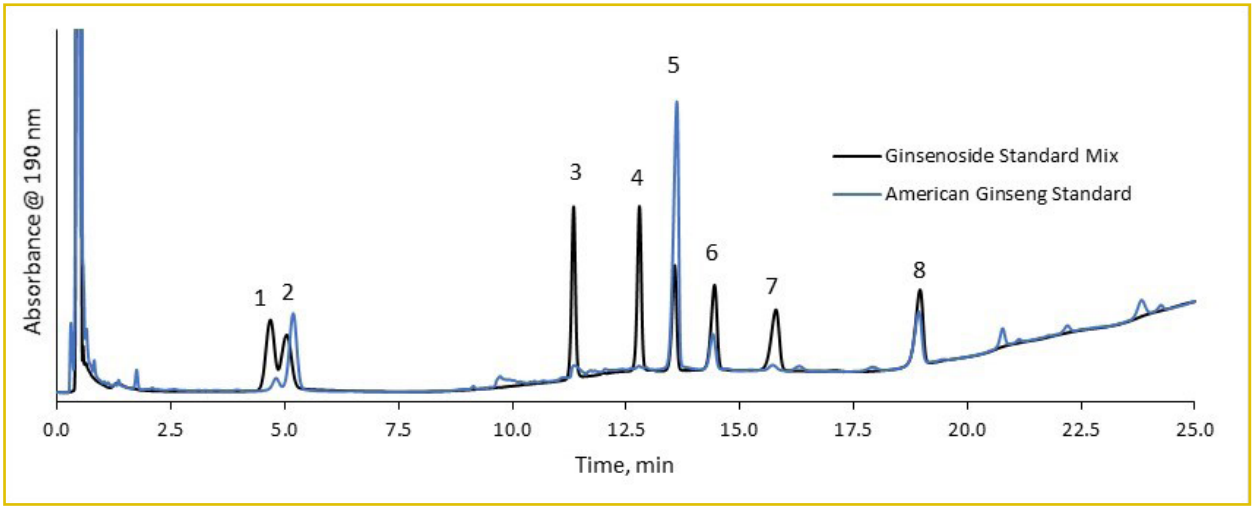
HALO®

FOOD / BEVERAGE

Ginseng Analysis using 5 µm HALO® C18

225-F



TEST CONDITIONS:

Column: HALO 90 Å C18, 5 µm 3.0 x 50 mm
Part Number: 95813-402
Mobile Phase A: Water
B: Acetonitrile

Gradient:

Time	%B
0.0	19
5.6	19
11.6	29
17.0	29
25.0	40

Flow Rate: 0.425 mL/min
Pressure: 60 bar
Temperature: 30 °C
Detection: 190 nm
Injection Volume: 4 µl
Sample Solvent: Methanol
Data Rate: 100 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µL
LC System: Shimadzu Nexera

PEAK IDENTITIES:

1. Ginsenoside Rg1
2. Ginsenoside Re
3. Ginsenoside Rf
4. Ginsenoside Rg2
5. Ginsenoside Rb1
6. Ginsenoside Rc
7. Ginsenoside Rb2
8. Ginsenoside Rd

Ginseng root has been used as a traditional medicine for centuries. It is believed to benefit the immune system, brain function, and act as an antioxidant that may reduce inflammation. Ginseng can be prepared as a dietary supplement, an herbal tea, or even used in cooking.

Ginsenosides are a class of natural product steroid saponins primarily found in ginseng root. Ginseng root from Panax quinquefolium (American ginseng) is overlayed with a standard mixture of eight ginsenosides on a 5 µm HALO® C18 column showing excellent resolution at low back pressures.

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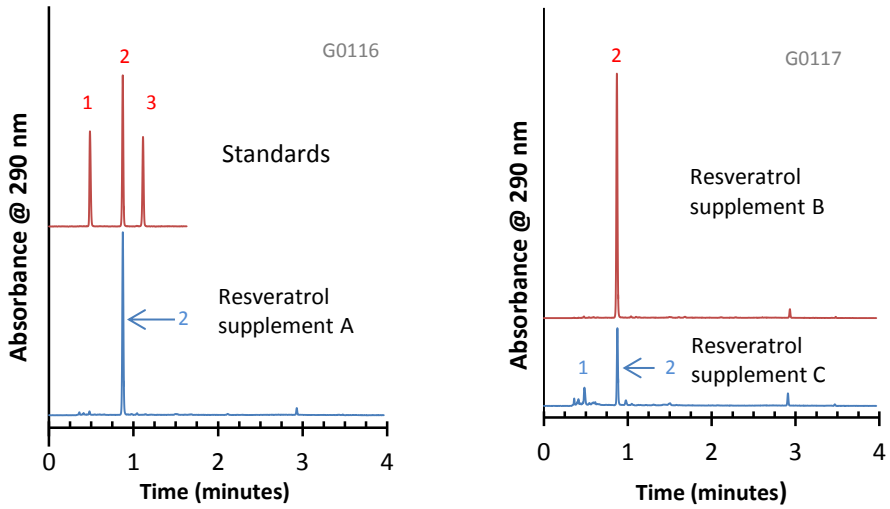
Application Note: 132-P

Separation of Resveratrols on HALO C18, 2.7 µm



PEAK IDENTITIES:

1. Polydatin
2. *trans*-Resveratrol
3. *cis*-Resveratrol

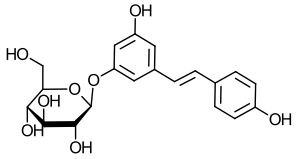
TEST CONDITIONS:

Column: 4.6 x 75 mm, HALO C18, 2.7 µm
Part Number: 92814-502
Mobile Phase: A= water/B= acetonitrile
Gradient:

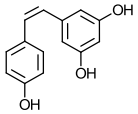
Time	%B
0.0	30
2.0	50
3.0	90
4.0	90

Flow Rate: 1.8 mL/min.
Pressure: 240 Bar
Temperature: 35°C
Detection: UV 290 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: 50/50-acetonitrile/methanol
Response Time: 0.02 sec.
Data rate: 25 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
ECV: ~14 µL

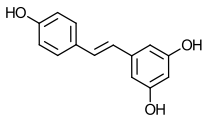
STRUCTURES:



Polydatin



cis-Resveratrol



trans-Resveratrol

Resveratrols are polyhydroxy compounds and have been reported to have antioxidant and anti-aging properties and are available as food supplements. These food supplements can be analyzed rapidly using short HALO Fused-Core C18 columns.

Resveratrol supplement tablets were extracted overnight using 15 mL of a 50/50 mixture of methanol and acetonitrile. The extracts were then filtered through a 0.45 µm porosity nylon membrane. This filtered solution was further diluted 1:10 using the 50/50 mixture of methanol and acetonitrile before analysis.

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TECHNICAL REPORT: AMT-TR022001

TITLE: **HIGH RESOLUTION LCMS SEPARATIONS OF EDIBLE OILS**

MARKET SEGMENT: FOOD / BEVERAGE

AUTHORS:
Andrew Harron PhD, Application Scientist

ABSTRACT

Edible oils, extracted from both plant and animal sources, have evolved into a multibillion-dollar industry, and are being used in new applications every year. In 2018 over 582.05 million metric tons of edible oils were consumed and utilized worldwide. Products such as biodiesel, pharmaceutical formulation applications, soaps, shampoos, and household cleaners are among a few. In recent years, the food industry has sought to incorporate new oils with higher nutritional value, but with often ambiguous results. The hydrophobic nature of oils often makes analysis problematic by C18 stationary phases due to limited selectivity. In this technical report we generate the TAG profile of four common edible oils, including corn, coconut, canola, and grape seed oil by LCMS, to demonstrate how the HALO® C30 column with its unique stationary phase offers superior specificity and higher shape selectivity. Thus, enabling better separation of the hydrophobic long-chain molecules, such as TAGs.

INTRODUCTION

Often thought of as an essential part of a healthy diet, the nutritional value of edible oils has been a topic of debate, primarily due to their composition. The major component of edible oils is triacylglycerols (TAGs), which comprises approximately 90-95% of the oil. The remaining 5-10% is a mixture of free acids, monoacylglycerols, diacylglycerols (DAGs), phospholipids, sterols, and various hydrocarbons, including alkanes and carotenes. (Gunstone 2006).

The analysis of edible oils by LCMS is difficult due to the high concentration of hydrophobic molecules, such as long chain fatty acids (FAs) and esters, as well as DAGs and TAGs. In the food industry, the analysis of TAGs in the oil is a critical step to determine nutritional value, for example amount of unsaturation in the oil, as well as suitability for non-food-based applications. C18 columns, the most widely used phase in HPLC analysis, lack the specificity to separate such molecules, therefore; the C30 phase has seen increased application and utility in this area (Rentel et al., 1998; Abidi 2000; Sander and Wise, 1993). The structure and longer chain of the C30 phase, compared to C18, provides better phase thickness to enhance the interaction between the stationary phase, and long chain molecules, such as TAGs and DAGs (Sander and Wise, 1993). In this application note we report the TAGs profile of 4 commercial edible oils, and compare with previously published data, to demonstrate the utility of the HALO® C30 for the analysis of long chain hydrophobic molecules, such as those found in edible oils.

KEY WORDS:
Edible oils, Triacylglycerides, Diacylglycerides, HALO C30, Hydrophobic, LCMS, TAG, DAG, corn oil, coconut oil, canola oil, grape seed oil

EXPERIMENTAL DATA

Canola oil, grape seed oil and coconut oil were purchased from a commercial food store. Corn oil was obtained from the USDA Eastern Regional Research Center (Wyndmoor, PA). Acetonitrile (HPLC grade), isopropyl alcohol (Omni Solv), and methanol (HPLC grade) and ammonium formate were purchased from Millipore Sigma (Burlington, MA). A HALO 160 Å C30, 2.7 µm, 2.1 x 150 mm (Advanced Materials Technology, Wilmington, DE) was used on a Shimadzu Nexera X2 (Shimadzu Scientific Instruments, USA). Mass spectra were acquired using a Thermo Scientific Orbitrap Exactive mass spectrometer (Bremen, Germany) using a heated electrospray (HESI-II) probe on the Ion Max source.

Sample prep

Stock solutions of the oil samples were made up at 1 mg/mL and dissolved in methanol. Samples were further diluted for analysis to a concentration of 10 µg/mL in methanol.

Instrument Parameters and Gradient

Column: HALO 160 Å C30, 2.7 µm, 2.1 x 150 mm

(Part Number: 92112-730)

Flow Rate: 0.3 mL/min

Initial Pressure: 325 bar

Temperature: Ambient

Injection Volume: 2 µL

Sample Solvent: MeOH

Mobile Phase A: (60/40) ACN/H2O 10mM ammonium formate/0.1% formic acid

Mobile Phase B: IPA/0.1% Formic acid

Table 1. Gradient conditions

Time	%B
0.0	5
10.0	20
15.0	80
25.0	80
25.5	5
32.0	END

Table 2. MS source conditions

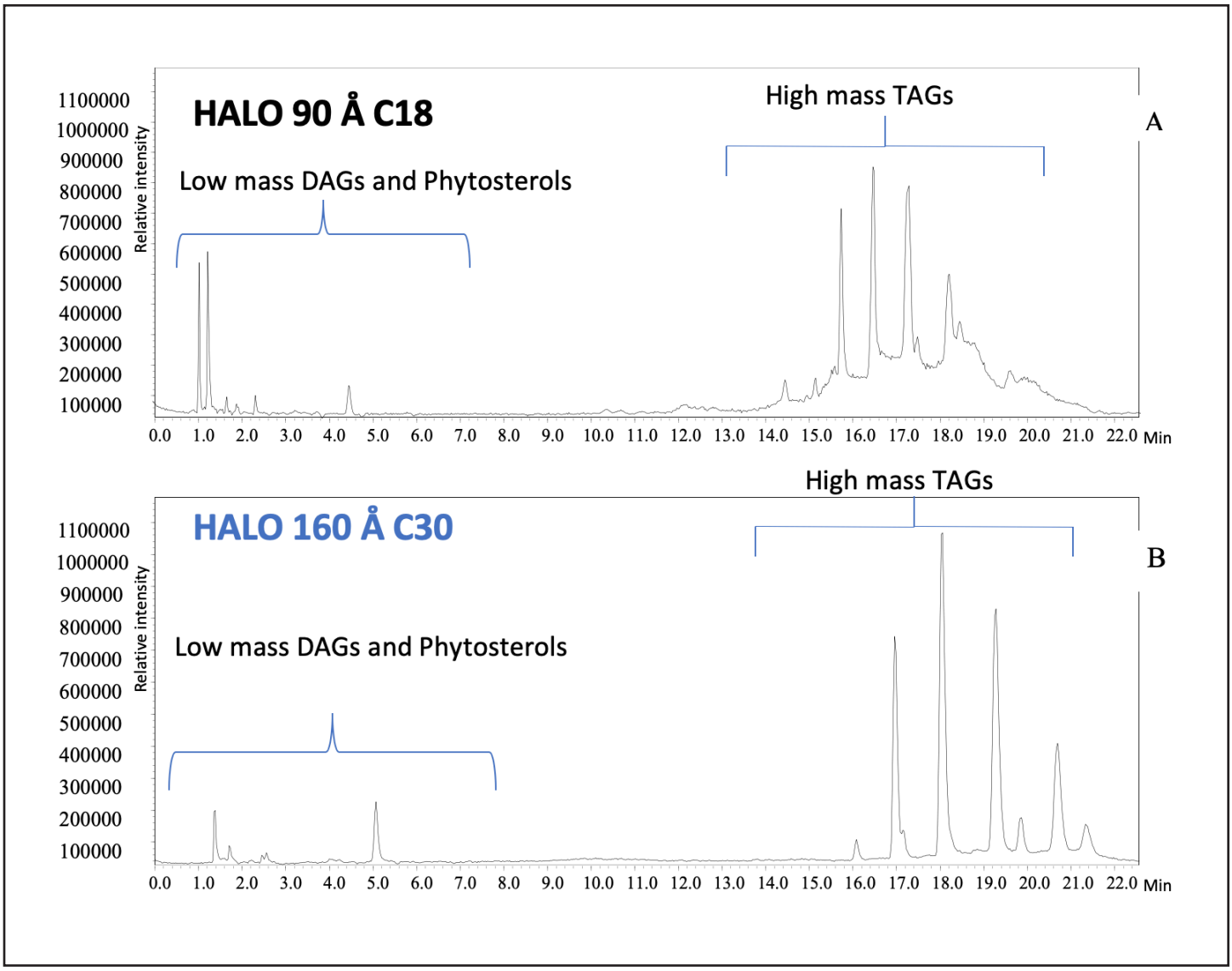
MD Source Conditions	Setting
Spray Voltage	3.5 kV
Sheath gas	30 arb units
Aux gas	3 arb units
Sweep gas	0 arb units
RF voltage	45 V
Aux heater temp	400° C

RESULTS

Column Selection

With the classification of monounsaturated and polyunsaturated fats as healthy fats, edible oils have generated a new level of interest due to their wide spread usage in the food industry. However, analysis is challenging and often requires the need to analyze very hydrophobic samples that have subtle positional isomerism. Traditional reversed phase chemistries, such as C18, are lacking in their ability to resolve some of these analytes. For this reason, the HALO® C30 phase has been developed, and provides both high hydrophobicity as well as a proclivity to separate isomeric forms that are required for routine analysis.

Figure 1. Comparison of a HALO C18 column (A) and a HALO



C30 column (B) for the separation of corn oil

Figure 1 shows the difference in selectivity between the HALO C18 phase (A) and the HALO C30 phase (B) for the separation of corn oil. Notice how the high mass TAGs are clearly resolved on the HALO C30 (B), while unresolved in the HALO C18 (A), demonstrating that the C30 phase offers superior specificity compared to the HALO C18 phase by exhibiting higher shape selectivity, thus enabling better separation of hydrophobic, long-chain, structures, and proving to be an ideal choice for the analysis of edible oils.

Corn oil

Corn oil, extracted from either wet milled or dry milled germ seed, is an edible oil that is mainly used in the food industry for cooking food, and as margarines and spreads. In recent years however, corn oil has found application in the biodiesel industry (Moreau et al. 1999). Corn oil TAG and FA content is dominated by long chain TAGs, composed of fatty acids consisting of (C10-C18) FA species.

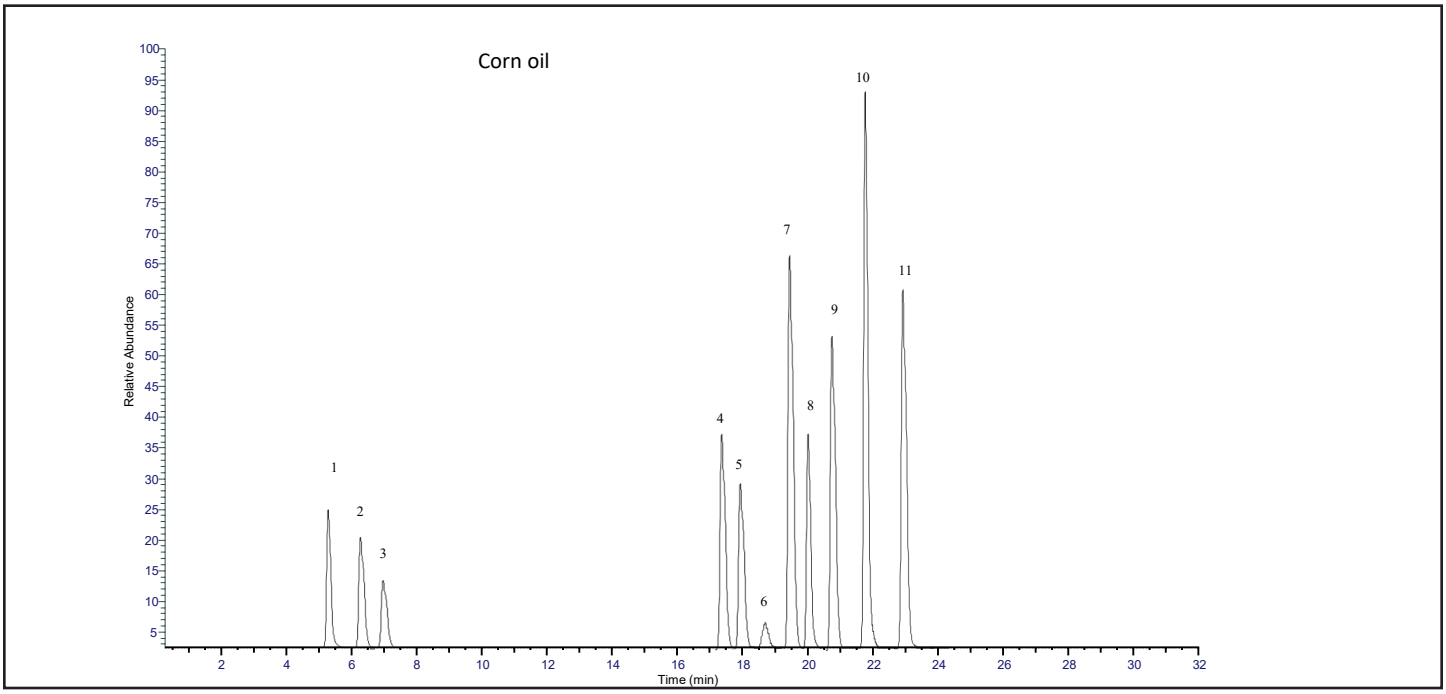


Figure 2. LCMS of corn oil on a HALO C30 column

Linoleic acid (L), Oleic acid (O), and Palmitic acid (P), are the primary fatty acids that comprise the TAGs species in corn oil. The major TAG composition of corn oil is LLL (25%), LLO (22%), LLP (15%), OOL (11%), and PLO (10%) (Strecker et al. (1990). The HALO C30 can clearly resolve the low mass DAGs and the high mass TAGs that are abundant in corn oil, and confirms the previously reported data on the profile of the DAGs and TAGs contained in the oil.

Table 3. Corn oil DAG and TAG Profile

Peak #	Major DAGs & TAGs in Corn Oil	Observed Ions (m/z)
1	DAG	634.325
2	DAG	636.375
3	DAG	638.405
4	TAG (PLO)	856.751
5	TAG (LLP)	858.767
6	TAG (LLO)	872.755
7	TAG (LLL)	879.405
8	TAG (LLO)	881.465
9	TAG (OOL)	885.432
10	TAG (LLL)	898.358
11	TAG (LLL)	900.478

linoleic acid, O = oleic acid, P = palmitic acid

Coconut oil

Coconut oil, used in the food and cosmetic industries, is extracted from the dried kernel, or copra, and contains high levels of medium- and long-chain saturated fatty acids. Coconut oil, unlike corn oil, is composed mainly of medium chain length TAGs, predominantly composed of (C32-C40).

The TAG profile for coconut oil is much different than corn oil, due to the medium chain length TAGs. TAG species are composed of primarily of the (C10-C18) FA species lauric (La), myristic (M), caprylic (C) and palmitic acid (P). C36 is the most abundant TAG in coconut oil at 20%, followed by C34 at 17%, C38 at 16%, C32 at 13% and C40 at 10%. Trilaurin, (LaLaLa) (20%), a C36 TAG, is the most abundant TAG in coconut oil, followed by MLaC and PCC. (Pantzaris and Basiron (2002))

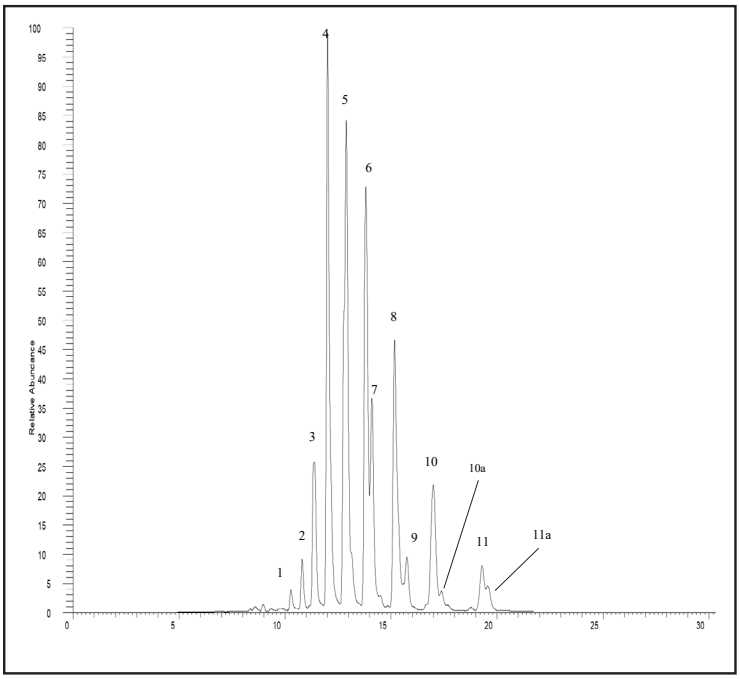


Figure 3. LCMS of coconut oil on a HALO® C30 column. Peaks differ by m/z 28 corresponding to difference in the number of –(CH2CH2)– groups on sidechains.

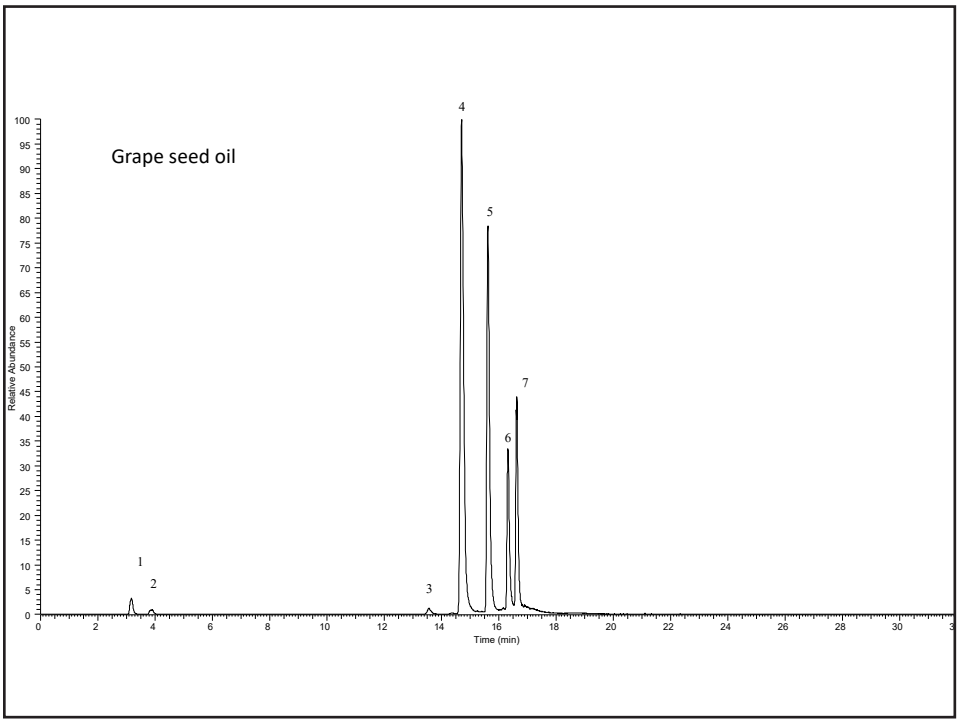
The most abundant TAG in coconut oil is trilaurin with a mass of 638.54852 g/mol, however with the addition of ammonium formate to the mobile phase, each triglyceride shows predominantly an M+18 adduct ion. In Figure 3 showing the TIC of coconut oil, notice how each peak differs by m/z 28 corresponding to difference in the number of –(CH2CH2)– groups on sidechains. The results of observed ions are summarized in Table 4. It is also of note that there were no DAGs detected in coconut oil, and the complex nature of the TAGs can clearly be seen in the chromatogram. In the higher mass species, the HALO® C30 column can pull apart isomeric species of the C36-C40 TAGs as noted in peaks 10a and 11a.

Table 4. Coconut oil TAG Profile

Peak #	TAG & DAG Profile of Coconut Oil	Observed Ions (m/z)
1	TAG (C32)	516.506
2	TAG (C34)	544.554
3	TAG (C34)	572.668
4	TAG (C36)	600.502
5	TAG (C36)	628.616
6	TAG (C36)	656.713
7	TAG (C36)	684.774
8	TAG (C38)	712.598
9	TAG (C38)	740.518
10	TAG (C40)	768.566
10a	TAG (C40) Isomeric species	768.566
11	TAG (C40)	796.614
11a	TAG(C40) Isomeric species	796.614

Grape seed oil

Grape seed oil is extracted from the seeds of grapes, and is usually as a byproduct of the wine making process. It is used primarily in the food and cosmetics industry, and has recently found application in the pharmaceutical industry. Grape seed oil TAG and FA profile consists of long chain TAGs, composed of fatty acids consisting of (C16-C18) FA species. The FA composition of grape seed oil is dominated by linoleic acid at approximately 68.4%, followed by oleic acid at approximately 18.3%. The TAGs are made up of combinations of linoleic (LLL), oleic (OLL, POL) and palmitic (PLL, POL) Beveridge et al. (2005).



The resulting chromatogram from grape seed oil shows similar high mass TAG content to corn oil, however substantially lower amounts. There is also the presence of DAGs as well in the low mass and early elution area. The results are summarized in Table 5. This is to be expected and matches previous analysis and reported data (Beveridge et al. 2005).

Figure 4. LCMS of grape seed oil on a HALO® C30 column

Table 5. Grape seed oil DAG and TAG Profile

Peak #	TAG & DAG Profile of Grape Seed Oil	Observed Ions (m/z)
1	DAG	634.475
2	DAG	636.491
3	TAG (POL)	894.718
4	TAG (LLL)	896.358
5	TAG (OLL)	898.471
6	TAG (PLL)	900.385
7	TAG (LLL)	900.485

L = linoleic acid, O = oleic acid, P = palmitic acid

Canola oil

Canola (rapeseed) oil, is extracted from a rapeseed which has been bred to contain low levels of erucic acid (Ackman 1990). Used mainly in the food and petroleum industry, the oil TAG profile consists of long chain TAGS, comprised mainly of (C18) FA species oleic (O), linoleic (L), palmitic (P) and linolenic (LN) (Neff et al. (1994, 1997).

The resulting chromatogram shows similar high mass TAG content to corn oil, but the DAGs are in substantially smaller quantities than corn oil. This matches with previously reported analysis (Neff et al. (1994, 1997). The HALO® C30 clearly shows the separation between the species. The results are summarized in Table 6.

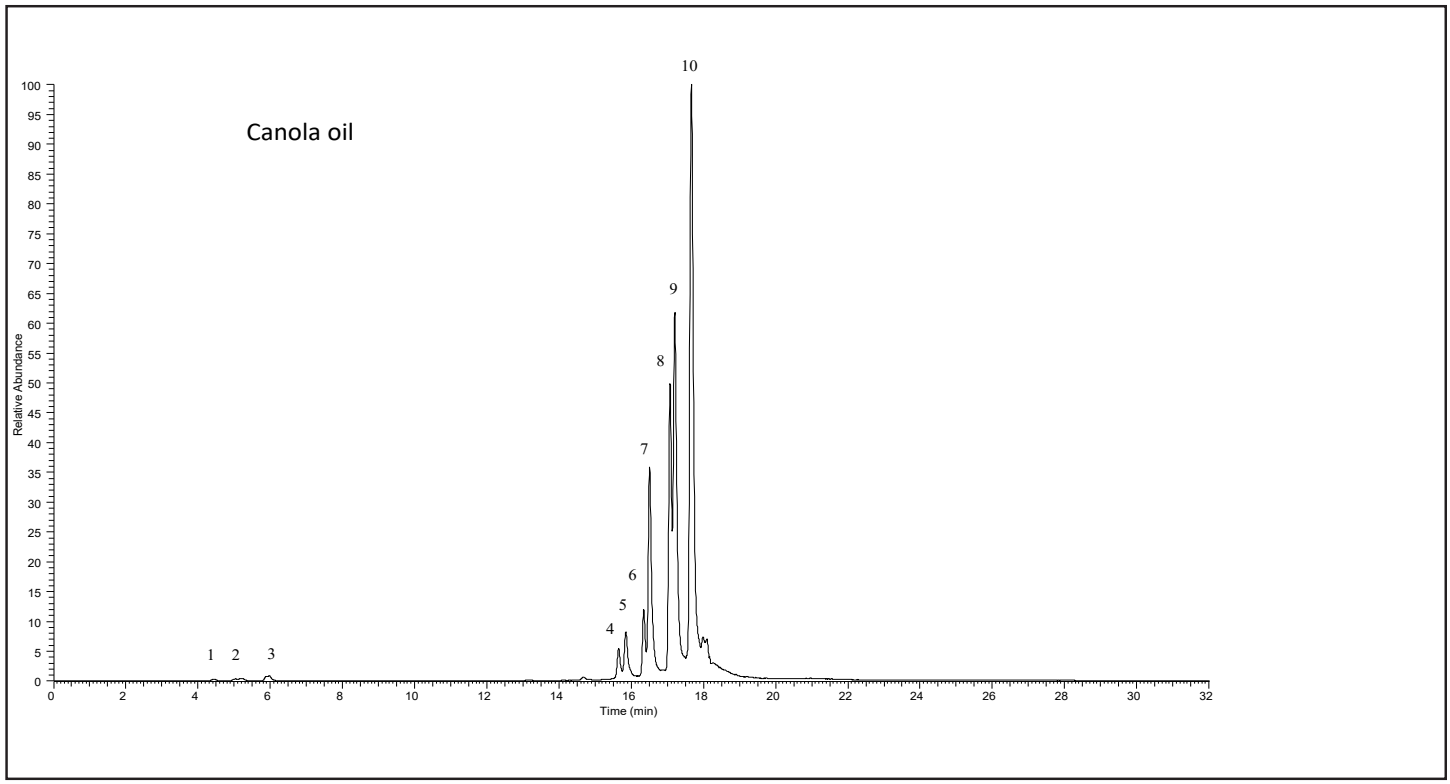


Figure 5. LCMS of canola oil on a HALO® C30 column

Table 6. Canola oil DAG and TAG Profile

Peak #	TAG & DAG Profile of Canola Oil	Observed Ions (m/z)
1	DAG	634.425
2	DAG	636.495
3	DAG	638.437
4	TAG (LnLO)	858.767
5	TAG (LLO)	872.755
6	TAG (POO)	874.355
7	TAG (LOO)	885.432
8	TAG (LnOO)	896.432
9	TAG (LOP)	900.734
10	TAG (OOO)	902.499

L = linoleic, Ln = linolenic, O = oleic, P = palmitic

CONCLUSION:

The results of the experiments on four edible oils: corn, coconut grape seed, and canola, clearly demonstrate that the HALO® C30 provided higher resolution separations of the components of the edible oils compared to the C18 stationary phase. In addition, the selectivity of the HALO® C30 was able to separate the isomeric TAG species in coconut oil, enabling a thorough characterization profile. This data will be especially useful to help determine the nutritional value of edible oils as they continue to be used in the food industry. The HALO® C30 is an ideal choice for the analysis of high mass triglycerides found in edible oils. Built on proven Fused-Core® technology, the HALO® C30 offers superior specificity and exhibits higher shape selectivity, enabling better separation of hydrophobic, long-chain, structures.

REFERENCES:

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Comparative results presented may not be representative for all applications.

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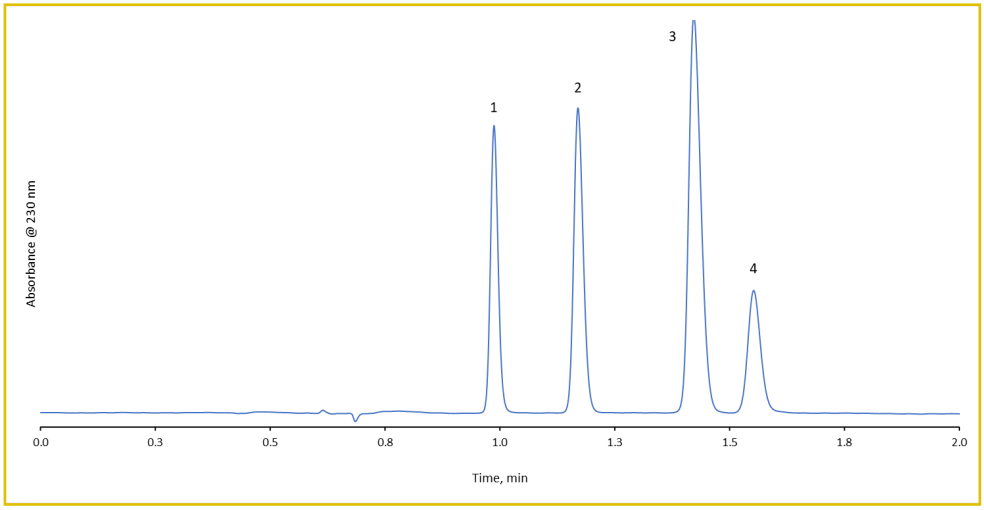
FOOD / BEVERAGE

Food Additives Assay using HALO® AQ-C18, 5µm

260-P



TEST CONDITIONS:

Column: HALO 90 Å AQ-C18 5 µm, 4.6 × 150 mm
Part Number: 95814-722
Mobile Phase A: 20 mM ammonium acetate
Mobile Phase B: Methanol
Isocractic: 90/10 A/B
Flow Rate: 2 mL/min
Pressure: 336 bar
Temperature: 30°C
Detection wavelength: 230 nm
Injection Volume: 10 µL
Sample Solvent: mobile phase
Data Rate: 100 hz
Response Time: 0.025 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

PEAK IDENTITIES:

1. Acesulfame
2. Benzoic acid
3. Sorbic acid
4. Saccharin sodium

A rapid and highly efficient assay <400 bar for food security and safety measurements is demonstrated with a HALO 90 Å AQ-C18 5 µm, 4.6 × 150 mm column. Determination of acesulfame, benzoic acid, sorbic acid and saccharin sodium food additives are specified in China's national standard regulation methods GB 5009.28-2016 and GB 5009.140-2016. These compounds are used as anti-septic/anti-microbial agents to prevent spoilage of food products by microorganisms. A baseline resolution separation is completed <1.7 min; modernization of this method is as easy as exploiting the 5 micron HALO® column - compatible with HPLC and UHPLC instruments.

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