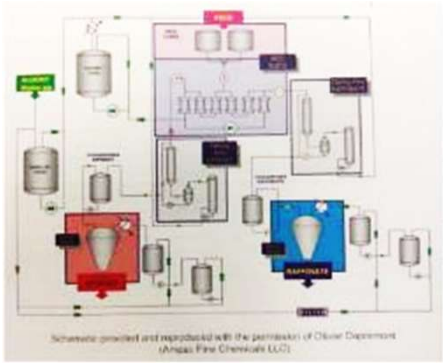
Fine Chemicals:

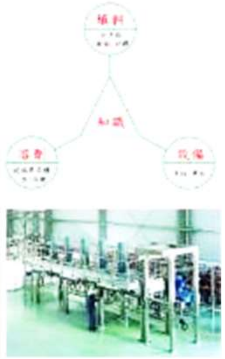
- Separation of Chiral compound,
- Synthesis of lipid A,
- Separation of Saturated and Unsaturated fatty acids,
- Separation of Alkaloids, Fatty acid esters, Herbmedicine, Monosaccharide, Quinones, etc.

Biotechnology:

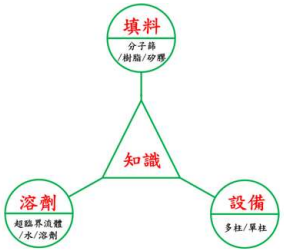
- Purification of antibiotics from fermentation broth,
- Enzymes from Yeast extract,
- Fungous toxin Nivalenol,
- Separation of salmon sperm DNA,
- Separation of serum Proteins.







Cost of Chromatography



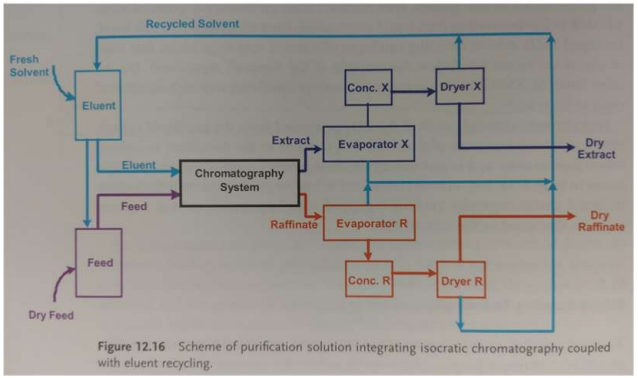
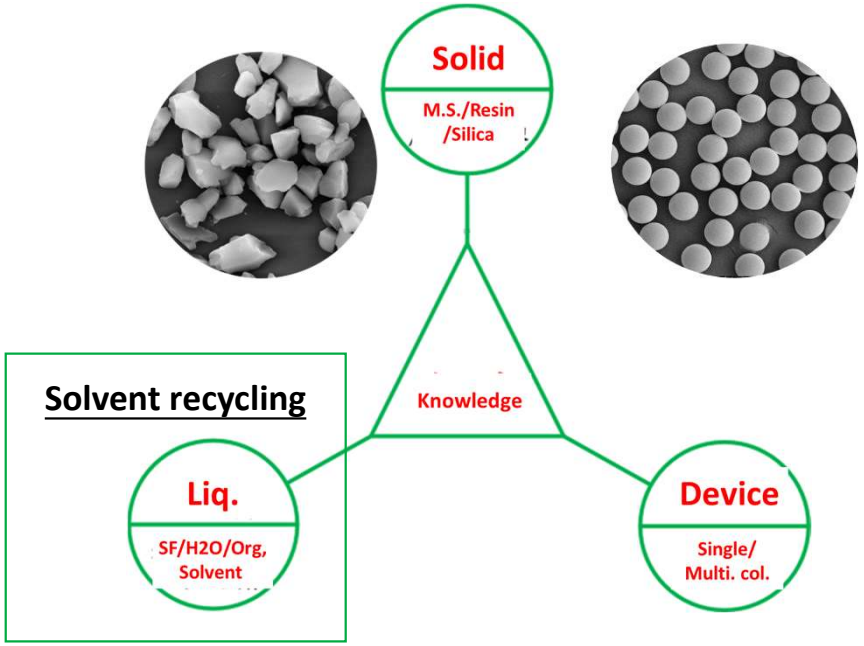
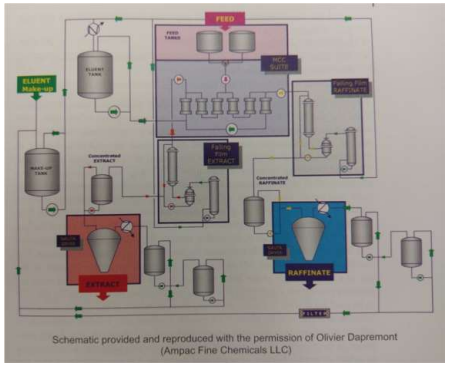


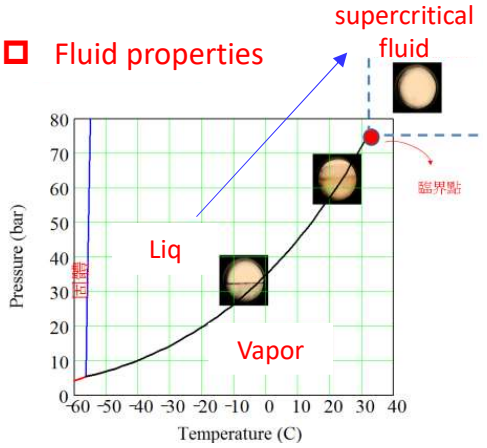
Figure 12.16 Scheme of purification solution integrating isocratic chromatography coupled with eluent recycling.





Schematic provided and reproduced with the permission of Olivier Dapremont (Ampac Fine Chemicals LLC)

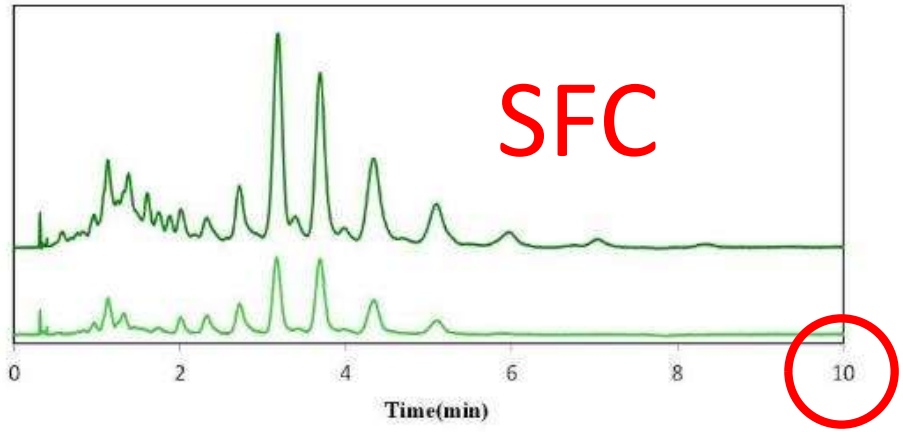
Fluid properties

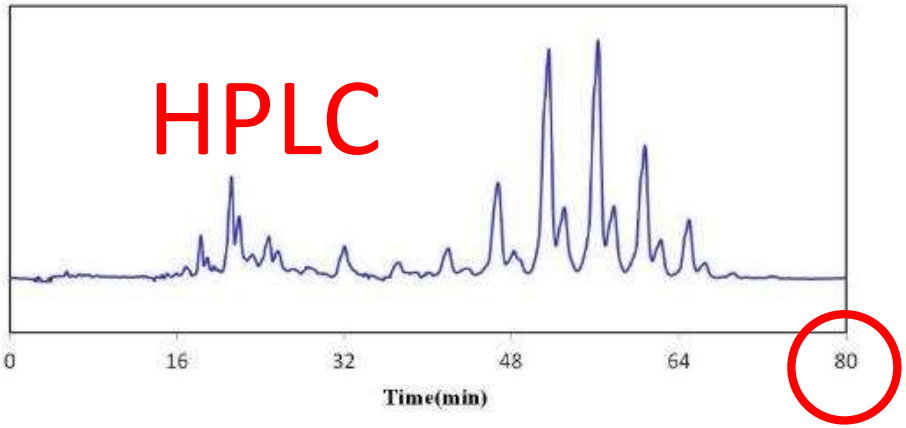


The SFC with Packed Column

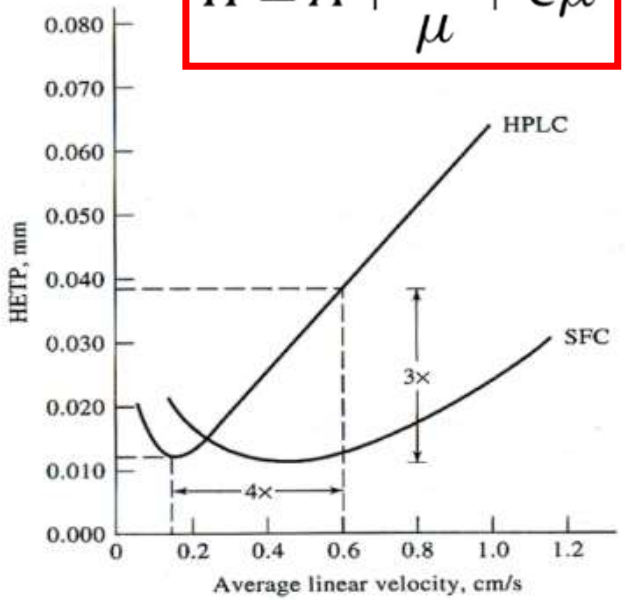
HPLC and SFC Chromatograms for Paparika red.

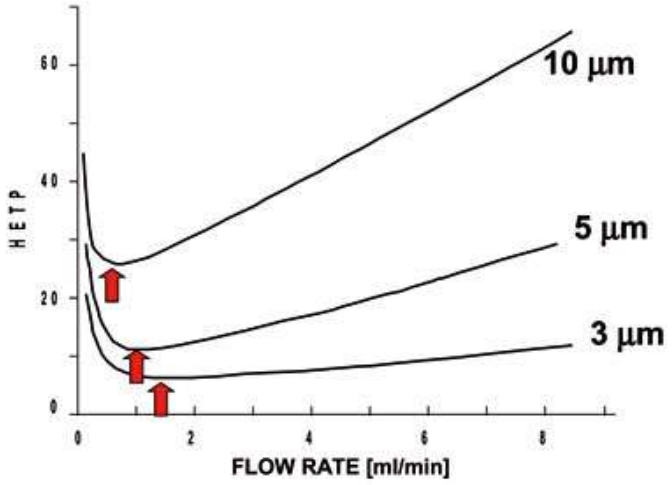
Shortened retention time and Higher Throughput !





$$H = A + \frac{B}{\mu} + C\mu$$





喬璞科技
JOPE TECHNOLOGY

❑ Pros and Cons of SF-SMB

喬璞科技 JOPE TECHNOLOGY

Advantages of using SF for SMB

- can simplify the downstream concentration, because **the carbon dioxide will evaporate immediately after exposed to the ambient**
- The easy recycling of desorbent allows the **elimination of fourth section** in SMB.
- Potentially creating gradient in elution power by changing pressure and cosolvent concentration in different sections.
- It is also recognized that both reversed phase and normal phase are suitable for supercritical carbon dioxide chromatography.
- **Pressure drop** is small, the flow rate can largely increased.

Disadvantages:

- Complicated pipe design
- High pressure operation and equipment
- Solubility is generally low for pure carbon dioxide

❑ Comparison of Chromatographic Methods

喬璞科技 JOPE TECHNOLOGY

		Productivity*		Solvent usage	
		g racemate/kg CSP/day	g racemate/kg CSP/day	L/g racemate	L/g racemate
Batch	HPLC (recycle)	128	64	3.6	7.2
Batch	SSR	398	199	1.73	3.46
Batch	SFC	1600	800	0.44	0.88
Continuous	SMB	1920	960	0.16	0.32
Continuous	SF-SMB	√	√	√	√

Yields for all techniques > 95%, product purity > 98%
HPLC: Close-loop recycling chromatography
SSR: Steady state recycling chromatography

Ref: SFC 2012 short course by Miller L. and Taylor L., 2012, Oct. 3, Brussels, Belgium

- SPICA 2008: Waters merged Thar's small scale SFC ; Jasco announced re-entering SFC business; Novasep announced that 60% of chiral separation were finished by SFC.
- SPICA 2014: Novasep announces all chiral separation used SFC, and 40% of achiral separation used SFC.
- SPICA 2012: Lilly published that 98% of achiral chromatography can be finished by Hilic or 2-EP.

❑ Cost Comparison among Chromatographic Methods

喬璞科技 JOPE TECHNOLOGY

Cost for production of fish oil by using different chromatography

Cost	SF-SMB	SMB	HPLC
Feed stock	10%	10%	10%
Personnel Cost	32%	38%	48%
depreciation	30%(28/2)	18%(16+2)	9%(8+1)
Solvent/Energy/Solid	17%(7/5/5)	100%(70/25/5)	400%(280/100/20)
Housing and Overhead	11%(4/7)	11%	11%
	100%	177%	478%

The saving mainly comes from the solvent recycling

❑ The State of the Art of SMB

SMB:

1950's- SMB begins with UOP
1970's- SMB for sugar industries
1993- First report for pharmaceutical industry
1997- First chiral application run at UCB on a production scale
2001- the first FDA inspection of an SMB unit for an API

1990's – Aerojet, Lundbeck, Carbogen and Bayer
2003- Aldrich invests in plant in Buchs
2006- Aerojet built up a 100 cm unit
2016-Novasep built up a 120 cm fish oil

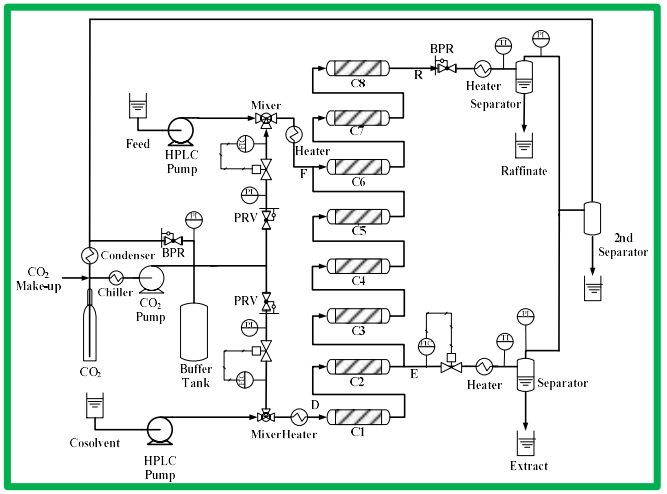
SFC and SF-SMB:

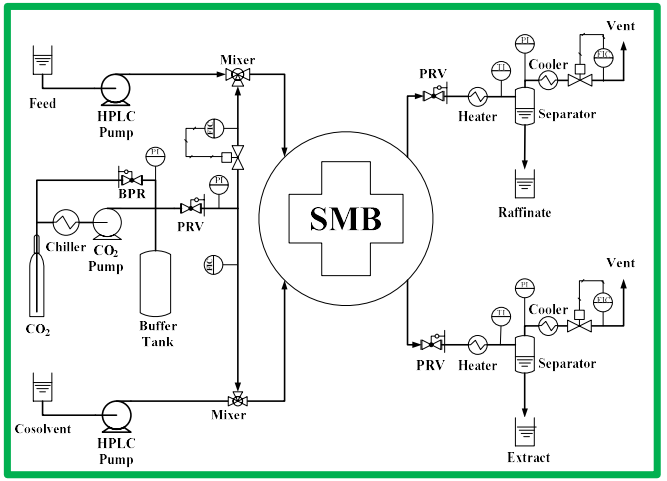
1962- first report of SFC by Klesper et al.
1970's – CO_2 become preferred fluid for packed column
1980's - open tubular capillary columns became popular
1990's – packed column regained popularity and began widely in chiral separation
1996- first report of SF-SMB by Dr. Perrut
2015- First pilot SF-SMB 80mmx8 by JOPE
2019- The largest SF-SMB 300mmx6 and SFC 600mm for CBD
2020- First Industrial Application in Taiwan for Fish Oil

喬璞科技
JOPE TECHNOLOGY

An Overlook of Production Scale SF-SMB

喬璞科技 JOPE TECHNOLOGY

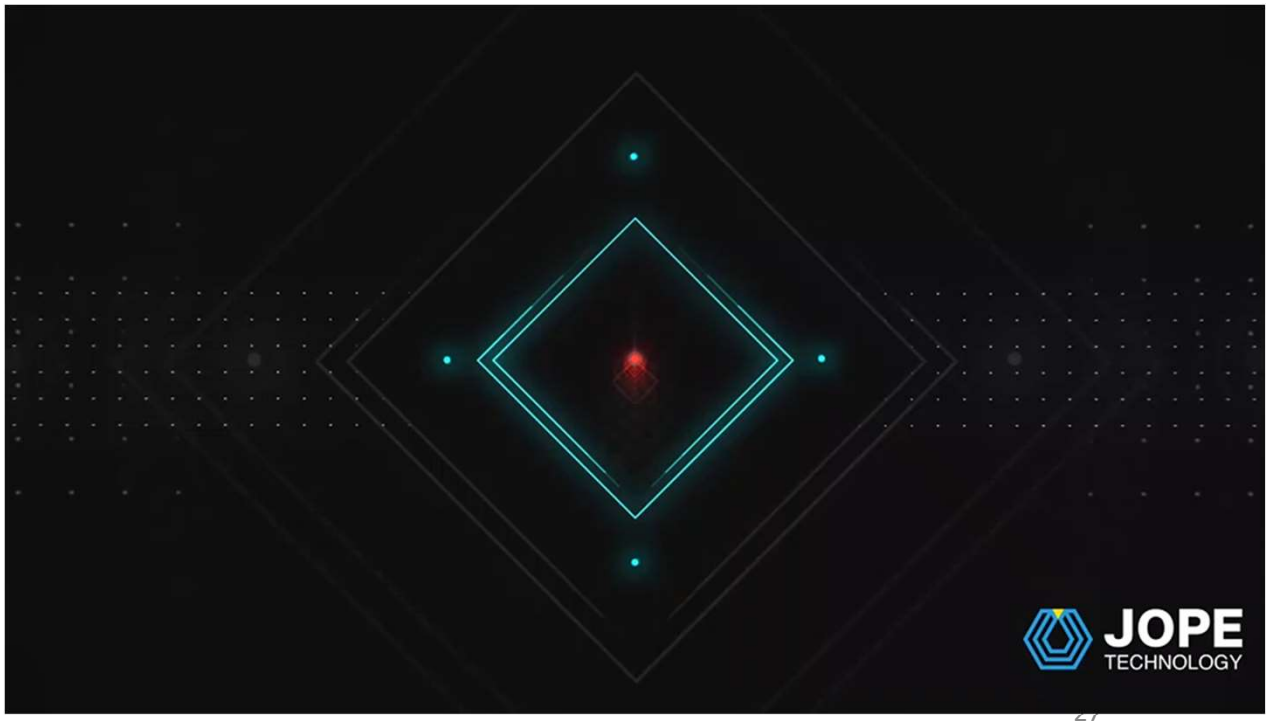




❑ Construction of SF-SMB

喬璞科技
JOPE TECHNOLOGY

◆ SF-SMB 300mmx6 and SFC 600mm

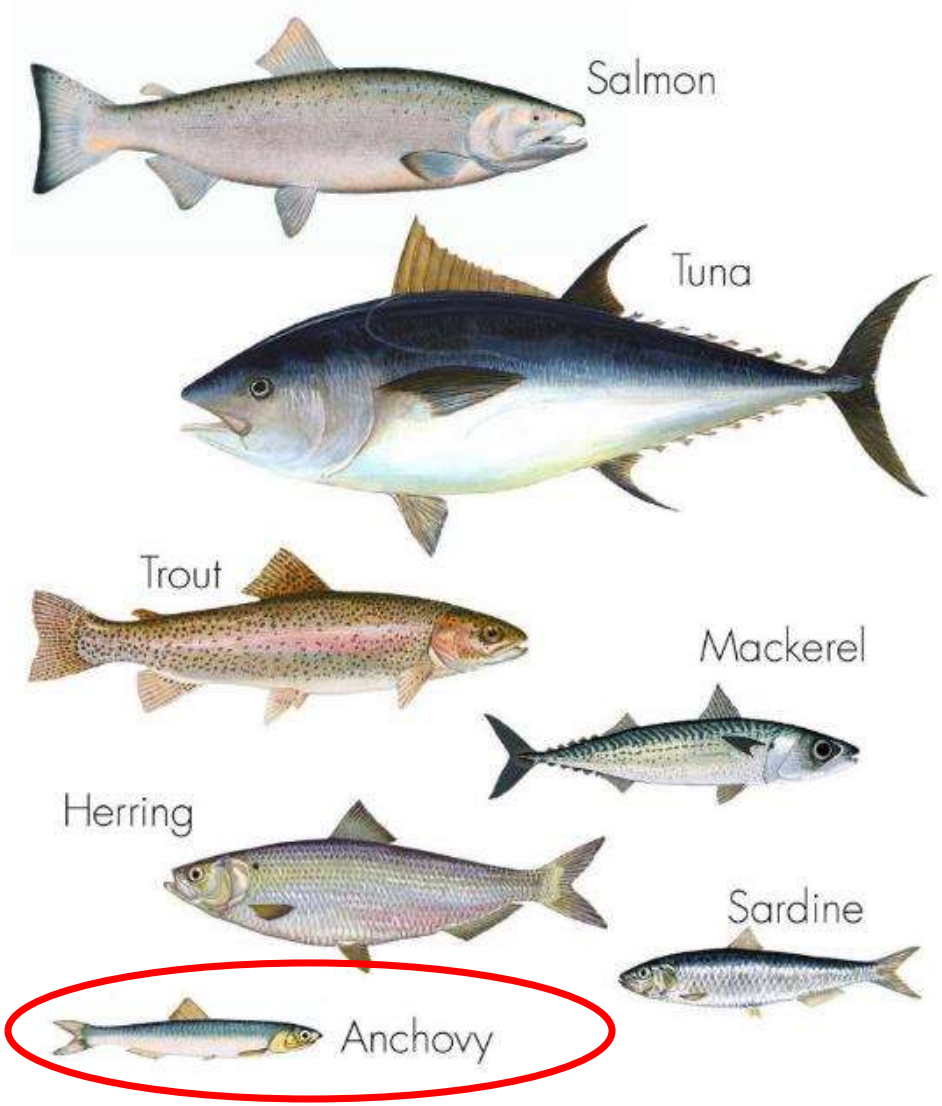


喬璞科技
JOPE TECHNOLOGY

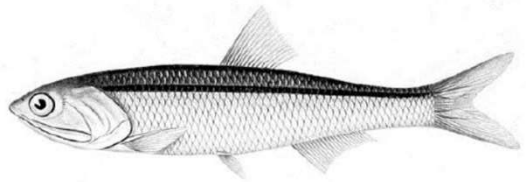
OUTLINES

- Industrial Application of SFE
- Introduction of SMB and SFC
- Isolation of EPA from Fish Oil
- Conclusion

❑ Oily Fish from Chile and Peru



喬璞科技
JOPE TECHNOLOGY



Anchovy in Taiwan

❑ Free Fatty Acids Composition in Fish Oil

喬璞科技 JOPE TECHNOLOGY

Fatty acid	Farmed Atlantic Salmon	Tuna	Jack mackerel	Herring	Atlantic cod liver	Atlantic menhaden	South American anchovy	Capelin	Sardine	Seal	Krill
14:0 (myristic acid)	4.2	3.9	7.3	7	3.3	7.3	7.5	7	8	5.0	9.5
16:0 (palmic acid)	15.7	17.6	15.7	16	13.4	19	17.5	10	18	11.3	20.8
16:1 n-7	5.1	5.4	5.1	6	9.6	9.1	9	10	10	14.3	9.9
18:0 (stearic acid)	4.2	4.1	3.1	-	2.7	4.2	4	-	-	1.1	0.9
18:1 n-9	16.5	12.4	9.9	13	23.4	13.2	11.6	14	11	22.3	10.5
18:1 n-7	3.5	2.4	2.9	-	-	-	-	-	-	4.9	10.3
20:1 n-9	3.3	1.3	8.3	12	7.8	2	1.6	17	4	7	<1
22:1	2.5	0.5	5.8	20	5.3	0.6	1.2	14	3	2.3	<0.5
18:2 n-6 [1] (LA)	6.6	1.9	1.7	-	-	1.3	1.2	-	-	1.1	2.3
20:5 n-3 [2] (EPA)	7.1	12.4	10.9	5	11.5	11	17	8	18	6.6	18.2
22:6 n-3 [3] (DHA)	15.7	27.8	11.5	6	12.6	12.6	8.8	6	9	8.7	9.5
22:5 n-3 [4] (DPA)	3.9	1.7	2	-	1.6	1.6	1.6	-	-	4.4	-
Total n-3 [5] LC-PUFA	**26.7**	**41.9**	**24.4**	**11**	**25.7**	**22**	**27.4**	**14**	**27**	**19.7**	**27.7**

[1] LA – linoleic. [2] EPA – eicosapentaenoic. [3] DHA – docosahexaenoic acid. [4] DPA – docosapentaenoic acid.
[5] LC-PUFA: long chained polyunsaturated fatty acids.

API from Fish Oil: the separation of EPA and DHA

喬璞科技 JOPE TECHNOLOGY

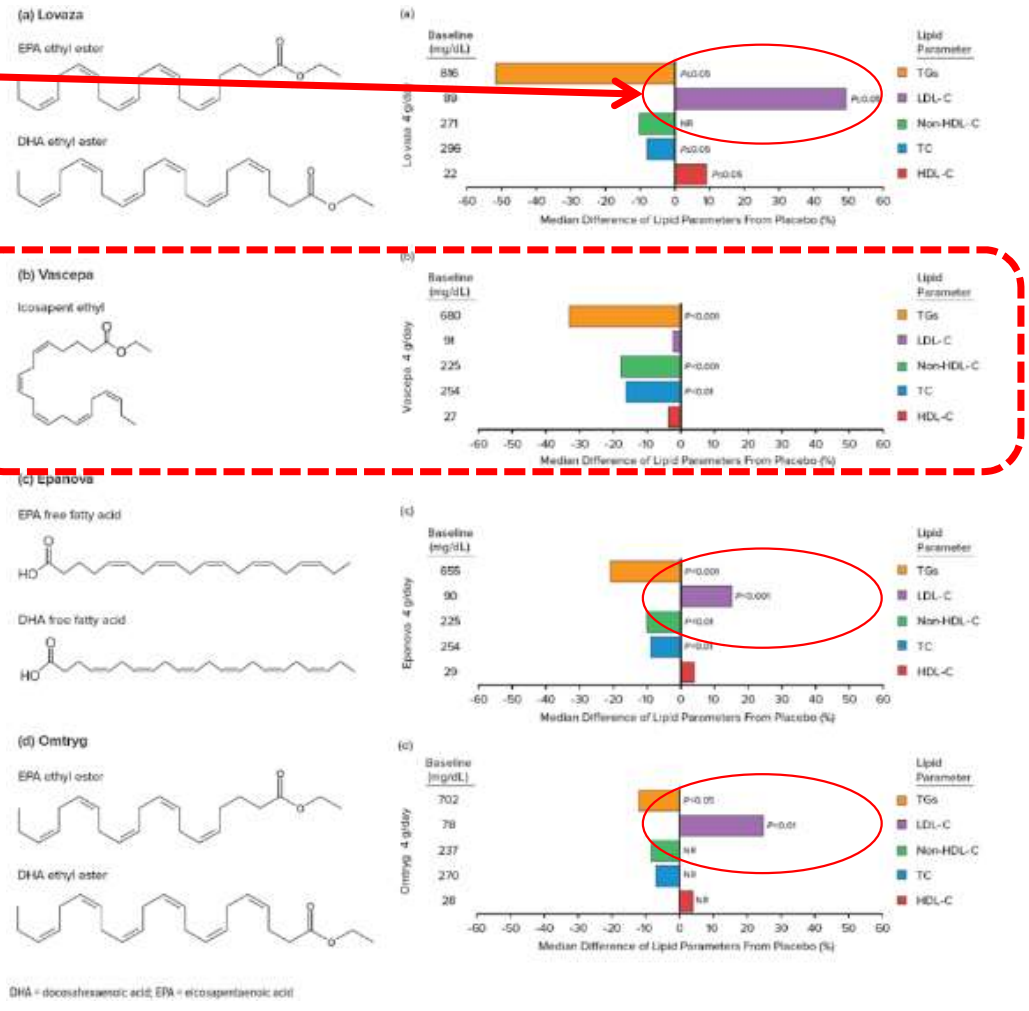
DHA leads the increase of LDL

1) Crandell JR, Tartaglia C, Tartaglia J., Lipid effects of switching from prescription EPA+DHA (omega-3-acid ethyl esters) to prescription EPA only (icosapent ethyl) in dyslipidemic patients, *Postgraduate Medicine*, 128 (2016) 859-864
2) Matthew K. Ito, A Comparative Overview of Prescription Omega-3 Fatty Acid Products, *Pharmact and Therapeutics*, 40 (2015) 826-836
3) Tajuddin N, Shaikh A, Hassan A. Prescription omega-3 fatty acid products: considerations for patients with diabetes mellitus, *Diabetes Metab Syndr Obes.*, 19 (2016) 109-118
4) Sperling LS, Nelson JR., History and future of omega-3 fatty acids in cardiovascular disease, *Curr Med Res Opin.*, 32 (2016) 301-11
5) Eliot A. Brinton, R. Preston Mason, Prescription omega-3 fatty acid products containing highly purified eicosapentaenoic acid (EPA), *Lipids in Health and Disease*, 16 (2017) 1-13

Vol. 40 No. 12 • December 2015 • P&T® 827

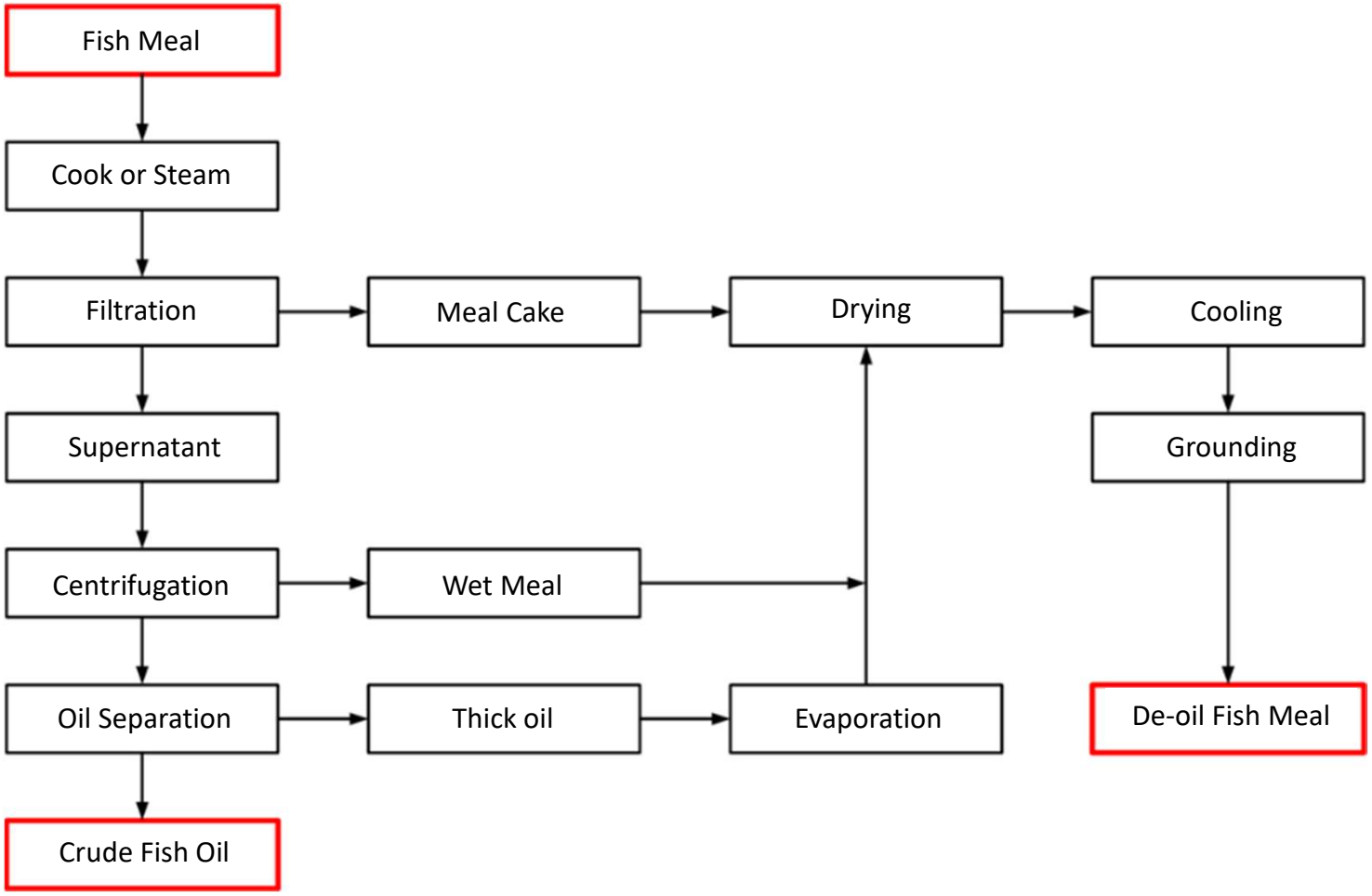
A Comparative Overview of Prescription Omega-3 Fatty Acid Products

Matthew K. Ito, PharmD, FCCP, FNLA, CLS



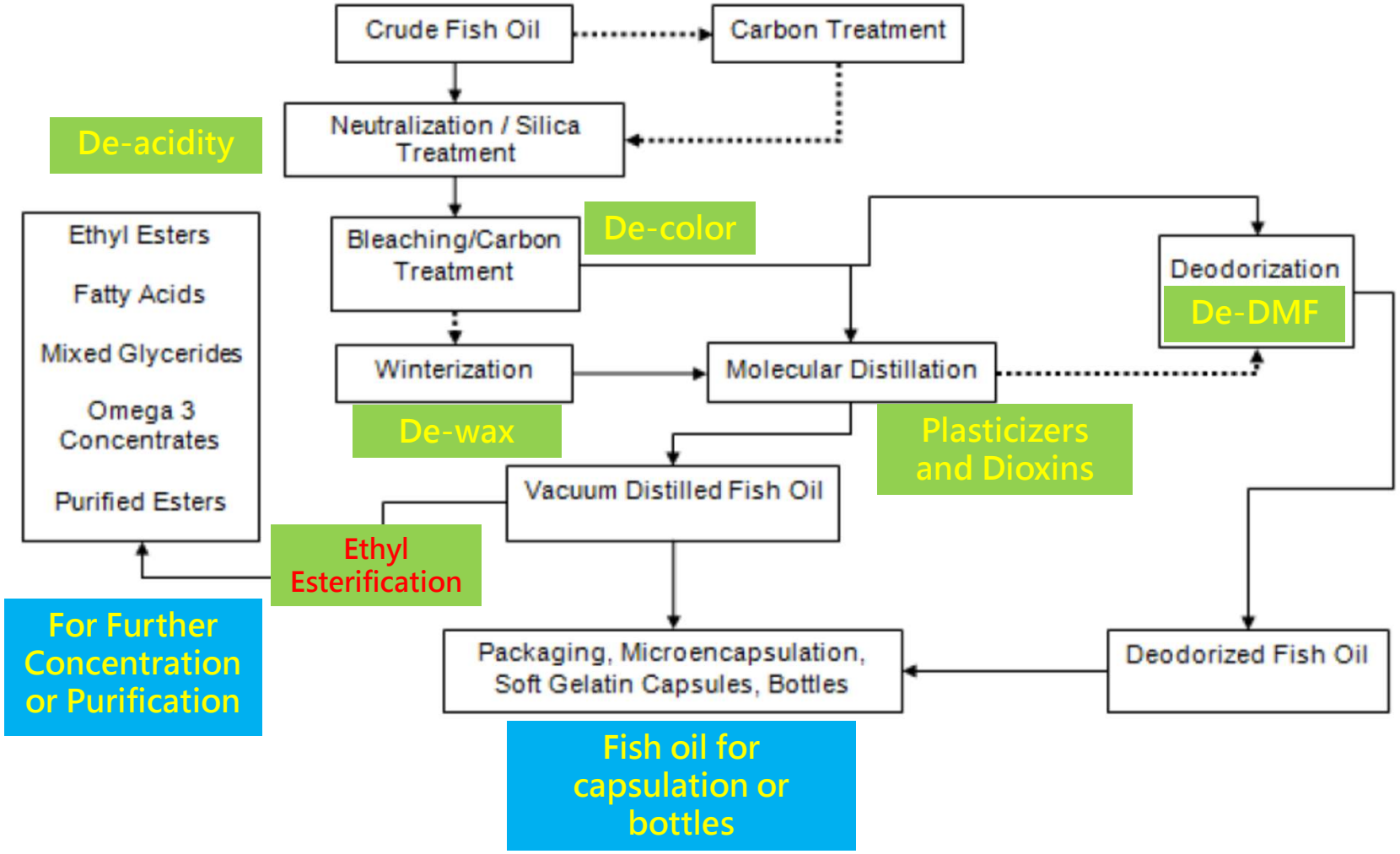
❑ Fish Oil Extraction

喬璞科技
JOPE TECHNOLOGY



❑ Fish Oil Refinery

喬璞科技 JOPE TECHNOLOGY



❑ Concentration and Isolation of Fish Oil

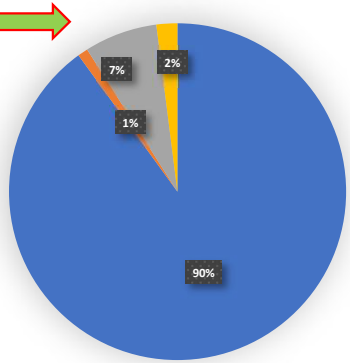
喬璞科技 JOPE TECHNOLOGY

Chromatography to isolate EPA

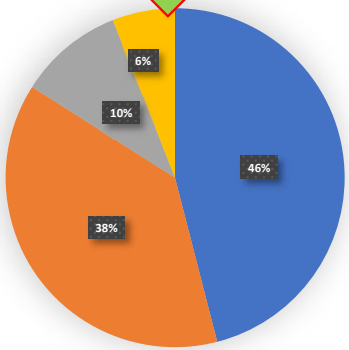
SFF or Vacuum Distillation to separate DHA and EPA

Molecular Distillation to remove C16 and C18 FA

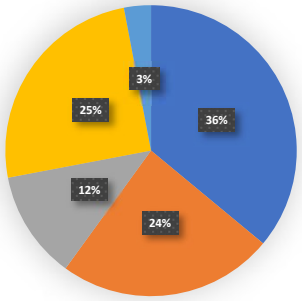
Crystallization to remove saturated and mono-unsaturated FA



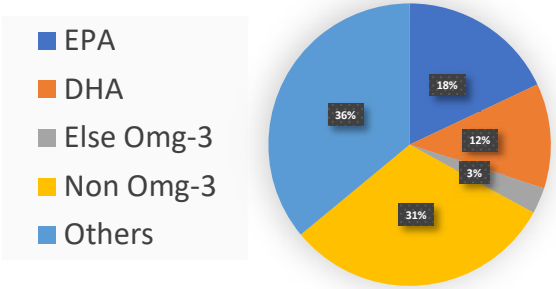
EPA~90



46/38



36/24



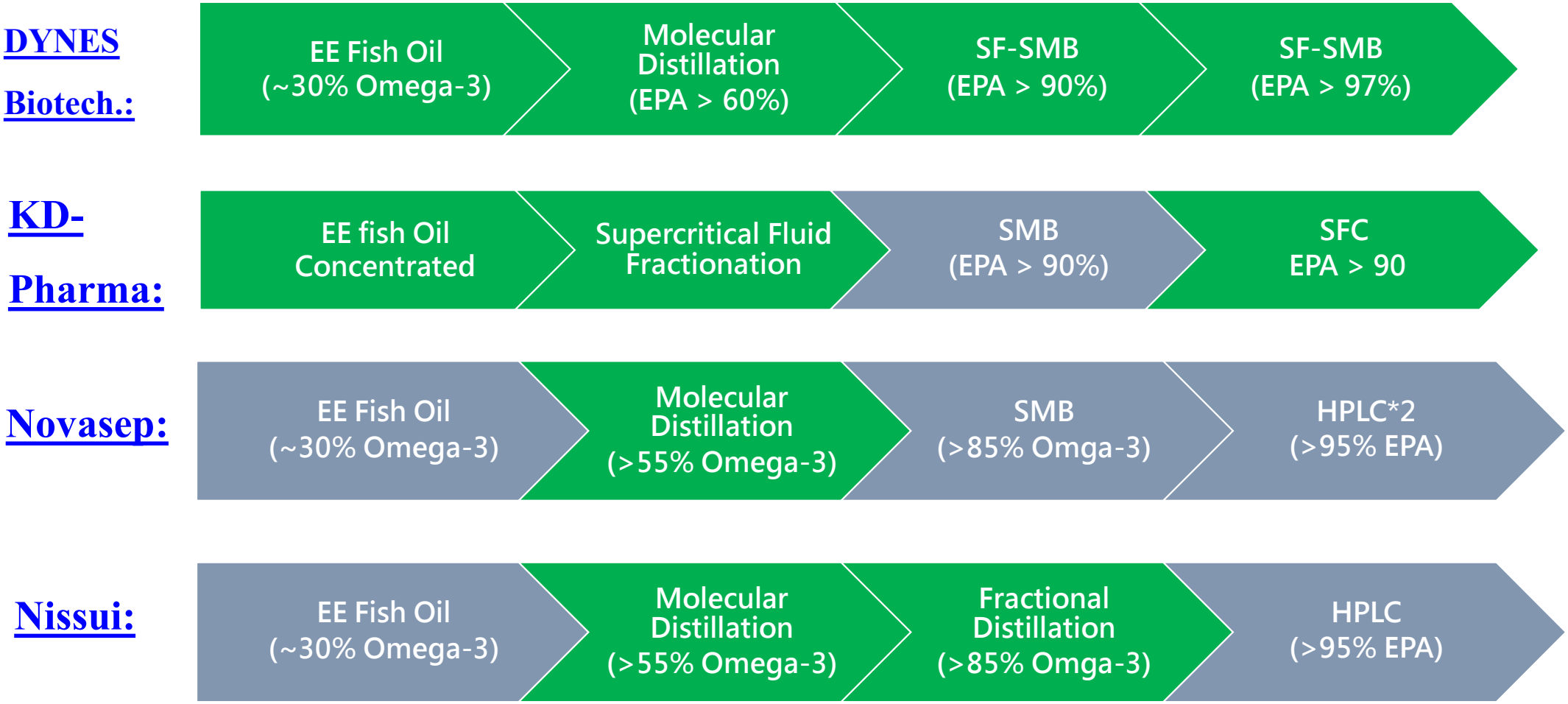
Original: EPA/DHA = 18/12

EPA95

Peak#	Ret. Time	Area		
1	1.625	86123099		
2	2.053	260633		
3	2.514	1495	0.12%	
4	14.616	15970	1.30%	C18:4, ω-3(SDA)
5	14.912	1258	0.10%	
6	17.379	2915	0.24%	
7	18.049	7546	0.62%	C20:4, ω-6
8	19.119	3952	0.32%	C20:4, ω-3
9	19.671	1172355	95.61%	C20:5,ω-3(EPA)
10	21.706	3325	0.27%	C21:5, ω-3
11	22.047	6225	0.51%	
12	24.836	9716	0.79%	C22:6, ω-3(DHA)
13	26.26	1421	0.12%	

❑ Production Technologies for EPA 97

喬璞科技 JOPE TECHNOLOGY



❑ EPA 97 by liq-SMB

喬璞科技 JOPE TECHNOLOGY

Novasep:

EE Fish Oil (~30% Omega-3) → Molecular Distillation (>55% Omega-3) → SMB (>85% Omga-3) → HPLC*2 (>95% EPA)

Varicol® 5-1200mm I.D./2 single-column chromatography systems of 1200 mm

❑ An overview of DYNES Biotech.

喬璞科技 JOPE TECHNOLOGY

DYNES Biotech.:

EE Fish Oil (~30% Omega-3) → Molecular Distillation (EPA > 60%) → SF-SMB (EPA > 90%) → SF-SMB (EPA > 97%)

❑ Vascepa and its generic drugs and Nuctriceuticals

喬璞科技 JOPE TECHNOLOGY

Amarin (prescription)	Epadel (prescription, OTC)	Hikma (prescription)	Dynes (Food Grade)

Generic Drugs:

- Manufacturer: APOTEX
  Approval date: June 30, 2021
- Manufacturer: DR REDDYS
  Approval date: August 7, 2020
- Manufacturer: HIKMA
  Approval date: May 21, 2020

EPA API Manufactures:

	Manufacturer	Launched	Technologies
Amarin (Brand Drug)	Nisshin(Japan)	2012	Vacuum Distillation + HPLC
	Chemport(Korea)	2012	Vacuum Distillation + HPLC
	Novasep(French)	2015	SMB + HPLC
	KD Pharma(German)	2019	SMB + HPLC
	BASF(German)	2013	SMB + HPLC
Generic	CCSB(Taiwan)	2020	Metal Complexed Extraction
Food Grade	DYNES(Taiwan)	2021	SF-SMB

Source: USFDA, Company filings, Jefferies

❑ EPA 97 API by CO2+MeOH; EPA 95 Nutriceuticals by CO2+EtOH

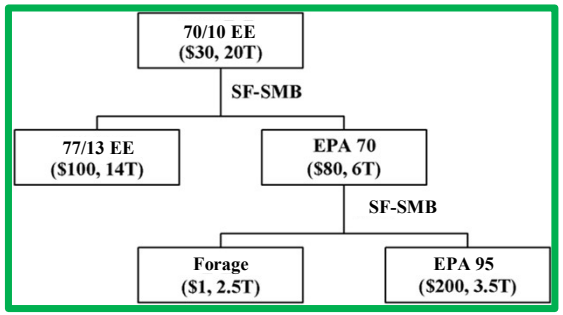
喬璞科技 JOPE TECHNOLOGY

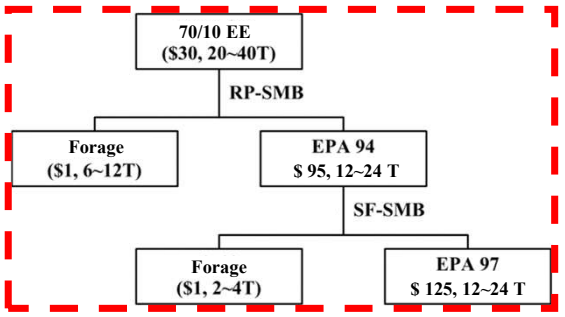
Nutriceuticals

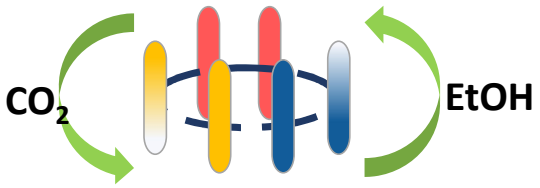
Pharmaceuticals

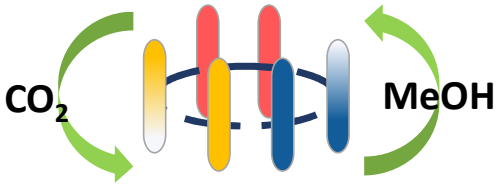
EPA95

Peak#	Ret. Time	Area		
1	1.625	86123099		
2	2.053	260633		
3	2.514	1495	0.12%	
4	14.616	15970	1.30%	C18:4, ω-3(SDA)
5	14.912	1258	0.10%	
6	17.379	2915	0.24%	
7	18.049	7546	0.62%	C20:4, ω-6
8	19.119	3952	0.32%	C20:4, ω-3
9	19.671	1172355	95.61%	C20:5,ω-3(EPA)
10	21.706	3325	0.27%	C21:5, ω-3
11	22.047	6225	0.51%	
12	24.836	9716	0.79%	C22:6, ω-3(DHA)
13	26.26	1421	0.12%	









EPA97

Peak#	R.Time	Area		
1	1.627	56539411		
2	2.079	385469		
3	9.183	1152	0.10%	
4	13.321	2127	0.18%	
5	13.79	1363	0.12%	
6	14.112	2900	0.25%	
7	14.756	4534	0.38%	C18:4,w-3(SDA)
8	18.196	5126	0.43%	C20:4,w-6
9	19.265	1795	0.15%	C20:4,w-3
10	19.814	1156678	98.01%	C20:5,w-3(EPA)
11	22.196	1747	0.15%	
12	24.984	2701	0.23%	C22:6,w-3(DHA)

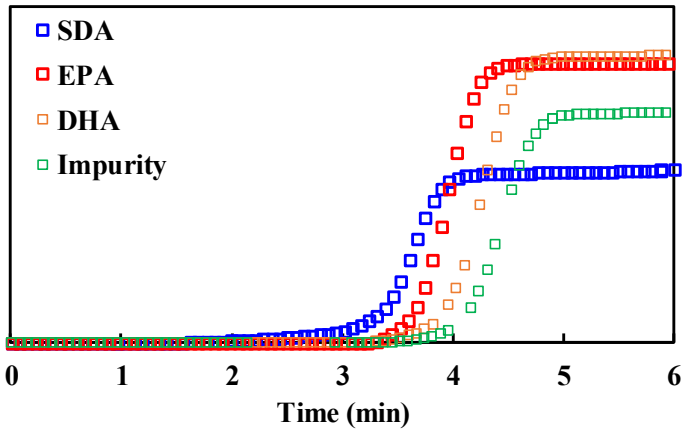
Process Development: Single Column Chromatography

STEP I: Single Column Chromatography

EPA is an intermediate retention component. Therefore, run the SMB twice.

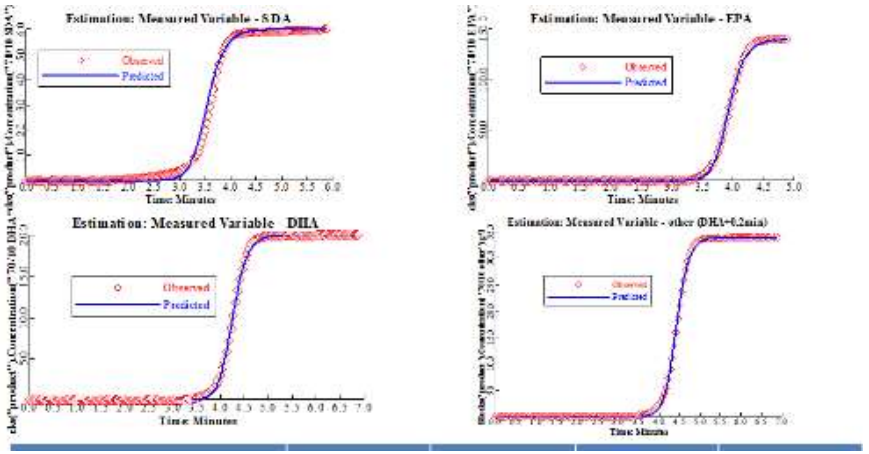
General rate model:

$$\frac{\partial c_{f,i}}{\partial t} + u\frac{\partial c_{f,i}}{\partial z} + \frac{(1-\varepsilon_{total})}{\varepsilon_{total}}\frac{\partial q_i}{\partial t} - D_{ax}\frac{\partial^2 c_{f,i}}{\partial z^2} + \frac{3k_i}{r_p}\cdot\frac{(1-\varepsilon_0)}{\varepsilon_0}\cdot\left(c_{f,i} - c_{p,i,r=r_p}\right) = 0$$

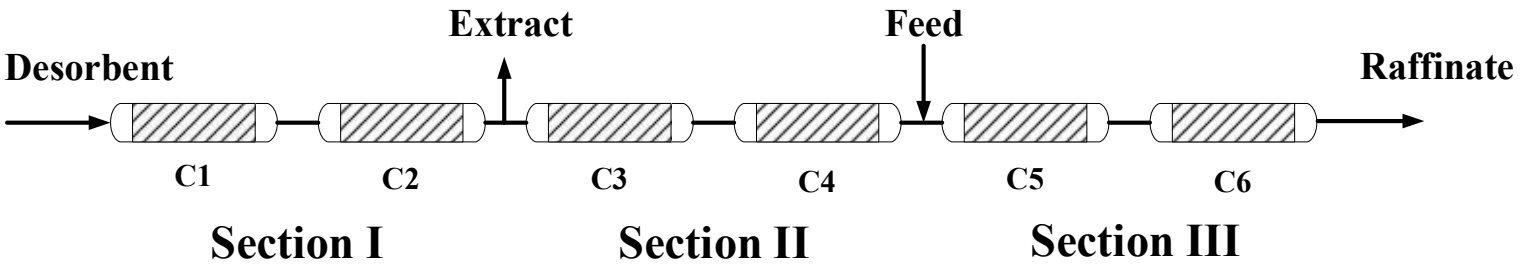


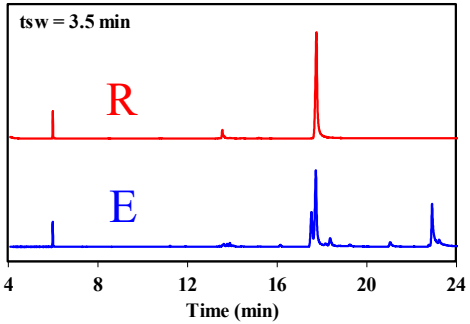
ASPEN Chromatography

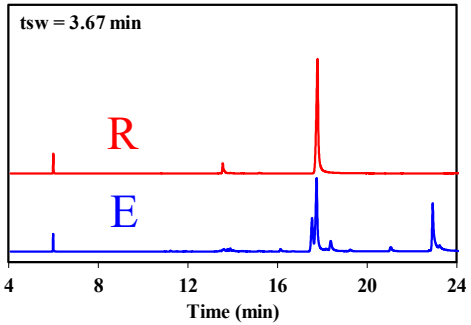
- k = mass transfer coefficient
- d = dispersion coefficient
- IP1, IP2 = adsorption constants

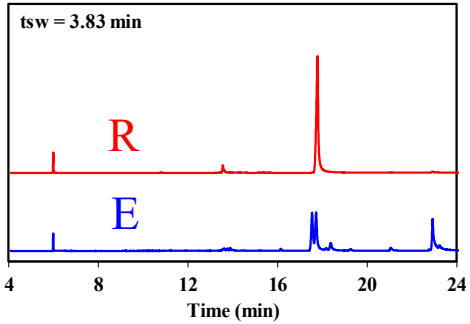


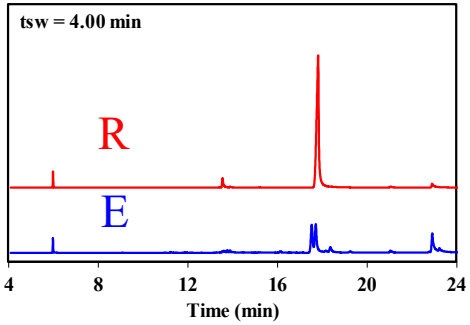
Process Development: Results from Lab SMB

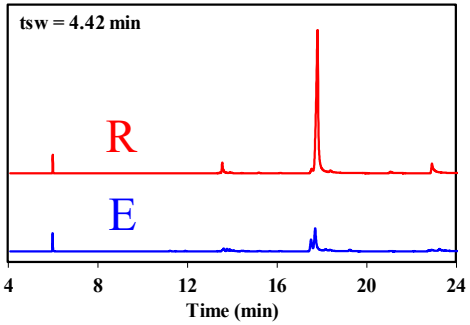






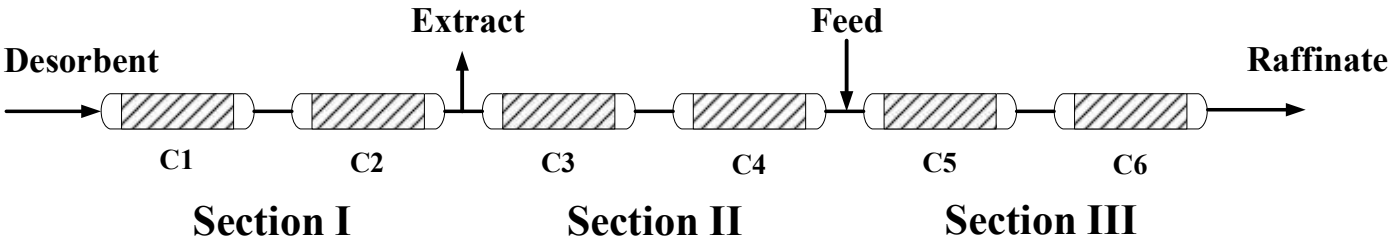




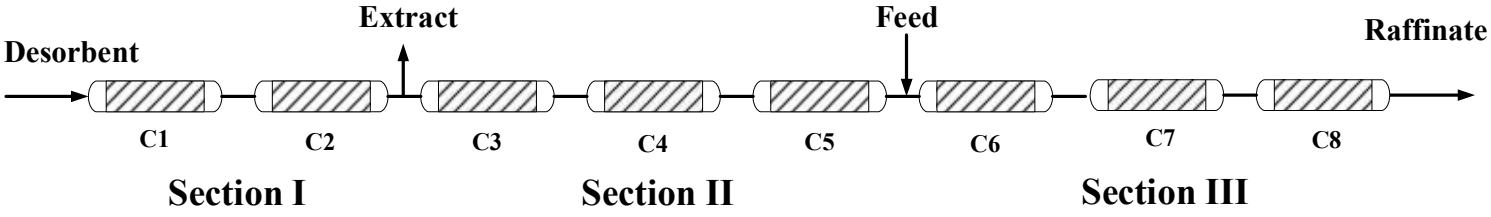


Process Development: SMB Equipment

Lab-scale SMB: 1 x 10 cm

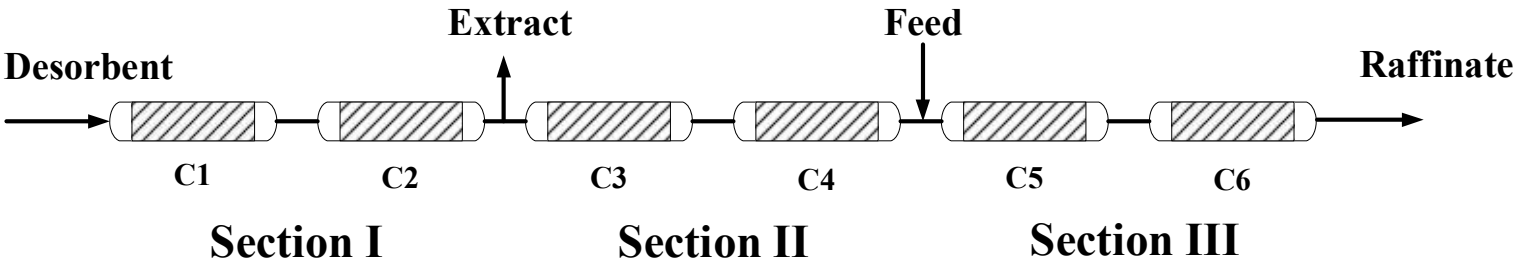


Pilot-scale SMB: 3 x 25 cm

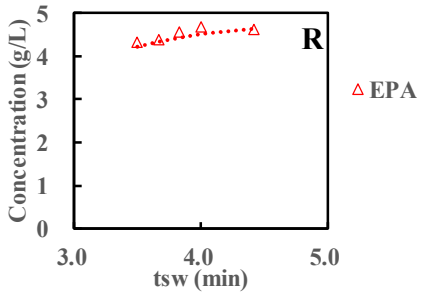


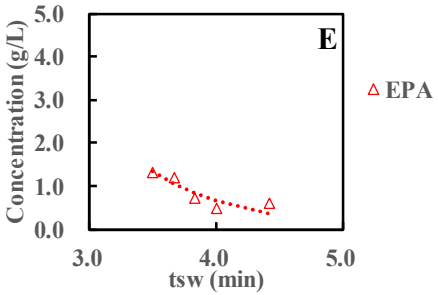
Process Development: Results from Lab SMB

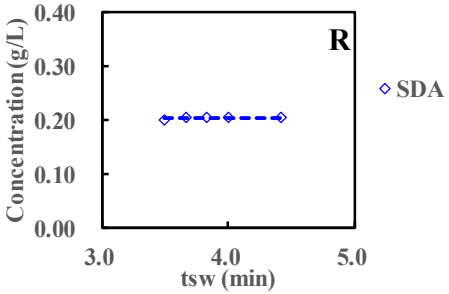
Lab SMB: 1 x 10 cm

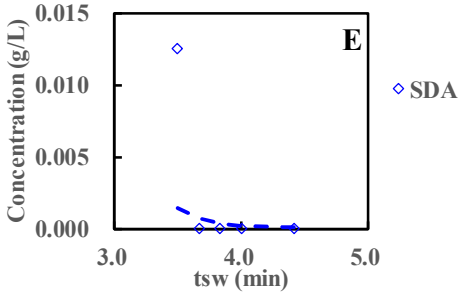


1st Run of SMB

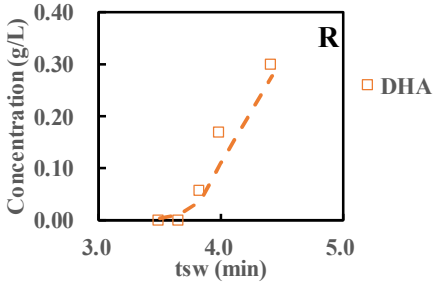


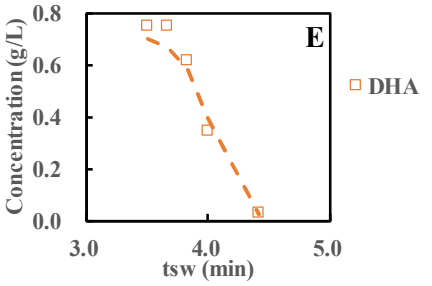


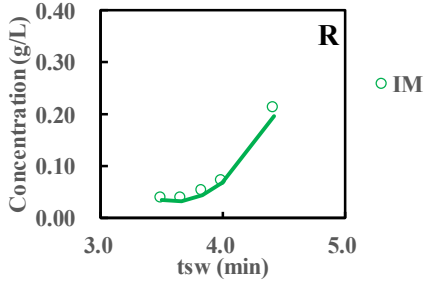


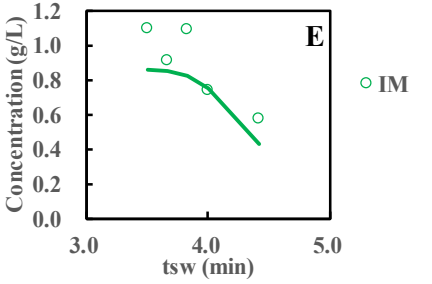


2nd Run of SMB



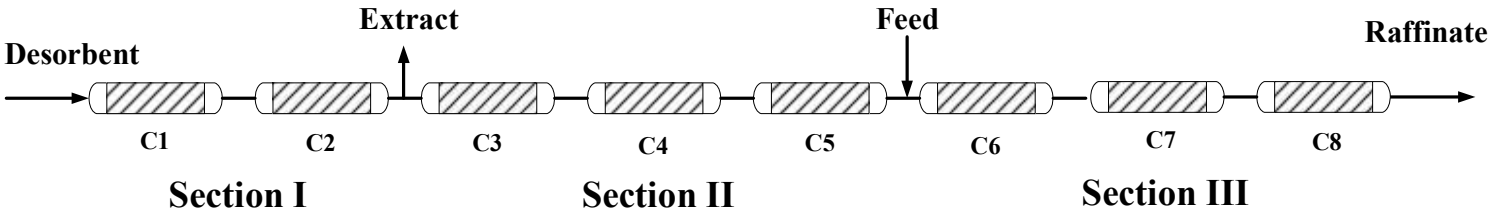




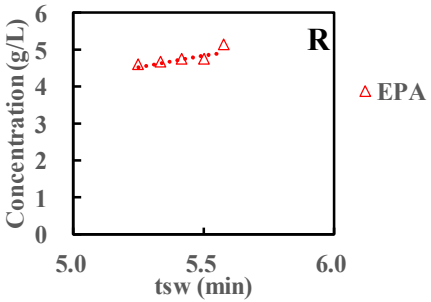


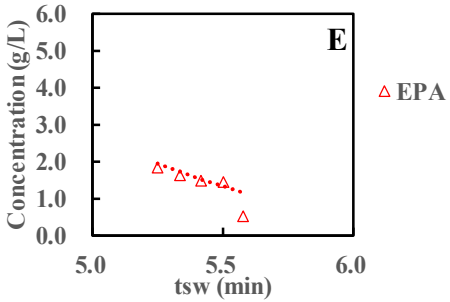
Process Development: Results from Pilot SMB

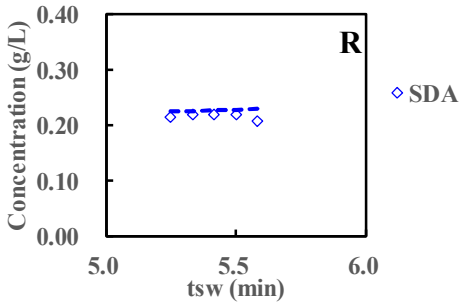
Pilot SMB: 3 x 25 cm

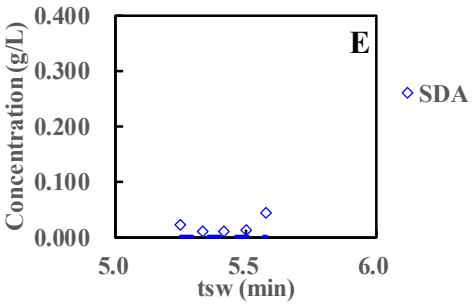


1st Run of SMB

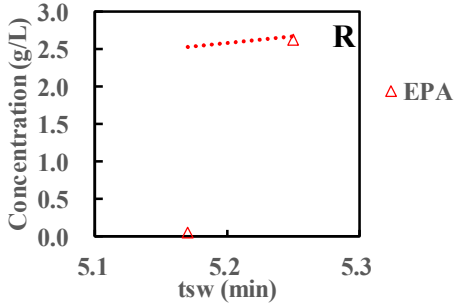


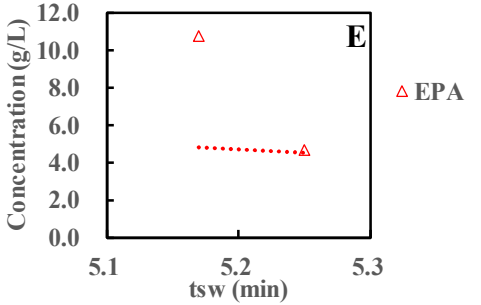


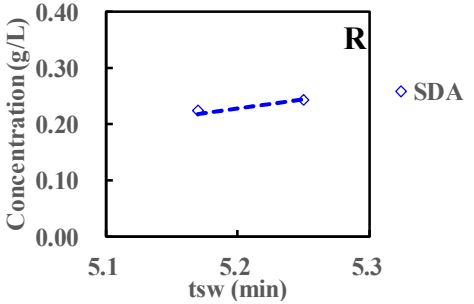


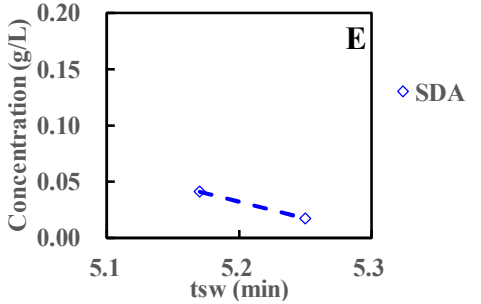


2nd Run of SMB









❑ Future Trends and Opportunities

喬璞科技 JOPE TECHNOLOGY

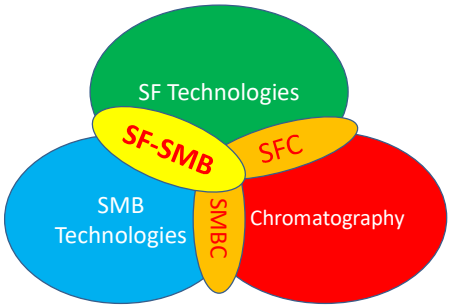
Conclusions:

- The first of its kind of industrial application on SF-SMB has been realized in Taiwan for fish oil nutriceuticals.
- SF-SMB can be used to produce API(Active Pharmaceutical Ingredient) or ANI (Active Nutriceutical Ingredient).

Challenges and Opportunities:

- Scale-up of the SF-SMB can further reduce the cost.
- SF-SMB needs sophisticated adsorbent for diversified application.
- Automation of SMB needs more studies and a new solid phase is always welcomed.
- **SF-SMB can also be applied in other products, such as chiral separation, natural product purification.**

Thanks for Listening



喬璞科技
JOPE TECHNOLOGY