Development of a bi-dimensional HPLC method for the stereospecific analysis of triacylglycerols

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Overview

- Problem statement
- Introduction
- Development of a HPLC method for Ag^+ column
  - Mobile phase selection
  - Column temperature
  - Flow rate
- Method validation
- Multidimensional analysis
- Conclusion

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Problem statement

- **Problem:**

  No accurate and reliable HPLC method for separation of TAG positional isomers with the same degree of unsaturation

- **Aim of the study:**
  - To develop and optimize Ag^+-HPLC method to separate positional isomers
  - Optimization of offline bi-dimensional HPLC method with C18-Ag^+ columns

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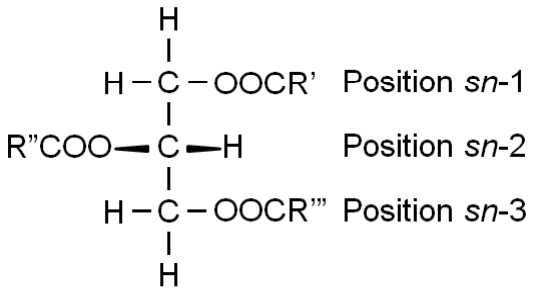
Introduction

- The TAG profile plays an important role in physical properties of an oil or fat

- Distribution of fatty acids between different stereospecific positions of TAGs have an effect on the physical, nutritional and biochemical characteristics of fats and oils



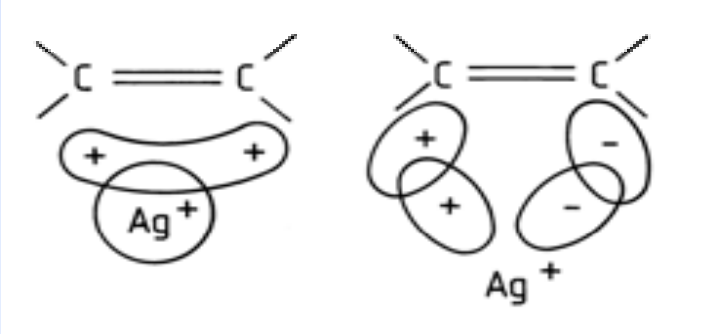
Fischer projection of triacyl-*sn*-glycerol

Introduction

- HPLC is the most common technique used in TAG analysis
  - Normal phase HPLC (NP-HPLC)
  - Reversed phase HPLC (RP-HPLC)
  - Silver ion HPLC (Ag^{+}-HPLC)

Principle

- **RP-HPLC and NP-HPLC**
  - Separation is based on chain lengths of the fatty acyl residues and on the total number of double bonds in the molecule
- **Silver ion HPLC (Ag^+-HPLC)**
  - Separation is based on the weak interaction between the silver ions and the pi (π)-electrons of the double or triple bonds of the carbon chain of the fatty acyl moieties



Silver ion HPLC (Ag^+-HPLC)

- **Elution order:**

SSS > SSM > SSD > MMM > SMD > MMD > SDD=SST > SMT=MDD > MMT > SDT=DDD > MDT ≥STT > DDT > MTT > DTT > TTT

Where:

S = Saturated fatty acid

M = Monounsaturated fatty acid

D = Diunsaturated fatty acid

T = Triunsaturated fatty acid

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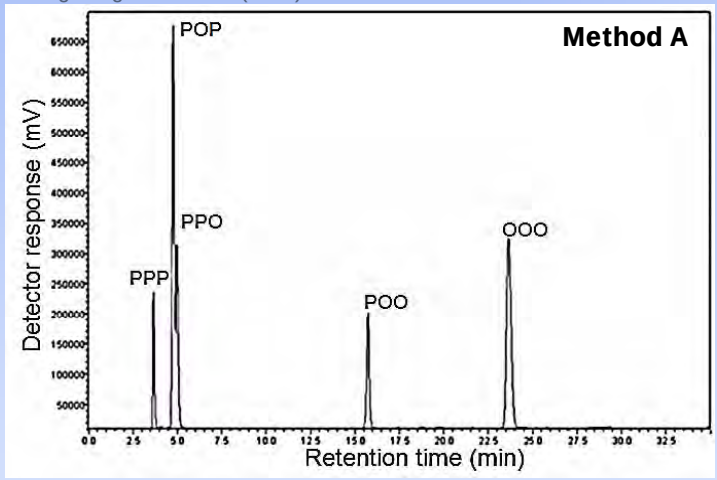
Mobile phase selection

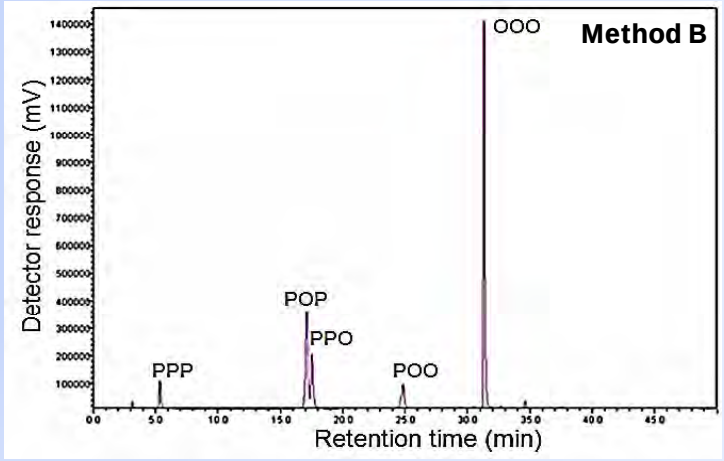
two-stepped linear gradient of dichloromethane : acetone (98:2)

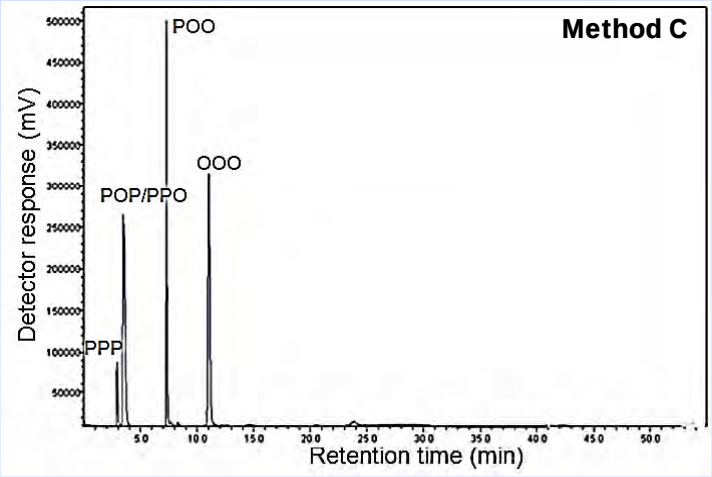
Ming-Lung Chen et al. (2004)



two-stepped linear gradient of acetone : heptane (2:98)

Maj-Britt Macher et al. (2001)

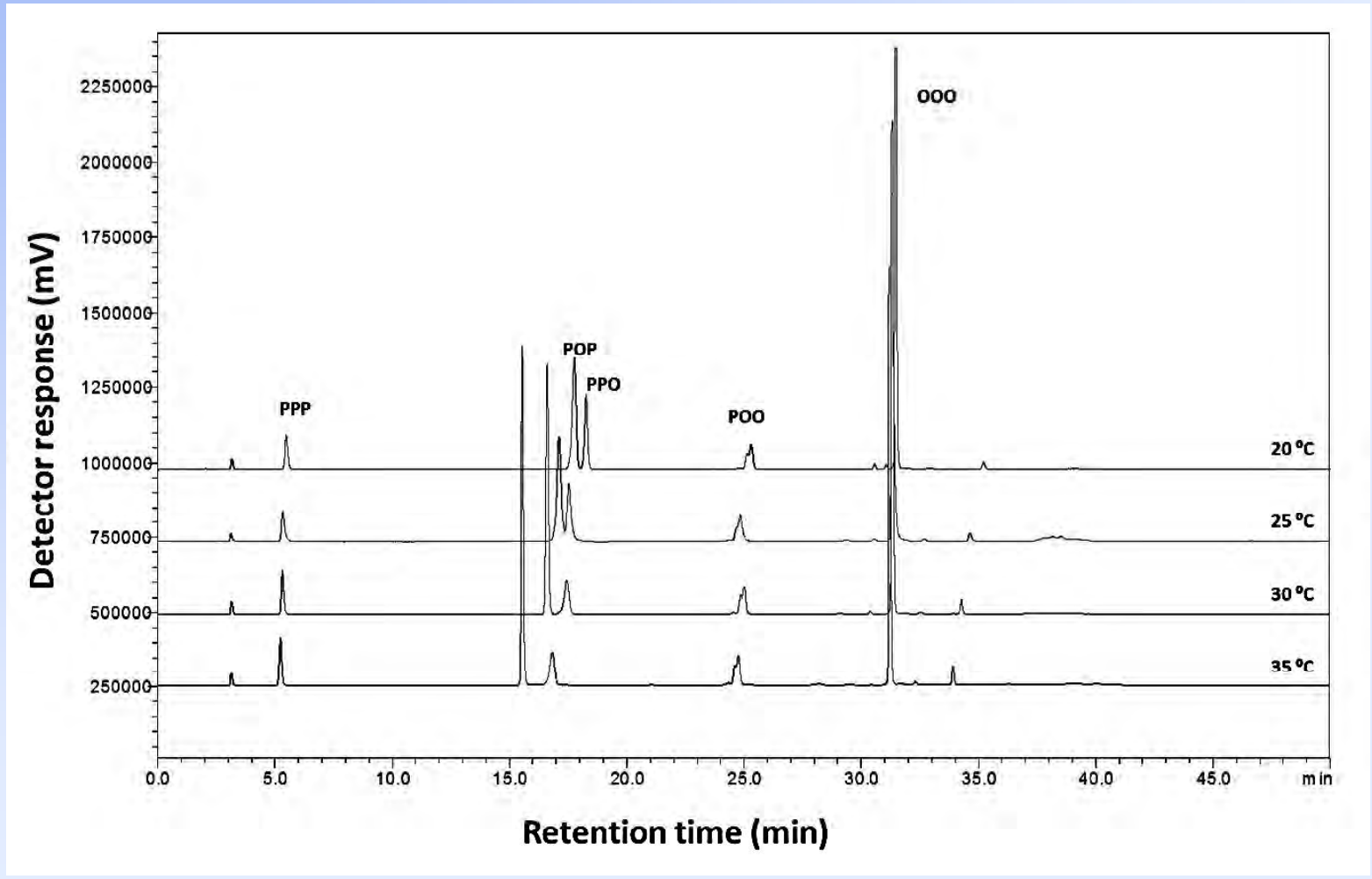




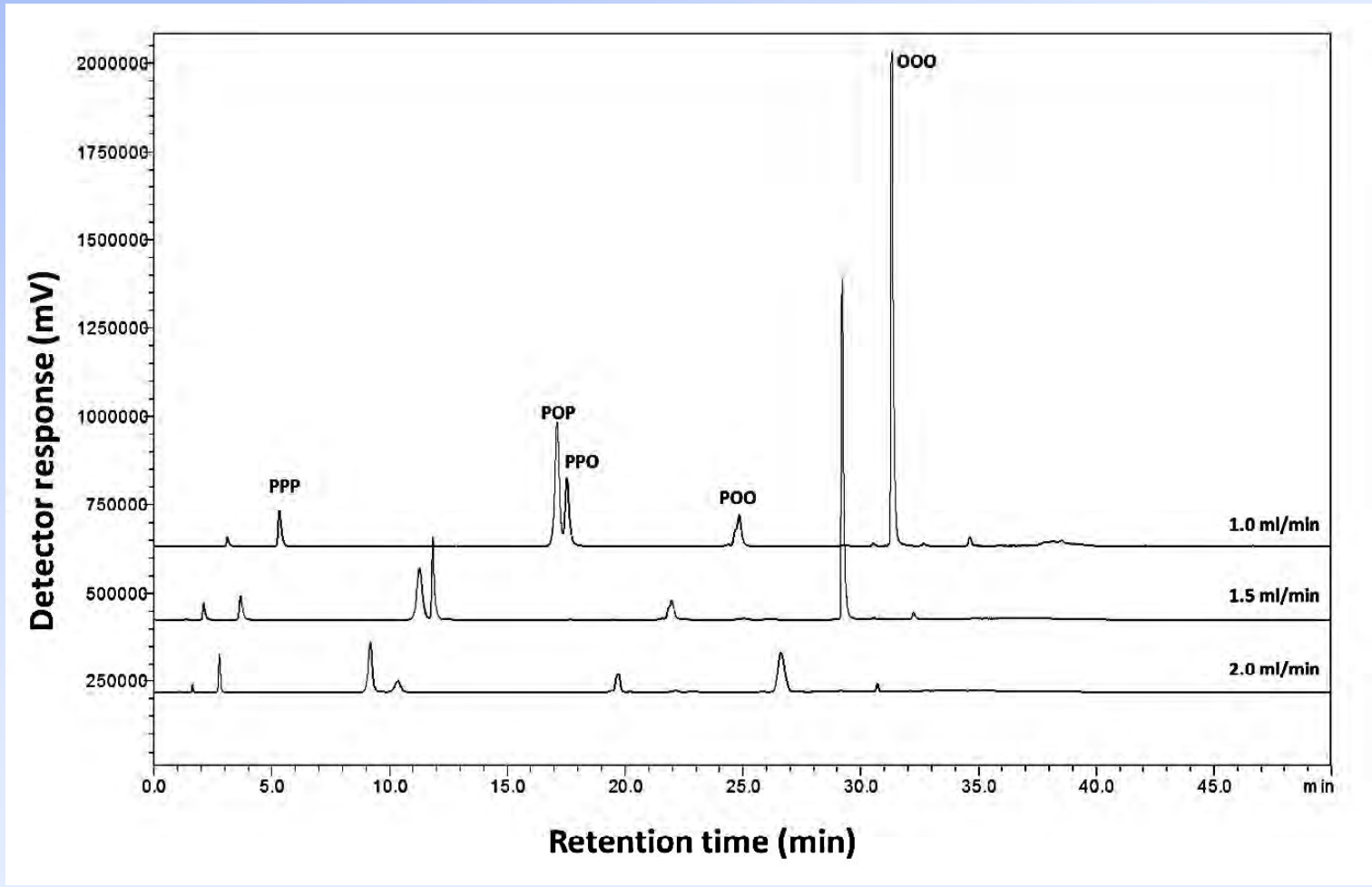
non linear gradient of acetonitrile, dichloromethane/1,2-dichloroethane (1:1 vol/vol), and acetone (0:98:2)

Smith et al. (1994)

Column Temperature



Flow rate


Detector response (mV)
2000000
1750000
1500000
1250000
1000000
750000
500000
250000
PPP
POP
PPO
POO
OOO
1.0 ml/min
1.5 ml/min
2.0 ml/min
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
min
Retention time (min)

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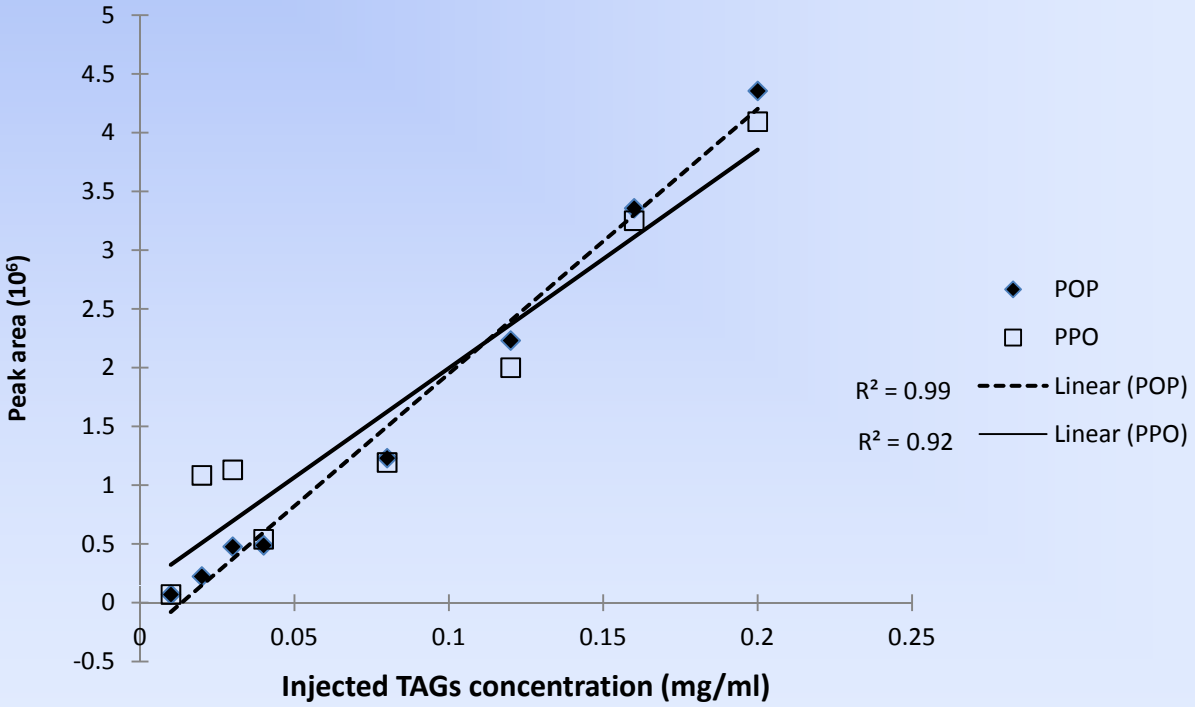
Method validation

- **Limit of detection** $LOD = 3.3 \times D/S$
- **Limit of quantification** $LOQ = 10 \times D/S$
- **Precision** $\%RSD = S/X \times 100$

TAG	LOD (mg/ml)	LOQ (mg/ml)	Precision
POP	0.01	0.04	2.38
PPO	0.02	0.06	6.32

Method validation

- **Linearity**



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Multidimensional analysis

First Dimension

Second Dimension

Fat Sample (Solve in ACN/DCM)

RP-HPLC (C18 Column)

Automatic Fraction Collector

Drying Fractions with N_2

TAG fractions (Solve in Heptane)

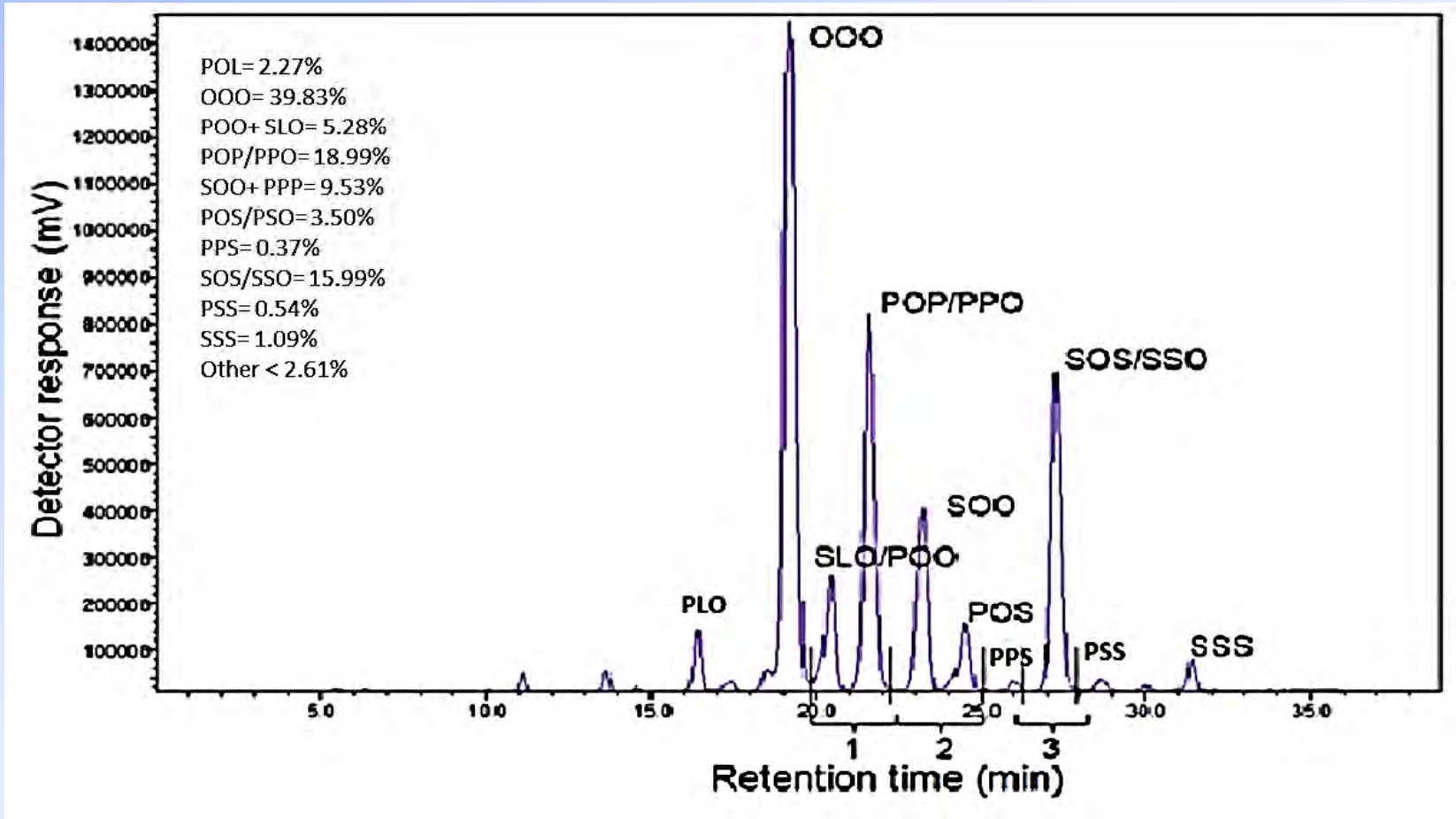
Ag^+-HPLC (Silver ion Column)

Detector (ELSD)

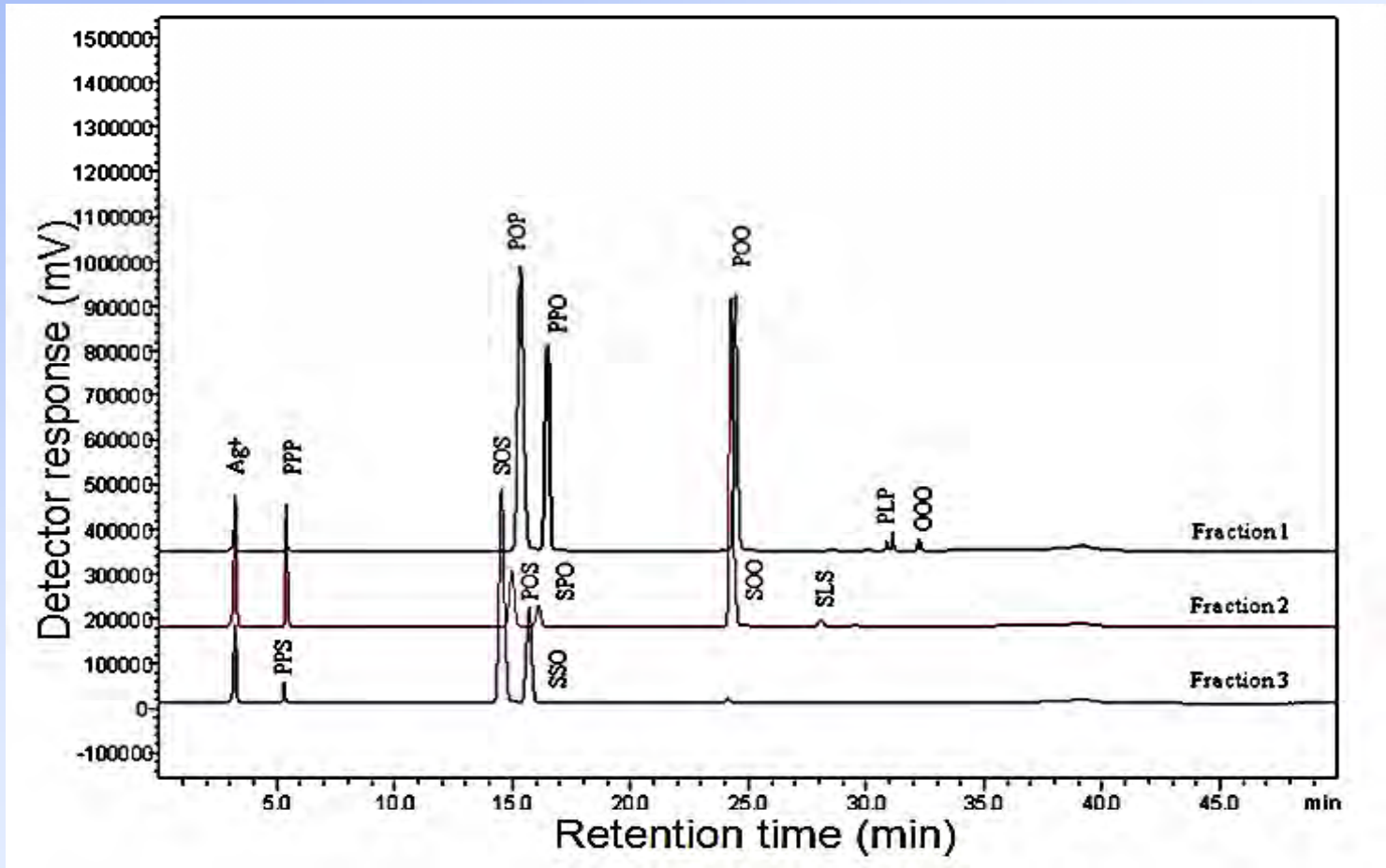
Peaks

First Dimension-C18 Column

Fat Blend



Second Dimension-Ag^+-Column



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Conclusion

- The method that has been developed is repeatable, accurate and easy to apply
- The combination of NARP and Ag^+-HPLC could give useful information about the important pairs of TAGs
- The analysis of positional isomers under Ag^+-HPLC conditions was greatly improved by the use of a pre-separation step

Thank you for your attention

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