Structural characterization of fatty acids and triacylglycerols by different mass spectrometric methods

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Content of the lecture

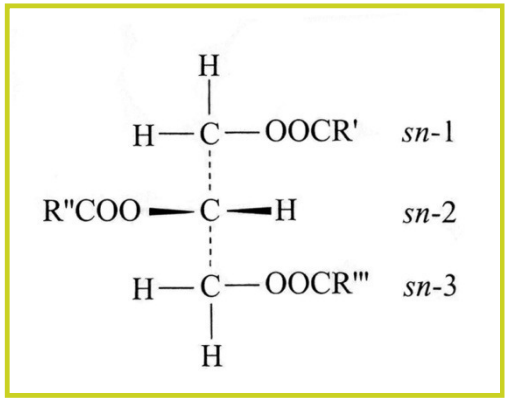
1) Structure of triacylglycerols (TAG)
   - Positional distribution of fatty acids (FA) in TAG
- **Mass spectrometry** as a tool to determine the **regiospecific positions of FA**

2) **EI-MS** (EI, electron impact ionization) methods in **FA structure** analysis
   - Different FA derivatives and methods
   - Fragmentation of 4,4-dimethyloxazoline derivatives (DMOX)

3) **CACI-MS/MS** (CACI, covalent adduct chemical ionization) in the determination of **double bond configuration** and position in FA

Structure of TAG

- Three FA are esterified to the glycerol backbone
  - ACN:DB (acyl carbon number : number of double bonds in the carbon chains), e.g. 52:2
- FA can be esterified in 3 different positions
  - *sn*-1 (*sn*, stereospecific numbering)
  - *sn*-2 (middle or secondary position)
  - *sn*-3



  - TAG A/B/C
    - FA combination is known, but not the positions
  - TAG *sn*-ABC
    - A is in the *sn*-1, B in the *sn*-2 and C in the *sn*-3 position

Why do we analyse regioisomerism?

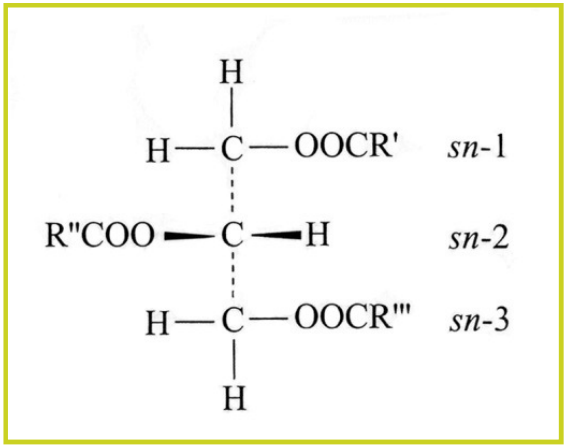
- Dietary fats consist mainly of TAG
- Positional distribution of FA affects the properties of fats
  - Nutritional properties
    - FA in the *sn*-2 position!
  - Technological and physical properties
  - Sensory properties
- In nature, the FA are not randomly esterified to the different *sn* positions
  - E.g. 16:0 in the middle position of TAG 16:0/18:1/18:1
    - In rapeseed and sunflower seed oil ca. 1-5 %
      - Generally in vegetable oils unsaturates are in the middle position
    - In lard ca. 95 %
      - Generally in lard 16:0 is predominant in the middle position
- Enzyme activity!
  - TAG synthesis, lipolysis and hydrolysis

MS methods for regioisomerism analysis

- MS is an efficient tool for the determination of regioisomerism of TAG
  - FA are cleaved off from the TAG molecule in the ionization or in CID (collision-induced dissociation)
  - FA in the *sn*-2 position is cleaved off less efficiently than the *sn*-1/3 FA

→ Regioisomerism of TAG (*sn*-2 vs. *sn*-1/3) can be resolved based on the intensities of the product ions

  - Usually diacylglycerol (DAG) product ions are used



An example of TAG fragmentation

HPLC/ESI-MS/MS spectra using ammonium adducts

(ESI, electrospray ionization)

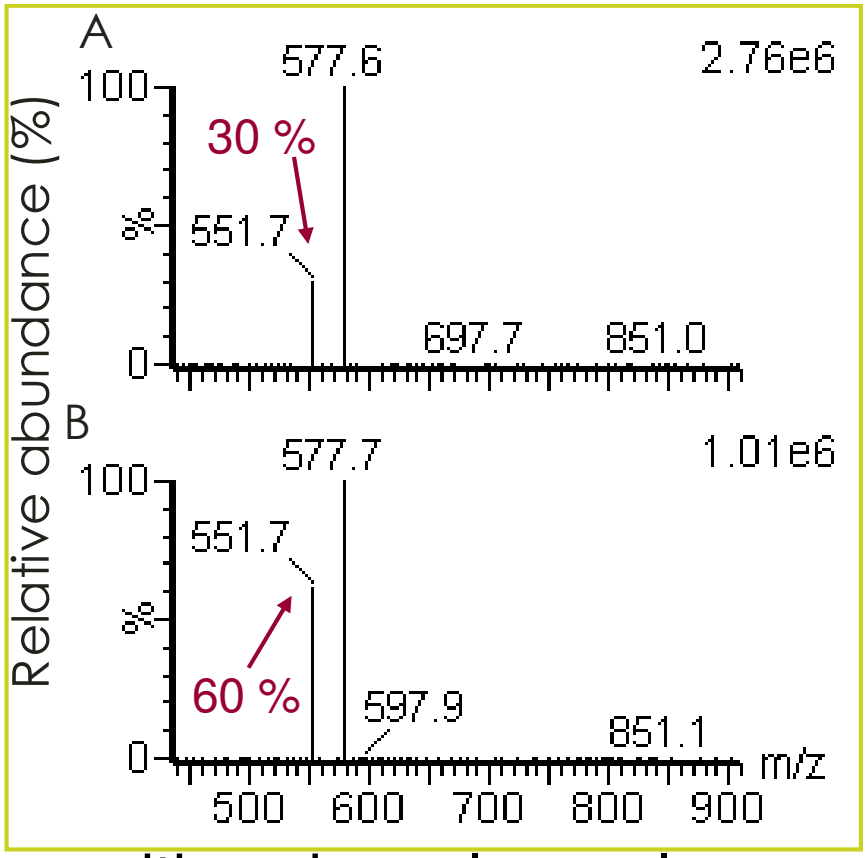
A) *sn*-16:0-18:1-16:0 (pure standard)

B) *sn*-16:0-16:0-18:1
+ *sn*-18:1-16:0-16:0 (pure standard)

- Diacylglycerol ions (DAG)

  m/z 551.7 $[M+NH_4\text{-}18{:}1\text{-}NH_4]^+$

  m/z 577.7 $[M+NH_4\text{-}16{:}0\text{-}NH_4]^+$



- Calculation of regioisomer compositions in real samples is done using calibration curves (DAG ion proportions vs. proportions of different regioisomers) prepared with pure TAG standards

Different MS methods in regioisomer analysis

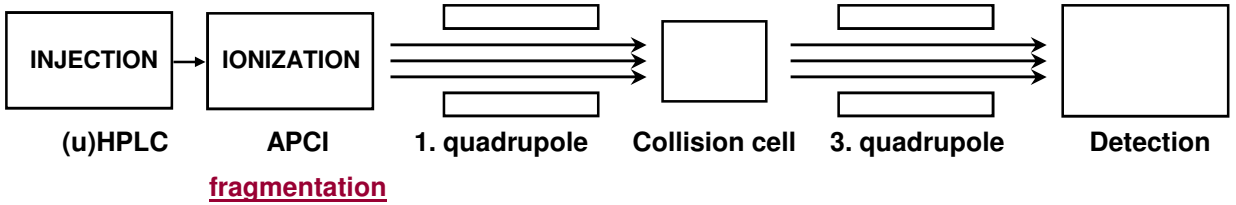
- ESI-MS/MS (also ESI-MS is possible)
  - Adducts or coordination ions are formed
    - e.g. $[M+NH_4]^+$, $[M+^{107}Ag]^+$ and $[M+^{109}Ag]^+$
- APCI-MS
  - APCI, atmospheric pressure chemical ionization
- NIAPCI-MS/MS using ammonia
  - NI, negative ion
  - $[M-H-RCOOH-100]^-$ ions are used instead of DAG ions
- Direct inlet ammonia NICI-MS/MS (direct inlet, suorasyöttö)
  - NICI, negative-ion chemical ionization
  - $[M-H-RCOOH-100]^-$ ions are used

HPLC in regioisomerism analysis

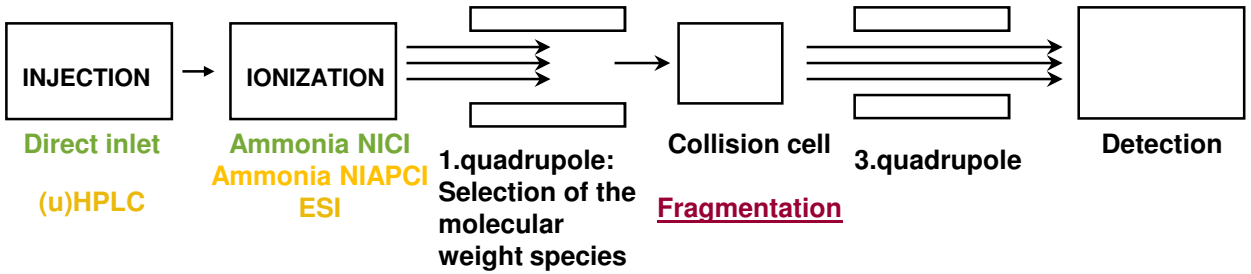
- (u)HPLC separation of TAG species prior MS analysis is important
  - Regiospecific analysis of FA is difficult if TAG have
    - the same molecular weight
    - the same fragmentation products
  - It is not possible to separate by MS those TAG that contain different FA isomers
    - different double bond position in FA, e.g. 18:3(n-3) and 18:3(n-6)
    - *cis*/*trans* isomers

Fragmentation in different MS methods

- In MS analyses, fragmentation occurs in the ionization (APCI-MS)



- In MS/MS analyses, fragmentation occurs in the collision cell (ammonia NI(AP)CI-MS/MS and ESI-MS/MS)

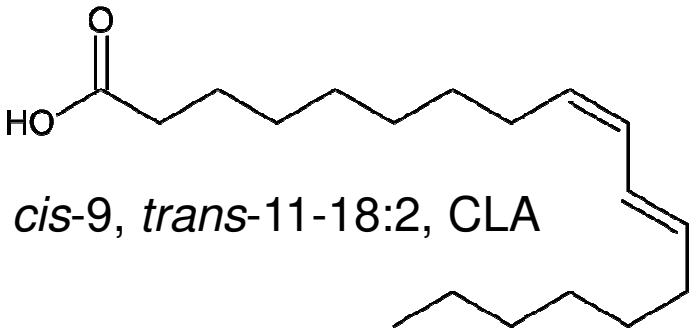


Fatty acid structure determination by GC/EI-MS

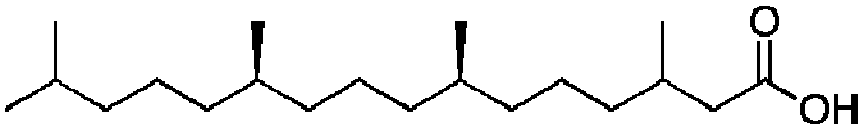
Fatty acid structure determination by GC/EI-MS

- Fatty acids are separated by gas chromatography (GC) prior to MS analysis

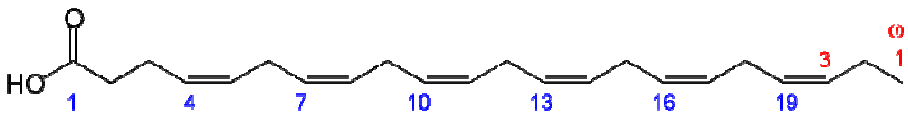
- Fatty acid methyl esters (FAME) can be used
  - FAME are commonly used in FA analysis and quantitation by GC-FID (FID, flame ionization detector)
  - Only information about the molecular weight is obtained by MS
- Different FA derivatives can be used
  - More detailed information about the FA structure is obtained



cis-9, *trans*-11-18:2, CLA



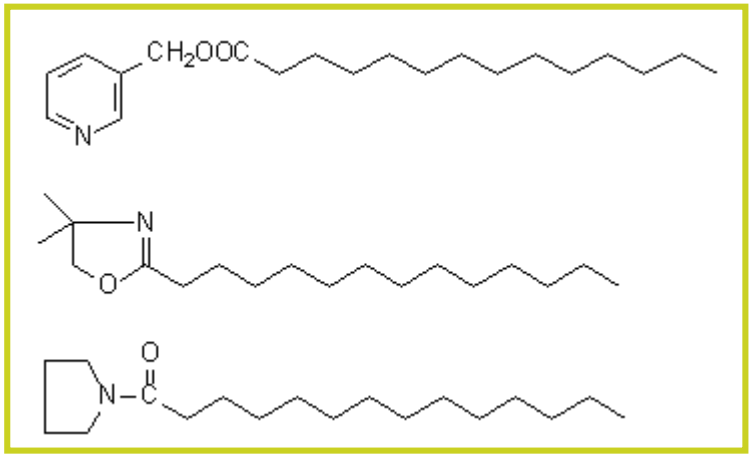
Phytanic acid



Docosahexaenoic acid, DHA

Fatty acid structure determination by GC/EI-MS

- Usage of pyrrolidide derivatives has been reported for over 30 years ago
- Currently mainly picolinyl esters (3-hydroxymethylpyridinyl) or 4,4-dimethyloxazoline (DMOX) derivatives are used



Picolinyl ester

DMOX derivative

Pyrrolidide derivative

Fatty acid structure determination by GC/EI-MS

- FA derivatives are ionized and the formed molecular ions and diagnostic fragment ions are used in the structure identification
  - Molecules are not fragmented randomly but the bonds break from the weakest points of the molecule

- Electron-impact ionization (EI)
  - Most commonly used in GC applications of FA analysis

Fatty acid structure determination by GC/EI-MS

- Derivatization of FA prevents transfer of double bonds along the carbon chain
- Radical induced fragmentation occurs evenly along the carbon chain
  - Series of high molecular weight ions is formed in the breakage of each C-C bond
  - When double bonds or other functional groups (e.g. branched FA, keto- or hydroxy groups) are present, specific ions are formed that help in the structure identification

Fatty acid structure determination by GC/EI-MS: DMOX

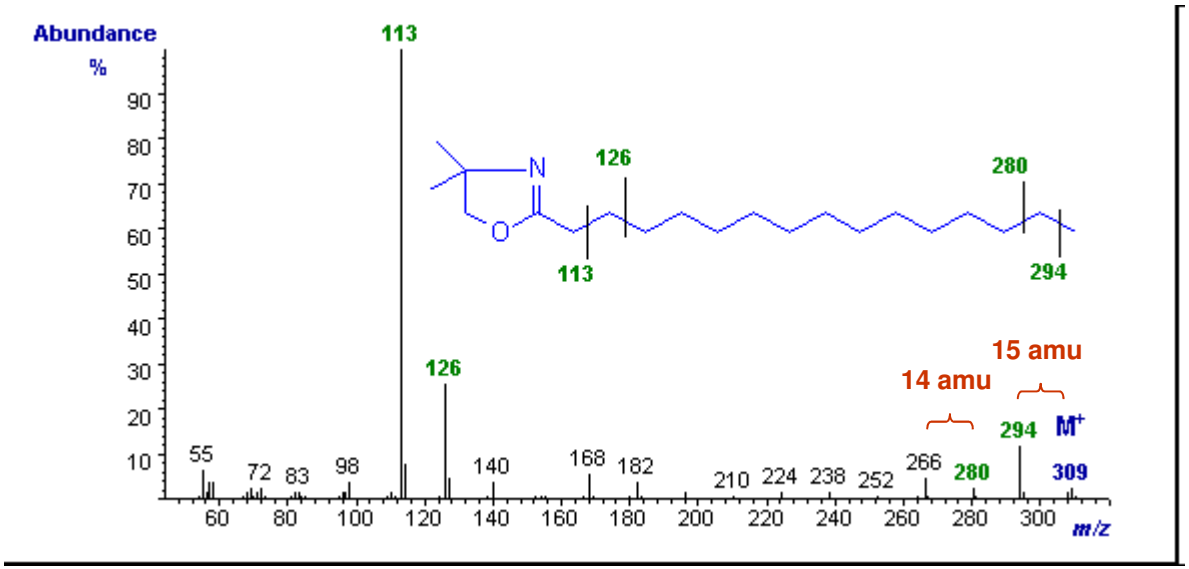
- DMOX derivatives are almost as volatile as FAME
- Polar stationary phases can be used in GC analysis

  → Almost equal conditions as in FAME analysis
    - Nearly equal resolution

- Few examples of the EI-MS fragmentation of DMOX is given in following slides…

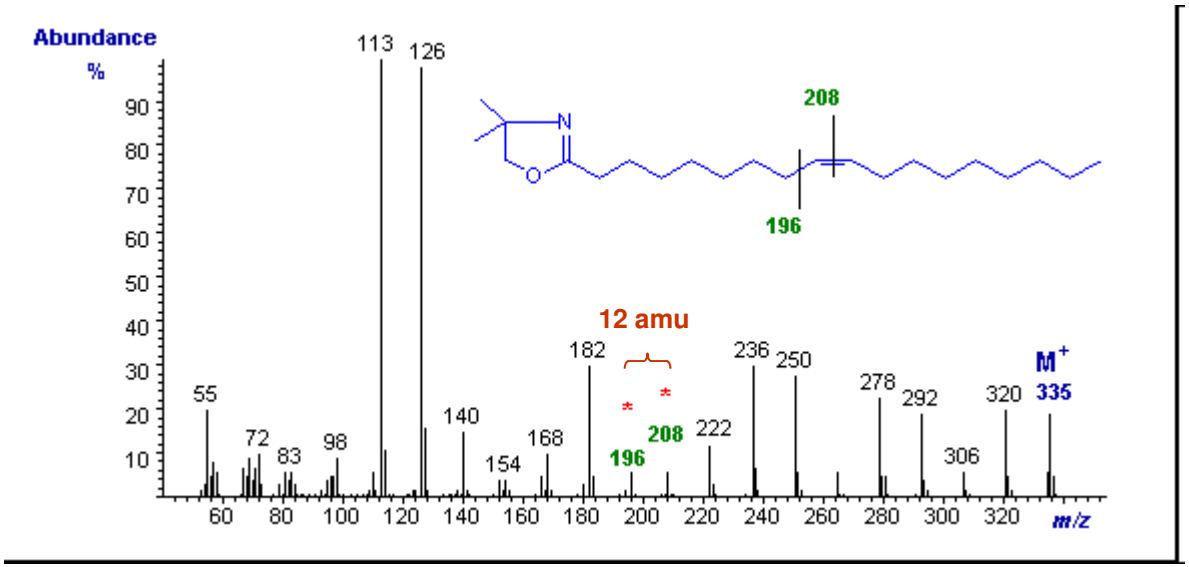
DMOX: saturated FA

- DMOX spectrum of palmitic acid, 16:0
  - Ion *m*/*z* = 113 has generally the highest intensity
  - Ion *m*/*z* = 126 is also predominant
  - Molecular ion is *m*/*z* = 309
  - Ions are formed in intervals of 15 amu and after that sequentially in intervals of 14 amu
    - Ions are formed when methylene groups are cleaved off



DMOX: monoenes

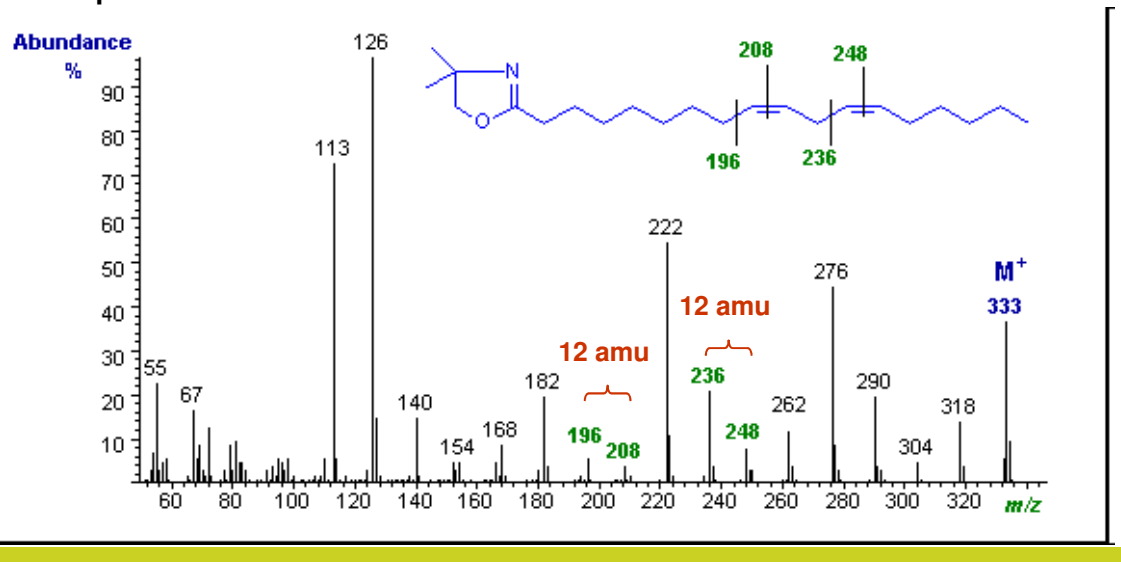
- DMOX spectrum of oleic acid, 18:1(n-9)
  - A gap of 12 amu between *m/z* = 196 and 208
  - → double bond (DB) in position 9
  - Structure identification is easy, if DB is located in the center parts of the carbon chain
  - If DB is located near either end of the chain, identification becomes more complicated



DMOX: dienes

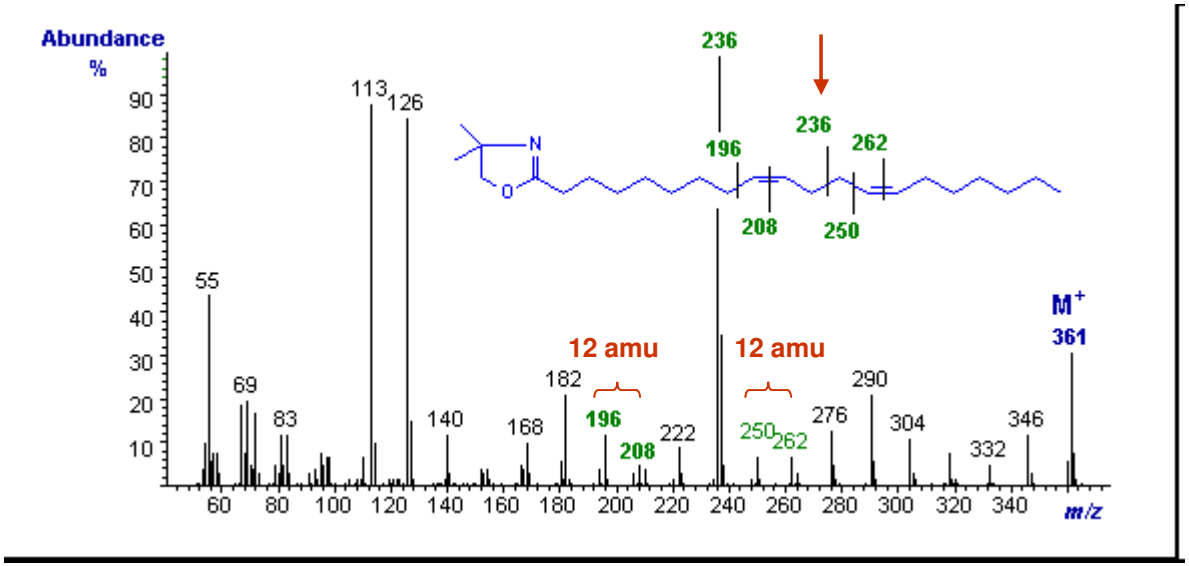
- DMOX spectrum of linolenic acid, 18:2(n-6)
  - A gap of 12 amu between ions *m*/*z* = 196 and 208 as well as 236 and 248

    → DB are located in positions 9 and 12
  - Predominant ions *m*/*z* = 222 and 276 are also diagnostic ions
  - Identification is easy, if DB is located centrally
    - In other cases compounds can be identified by comparison with authentic reference spectra



DMOX: dienes

- *Bis*-methylene-interrupted FA
- 9,13-eicosadienoic acid (9,13-20:2)
  - Strong diagnostic ion *m/z* = 236 is charasteristic and reveals the DB positions
  - Generally 12 amu gaps are also present



GC/EI-MS analysis of DMOX derivatives: limitations

- Interpretation of mass spectra becomes complicated if several FAs are co-eluted in the same chromatographic peak
- FA quantification is done by GC/FID as FAME
  - ”Transfer” of the identified GC/MS chromatogram peaks of DMOX derivatives to GC/FID chromatogram of FAME can be challenging, because the elution order is not always comparable (FAME vs. DMOX)

FA structure determination by GC/CACI-MS/MS

FA structure determination by GC/CACI-MS/MS

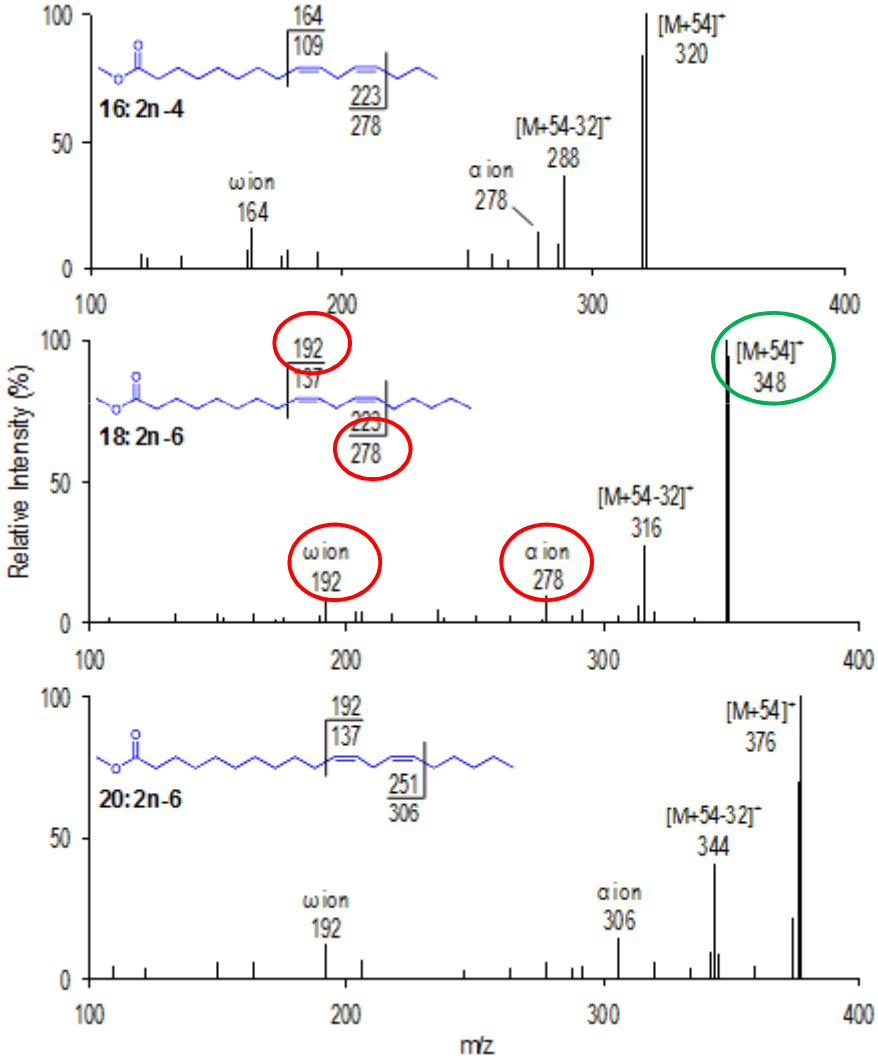
- Positive chemical ionization (CI) is used
- Fatty acids are analyzed as FAME
  - No further derivatization or chemical treatment is needed
  - GC conditions (e.g. column, temperature program) optimized for FAME analysis can be used

  → Elution order of FA are similar for GC-FID and GC-MS analyses
- Acetonitrile (CH_3CN, m/z 41) as a reagent gas in CI
  - Acetonitrile reacts with itself and ion at m/z 54 is formed

    (1-methyleneimino) -1-ethenylium (MIE) ion, $CH_2=C=N^+=CH_2$
  - MIE ion reacts rapidly with double bonds of FAME and produces $[M+54]^+$ ion.
- GC/CACI-MS and -MS/MS spectra together give an indication about the hydrocarbon chain length, degree of unsaturation and double bond position of FA

FA structure determination by GC/CACI-MS/MS

- In CACI-MS/MS the α and ω diagnostic ions result from predictable fragmentation of molecular ion $[M+54]^+$ revealing the double bond positions
  - NB! The FA structures and cleavage points in the right hand figures are drawn without the MIE

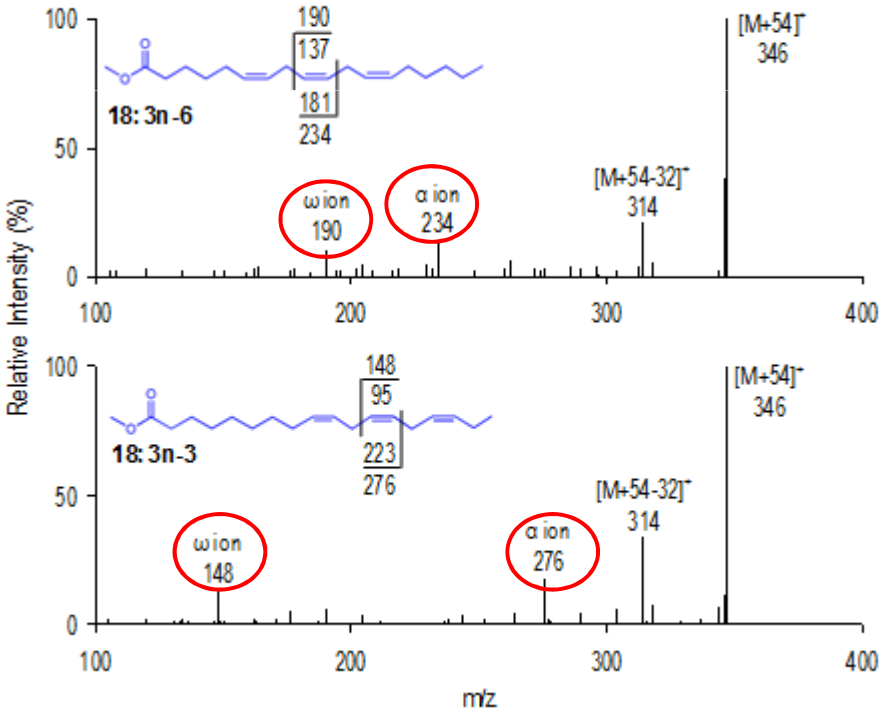


FA structure determination by GC/CACI-MS/MS

Isomers with different double bond positions

- Fatty acids 18:3(n-3) and 18:3(n-6)

- As the double bonds shift between the ω3 (n-3) and ω6 (n-6) positions, both diagnostic ions (α and ω) shift and enable facile identification of the two isomers

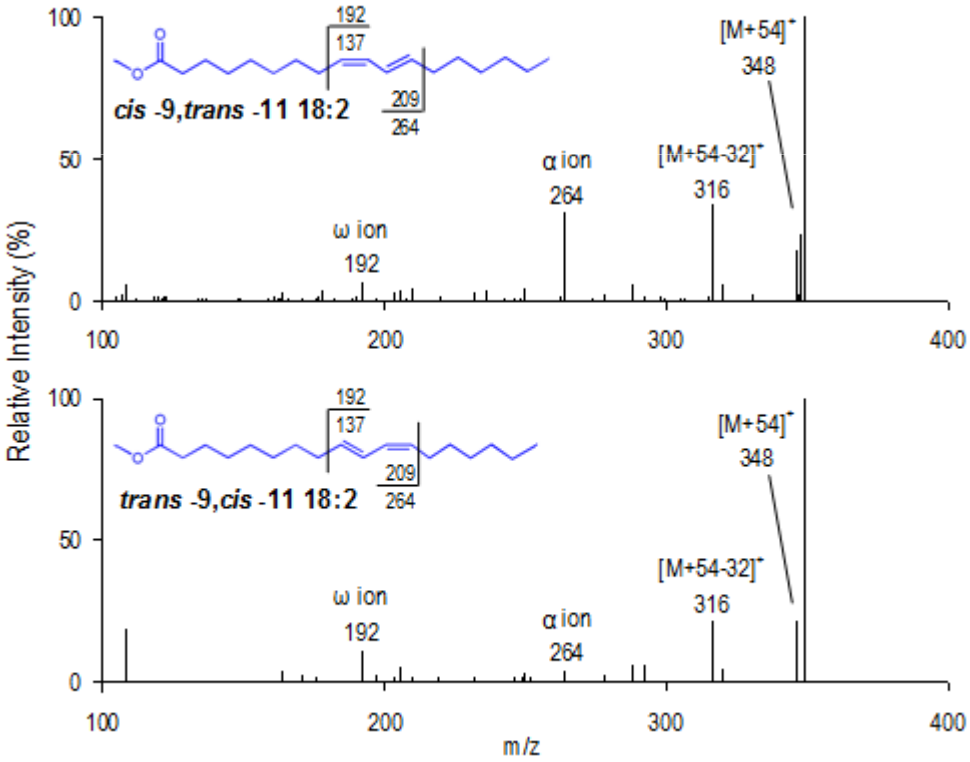


FA structure determination by GC/CACI-MS/MS

Double bond position and geometry (*cis*/*trans*)

- CLA isomers *cis*-9,*trans*-11-18:2 and *trans*-9, *cis*-11-18:2

- The ratio of α/ω diagnostic ions for these isomers is 5.0 and 0.4, respectively, indicative of *cis,trans* and *trans,cis* double bond geometry



References and further reading

- http://lipidlibrary.aocs.org/ (Christie, W.W.)
  - GC/MS and GC/MS/MS spectra used in this presentation are taken from the AOCS Lipid Library
- Christie, W.W. (2010) Lipid Analysis, 4th edition, The Oily Press, Bridgwater, England

- Publications
  - Leskinen, H., Suomela, J.-P., Pinta, J. and Kallio, H. (2008) Regioisomeric structure determination of α- and γ-linolenoyldilinoleoylglycerol in blackcurrant seed oil by silver ion high performance liquid chromatography and mass spectrometry. Analytical Chemistry 80, 5788-5793.
  - Leskinen, H., Suomela, J.-P. and Kallio, H. (2007) Quantification of triacylglycerol regioisomers in oils and fat using different mass spectrometric and liquid chromatographic methods. Rapid Communications in Mass Spectrometry 21, 2361-2373.