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Technology Brief

Automation of FAME Derivatization for Fatty Acid Profiling and Determination of Erucic Acid in Canola Oil Using AOC-6000 Plus

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Highlights

- Full automation of canola oil sample preparation for fatty acid profiling and determination of erucic acid
- All samples are freshly prepared and injected into GC-MS in an identical manner
- Highly robust, reproducible and reliable results are obtained due to automation

Technologies Featured

Automated Sample Preparation With AOC-6000 Plus

GCMS-QP2020 NX

Introduction

Rapeseed oil originally contains about 25% to 50% erucic acid [1]. Links between erucic acid and possible risks in cardiac related diseases were first established and reported in rodents [1]. Since then, various regions started establishing legal limits on erucic acid in edible oil [2][3][4].

High erucic acid rapeseed (HEAR) is rapeseed oil containing a high content of erucic acid which is widely used in various industrial applications. Low erucic acid rapeseed (LEAR) is produced through artificial selection of rapeseed to produce oil with less than 5% erucic acid [1]. Canola oil is the result of further genetic selection from LEAR, which successfully bred seeds capable of yielding oil that are low in both erucic acid (<2%) and glucosinolates [1], making it suitable for human consumption.

LEAR crops have a high risk of erucic acid contaminations from various sources: accidental mixing of HEAR-LEAR seed varieties, and cross-pollination between LEAR with HEAR, feral plants that are closely related (e.g. mustard), and with volunteer plants that escaped from the previous harvesting cycle. Therefore, canola oil trade faces the unique risk of having erucic acid contamination which can compromise the safety of consumers [1] [5].

In this technology brief, the content of erucic acid and other fatty acids content in canola oil were analyzed using GCMS-QP2020 NX. Prior to GC-MS analysis, triglycerides and free fatty acids (including erucic acid) need to be derivatized to fatty acid methyl esters (FAME). For this purpose, AOC-6000 Plus was used to automate the derivatization and the injection process.

Principles Behind The Experiment

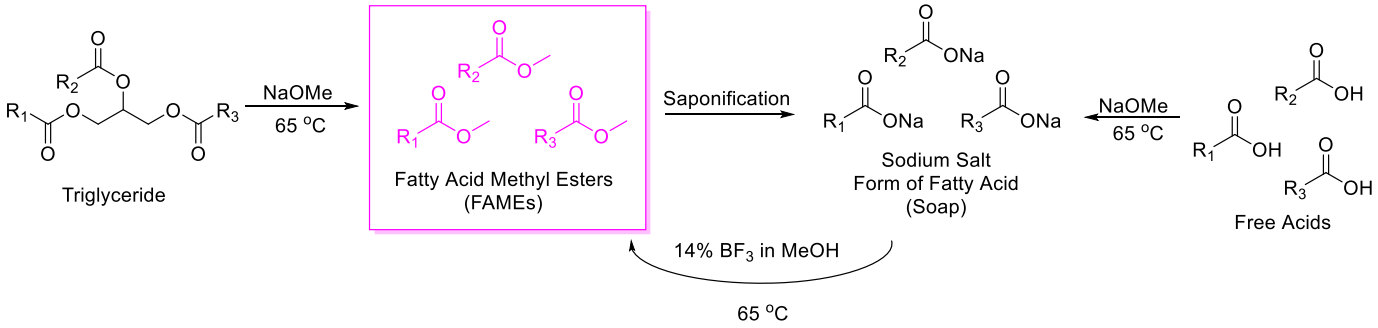

Fig. 1 Reaction scheme of the different chemical species upon addition of NaOMe and BF_3

Fig.1 depicts the derivatization reaction to be automated in this experiment. NaOMe (NaOH dissolved in MeOH) was first added to the prepared edible oil sample and incubated at 65 °C, the triglycerides in the sample were then hydrolyzed to FAME. It was challenging to precisely stop the reaction at the conversion to FAME as saponification will occur upon further incubation. Free fatty acids that were already present in the sample will also be neutralized to its sodium salt form (soap species). A strong Lewis acid, BF_3, was then added to catalyze the conversion of all side products (soap) to FAME before injection of the samples into GC-MS.

Experiment Design

Reagents Preparation

0.5 M NaOMe

2 grams (±0.1 gram) of NaOH (Merck V800383) was weighed and transferred to a 250 mL container. 100 mL of MeOH (Kanto Chemical, 25183-4B, for pesticide residue and PCB tests) was added and sonicated for approximately an hour to ensure complete dissolution. White precipitate (sodium carbonate) could be observed to form at the bottom of the container (over time) due to reaction with atmospheric CO_2; this could be ignored.

Saturated NaCl

40 grams (±0.1 gram) of NaCl (Merck 31434) was weighed and transferred to a 250 mL container. 100 mL of ultrapure water was then added, followed by sonication for approximately an hour. An additional one spoonful of NaCl was then added to the solution to ensure it remained saturated throughout the experiment.

14% BF_3 in MeOH and Hexane

14% BF_3 in MeOH (Merck B1252) and hexane (Kanto Chemical, 18041-3B, for pesticide residue and PCB tests) were purchased commercially.

Approximately 10 mL of each prepared reagent (NaOMe, Saturated NaCl, and 14% BF_3 in MeOH) was transferred to clean 10 mL vials and placed them in the Standard Wash Modules in the AOC-6000 Plus autosampler.

Erucic Acid Stock Solution (10 mg/mL)

Approximately 10 mg of erucic acid was transferred (record down the exact mass transferred) into a 1.5 mL vial. Appropriate amount of hexane was then added to prepare a solution of 10 mg/mL of erucic acid solution. The mixture was then homogenized via vortexing. This stock solution would then be diluted into different appropriate concentrations as spiking solutions.

Unspiked Canola Oil Sample

5 (±0.5) mg of the oil sample was first transferred into a 1.5 mL vial (P/N: 226-54110-33). The sample vial was then capped with a magnetic screw cap (P/N: 227-34158-01) and placed it on AOC-6000 Plus autosampler sample rack.

Spiked Canola Oil Sample

To illustrate the presence of erucic acid in the canola oil sample, various amounts of erucic acid were added to the blank canola oil samples.

The viscous oil sample was first mixed well by drawing it up and down using a disposable glass

Pasteur pipette. Using the same disposable glass Pasteur pipette, 5 (±0.5) mg the oil samples were then transferred into 3 separate 1.5 mL vials (P/N: 226-54110-33) and spiked manually with different amounts of erucic acid using the prepared spiking solutions. Each sample vial was subsequently capped with a magnetic screw cap (P/N: 227-34158-01) and placed on the AOC-6000 Plus autosampler sample rack.

The aim of spiking the blank canola oil sample with various concentrations of erucic acid is to simulate oil samples containing various amounts of erucic acid. Both unspiked and spiked samples would undergo automated derivatization (**Fig. 2**) that converts erucic acid to methyl erucate, then subject to GC-MS analysis. The amount of methyl erucate determined would be a representative of the percentage of erucic acid in the canola oil. Currently, the legal limit of erucic acid is set as 2% in the EU and the US, and this limit is closely followed by others around the world [2][3][4].

Automation of Sample Preparation by AOC-6000 Plus

Hydrolysis
- Transfer 150 µL of 0.5 M NaOMe to the oil sample in the sample vial using a 1000 µL syringe.
- Transfer the sample vial to the agitator and incubate at 65 °C, 300 rpm for 10 minutes.
- Return the vial to the sample rack.

Esterification
- Transfer 150 µL of 14% BF_3 in MeOH to the sample vial.
- Transfer the sample vial to the agitator and incubate it at 65 °C, 300 rpm for 10 minutes.
- Return the vial to the sample rack.

Extraction
- Transfer 600 µL of hexane to the sample vial.
- Transfer 150 µL of saturated NaCl solution to the sample vial.
- Transfer the sample vial to the vortex mixer and mix it at 1200 rpm for 20 seconds.
- Return the vial to the sample rack and wait for 1 minute and 30 seconds to allow for phase separation.

Injection
- Transfer 150 µL of the supernatant into a clean vial.
- Park the 1000 µL syringe and take a 10 µL syringe.
- Injection into the GC-MS.

Fig. 2 Workflow of FAME derivatization steps performed by AOC-6000 Plus

Experimental Measurement Conditions

AOC-6000 Plus autosampler was used to perform automated sample preparation and injection as depicted on **Fig. 2**. Modules necessary for automation of FAME derivatization are shown in **Fig. 3**. Subsequent analysis was performed by GCMS-QP2020 NX using the analytical conditions depicted in **Table 1.**

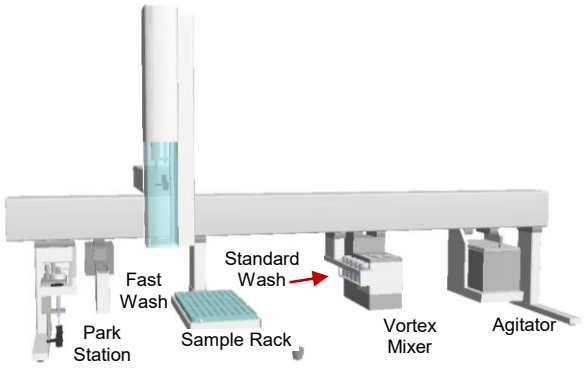

Fig. 3 Overall experimental design and AOC-6000 Plus modules for automation of FAME derivatization

Table 1. GC-MS Configuration & Parameters

Instrument	GCMS-QP2020 NX
Autosampler	AOC-6000 Plus with PAL Method Composer Software
Flow Control Mode	Linear Velocity
Linear Velocity	20 cm/s
Injection Mode	Split (Split Ratio = 100)
Injection Port Temp.	225 °C
Carrier Gas	He
Column	SH-2330 P/N: 227-36239-03 105 m x 0.25 mm I.D. x 0.20 µm df
Interface Temp.	250 °C
Solvent Cut Time	11 min
Acquisition Mode	Scan *m/z* 35 to 500

The concentration of methyl erucate would be determined using the peak area normalization based on the GC-MS result. This approach calculates the relative percentage of methyl erucate by dividing its peak area with respect to the total peak area of all FAME species detected. Thus, only the FAME species are accounted and excludes other components in the oil. Hence, the percentage of methyl erucate detected will not directly correspond to the mass percent of erucic acid spiked into the canola oil.

In this technology brief, MS was used as the detector instead of the conventional FID approach. This was to leverage the advantage of having additional dimension of mass spectrum which enables the identification and confirmation of the targeted contamination peaks with ease. Together with our earlier published data [6], we have successfully demonstrated suitability of this approach as the data generated was in strong agreement with the literatures that used conventional FID approach [6][7].

Results & Discussion

Unspiked Canola Oil Sample

Fig. 4 depicts the FAME profile of an unspiked canola oil. Methyl erucate, C22:1(n9), was not detected. **Table 2** summarized the results obtained for the unspiked canola oil sample on 2 different days. The data obtained was in strong agreement with the reported literature value [7] and had excellent reproducibility. This strongly demonstrated the stability and robustness of GCMS-QP2020 NX and the proposed workflow. The slight variations of the results obtained with respect to the literature could be attributed to the sources/subspecies of the rapeseed used, and possibly, also due to the difference in the extraction process.

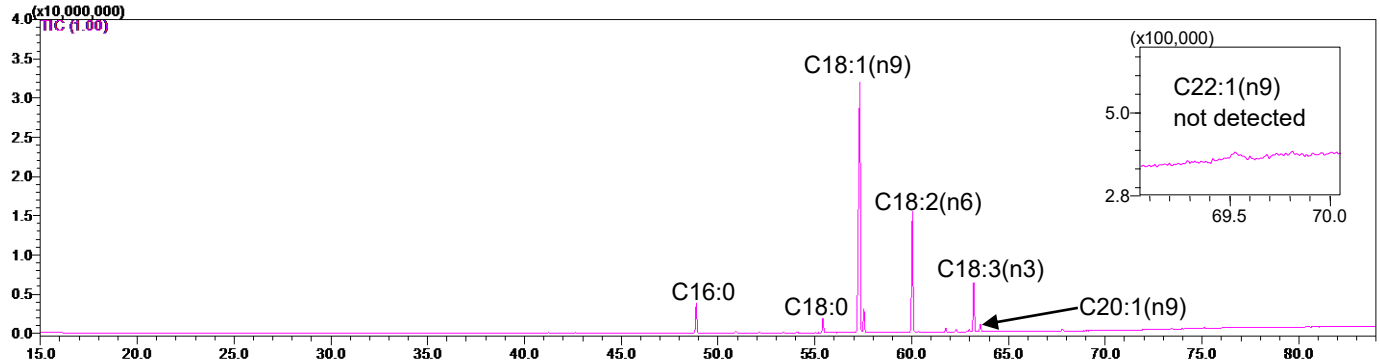

Fig. 4 TIC FAME profile of unspiked canola oil

Table 2. Canola Oil FAMEs Profile (TIC Area%) Obtained vs Literature Value (FID Area%) [7]

Fatty Acid Methyl Ester	Day 1 Canola Oil /Area%	Day 2 Canola Oil /Area%	Reference Value [7] /Area%
Methyl Palmitate, C16:0	5	5	3
Methyl Stearate, C18:0	2	2	2
Methyl Oleate, C18:1 (n9)	59	59	59
Methyl Linoleate, C18:2 (n6)	22	22	22
Methyl Arachidate, C20:0	1	1	1
Methyl α-Linolenate, C18:3 (n3)	8	8	12
Methyl cis-11-Eicosenoate, C20:1 (n9)	1	1	1
Methyl Erucate C22:1 (n9)	0	0	0

All the data in the table above are rounded off to the nearest percentage.

Spiked Canola Oil Sample

Fig. 5a-c depict the FAME profiles for canola sample spiked with various concentrations of erucic acid. **Fig. 5b** represents the FAME profile of the canola oil that contained methyl erucate, C22:1(n9), at a level close to the legal limit of 2% (detected as 1.92%). **Fig. 5a** depicts the FAME profiling for sample with methyl erucate that is below the legal limit. And lastly, **Fig. 5c** depicts sample with methyl erucate that is above the legal limit. With this set of data (**Fig 5a-c**), the capability to perform screening of canola oil sample for erucic acid that are below, close to and above the legal limits had been successfully demonstrated.

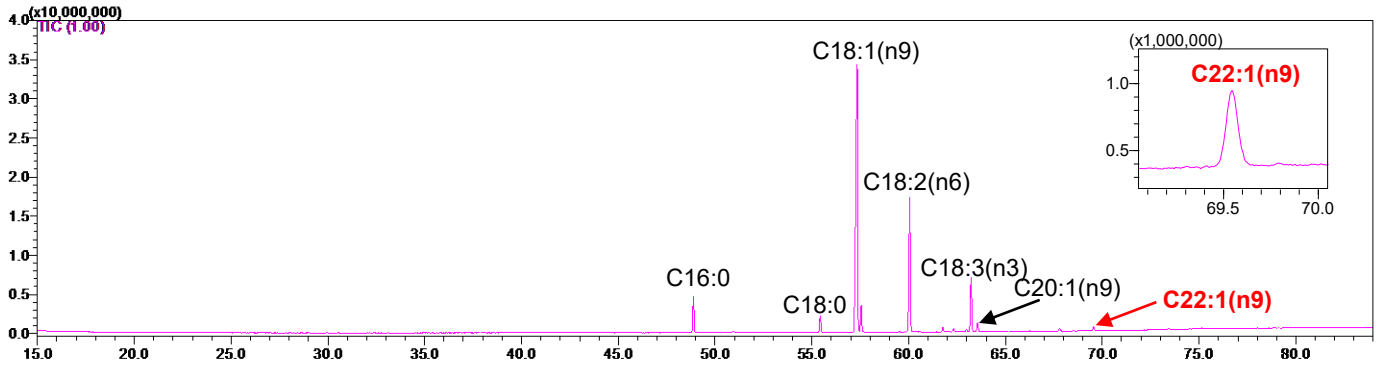

Fig. 5a TIC FAME profile of canola oil containing 0.64% of erucic acid

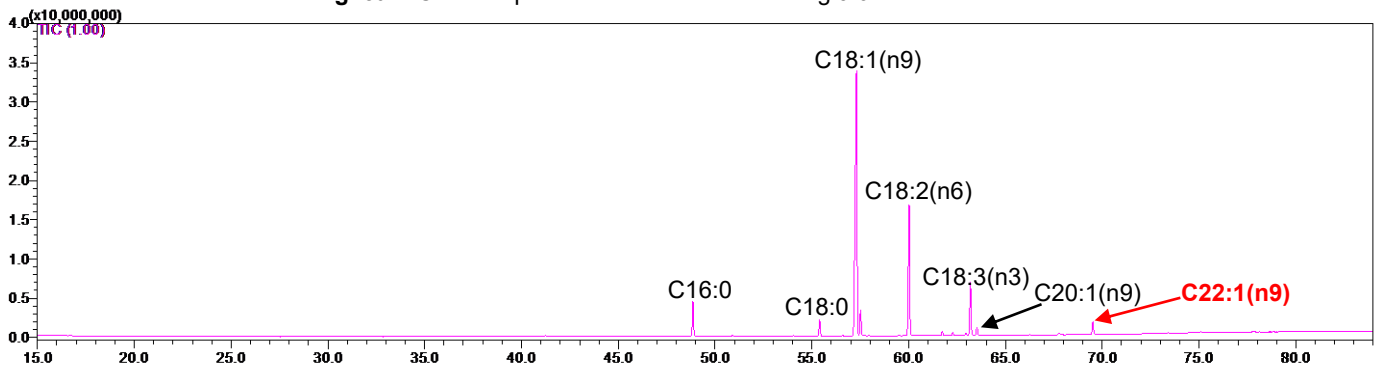

Fig. 5b TIC FAME profile of canola oil containing 1.92% of erucic acid

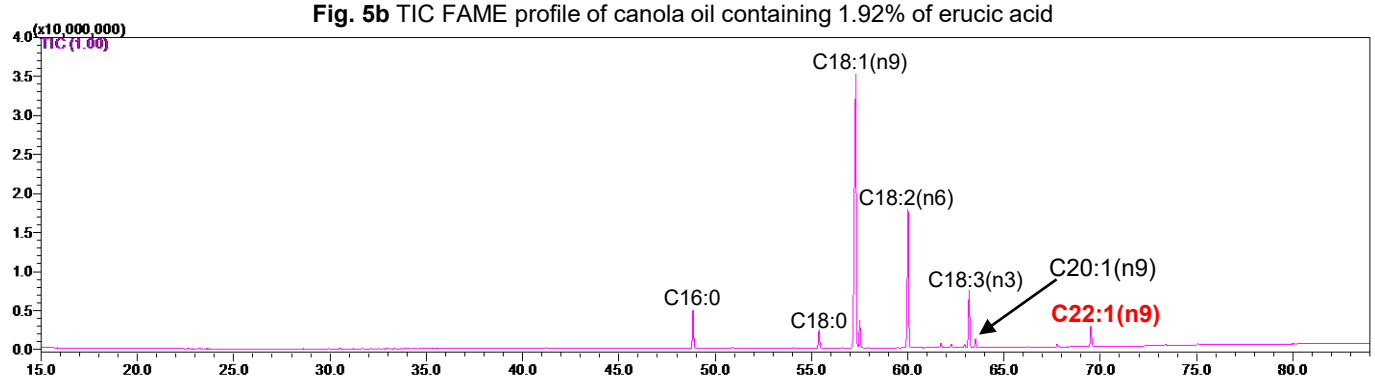

Fig. 5c TIC FAME profile of canola oil containing 2.84% of erucic acid

Conclusion

The results presented in this Technology Brief demonstrate the convenience of automated workflow using Shimadzu GCMS-QP2020 NX, coupled with the AOC-6000 Plus. Using the workflow, the data generated could easily differentiate samples with erucic acid levels that are below, close to and above the legal limit.

Acknowledgement

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