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
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

![Graph showing the effect of temperature on detector response and retention time.](image)
Flow rate
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Method validation

- **Limit of detection**
  - LOD = $3.3 \times D/S$

- **Limit of quantification**
  - LOQ = $10 \times D/S$

- **Precision**
  - $%\text{RSD} = S/X \times 100$

<table>
<thead>
<tr>
<th>TAG</th>
<th>LOD (mg/ml)</th>
<th>LOQ (mg/ml)</th>
<th>Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>POP</td>
<td>0.01</td>
<td>0.04</td>
<td>2.38</td>
</tr>
<tr>
<td>PPO</td>
<td>0.02</td>
<td>0.06</td>
<td>6.32</td>
</tr>
</tbody>
</table>
Method validation

- **Linearity**

- **POP**
  - $R^2 = 0.99$

- **PPO**
  - $R^2 = 0.92$
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- **Multidimensional analysis**
- Conclusion
Multidimensional analysis

First Dimension
- Fat Sample (Solve in ACN/DCM)
- RP-HPLC (C18 Column)
- Automatic Fraction Collector
- Drying Fractions with $N_2$
- TAG fractions (Solve in Heptane)

Second Dimension
- Ag$^+$-HPLC (Silver ion Column)
- Detector (ELSD)
- Peaks

LABORATORY of FOOD TECHNOLOGY and ENGINEERING www.fte.ugent.be
First Dimension-C18 Column

Fat Blend

PLO = 2.27%
OOO = 39.83%
POO + SLO = 5.28%
POP/PPO = 18.99%
SOO + PPP = 9.53%
POS/PSO = 3.50%
PPS = 0.37%
SSO/SSO = 15.99%
PSO = 0.54%
SSS = 1.09%
Other < 2.61%
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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