TECHNOLOGY BRIEF | NO. MST-202

GC/MS – Polymode Ionization – Food and Beverage

A Sample Prep-Free Analysis of Triglycerides and Fatty Acids for Authentication of Vegetable Oil with *“Smart IS+”* and *“SMCI+”*

Written by:

Chua Chun Kiang
Tan Yu Jun
Jackie Jackie
Loo Lai Chin

Abstract

The gas chromatography/mass spectrometry (GC/MS) technique has long been used for the analysis of triglycerides and fatty acids. The analysis is usually achieved by detecting esters of fatty acids, which usually requires extensive transesterification and clean-up steps. This Technology Brief introduces a direct and effortless analysis technique that does away with tedious sample pretreatment steps. By coupling a direct sample probe with innovative ion sources and reagent gas sources that are capable of polymode ionization, both electron and chemical ionization analysis modes for triglycerides and fatty acids can be achieved. The new polymode ionization setup further enables any laboratories to quickly develop the capability of conducting preliminary authentication investigation of vegetable oil samples with GC/MS.


Applications include authentication of regular sunflower oil and coconut oil

Keywords:
Polymode Ionization, Chemical Ionization, Triglycerides, Fatty Acids, Direct Sampling

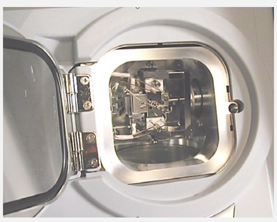
Highlights

- *“Smart IS+”* and *“SMCI+”* enable derivatization-free procedures for qualitative GC/MS analysis of fatty acids and triglycerides
- Easily switch between both electron ionization and positive chemical ionization modes with *“Smart IS+”* setup
- Experience a convenient and safe positive chemical ionization setup with *“SMCI+”* as the setup requires only common laboratory solvents as reagent gas
- *“SMCI+”* with methanol reagent gas produces characteristic mass spectra for saturated fatty acids
- *“Smart IS+”* and *“SMCI+”* support quick preliminary authentication study of vegetable oil, e.g., coconut oil and sunflower oil

Technologies Featured

Direct Sample Inlet

Smart EI/CI Ion Source (Smart IS)



Solvent Mediated Chemical Ionization (SMCI)

1. INTRODUCTION

Triglycerides are the primary constituents of body fat in animals as well as vegetable fat. Triglycerides are tri-esters that constitute glycerol and three fatty acids. The analysis of triglycerides and fatty acids is crucial to refining the current physiological understanding of animals and plants.

The direct analysis of free fatty acids (FFAs) and triglycerides (TGs) with gas chromatography/mass spectrometry (GC/MS) technique requires specialized columns. Otherwise, multi-step transesterification or derivatization procedures are necessary to enable GC/MS analysis. The usage of a direct probe as a sample inlet in GC/MS provides an alternative technique that eliminates the need for tedious sample preparation. This benefit paves the way for a quick and direct method to achieve preliminary detection and identification of FFAs and TGs.

This article demonstrates the application of a direct probe in conjunction with a Smart EI/CI ion source (Smart IS) or solvent mediated chemical ionization (SMCI) unit for the analysis of FFAs and TGs. The mass spectra of FFAs and TGs generated by Smart IS and SMCI units will be evaluated, followed by examples on their usage for preliminary authentication study of vegetable oils.

2. EXPERIMENT

2.1 Analytical Setup

The analytical results in this report were generated using a Direct Sample Inlet (DI) probe in conjunction with a Smart IS or SMCI unit. The combination of DI with Smart IS *or* SMCI unit is hence known as Smart IS and *"SMCI+"* in this article, respectively (Figure 1).

The DI probe is designed to be able to fit a miniature sample vial at its tip. The sample vial is thereafter placed close to the ion source and subsequently heated up according to a temperature program. The chemicals in the sample vial are hence volatilized and ionized in the ion source.

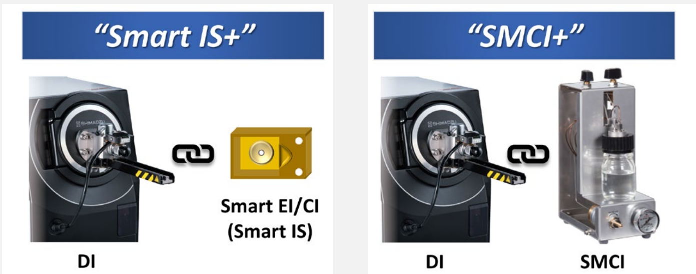


Figure 1. *Polymode Ionization* setup inclusive of *"Smart IS+"* and *"SMCI+"*

Smart IS is a 2-in-1 ion source that enables both electron ionization (EI) and positive chemical ionization (PCI) modes. PCI is achieved with the usage of isobutane gas as a reagent gas. Due to the simplicity of switching between two different ionization modes with Smart IS, the PCI mode attained with Smart IS is referred to as quick chemical ionization (QCI).

On the other hand, the SMCI unit enables PCI mode with conventional PCI ion source and methanol as the reagent gas. Usage of methanol allows safe (i.e., it eliminates the use of flammable and toxic reagent gases such as methane, isobutane, and ammonia) and convenient adoption of PCI mode in routine GC/MS analysis.

A total of 6 free fatty acids (i.e., decanoic acid C10:0, lauric acid C12:0, myristic acid C14:0, palmitic acid C16:0, stearic acid C18:0, and oleic acid C18:1) and 5 triglycerides (i.e., trilaurin LLL, trimyristin MMM, tripalmitin PPP, tristearin SSS, and triolein OOO) were analyzed in this study.

Name	Abbreviation	Formula	MW
	Fatty Acids		
Decanoic Acid	C10:0	$C_{10}H_{20}O_2$	172
Lauric Acid	C12:0	$C_{12}H_{24}O_2$	200
Myristic Acid	C14:0	$C_{14}H_{28}O_2$	228
Palmitic Acid	C16:0	$C_{16}H_{32}O_2$	256
Stearic Acid	C18:0	$C_{18}H_{36}O_2$	284
Oleic Acid	C18:1	$C_{18}H_{34}O_2$	282

Name	Abbreviation	Formula	MW
	Triglycerides		
Trilaurin	LLL	$C_{39}H_{74}O_6$	639
Trimyristin	MMM	$C_{45}H_{86}O_6$	723
Tripalmitin	PPP	$C_{51}H_{98}O_6$	807
Tristearin	SSS	$C_{57}H_{110}O_6$	891
Triolein	OOO	$C_{57}H_{104}O_6$	885

2.2 Experimental Condition

Standard solutions of FFAs and TGs were prepared to a concentration of 5000 ppm in ethanol and acetone, respectively. 1 µL of the standard solution was introduced into the DI sample vial for analysis. A mixture sample of FFAs and TGs were prepared by introducing 1 µL of each standard solution into the DI sample vial. The samples were left to dry before analysis.

The DI probe was heated at 20°C/min to 100°C, then 40°C /min to 450°C and held for 7 min. The ion source temperature was set to 230°C. Ionization mode used included EI, QCI (isobutane), and SMCI (methanol). Scan mode was performed in the range of *m/z* 50-900 with a scan speed of 3333.

Cold-pressed coconut oil and regular sunflower oil purchased from a local supermarket were used for the analysis.

3. RESULTS AND DISCUSSION

3.1. "Smart IS+" and "SMCI+" Mass Spectra of FFAs

Using *"Smart IS+"* and *"SMCI+"*, the FFAs were first analyzed with three different ionization modes, EI, QCI, and SMCI, to establish the mass spectra. The consolidated mass spectra of the FFAs collected with the three ionization modes are shown in Figure 2.

The EI mass spectra of the FFAs were successfully matched to the NIST mass spectral library with high similarity index scores of >85. Despite that, the intensities of molecular ion peaks for C10:0 and C18:1 were rather low. The base peaks in the mass spectra were small fragmentation products.

In the QCI mass spectra of saturated FFAs, the protonated molecule, $[M+H]^+$ ion, was detected as the base peak. The mass spectrum of unsaturated FFA C18:1 indicated the $[M-OH]^+$ ion as the base peak at *m/z* 265. These characteristic ions could potentially be exploited to enable the rapid identification of FFAs.

Subsequent analysis with SMCI mode revealed the presence of three characteristic ions in the mass spectra of saturated FFAs. Peaks corresponding to $[M-OH]^+$, $[M+H]^+$, and $[M-OH+OMe+H]^+$ ions were found in the mass spectra. The presence of $[M-OH+OMe+H]^+$ ion peak suggested a possible reaction between the FFAs and methanol reagent gas, resulting in the formation of fatty acid methyl ester (FAME).

In contrast, the esterification product was not observed for C18:1. All in all, the characteristic ions found in QCI and SMCI mass spectra could also serve as confirmatory signals for the rapid identification of FFAs. Furthermore, the concurrent presence of $[M-OH]^+$, $[M+H]^+$, and $[M-OH+OMe+H]^+$ ions in SMCI mass spectra could further serve as an indicator for the presence of saturated FFAs.

A mixture of FFAs was subsequently analyzed to evaluate their elution profile. The extracted ion thermogram (EIT) profiles of $[M+H]^+$ ion for the FFAs collected with QCI mode are shown in Figure 3. The FFAs eluted within 225°C with a visible resolution, starting from shorter carbon chain species. The order of elution of the saturated FFAs was from C10:0, C12:0, C14:0, C16:0 to C18:0. C18:1 eluted slightly earlier than C18:0, possibly due to lower volatilization temperature as a result of unsaturation in the carbon chain.

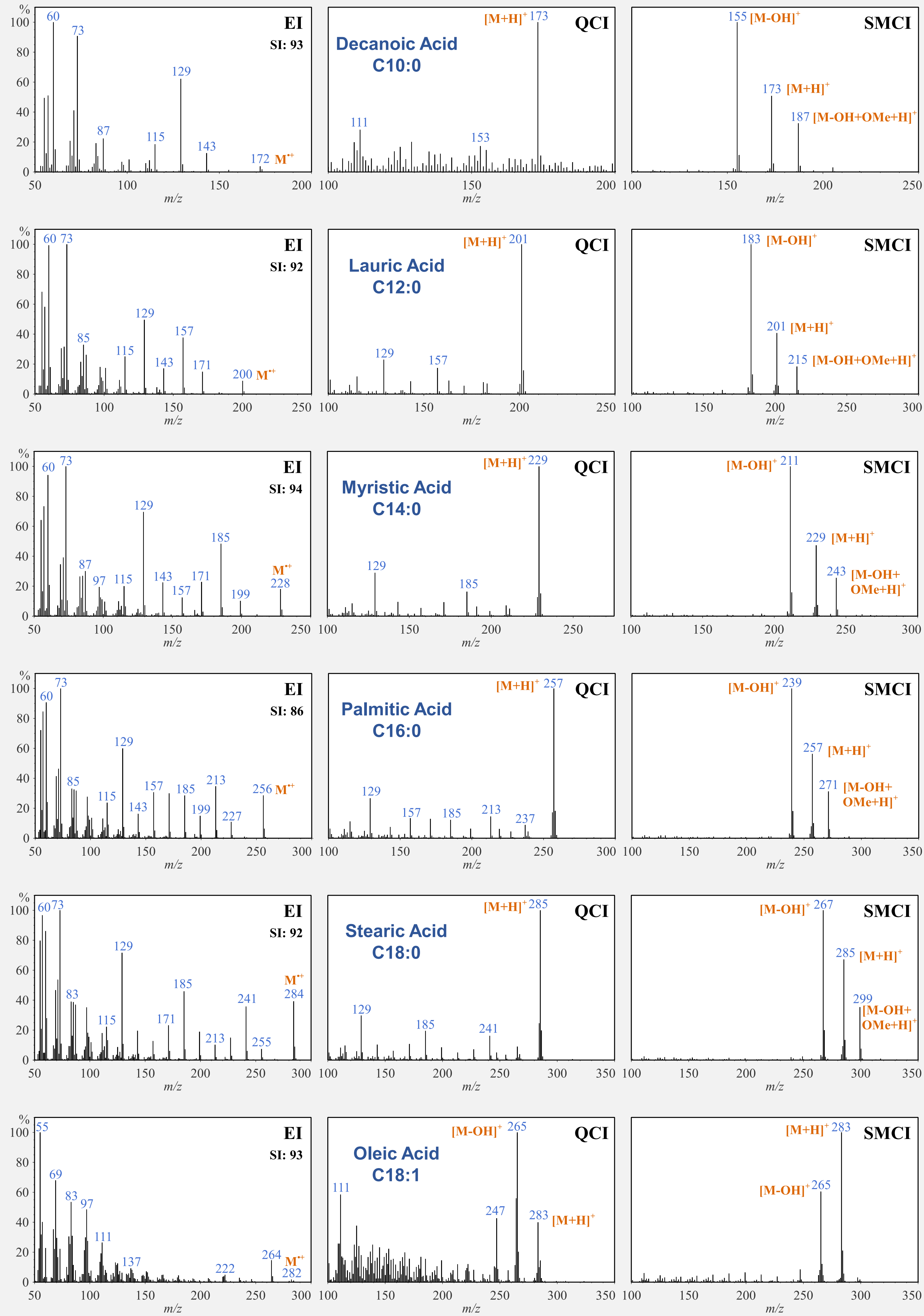


Figure 2. EI, QCI, and SMCI mass spectra of FFAs collected with “Smart IS+” and “SMCI+”. SI: Library similarity index

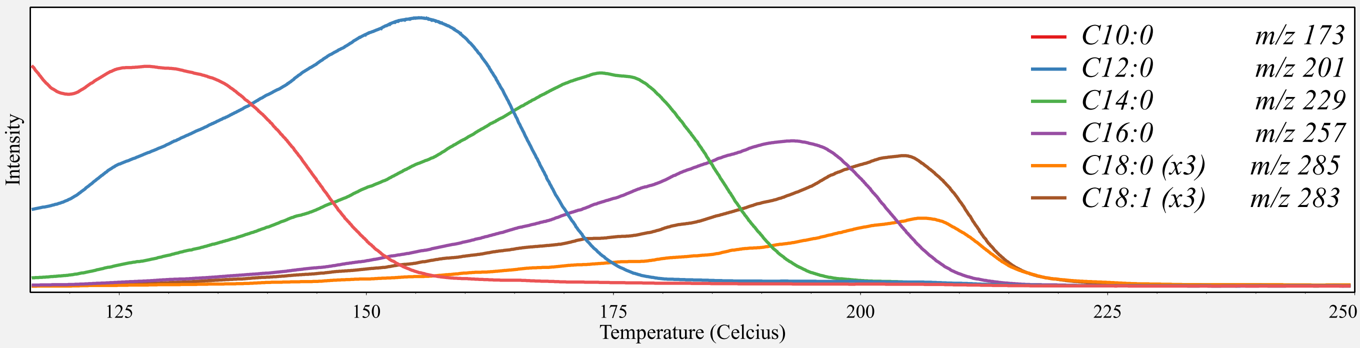
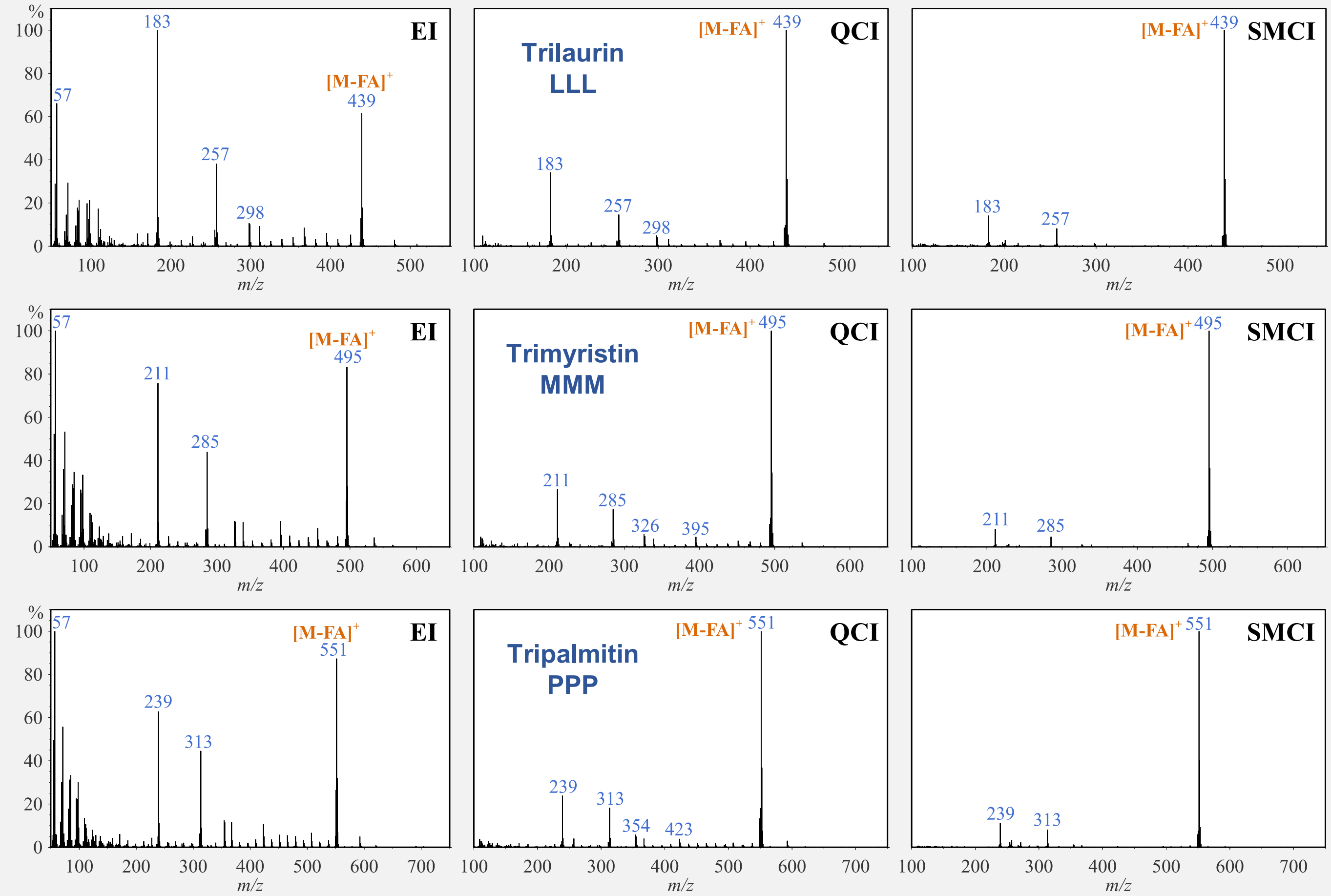


Figure 3. EIT profiles of QCI mode for a mixture of FFAs

3.2. "Smart IS+" and "SMCI+" Mass Spectra of TGs

The consolidated mass spectra of the TGs collected with the three ionization modes are shown in Figure 4. The EI mass spectra of the TGs contained distinct peaks corresponding $[M\text{-}FA]^+$ ion peak resulting from the α-cleavage of a fatty acid chain from the triglyceride. In addition, each triglyceride has its characteristic ions, such as *m/z* 183 and 257 for LLL, *m/z* 211 and 285 for MMM, *m/z* 239 and 313 for PPP, *m/z* 267 and 341 for SSS, and *m/z* 265 and 339 for OOO. These characteristic peaks were also observed in the QCI and SMCI mass spectra. The $[M\text{-}FA]^+$ ion peaks in QCI mass spectra were detected as base peak, which was also the case for SMCI mass spectra, except for SSS.

A mixture of TGs was then analyzed to evaluate their elution profile. The extracted ion thermogram profiles of $[M\text{-}FA]^+$ ion for the TGs collected with QCI mode are shown in Figure 5. Like the elution order of FFAs, the TGs eluted with a visible resolution, starting from the shorter carbon chain species within the temperature range of 150 to 350°C. The order of elution was LLL, MMM, PPP, OOO, and SSS. OOO eluted at an almost similar temperature as SSS.



(continued on next page)

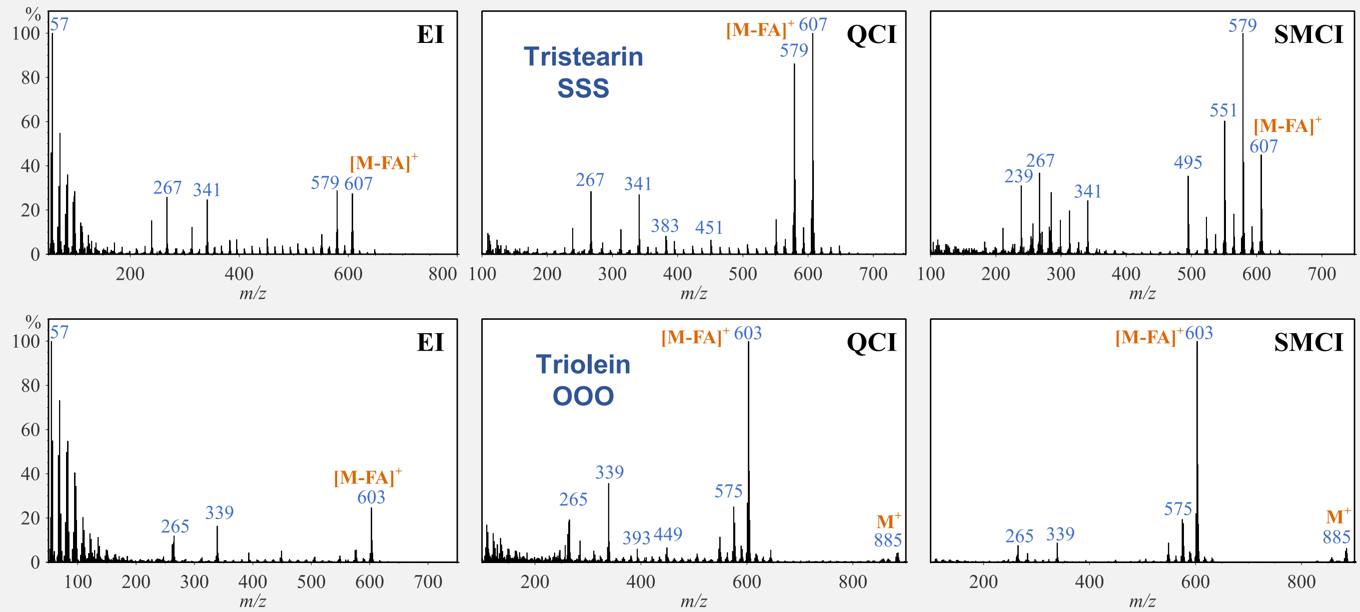


Figure 4. EI, QCI, and SMCI mass spectra of TGs collected with "Smart IS+" and "SMCI+". The formula of FA refers to $C_{12}H_{24}O_2$ for LLL, $C_{14}H_{28}O_2$ for MMM, $C_{16}H_{32}O_2$ for PPP, $C_{18}H_{36}O_2$ for SSS, and $C_{18}H_{34}O_2$ for OOO

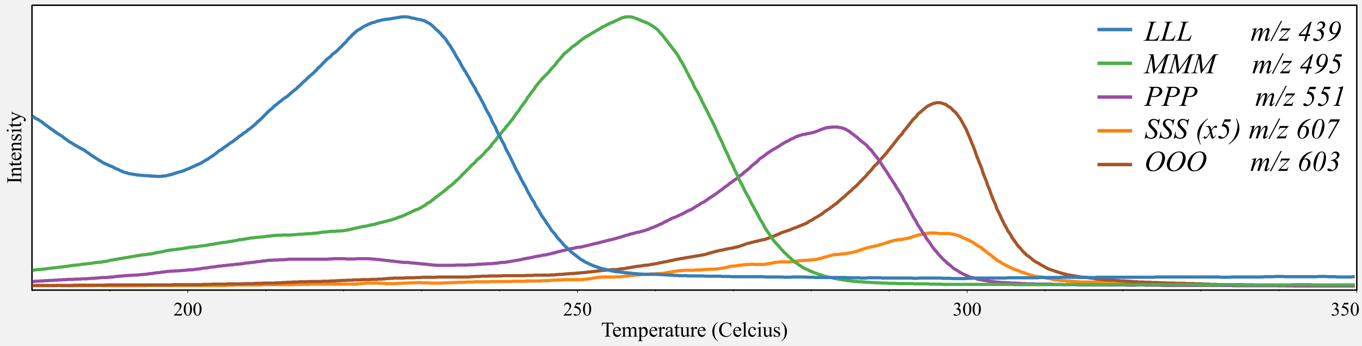


Figure 5. EIT profiles of SMCI mode for a mixture of TGs

3.3 Authentication of Vegetable Oils

By using the EIT of the unique [M-FA]+ ion of each TGs on GC/MS data of vegetable oil, it is possible to determine the authenticity of the oil. Figure 6 shows the TIT profiles of coconut and sunflower oil typically used for cooking. Coconut oil is known to contain a high amount of saturated fatty acids while sunflower oil is known for its high content of unsaturated fatty acids. The fatty acids are usually present in the form of triglycerides. The most prevalent saturated fatty acid in coconut oil is C12:0, which correlated to LLL. On the other hand, the most prevalent unsaturated fatty acid in sunflower oil is C18:1, which correlated to OOO.

As a result of analyzing coconut oil with SMCI mode (Figure 6, top), distinctive [M-FA]+ ion peaks of LLL and MMM were observed while only a slight amount of PPP was present. As expected, unsaturated triglycerides, i.e., OOO, was barely observed. A closer look at the mass spectrum at the peak apex of the TIT profile, within the range of *m/z* 300-600, indicated the [M-FA]+ ions of LLL and MMM. The remaining peaks, i.e., *m/z* 383, 411, 467, and 523 could arise from isomers of triglycerides.

Subsequent analysis of sunflower oil with SMCI mode (Figure 6 bottom) indicated the distinctive presence of [M-FA]+ ion for OOO and a trace amount of PPP. LLL and MMM species were not observed. Similarly, a closer look at the mass spectrum at the peak apex of the TIT profile revealed the presence of peaks such as *m/z* 599, 601, and 604, which could arise from isomers of triglycerides.

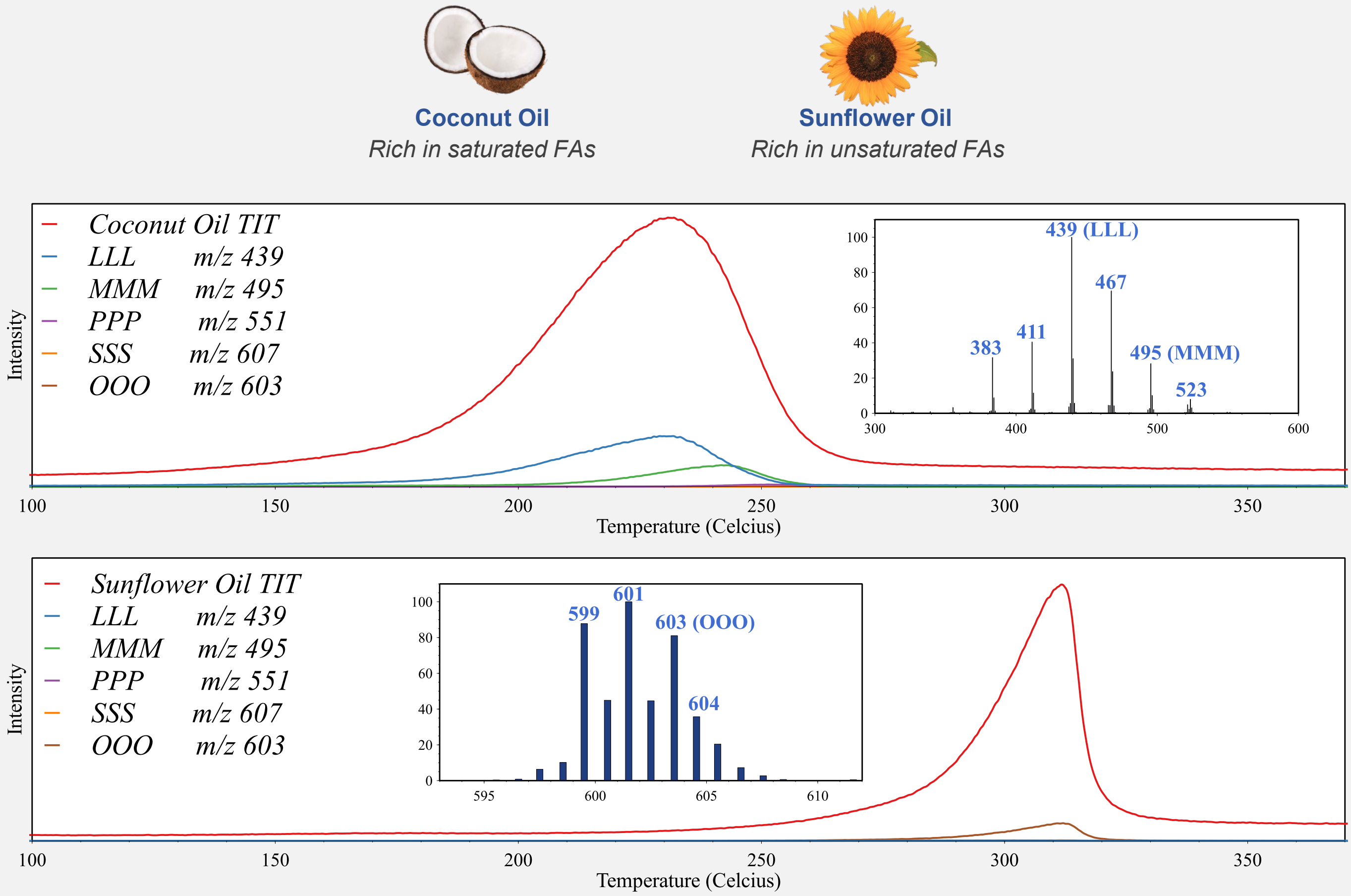


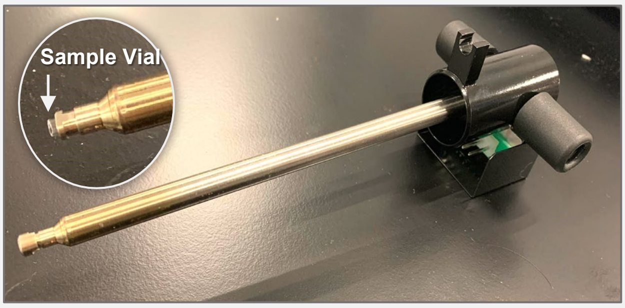
Figure 6. TIT and EIT profiles of SMCI mode for (top) coconut oil and (bottom) sunflower oil. Inset shows the mass spectrum at peak apex of the corresponding TIT profile

4. CONCLUSION

The *"Smart IS+"* and newly introduced *"SMCI+"* enable a direct and quick qualitative analysis of fatty acids and triglycerides, which conventionally require tedious derivatization steps prior to GC/MS analysis. The *"Smart IS+"* setup delivers convenience in switching between electron ionization and positive chemical ionization mode of analysis. On the other hand, the *"SMCI+"* setup delivers utmost convenience and safety to carry out positive chemical ionization since it utilizes methanol, which is a common laboratory solvent, as the reagent gas. The characteristics ion in the mass spectra of triglycerides further enabled quick preliminary authentication study of coconut oil and sunflower oil, supported by the *"Smart IS+"* and *"SMCI+"* setups.

READ MORE

Direct Sample Inlet (DI)



The DI unit enables samples to be injected directly into the ion source without passing through the gas chromatograph. The DI probe can be heated up to 500°C under vacuum conditions. As a result, mass spectra of high polarity and high boiling point compounds can be generated easily. The analysis is performed by simply placing the sample in a sample vial that is secured in the DI probe.

Learn More

Solvent Mediated Chemical Ionization (SMCI)

SMCI introduces headspace reagent gas from a bottle into the GC/MS ionization unit for use in positive or negative chemical ionization. Conventional chemical ionization techniques require the use of flammable reagent gas cylinders, but SMCI can be carried out with general organic solvents, such as methanol or acetonitrile, together with nitrogen or argon gas. This results in greater safety and lower running costs.

Learn More

Smart Metabolites Database

The Smart Metabolites Database includes more than 500 mass spectra and 475 MRM transitions of metabolites commonly found in biological samples. The availability of retention indices information further increases the confidence of identification even in the most complex samples. Move ahead with your rigorous analysis in three different ready-to-use analysis methods, i.e. Scan, MRM, or Scan/MRM, effortlessly.

Learn More

Lipids Library v1.0

The Lipids Library encompasses the mass spectral and retention indices information for more than 400 lipid-like molecules for food analysis, clinical and medical applications. The library is carefully curated by Prof. Luigi Mondello and his team at the University of Messina, Italy on Shimadzu GC-MS.

Learn More

SHIMADZU

Shimadzu (Asia Pacific) Pte. Ltd
www.shimadzu.com.sg