Quantification of Triacylglycerol Positional Isomers in Edible Oil by Recycle High-Performance Liquid Chromatography Coupled to Atmospheric Pressure Chemical Ionization Mass Spectrometry

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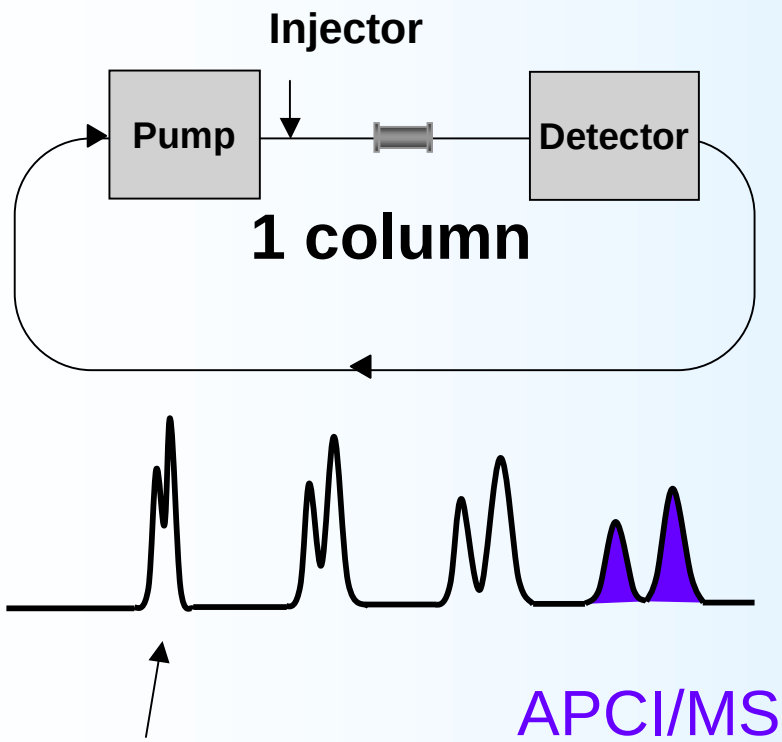
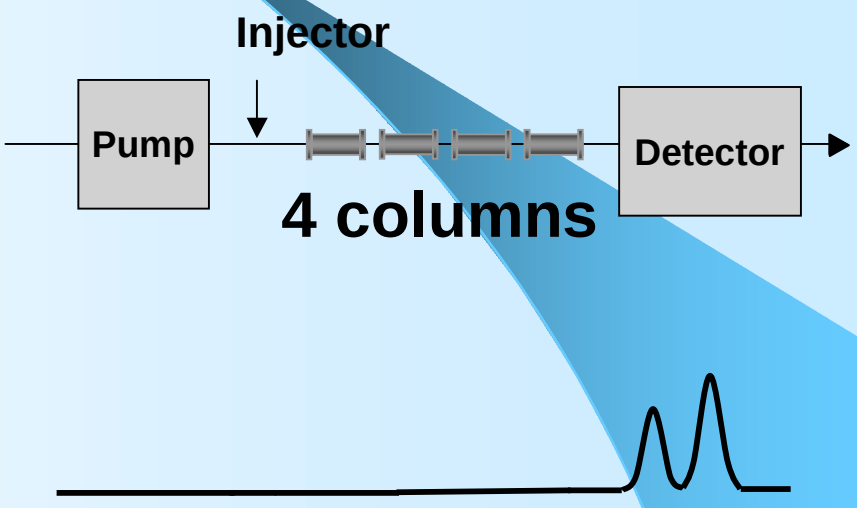
Introduction:

Scheme of recycle HPLC



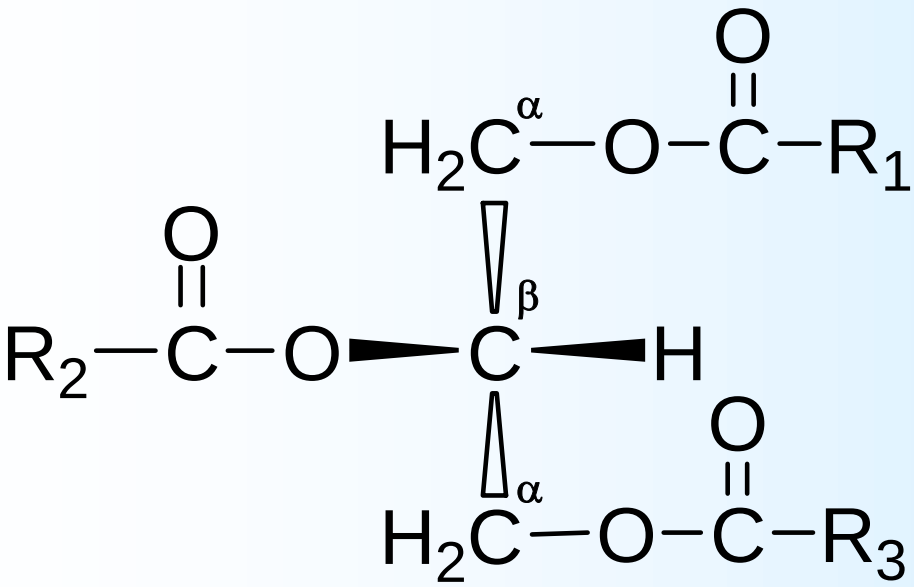


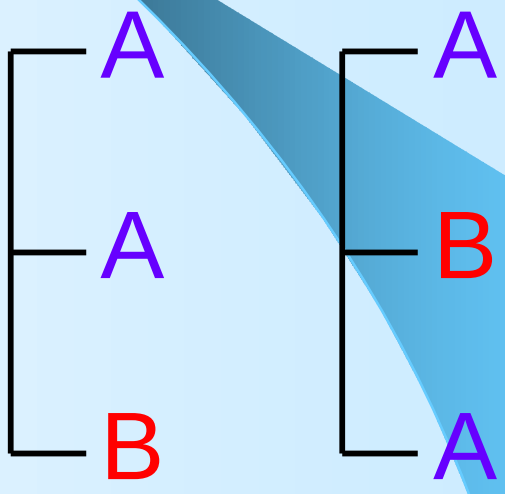
APCI/MS-MS

Triacylglycerol positional isomers (TAG-PIs)

Introduction:

The structure of triacylglycerol positional isomer (TAG-PI)





A, B = Fatty acid

Introduction:

TAG composition of palm oil

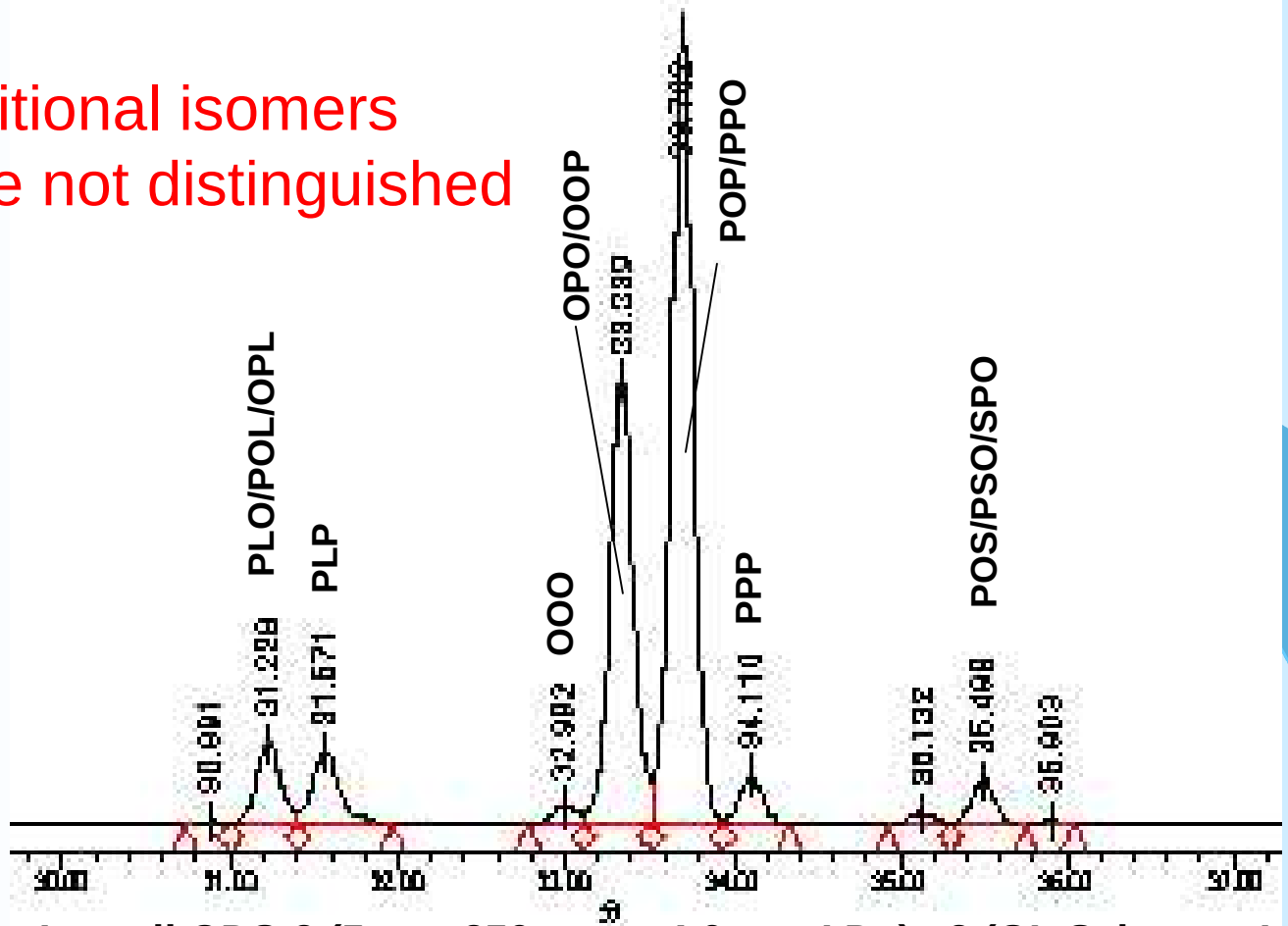
Triacylglycerol Composition (%)										
Double Bond	0		1		2		3		more than 4	
	MPP	0.5	MOP	1.4	MOO	0.7	MOL	0.2	PLL	0.8
	PMP	0.2	MPO	0.2	PLP	6.3	PLO	6.0	OLO	1.4
	PPP	7.2	**POP**	**23.7**	PLS	0.8	POL	3.1	OOL	1.5
	PPS	1.0	POS	3.1	PPL	1.0	SLO	0.4	LOL	0.1
	PSS	0.1	**PPO**	**6.9**	SPL	0.1	SOL	0.2		
	PSP	0.7	PSO	0.6	POO	21.5	OOO	5.1		
			SPO	0.5	SOO	1.4	OPL	0.5		
			他	0.3	OPO	1.6	MOL	0.1		
					OSO	0.2				
					PSL	0.1				
					他	0.5				
Total		9.7		36.7		34.2		15.6		3.8

M: Myristic acid (C14:0), P: Palmitic acid (C16:0), S: Stearic acid (C18:0),
O: Oleic acid (C18:1), L: Linoleic acid (C18:2)

M. MacLellan, *PORIM Bulletin, 11:* 40 (1984)

Introduction:
Palm oil TAG chromatogram by RP-HPLC

Positional isomers
were not distinguished

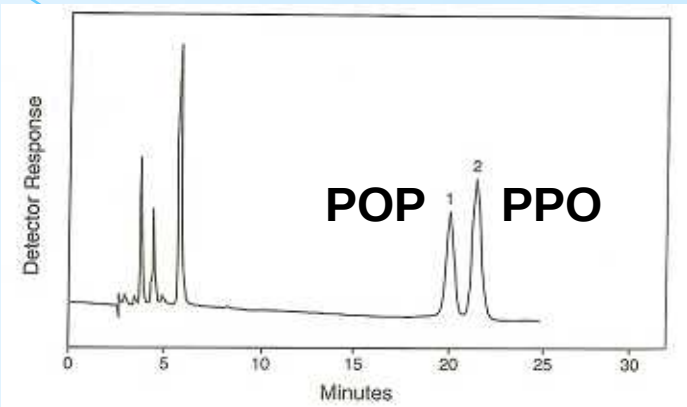


Column: Inertsil ODS-3 (5 μm, 250 mm x 4.6 mm I.D.) x2 (GL Sciences Inc.)
Column temperature: 35 ºC
Mobile phase: Acetonitrile-Acetone 50:50→0:100 (30min) →0:100 (45min)
Flow rate: 1.0 mL /min
Detector: ELSD

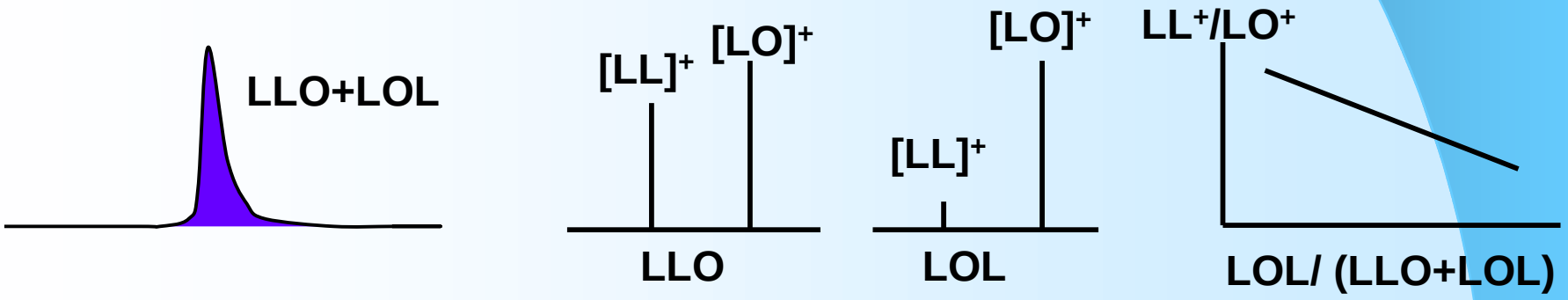
Introduction:
Previous studies for quantification of TAG-PIs

1) Separation of POP and PPO mixture by dual–column Ag-HPLC.

R. O. Adlof, *New Techniques and Applications in Lipid Analysis (AOCS PRESS)*, p.256-265 (1997)



2) The ratio of TAG-PIs are calculated from DAG ions caused by in-source CID of mass spectrometer.



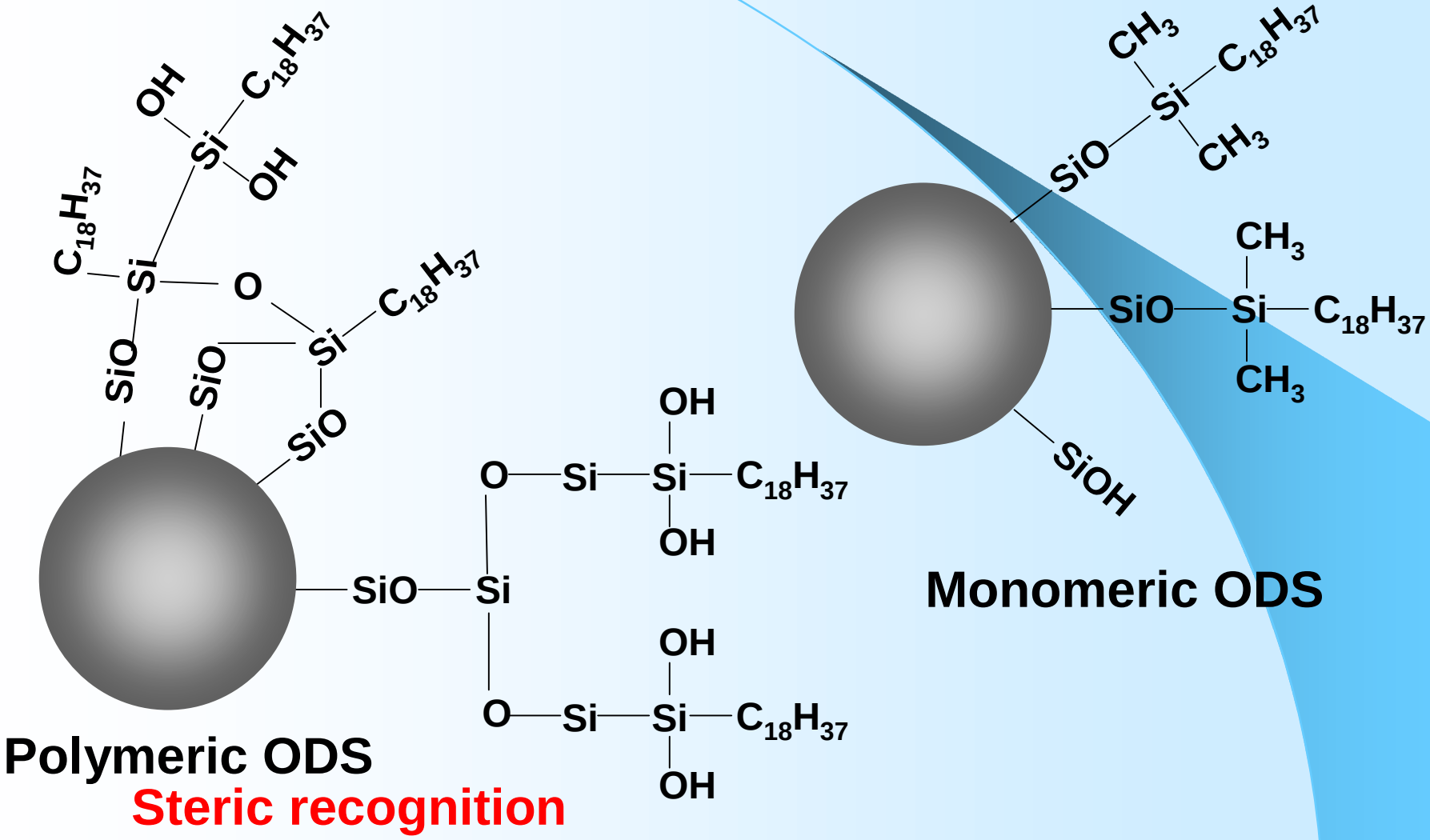
Jakab, A. *et al*., *Rapid Commun. Mass Spectrom. 17:* 2295-2302 (2003)

Introduction:

The comparison between RP-HPLC and Ag^+-HPLC

	RP-HPLC	Ag^+-HPLC
Chemical and mechanical durability	good	poor
Reproducibility	good	poor
Separation of TAG-PI	not good	good

Introduction:
Polymeric and monomeric ODS stationary phase



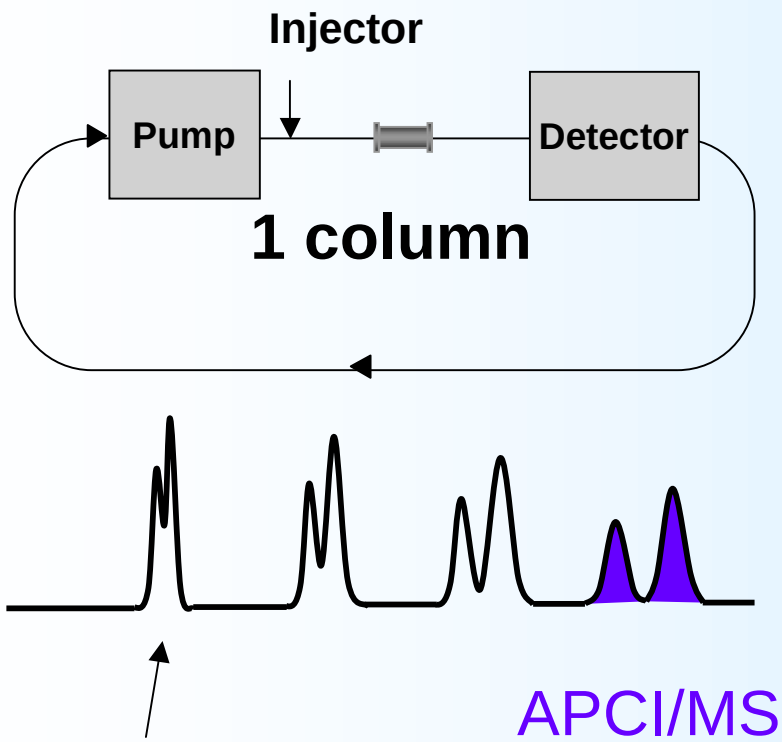
Polymeric ODS

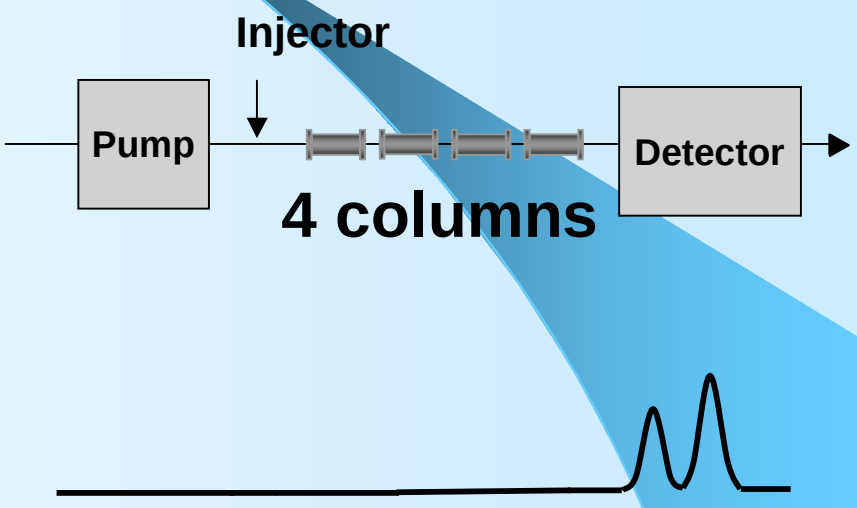
Steric recognition

Monomeric ODS

Introduction:

Scheme of recycle HPLC

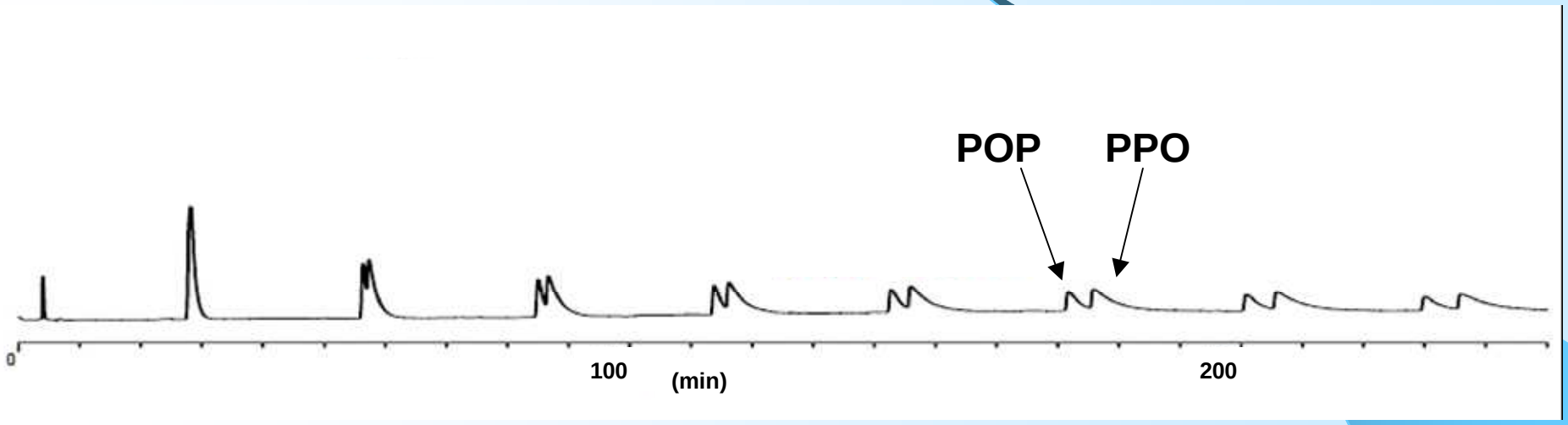




APCI/MS-MS

Triacylglycerol positional isomers (TAG-PIs)

Introduction:
POP / PPO were resolved by non-endcapped polymeric ODS columns

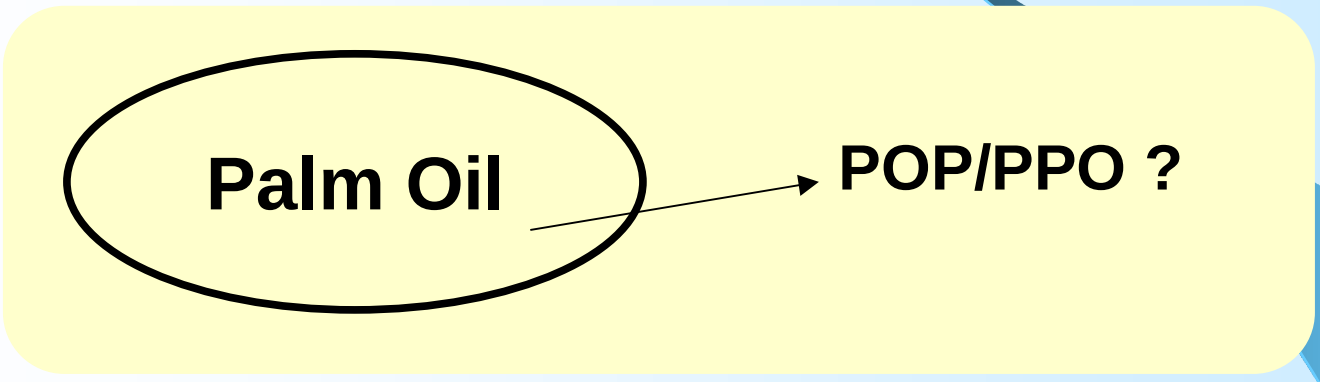


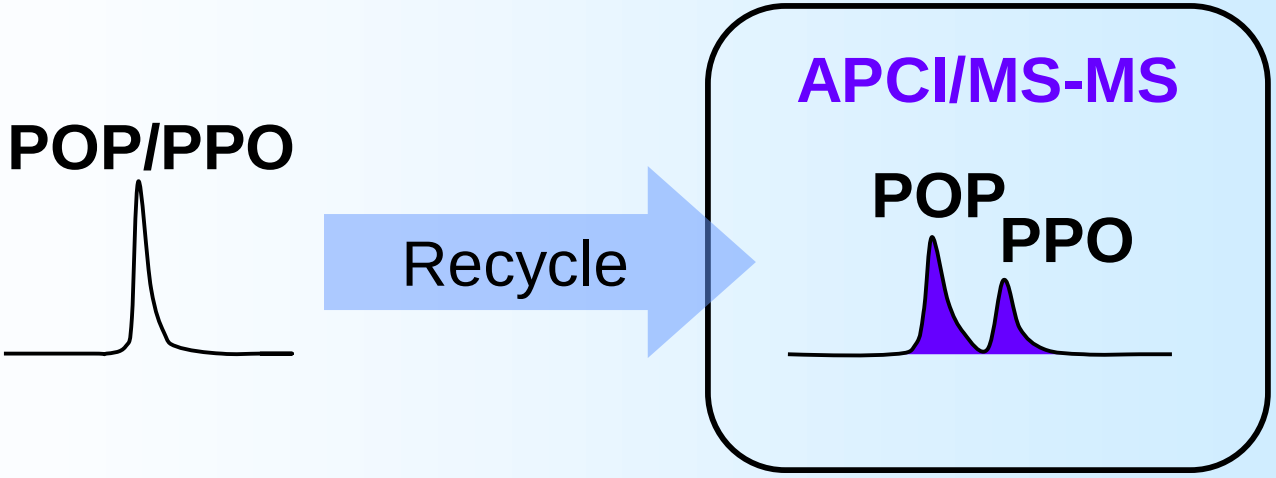
Column: Inertsil ODS-P (5 μm, 250 mm x 4.6 mm I.D.) x2 (GL Sciences Inc.)
Column temperature: 25 ºC
Mobile phase: Acetonitrile- 2-Propanol- Hexane (3:2:1, v/v/v)
Flow rate: 1.0 mL /min
Detector: UV 205 nm

I. Kuroda, T. Nagai, H. Mizobe, N. Yoshimura, N. Gotoh, and S. Wada, *Analytical Sciences, 24:* 865-869 (2008)

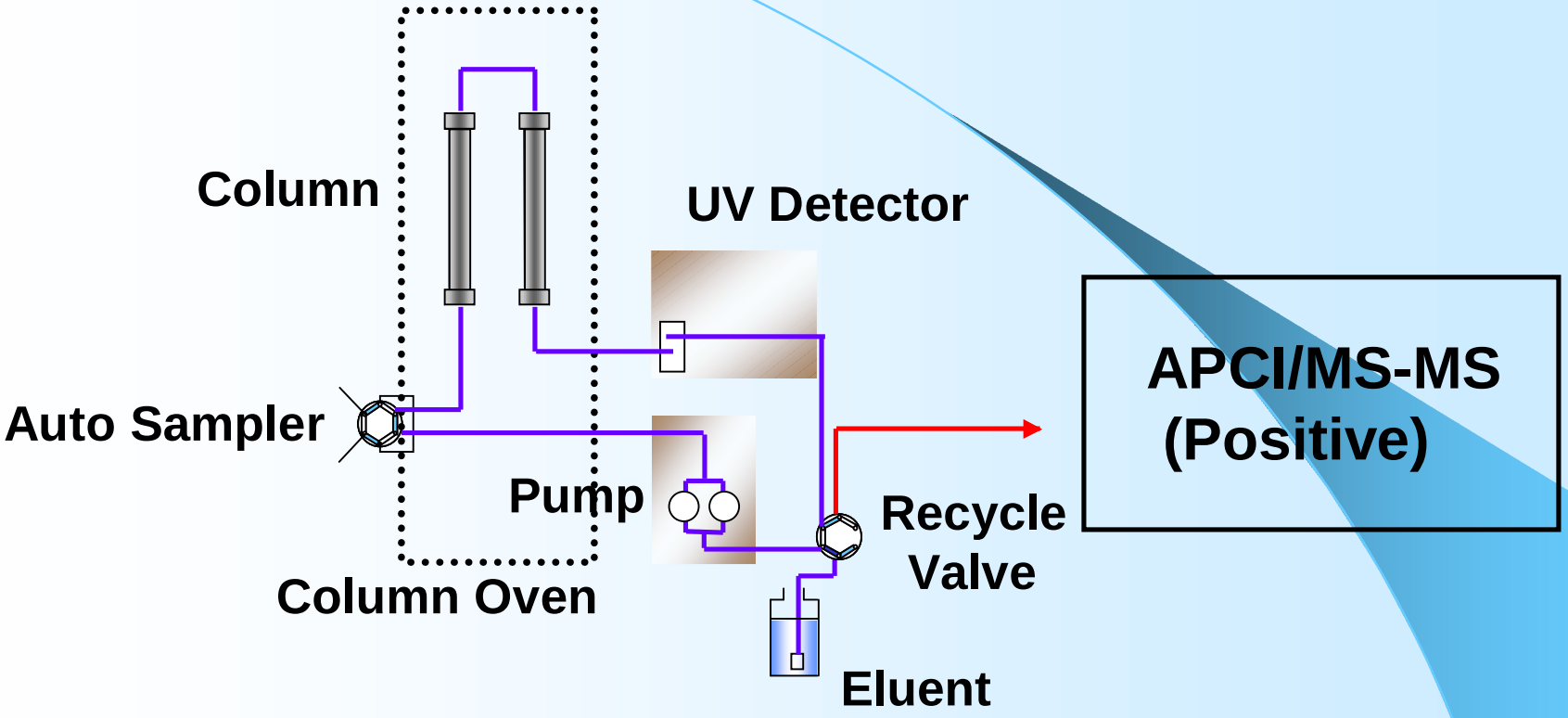
Objective:

Quantification of TAG-PI, POP/PPO in palm oil by recycle HPLC coupled to APCI/MS-MS





Methods:
Recycle HPLC and APCI/MS-MS

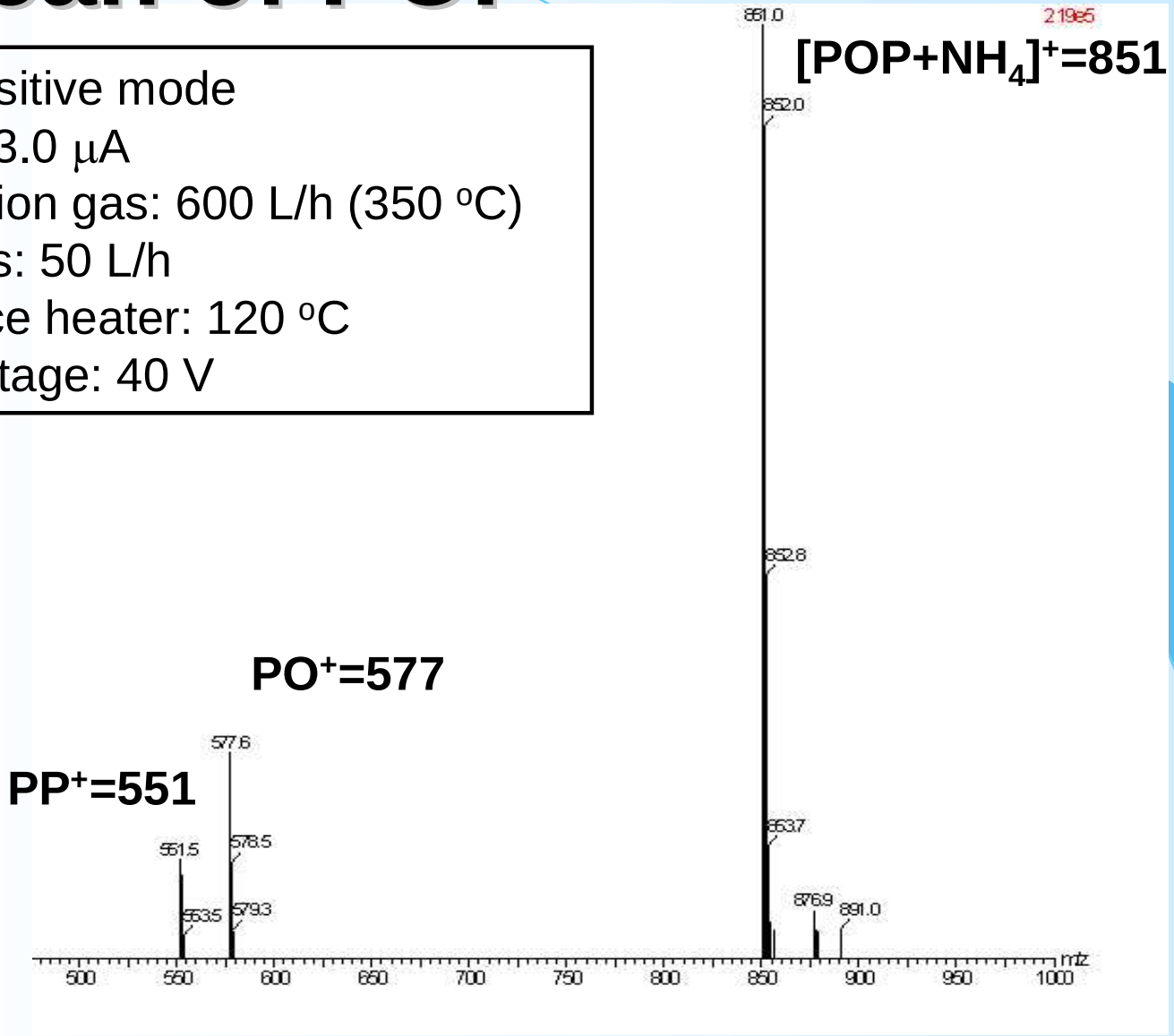


HPLC: Recycle HPLC System (GL Sciences Inc.)
Column: Inertsil ODS-P (5 μm, 250 mm x 4.6 mm I.D.) x2 (GL Sciences Inc.)
Column temperature: 25 °C
Mobile phase: Acetonitrile- 2-Propanol- Hexane (3:2:1, v/v/v)
Flow rate: 1.0 mL /min
Injection Volume: 20 μL
Detector: UV 210 nm, APCI/MS-MS (Quattro micro API, Waters)

Methods: Optimization of MS parameters

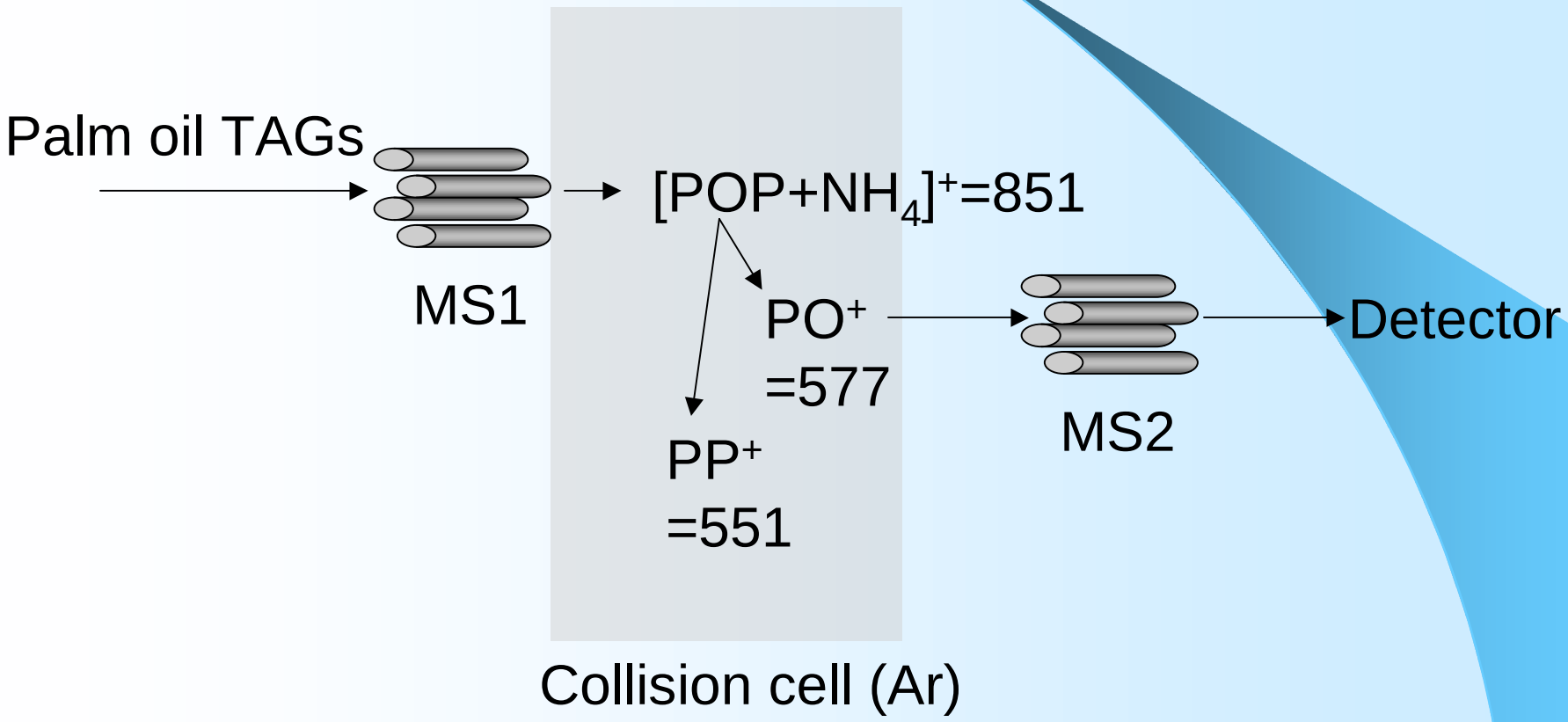
MS scan of POP

APCI-Positive mode
Corona: 3.0 μA
Desolvation gas: 600 L/h (350 °C)
Cone gas: 50 L/h
Ion source heater: 120 °C
Cone voltage: 40 V



Methods: Optimization of MS parameters

SRM transition of triacylglycerol, POP



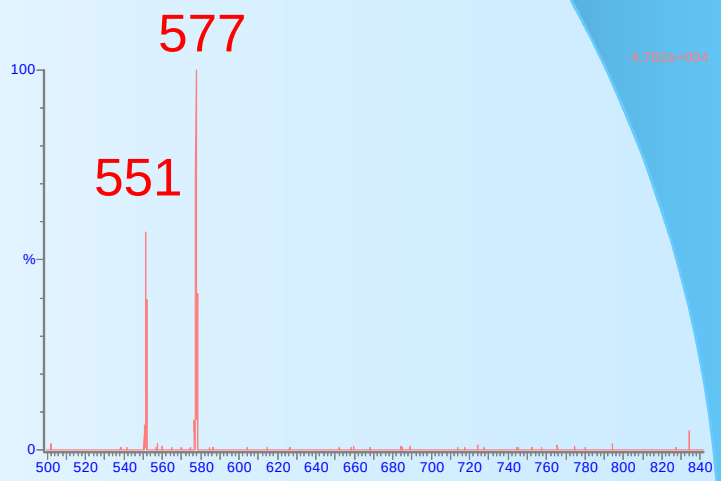
Methods: Optimization of MS parameters

Development of SRM channel

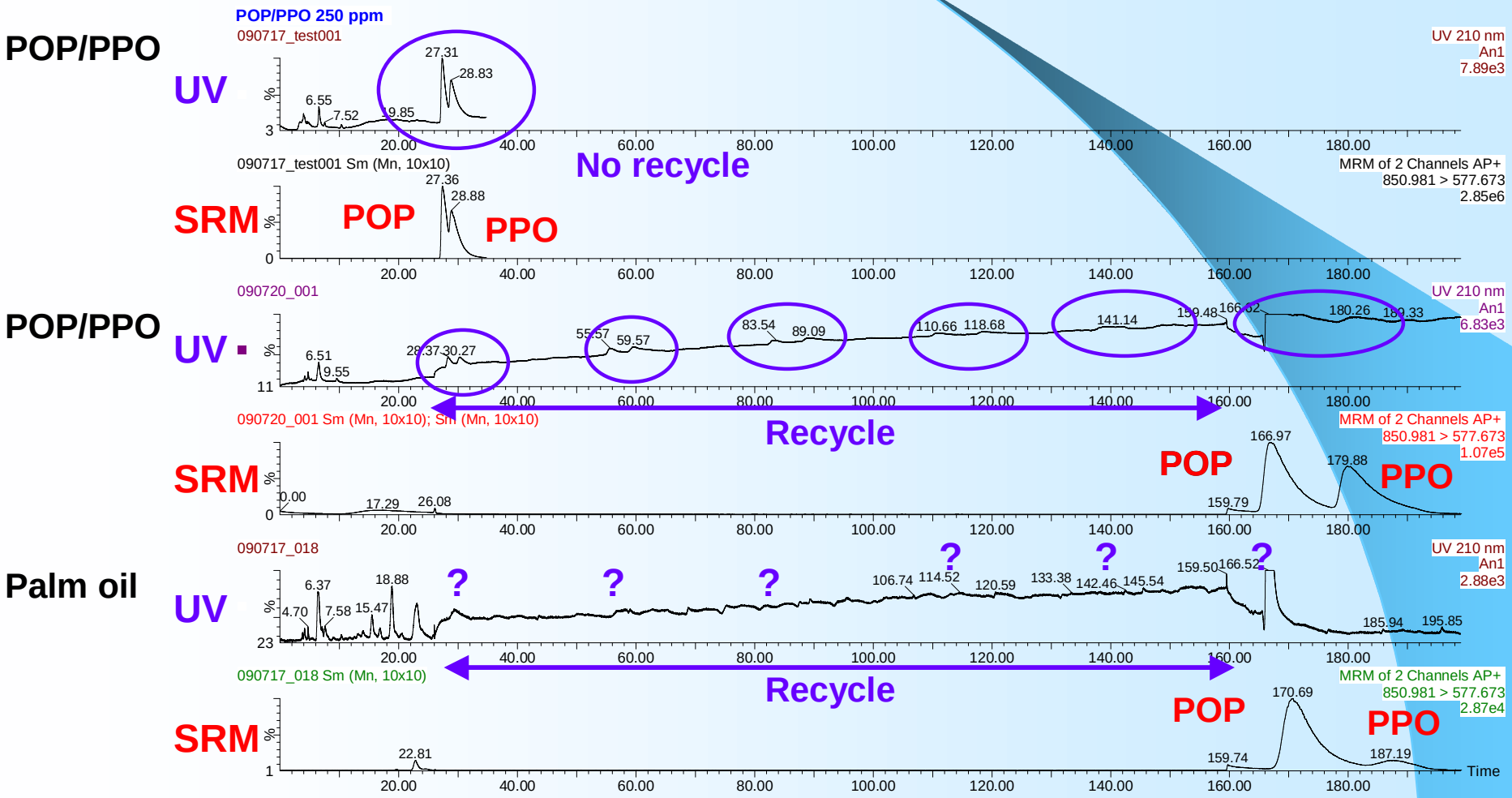
Precursor Mass	Product Mass	Cone Voltage	Collision Energy
850.98	551.5	40.00	26.00
850.98	577.7	40.00	24.00

Collision Energy Optimize Chromatogram for m/z 850.98 -> 577.7

Collision Energy Optimize Spectrum for m/z 850.98 -> 577.7



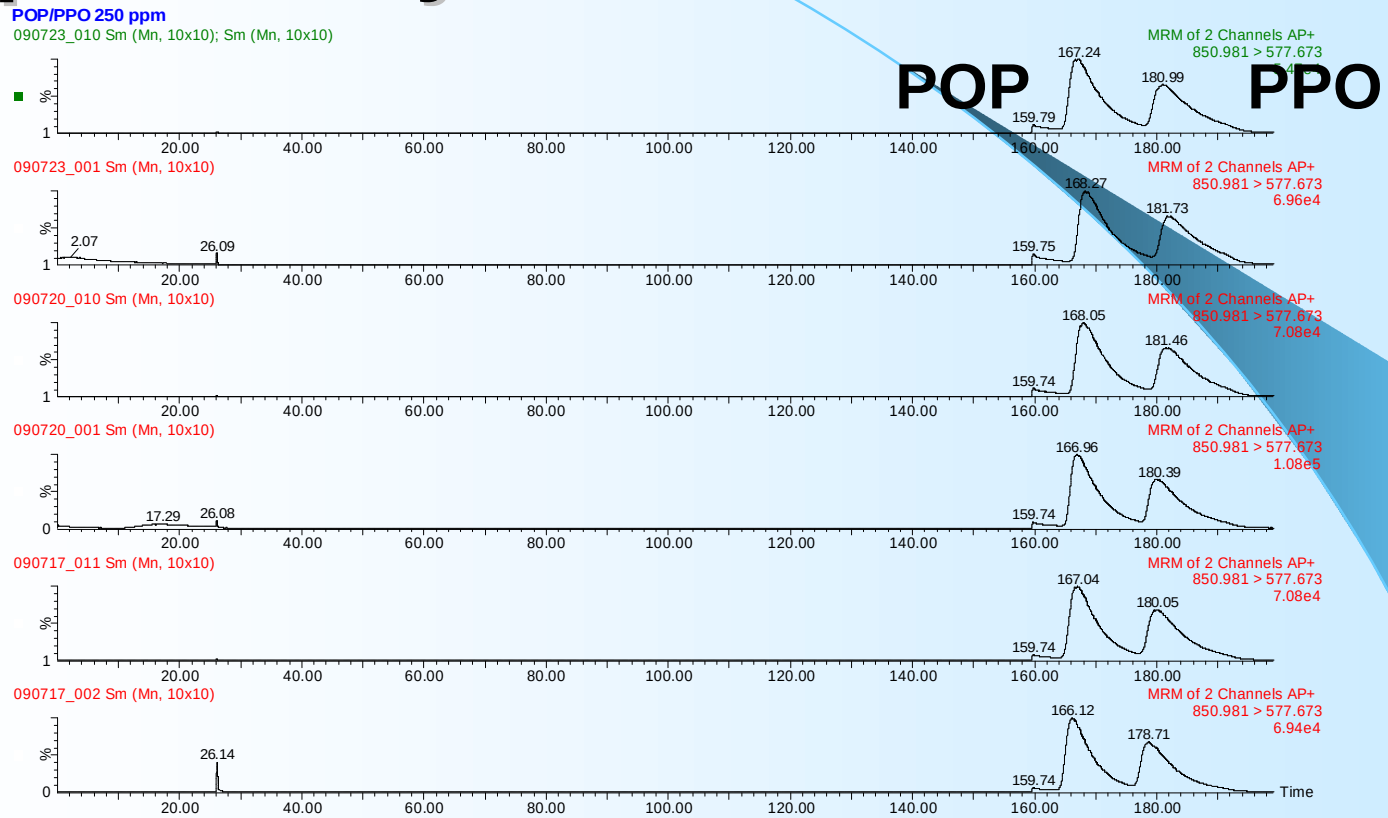
Results:
UV and SRM chromatograms of authentic POP/PPO and palm oil obtained by recycle separation



Recycle x 5 = 12 columns

Results:

Repeatability of retention times

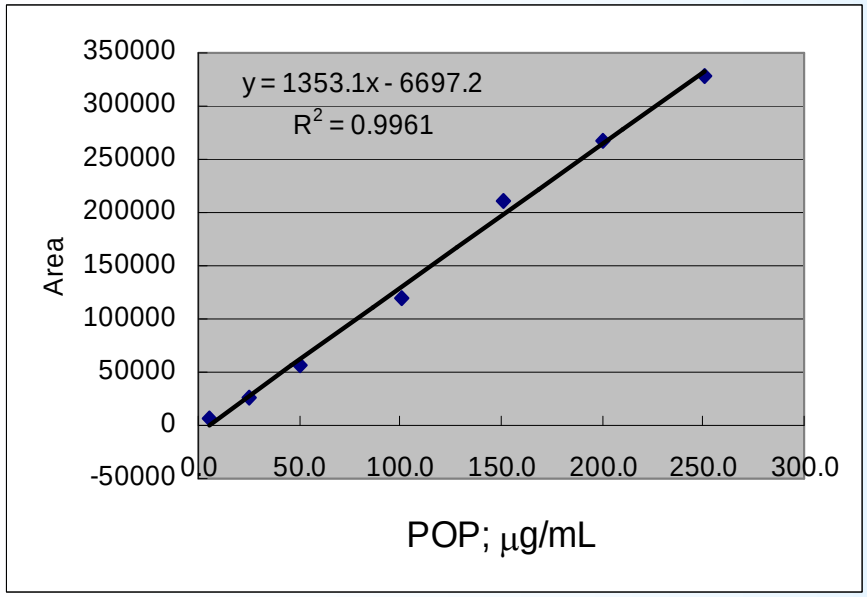


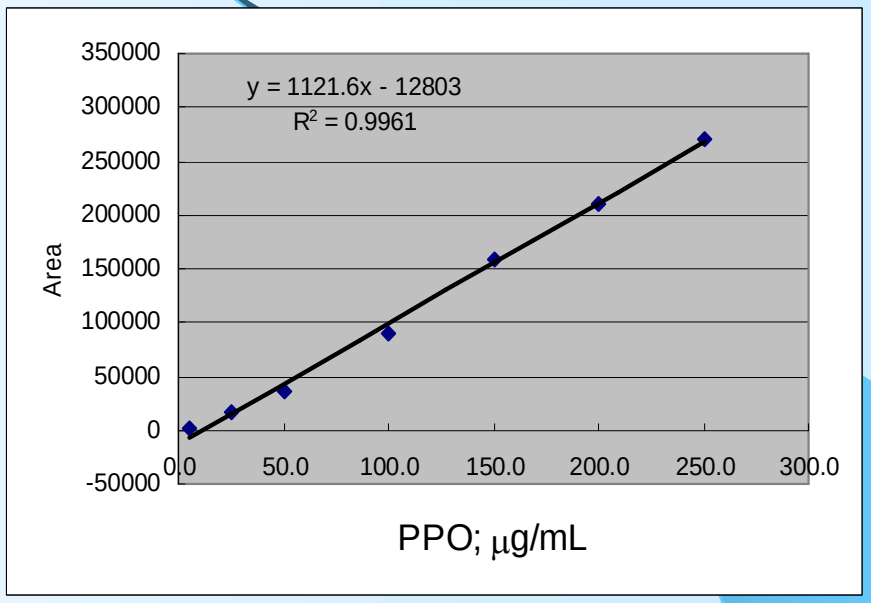
The precision of retention times of POP and PPO (min)

	POP	PPO
MEAN	167.3	180.6
SD	0.8	1.1
RSD; %	0.47%	0.61%

Results:

The calibration curves and recovery of POP and PPO

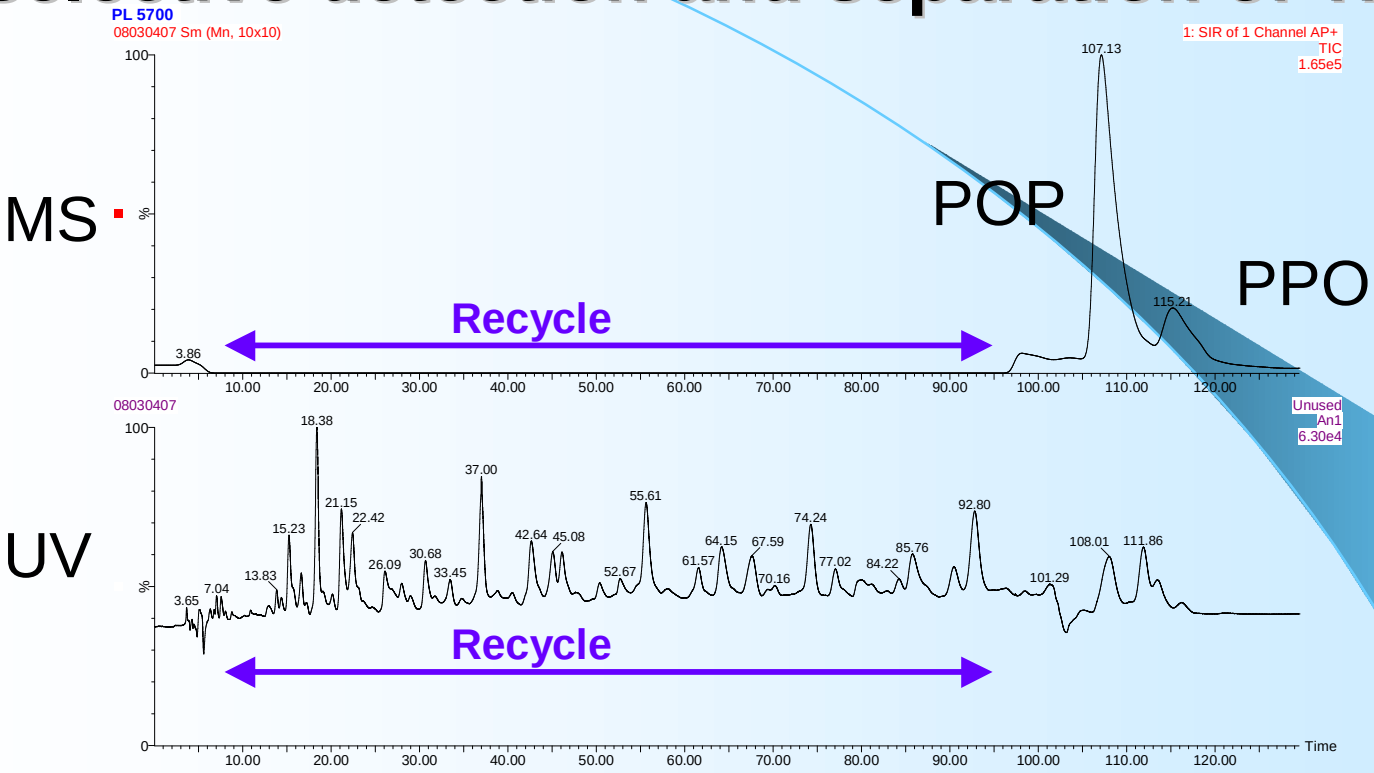




	POP			PPO		
	MEAN	SD	RSD (%)	MEAN	SD	RSD (%)
Rate of TAG-PI in palm oil; %	22.4%	2.7%	12.2%	4.6%	2.9%	63.4%
Correlation coefficient (R^2)	0.983	0.014	1.5%	0.985	0.016	1.6%
Recovery rate; %	105%	9%	8.7%	99%	16%	16.0%

(n=4)

Merits of recycle HPLC coupled to APCI/MS-MS for selective detection and separation of TAG-PIs



Target TAG-PIs and the other TAGs	Selective detection by SRM chromatogram
TAG-PIs ex.) POP and PPO	Recycle separation

Quantification of TAG-PIs in edible oils and fats

Conclusion:

The target TAG-PIs, POP and PPO in palm oil can be selectively quantified by recycle HPLC-APCI/MS-MS.

Thank you for your kind attention.