Capillary Supercritical Fluid Chromatography–Atmospheric Pressure Chemical Ionization Mass Spectrometry of Triacylglycerols in Berry Oils

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ABSTRACT: A capillary supercritical fluid chromatograph (SFC), combined with a triple-quadrupole mass spectrometer (MS) *via* a liquid chromatography–atmospheric pressure chemical ionization (LC–APCI) interface, was utilized in the analysis of berry oil triacylglycerols. No modification of the commercially available interface was required. Vapor of different solvents, such as methanol, isopropanol, water, or ammonium hydroxide in methanol, was introduced in the sheath gas flow in the APCI source to achieve adequate ionization of triacylglycerols. The separation of triacylglycerols according to acyl carbon number and degree of unsaturation was accomplished on a 20 m × 50 μm i.d. SB-Cyanopropyl-25 column. The resolution of triacylglycerols in the reconstructed ion chromatogram and the sensitivity of the SFC–(APCI)MS system was comparable to or slightly better than that obtained with a flame ionization detector. No baseline drifting was observed during the SFC density programming. Triacylglycerols formed diagnostic $[M + H]^+$ and $[M - RCOO]^+$ ions with all tested reactant ion solvents except with ammonium hydroxide in methanol, which formed abundant $[M + 18]^+$ ions instead of $[M + H]^+$ ions. The abundance of the $[M + H]^+$ ion increased with increasing degree of unsaturation of a triacylglycerol, whereas the abundance of the $[M - RCOO]^+$ ion depended on the regiospecific distribution of the fatty acid moiety between the *sn*-1/3 positions and the *sn*-2 position and on the number of double bonds.

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KEY WORDS: Atmospheric pressure chemical ionization, berry oils, capillary supercritical fluid chromatography, cloudberry, mass spectrometry, sea buckthorn, triacylglycerols.

The chromatographic separation of triacylglycerols most typically involves high-performance liquid chromatography (HPLC) with reversed-phase or silver-ion columns (1–5). Also, gas chromatography (GC) has been used to separate triacylglycerols (3,6,7). However, the technique is not suitable for analysis of highly unsaturated molecules. In general, mass spectrometry (MS) is considered to be the best method for structural elucidation of triacylglycerols (5,8,9). Most often, the identification has been accomplished by HPLC–MS with chemical ionization (CI) or by GC–MS with either electron ionization (EI) or CI. Recently, good results for the identification of triacylglycerols has been achieved by atmospheric pressure chemical ionization (APCI), combined with both reversed-phase HPLC (10–12) and silver-ion HPLC separation (13).

In contrast to HPLC and GC, interfacing supercritical fluid chromatography (SFC) with MS is often problematic. SFC has been combined with MS by various interfaces and often involves solvent elimination or direct fluid introduction (DFI) techniques (14). Usually, successful SFC–MS requires specific design or modification of commercially available HPLC–MS interfaces. The modifications of the interfaces most frequently involve the installation of an additional heating device at the sample outlet to compensate for the strong cooling effect of the expanding supercritical fluid carrier. The advantages, drawbacks, and prospects of interfacing SFC with MS have been reviewed by Arpino *et al*. and Niessen *et al*. (14–16). The major problem of DFI, the most popular interfacing technique in SFC–MS, concerns the carrier fluid-flow variations during density/pressure programming, especially at high pressures, which leads to unsatisfactory sensitivity of later-eluting higher molecular weight components. In addition, the low capacity of SFC capillary columns is often claimed to be responsible for the lack of sensitivity of capillary SFC–MS systems. CI is considered favorably for SFC–MS because the source vacuum is lower than in EI.

Ionization at atmospheric pressure is a preferred choice for SFC–MS because it is almost independent of carrier fluid-flow variations during density/pressure programming. Some articles concerning applications for both packed and capillary column SFC–(APCI)MS have been published and deal with the analysis of such components as steroids, biological matrices, and polyaromatic compounds (17–23). All these applications involved the installation of an additional heating source at the SFC sample outlet. One of the reports described glycerolipids in arils (23), another the ionization of triacylglycerols with one reference compound as an example (19). In general, no detailed studies concerning SFC–MS of triacylglycerols have been published.

Recently, capillary SFC has been reported to provide more information about triacylglycerol mixtures than just the acyl

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carbon number distribution (24,25). Unfortunately, co-elution of even three triacylglycerols in a single peak disabled the reliable identification of existing compounds in that peak. In this study, capillary SFC was combined with (APCI)MS to achieve reliable identification and additional information concerning the molecular structure of the berry oil triacylglycerols with a 25% cyanopropyl/25% phenyl/50% methylpolysiloxane stationary phase. This study also introduces a fast and simple capillary SFC interface with MS by using a commercial LC–(APCI) interface. In addition, the effect of some APCI parameters and different reactant ion solvents on ion formation and sensitivity is discussed.

EXPERIMENTAL PROCEDURES

Materials. Trioctanoylglycerol (3 × 8:0), tripalmitoylglycerol (3 × 16:0), and tri-α-linolenoylglycerol (3 × 18:3n-3) were used to test the SFC–(APCI)MS system. Two mixtures of reference components were used to study the mass-spectrometric fragmentation pattern of triacylglycerols: Mixture #1 consisted of *rac*-1,2-dipalmitoyl-3-oleoyl-*sn*-glycerol (*rac*-16:0-16:0-18:1), *rac*-1,2-dioleoyl-3-palmitoyl-*sn*-glycerol (*rac*-18:1-18:1-16:0), *rac*-1,2-dipalmitoyl-3-linoleoyl-*sn*-glycerol (*rac*-16:0-16:0-18:2), *rac*-1,2-dioleoyl-3-γ-linolenoyl-*sn*-glycerol (*rac*-18:1-18:1-18:3n-6), *rac*-1-stearoyl-2-oleoyl-3-linoleoyl-*sn*-glycerol (*rac*-18:0-18:1-18:2), and *rac*-1-oleoyl-2-linoleoyl-3-eicosanoyl-*sn*-glycerol (*rac*-18:1-18:2-20:0). Mixture #2 consisted of 1,3-dipalmitoyl-2-oleoyl-*sn*-glycerol (*sn*-16:0-18:1-16:0), 1,3-dioleoyl-2-palmitoyl-*sn*-glycerol (*sn*-18:1-16:0-18:1), 1,3-distearoyl-2-linoleoyl-*sn*-glycerol (*sn*-18:0-18:2-18:0), and 1,3-dioleoyl-2-γ-linolenoyl-*sn*-glycerol (*sn*-18:1-18:3n-6-18:1). Mixtures #1 and #2 contained approximately 0.2 mg of each reference component dissolved in 1 mL 1,2-dichloroethane (Rathburn, Walkerburn, Scotland). All triacylglycerol standards were purchased from Larodan (Malmö, Sweden).

Cloudberry (*Rubus chamaemorus*) seed oil and sea buckthorn (*Hippophaë rhamnoides*) pulp and seed oil were extracted with supercritical carbon dioxide at Flavex (Rehlingen, Germany). Each oil (10.2 mg) was diluted in 1 mL dichloromethane (Rathburn) for the chromatography.

SFC. Supercritical-fluid-chromatographic separations were achieved with a Lee Scientific Series 600 supercritical fluid chromatograph (Dionex, Salt Lake City, UT), equipped with a flame-ionization detector (FID). The temperature of the FID was held at 340°C during SFC–FID operation and at 135°C during SFC–(APCI)MS as the analytical column was not connected to the FID. A pneumatic and electrically controlled Valco switching valve (Valco Instruments, Inc., Houston, TX) with an internal loop size of 1.0 µL was used for timed/dynamic split injection. The timed split loop open time was 2.0 s. Frit restrictors (30 cm × 50 µm i.d., 195 µm o.d.; Dionex) were installed both at the dynamic split outlet and after the analytical column. An SB-Octyl-50 (50% octyl/50% methylpolysiloxane, 10 m × 50 µm i.d.; Dionex) column or two 10 m × 50 µm i.d. SB-Cyanopropyl-25 (25% cyanopropyl/25% phenyl/50% methylpolysiloxane; Dionex) columns combined in series with a zero dead volume butt connector (SGE, Austin, TX) were used for the chromatographic separations. Separations were performed at constant temperature of 135°C with a linear density ramp from 0.15 to 0.62 g/mL at a rate of 0.010 g/mL/min. SFC-grade CO_2 (Scott Specialty Gases, Plumsteadville, PA) was used as a carrier fluid with a column flow rate of 0.37 mL/min with the SB-Octyl-50 column and 0.17 mL/min with the SB-Cyanopropyl-25 column, measured with propane at the initial conditions of the chromatographic program when connected to the FID.

SFC–(APCI)MS. Capillary SFC was combined with a Finnigan MAT TSQ-700 triple-quadrupole mass spectrometer (Finnigan MAT, San Jose, CA) simply by installing the SFC frit restrictor capillary through the sample inlet of the manifold of the Finnigan MAT APCI probe assembly (Fig. 1). The restrictor capillary was attached to the manifold with a PEEK ferrule and nut by using a piece of 0.008″ i.d. PEEK tubing as a sleeve. The attachment could be tightened and loosened several times without damage to any of the PEEK parts. The no-frit end of the restrictor was coupled to the SFC analytical column with a 1/16″ zero dead volume butt connector (SGE). The sample inlet could be removed by hand without removing the APCI manifold, and allowed a rapid change of the position of the frit end of the restrictor capillary in the vaporizer tube or replacement of a damaged restrictor. No additional devices were installed to heat the restrictor because it was assumed that a high enough temperature could be achieved with the APCI-vaporizer (T_{max} = 600°C) and preheated sheath and auxiliary gases. The preheating of APCI gases (N_2) was obtained by placing the poly(tetrafluoroethylene) gas-tube coils between the SFC oven outer wall and the oven cover. Introduction of the reactant ion solvent vapor into the APCI source was performed by leading the sheath gas line through a 200-mL solvent bottle that contained 50 mL methanol (gradient grade, Merck, Darmstadt, Germany), isopropanol (gradient grade, Merck), water (MilliQ PLUS, Millipore Corp., Bedford, MA), or 0.5% ammonium hydroxide (p.a.-plus, Riedel-de Haën, Germany) in methanol, and the inlet tube was just beyond the solvent surface. The reactant ion solvent container was kept at ambient temperature.

The MS was operated in the full scan mode by using quadrupole 1 for scanning; quadrupoles 2 and 3 were operated in *rf*-only mode. Positively charged ions with *m/z* values of 450–950 were scanned with a scan time of 0.7 s. The corona needle current was set at 5.0 µA. Tuning of the MS was accomplished in the electrospray mode by the autotune procedure by using a mixture of horse skeletal muscle apomyoglobin (Sigma, St. Louis, MO) and L-methionyl-arginyl-phenylalanyl-alanine acetate·H_2O as a tuning solution. After changing the system to the (APCI)MS mode, it was necessary to retune only the values for tube lense and APCI-capillary voltage for the analyte molecules. The voltages were optimized for triacylglycerols (3 × 8:0, *rac*-18:1-

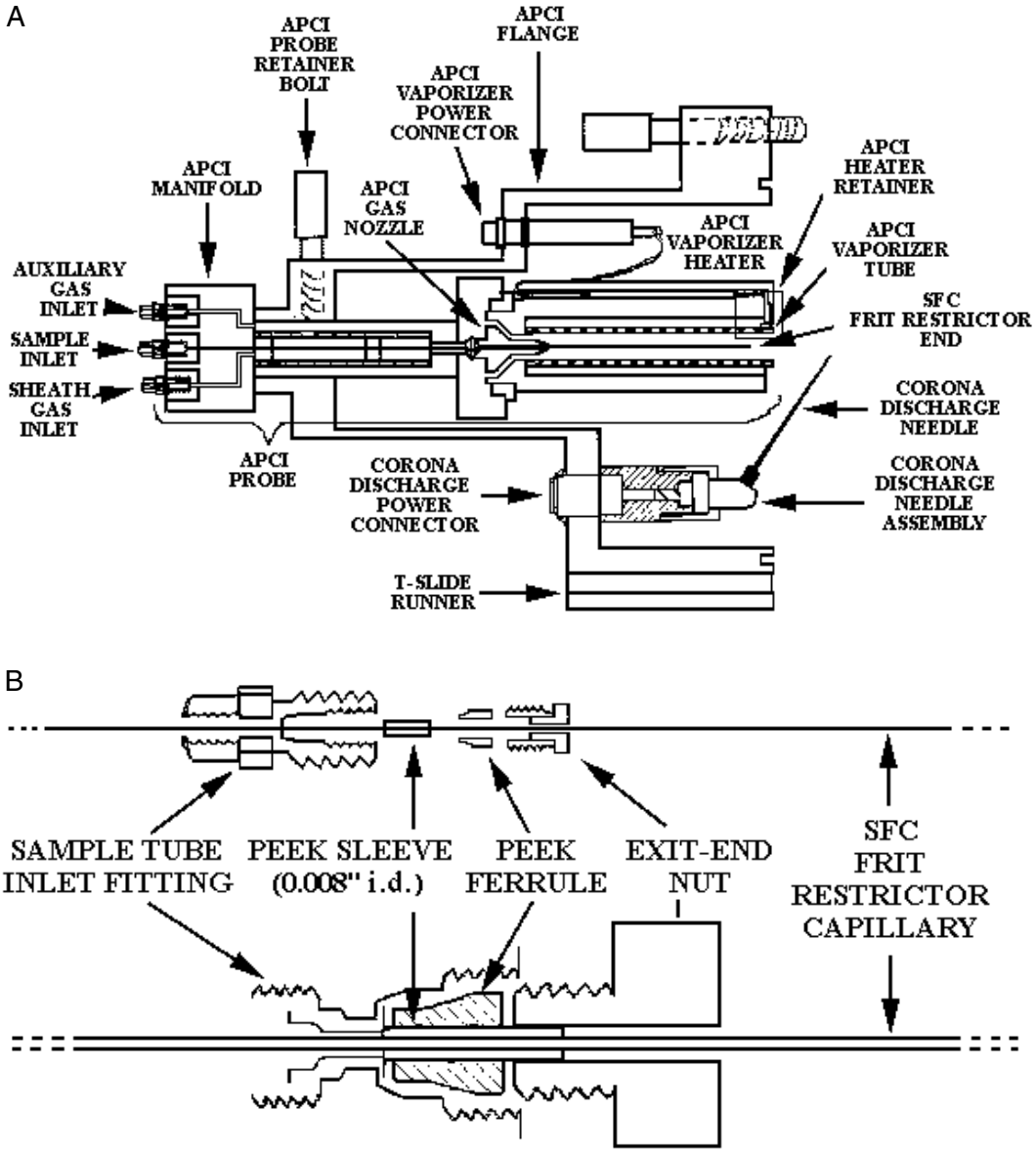

FIG. 1. (A) The position of supercritical fluid chromatography (SFC) frit restrictor in liquid chromatography–atmospheric pressure chemical ionization–mass spectrometry [LC–(APCI)MS] interface (Finnigan MAT, San Jose, CA) utilized in capillary SFC-(APCI)MS, and (B) the attachment of the SFC restrictor to the APCI manifold (reproduced and modified by permission of Finnigan MAT).

18:1-16:0 and *rac*-18:1-18:1-18:3n-6) by loop injection technique using methanol-isopropanol (3:2, vol/vol) as the eluent with a flow rate of 0.6 mL/min.

RESULTS AND DISCUSSION

The first attempts to produce a mass spectrum of triacylglycerol reference components by capillary SFC-(APCI)MS were accomplished by placing the frit restrictor end at the same level as the APCI gas nozzle outlet, or pushing it 2 mm forward or 1 mm backward [in LC–(APCI)MS operation, the sample capillary head is 1 mm beyond the nozzle outlet]. The SFC was operated in timed/dynamic split injection mode with an SB-Octyl-50 column, and water was used as the reactant ion solvent. No peaks were seen in the reconstructed ion chromatogram (RIC) at any sheath gas or auxiliary gas flow rates. Efficient ionization of triacylglycerols and reasonable resolution of the chromatographic peaks was not achieved until the frit restrictor end was pushed 6.0 cm forward from the level of the APCI gas nozzle head. No visual bending of the restrictor capillary was observed. The original function of the sheath gas as a nebulizer in LC–(APCI)MS operation was no longer valid in the system. SFC remains a good option to introduce column effluent in the APCI source because the presence of pure CO_2 will ensure that the effluent is in the gaseous phase when exiting the frit restrictor as a fine spray. In addition, the column flow rate does not appear to be a problem. Therefore, the function of sheath gas as a nebulizer is not so important

in capillary SFC operation, but it seemed to be the best way to introduce the reactant ion solvent in the APCI source.

This design was tested by injecting a cloudberry oil sample (10.2 mg/mL) with methanol as the reactant ion solvent and provided an acceptable RIC at the first attempt. The column was changed to an SB-Cyanopropyl-25 column, 20 m in length, to achieve separation according to acyl carbon number and degree of unsaturation (24,25). The APCI-gas flow rates affected the overall sensitivity, whereas the vaporizer and APCI-capillary temperatures principally affected the fragmentation of the triacylglycerols. The optimized values for APCI-parameters with the instrumentation used were as follows: sheath gas pressure 15 psi, auxiliary gas flow rate 5 mL/min, vaporizer temperature 425°C, and APCI-capillary temperature 200°C. Higher vaporizer temperatures (>500°C) resulted in burning of the polyimide coating of the restrictor capillary and destruction of the frit section. According to the authors' optimization experiences, it seems that the need of an extra heating device may be over-emphasized in capillary SFC–(APCI)MS. However, in all other studies concerning SFC–(APCI)MS, other manufacturers' LC–APCI-interfaces have been utilized.

The optimal distance between the frit and the corona needle was found to be 2.2–2.4 cm for efficient molecule transportation to the ionization region. In this position, the frit end was 0.7–0.9 cm inside the APCI-vaporizer tube; thus, the auxiliary gas could still effectively focus the SFC effluent spray and provide enough time for the triacylglycerols to be volatilized at the temperature of the vaporizer tube. The injection of 4.2 μg/mL of *sn*-16:0-18:1-16:0 resulted in a peak with S/N = 5 in the RIC when methanol was used as the reactant ion solvent. This corresponded to the detection of approximately 200 pg of the compound when the injection volume and dynamic split ratio were taken into account.

Four different reactant ion solvents, *viz.,* methanol, isopropanol, water, and 0.5% ammonium hydroxide in methanol, were tested to achieve abundant ions to provide information on the molecular weight as well as on the fatty acid moieties of triacylglycerols. The best overall sensitivity and the most stable ion formation were achieved with methanol, whereas the lowest ion abundances were formed by isopropanol. The mass spectra of triacylglycerols exhibited abundant $[M + H]^+$ and $[M - RCOO]^+$ ions with methanol, isopropanol, and water as the reactant ion solvent. In addition to $[M + H]^+$ and $[M - RCOO]^+$ ions, methanol also yielded $[M + 18]^+$ molecular adduct ions. $[M + H]^+$ ions were not usually detected for triacylglycerols with only one double bond, and for triacylglycerols with two double bonds, the abundance of $[M + H]^+$ ions was low (1.0–3.0% in relative abundance). Ammonium hydroxide in methanol mostly yielded $[M + 18]^+$ ions and abundant $[M - RCOO]^+$ ions, whereas only a low abundance of $[M + H]^+$ ions was obtained from molecules that typically have four or more double bonds in the acyl chains. The use of ammonium hydroxide might be an advantage compared with the ionization properties of other reactant ion solvents tested, especially in the analysis of relatively saturated triacylglycerol mixtures. Water could be a good alternative for methanol; however, ion formation was somewhat less stable and the water quality available was a problem. In principle, each of the reactant ion solvents tested could be applied for adequate ionization of triacylglycerols by SFC–(APCI)MS. For the purpose of berry oils, methanol was selected for further studies.

The analyses of two reference mixtures, containing different regioisomers of triacylglycerols, revealed that the abundance of the $[M + H]^+$ ion increased with increasing number of double bonds in the triacylglycerol acyl chains (Table 1). The results indicate that double bonds might stabilize the triacylglycerol and thus reduce fragmentation similarly as in LC–(APCI)MS (11). A fatty acid moiety in the *sn*-2 position was found to form less abundant $[M - RCOO]^+$ ions than in the *sn*-1/3 positions (Fig. 2). $[M - RCOO]^+$ and $[M + H]^+$ ion

TABLE 1
Ion Species of Triacylglycerols Produced by Capillary SFC-(APCI)MS

Triacylglycerol	$[M + H]^+$ *m/z* (abundance)[a]	$[M + 18]^+$ *m/z* (abundance)[a]	$[M - RCOO]^+$ *m/z* (abundance)[a] [ion]		
rac-16:0-16:0-18:1	833.9 (1.4)	850.9 (1.9)	577.7 (100.0) $[M - 16:0]^+$	551.6 (79.2) $[M - 18:1]^+$	
sn-16:0-18:1-16:0	833.9 (<1.0)	850.8 (1.3)	577.7 (100.0) $[M - 16:0]^+$	551.6 (41.1) $[M - 18:1]^+$	
rac-16:0-16:0-18:2	831.7 (1.6)	848.7 (1.5)	575.8 (70.0) $[M - 16:0]^+$	551.6 (100.0) $[M - 18:2]^+$	
rac-16:0-18:1-18:1	859.8 (2.1)	876.8 (2.1)	603.7 (47.5) $[M - 16:0]^+$	577.6 (100.0) $[M - 18:1]^+$	
sn-18:1-16:0-18:1	859.8 (2.9)	877.0 (1.3)	603.7 (16.3) $[M - 16:0]^+$	577.6 (100.0) $[M - 18:1]^+$	
sn-18:0-18:2-18:0	887.8 (<1.0)	904.9 (1.8)	607.6 (40.9) $[M - 18:2]^+$	603.5 (100.0) $[M - 18:0]^+$	
rac-18:0-18:1-18:2	885.7 (25.7)	902.8 (4.7)	605.7 (76.1) $[M - 18:2]^+$	603.6 (34.2) $[M - 18:1]^+$	601.8 (100.0) $[M - 18:0]^+$
rac-18:1-18:1-18:3n-6	881.9 (66.2)	898.8 (4.0)	603.5 (100.0) $[M - 18:3]^+$	599.6 (77.8) $[M - 18:1]^+$	
sn-18:1-18:3n-6-18:1	881.9 (53.9)	898.8 (3.0)	603.6(62.7) $[M - 18:3]^+$	599.5 (100.0) $[M - 18:1]^+$	
rac-18:1-18:2-20:0	913.8 (36.0)	930.8 (2.1)	633.8 (53.5) $[M - 18:2]^+$	631.6 (59.9) $[M - 18:1]^+$	601.5 (100.0) $[M - 20:0]^+$

[a]Average of two chromatographic runs.

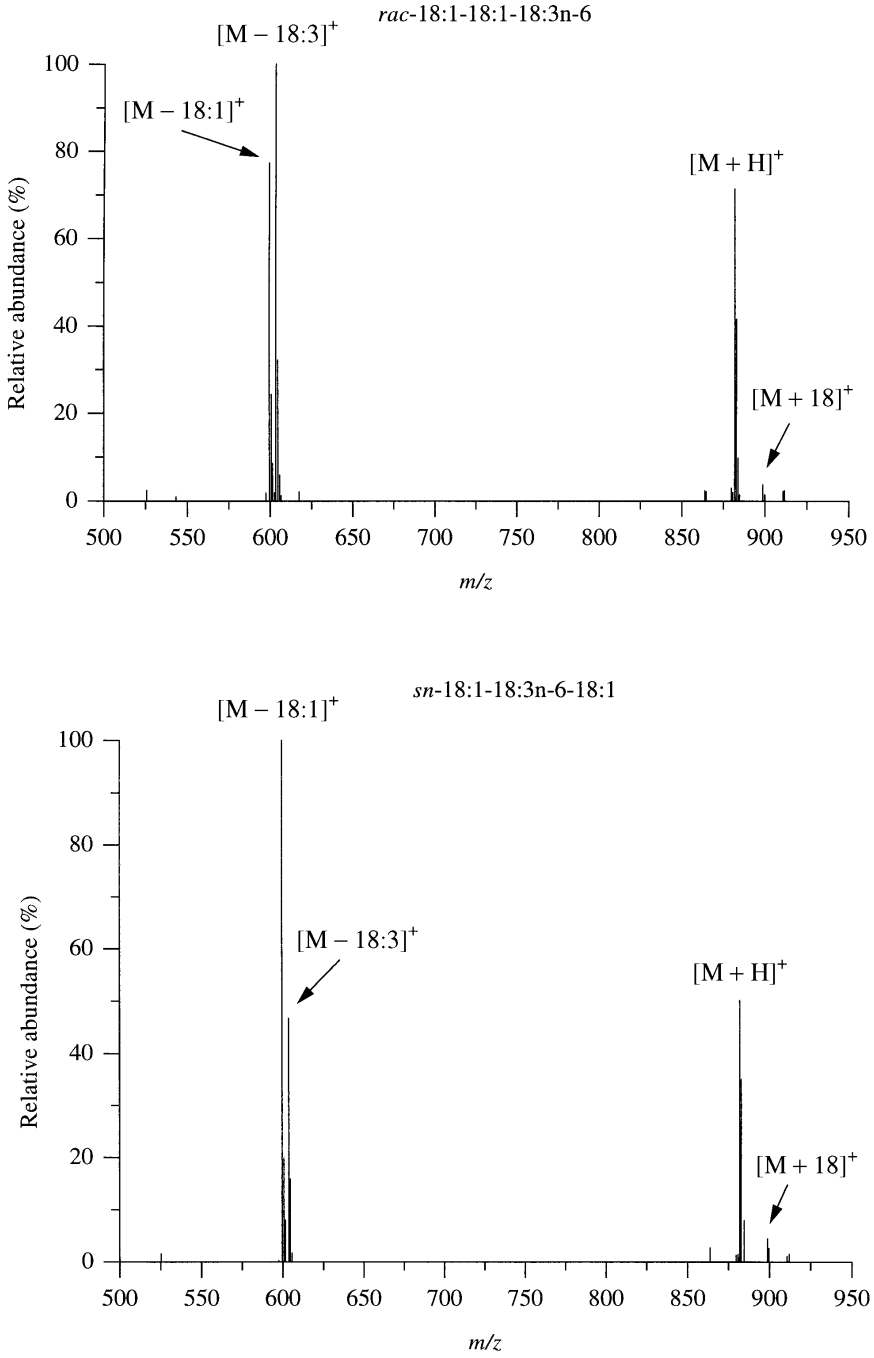

FIG. 2. The mass spectra of regioisomeric triacylglycerols achieved by capillary SFC–(APCI)MS. See Figure 1 for abbreviations.

abundances depended on the molecular environment; in addition to the regiospecific positions of fatty acids, ion abundances were affected by the acyl chainlength and the number of double bonds of the fatty acid moieties. The *m/z* values of the $[M - RCOO]^+$ ions define the molecular association of the fatty acids of a triacylglycerol. Furthermore, dependence of the $[M - RCOO]^+$ ion abundance on the regiospecific position of a fatty acid moiety provides additional structural information of triacylglycerols. However, when the SFC separation method is used, a single chromatographic peak may include four triacylglycerols. If the co-eluting triacylglycerols do not have abundant common $[M - RCOO]^+$ ions, then the regioisomeric composition of a triacylglycerol can be estimated.

Figure 3 presents the RIC of the oils studied. The sensitivity was comparable to or slightly better than that with FID (24). The SFC–(APCI)MS installation used did not affect the chromatographic resolution. In addition, no baseline drift was observed (the final pressure of the chromatographic programming was 362 atm). The mass-spectrum data obtained from each of the chromatographic peaks allowed the structural

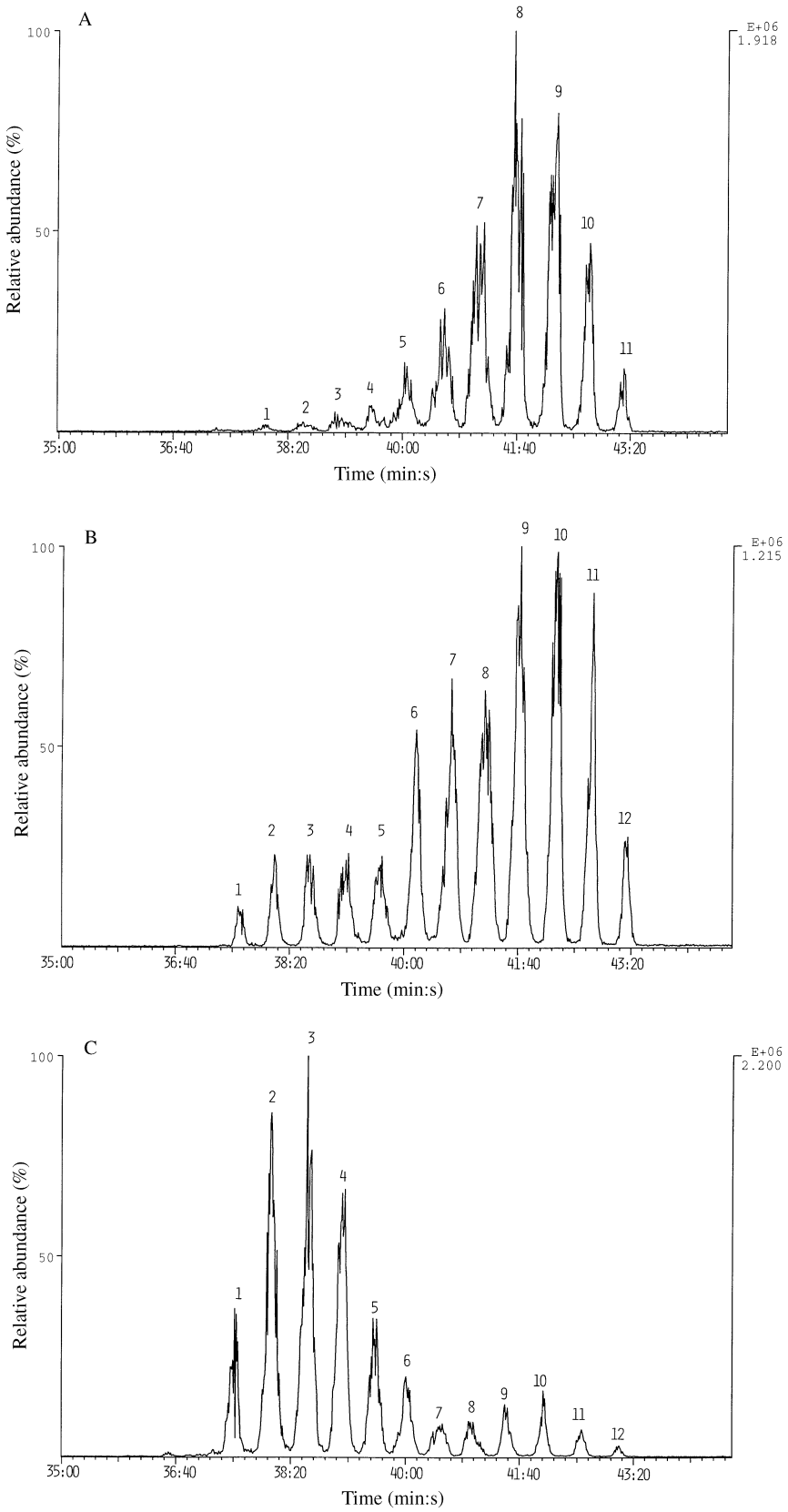

FIG. 3. Reconstructed ion chromatograms (RIC) of the triacylglycerols of (A) cloudberry seed oil, (B) sea buckthorn seed oil, and (C) sea buckthorn pulp oil, obtained by capillary SFC on a 20 m × 50 μm i.d. SB-Cyanopropyl-25 column combined with (APCI)MS detection. (No smoothing was used for RIC.) See Figure 1 for abbreviation.

compositions of the triacylglycerols to be established (Table 2). The $[M + H]^+$ ion provided the molecular weight information on a triacylglycerol, whereas the $[M - RCOO]^+$ ion gave information on the fatty acid residues. In the most favorable case, the regiospecific positions of the fatty acid moieties of a triacylglycerol could be judged. For example, the main fatty acid combination of the triacylglycerol 50:1 in cloudberry seed oil was judged to be *sn*-16:0-18:1-16:0. Unfortunately, this kind of identification was not always possible because one $[M + H]^+$ ion can represent a mixture of isobaric triacylglycerols that consist of more than three different fatty acid moieties. Figure 4 shows an example of the mass spectra of the chromatographic peak representing the triacylglycerols 52:5, 54:4, and 56:3 in each oil studied. There were clear differences, both at the molecular ion region ($m/z > 850$) and at region of the $[M - RCOO]^+$ ions ($m/z < 650$), indicating differences in the amounts of the triacylglycerols as well as in the fatty acid compositions. The suggested fatty acid combinations of the triacylglycerols of sea buckthorn seed and pulp oil are presented in Table 3. In general, the main molecular weight species of triacylglycerols (52:3-52:5, 54:4-54:8) were the same in both cloudberry seed oil and sea buck-

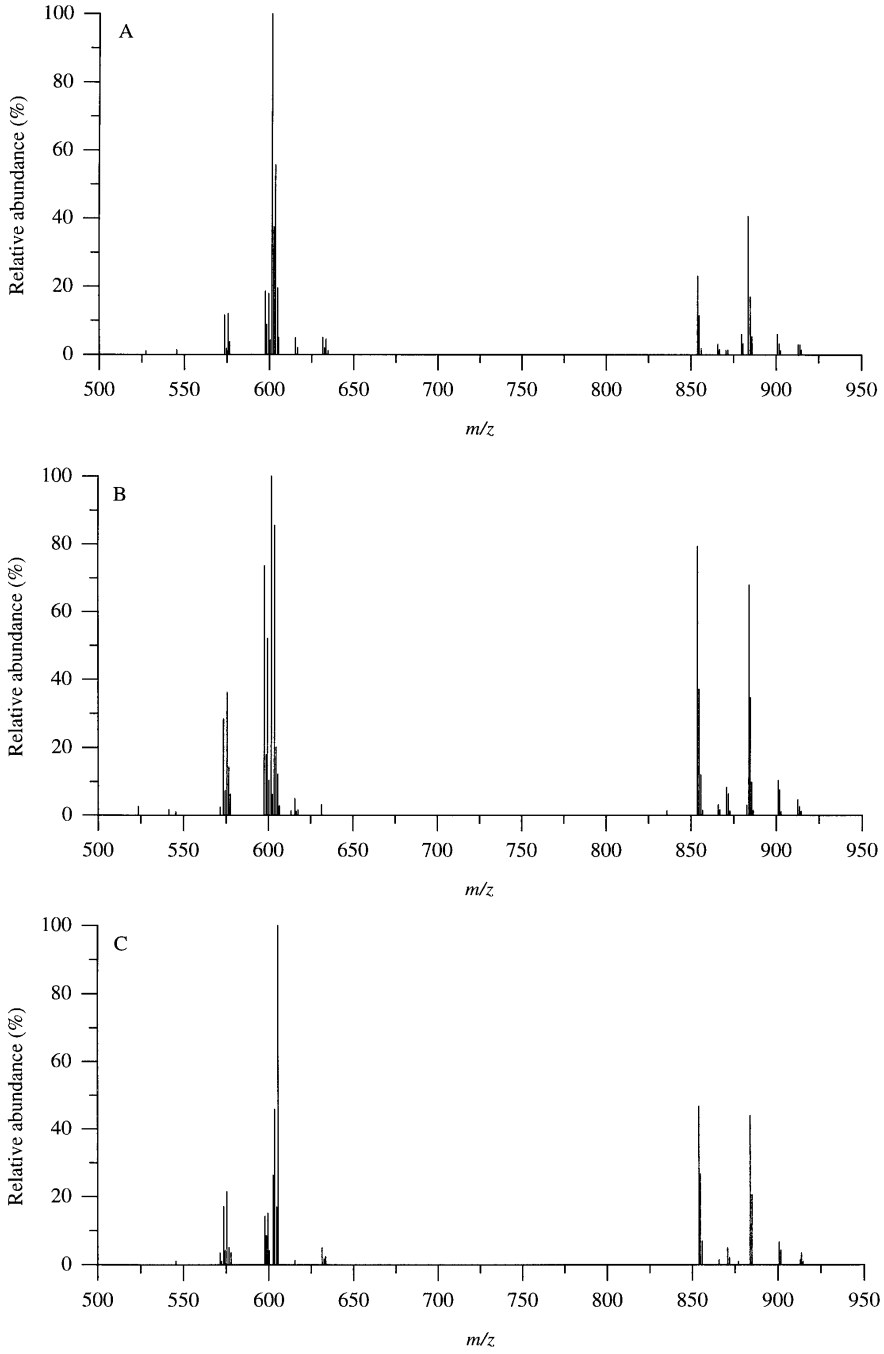

FIG. 4. Mass spectra of the chromatographic peak that contained triacylglycerols 52:5, 54:4, and 56:3 of (A) cloudberry seed oil, (B) sea buckthorn seed oil, and (C) sea buckthorn pulp oil.

TABLE 2
SFC–(APCI)MS Data, Identification, and Proposed Fatty Acid Combinations of Cloudberry Seed Oil Triacylglycerols

Peak no.	$[M + H]^+$ *m/z*(abund.)	Triacyl-glycerol	$[M - RCOO]^+$ *m/z*(abundance)[ion][a]						Molecular structure[b,c]
1	803.7(—)	48:2	575.6(100)[M – 14:0]$^+$	*549.7(21.7)*[M – 16:1]$^+$	547.8(14.8)[M – 16:0]$^+$	523.6(3.4)[M – 18:2]$^+$			**14:0-16:0-18:2**, 16:0-16:1-16:1
	833.8(—)	50:1	577.4(41.9)[M – 16:0]$^+$	551.6(14.3)[M – 18:1]$^+$	*549.7(21.7)*[M – 18:0]$^+$				16:0-16:0-18:1
2	801.7(2.1)	48:3	573.5(43.2)[M – 14:0]$^+$	*547.6(10.7)*[M – 16:1]$^+$	545.6(3.6)[M – 16:0]$^+$	523.6(3.5)[M – 18:3]$^+$			16:1-16:1-16:1, 14:0-16:0-18:3
	831.7(1.0)	50:2	603.7(100)[M – 14:0]$^+$	577.6(40.0)[M – 16:1]$^+$	575.5(81.2)[M – 16:0]$^+$	551.6(18.4)[M – 18:2]$^+$	549.5(25.8)[M – 18:1]$^+$	*547.6(10.7)*[M – 18:0]$^+$	16:0-16:1-18:1, 16:0-16:0-18:2, 14:0-18:0-18:2, 14:0-18:1-18:1
	861.8(<1.0)	52:1	605.6(17.2)[M – 16:0]$^+$	579.5(7.8)[M – 18:1]$^+$	577.6(40.0)[M – 18:0]$^+$				16:0-18:0-18:1
3	829.8(6.2)	50:3	601.7(96.9)[M – 14:0]$^+$	*575.6(23.6)*[M – 16:1]$^+$	573.6(30.0)[M – 16:0]$^+$	551.7(7.3)[M – 18:3]$^+$	549.6(8.6)[M – 18:2]$^+$	545.4(2.9)[M – 18:0]$^+$	16:0-16:1-18:2, 16:0-16:0-18:3, 14:0-18:0-18:3
	859.7(2.0)	52:2	603.6(86.6)[M – 16:0]$^+$	577.7(100)[M – 18:1]$^+$	*575.6(23.6)*[M – 18:0]$^+$				16:0-18:1-18:1
4	827.7(6.3)	50:4	*573.6(12.0)*[M – 16:1]$^+$	571.4(2.9)[M – 16:0]$^+$	549.6(6.2)[M – 18:3]$^+$				16:0-16:1-18:3
	857.8(33.4)	52:3	*603.7(33.3)*[M – 16:1]$^+$	601.5(100)[M – 16:0]$^+$	577.7(91.7)[M – 18:2]$^+$	575.7(80.5)[M – 18:1]$^+$	*573.6(12.0)*[M – 18:0]$^+$		16:0-18:1-18:2, 16:1-18:1-18:1, 16:1-18:0-18:2
	887.9(1.8)	54:2	*605.5(26.7)*[M – 18:1]$^+$	*603.7(33.3)*[M – 18:0]$^+$					18:0-18:1-18:1
5	855.7(40.6)	52:4	599.5(62.6)[M – 16:0]$^+$	577.6(15.3)[M – 18:3]$^+$	575.6(23.7)[M – 18:2]$^+$	573.6(15.4)[M – 18:1]$^+$			16:0-18:1-18:3, 16:0-18:2-18:2
	885.8(9.9)	54:3	*603.6(100)*[M – 18:1]$^+$						18:1-18:1-18:1
	915.8(<1.0)	56:2	633.6(3.3)[M – 18:1]$^+$	*603.6(100)*[M – 20:0]$^+$					18:1-18:1-20:0
6	853.9(23.1)	52:5	*599.6(18.0)*[M – 16:1]$^+$	597.6(18.6)[M – 16:0]$^+$	575.6(12.0)[M – 18:3]$^+$	573.5(11.6)[M – 18:2]$^+$			16:1-18:2-18:2, 16:0-18:2-18:3
	883.7(40.6)	54:4	*603.6(55.6)*[M – 18:2]$^+$	*601.5(100)*[M – 18:1]$^+$	*599.6(18.0)*[M – 18:0]$^+$				**18:1-18:1-18:2**, 18:0-18:2-18:2
	914.1(2.9)	56:3	633.6(4.6)[M – 18:2]$^+$	631.5(5.1)[M – 18:1]$^+$	*603.6(55.6)*[M – 20:1]$^+$	*601.5(100)*[M – 20:0]$^+$			18:1-18:2-20:0, 18:1-18:1-20:1
7	851.7(<1.0)	52:6	*597.7(5.7)*[M – 16:1]$^+$	595.5(3.7)[M – 16:0]$^+$	573.5(3.2)[M – 18:3]$^+$				16:0-18:3-18:3
	881.9(100)	54:5	*603.6(18.4)*[M – 18:3]$^+$	*601.6(69.6)*[M – 18:2]$^+$	*599.7(70.0)*[M – 18:1]$^+$	*597.7(5.7)*[M – 18:0]$^+$			**18:1-18:2-18:2**, 18:1-18:1-18:3, 18:0-18:2-18:3
	911.8(10.2)	56:4	633.7(1.3)[M – 18:3]$^+$	631.8(4.3)[M – 18:2]$^+$	*603.6(18.4)*[M – 20:2]$^+$	*601.6(69.6)*[M – 20:1]$^+$	*599.7(70.0)*[M – 20:0]$^+$		18:2-18:2-20:0
8	879.8(100)	54:6	*601.6(28.4)*[M – 18:3]$^+$	*599.5(81.4)*[M – 18:2]$^+$	*597.5(37.0)*[M – 18:1]$^+$				18:1-18:2-18:3, 18:2-18:2-18:2
	909.8(9.5)	56:5	*631.6(1.3)*[M – 18:3]$^+$	*629.5(3.5)*[M – 18:2]$^+$	*627.7(1.4)*[M – 18:1]$^+$	*601.6(28.4)*[M – 20:2]$^+$	*599.5(81.4)*[M – 20:1]$^+$	*597.5(37.0)*[M – 20:0]$^+$	18:2-18:2-20:1, 18:2-18:3-20:0,18:1-18:2-20:2, 18:1-18:3-20:1
	939.8(1.3)	58:4	*631.6(1.3)*[M – 20:2]$^+$	*629.5(3.5)*[M – 20:1]$^+$	*627.7(1.4)*[M – 20:0]$^+$				—[d]
9	877.8(100)	54:7	*599.7(48.7)*[M – 18:3]$^+$	*597.6(60.7)*[M – 18:2]$^+$	*595.7(14.4)*[M – 18:1]$^+$				**18:2-18:2-18:3**, 18:1-18:3-18:3
	907.9(9.4)	56:6	629.7(1.8)[M – 18:3]$^+$	627.6(1.6)[M – 18:2]$^+$	*599.7(48.7)*[M – 20:2]$^+$	*597.6(60.7)*[M – 20:1]$^+$	*595.7(14.4)*[M – 20:0]$^+$		**18:2-18:2-20:2**, **18:2-18:3-20:1**, 18:3-18:3-20:0
10	875.8(100)	54:8	*597.6(89.9)*[M – 18:3]$^+$	*595.6(39.3)*[M – 18:2]$^+$					18:2-18:3-18:3
	905.9(7.0)	56:7	627.7(1.6)[M – 18:3]$^+$	*597.6(89.9)*[M – 20:2]$^+$	*595.6(39.3)*[M – 20:1]$^+$				18:3-18:3-20:1
11	873.7(100)	54:9	*595.5(81.6)*[M – 18:3]$^+$						18:3-18:3-18:3
	903.7(1.0)	56:8	*595.5(81.6)*[M – 20:2]$^+$						—[d]

[a] *m/z* values and ion abundances typed in italic may be common ions with the triacylglycerols eluting in the same peak.
[b] Fatty acid combinations typed in bold are regarded as the main combinations of the triacylglycerol.
[c] The description does not make a judgment of the regiospecific positions of the fatty acid residues.
[d] Molecular structure could not be evaluated.

TABLE 3
Fatty Acid Combinations of the Triacylglycerols of Sea Buckthorn Seed and Pulp Oil Based on the SFC-(APCI)MS Data

Peak no.	Triacyl-glycerol	Sea buckthorn seed oil[a,b]	Sea buckthorn pulp oil[a,b]
1	48:1	16:0-16:0-16:1	16:0-16:0-16:1
2	48:2	16:0-16:1-16:1	16:0-16:1-16:1
	50:1	16:0-16:0-18:1	16:0-16:0-18:1
3	48:3	16:1-16:1-16:1	16:1-16:1-16:1
	50:2	**16:0-16:1-18:1**, 16:0-16:0-18:2, 16:1-16:1-18:0, 14:0-18:1-18:1, 14:0-18:0-18:2	**16:0-16:1-18:1**, 16:1-16:1-18:0, 16:0-16:0-18:2
	52:1	Not detected	16:0-18:0-18:1
4	50:3	16:1-16:1-18:1, 16:0-16:1-18:2, 16:0-16:0-18:3, 14:0-18:1-18:2	16:1-16:1-18:1, 16:0-16:1-18:2
	52:2	16:0-18:1-18:1	**16:0-18:1-18:1**, 16:1-18:0-18:1
5	50:4	16:0-16:1-18:3, 16:1-16:1-18:2	**16:0-16:1-18:3**, 16:1-16:1-18:2
	52:3	16:0-18:1-18:2, 16:1-18:1-18:1, 16:0-18:0-18:3	16:1-18:1-18:1, 16:0-18:1-18:2,16:1-18:0-18:2
	54:2	18:0-18:1-18.1, 18:0-18:0-18:2	18:0-18:1-18:1
6	52:4	16:0-18:2-18:2, 16:0-18:1-18:3	16:0-18:2-18:2, 16:0-18:1-18:3, 16:1-18:1-18:2
	54:3	**18:1-18:1-18:1**, 18:0-18:1-18:2	18:1-18:1-18:1
	56:2	Not detected	18:1-18:1-20:0
7	52:5	**16:0-18:2-18:3, 16:1-18:2-18:2**, 16:1-18:1-18:3	**16:0-18:2-18:3**, 16:1-18:1-18:3, 16:1-18:2-18:2
	54:4	**18:1-18:1-18:2**, 18:0-18:2-18:2	**18:1-18:1-18:2**,18:0-18:2-18:2
	56:3	18:1-18:1-20:1	18:1-18:2-20:0,18:1-18:1-20:1
8	52:6	**16:0-18:3-18:3**, 16:1-18:2-18:3	16:0-18:3-18:3
	54:5	**18:1-18:2-18:2,18:1-18:1-18:3**, 18:0-18:2-18:3	**18:1-18:2-18:2**, 18:0-18:2-18:3
	56:4	18:2-18:2-20:0	18:2-18:2-20:0
9	54:6	**18:1-18:2-18:3**, 18:2-18:2-18:2	18:1-18:2-18:3, 18:2-18:2-18:2
	56:5	—[c]	18:2-18:2-20:1
10	54:7	**18:2-18:2-18:3**, 18:1-18:3-18:3	**18:2-18:2-18:3**, 18:1-18:3-18:3
	56:6	—[c]	Not detected
11	54:8	18:2-18:3-18:3	18:3-18:2-18:3
	56:7	—[c]	—[c]
12	54:9	18:3-18:3-18:3	18:3-18:3-18:3
	56:8	—[c]	Not detected

[a]Fatty acid combinations do not make a judgment of the regiospecific positions.
[b]Fatty acid combinations typed in bold are regarded to be the main combinations of the triacylglycerols.
[c]Molecular structure could not be evaluated.

thorn seed oil, with the exception that triacylglycerols with 52 acyl carbons were less abundant in cloudberry seed oil. However, differences in the combinations of fatty acid moieties of the triacylglycerol molecular species were found. The sea buckthorn pulp oil was different from the seed oils in composition; the most abundant triacylglycerols were those with 48–52 acyl carbons and three or fewer double bonds in the acyl chains, excluding the triacylglycerol 48:3.

These results confirmed the tentative identification of molecular weight species and the suggested elution patterns of the triacylglycerols of the oils presented in a previous study with capillary SFC–FID (24). The general elution order of triacylglycerols by reversed-phase LC is according to the increasing value of the equivalent carbon number (ECN; ECN = ACN − 2*n*, where ACN is the acyl carbon number of the triacylglycerol and *n* is the combined number of double bonds in the acyl chains). Triacylglycerols with the same ECN values typically elute in the same region in LC. In capillary SFC on the 25% cyanopropyl/25% phenyl/50% methylpolysiloxane stationary phase, triacylglycerols with the same ACN + 2*n* values coeluted, and the general elution order of triacylgycerols was according to increasing values of ACN + 2*n*.

This study introduces a fast and simple capillary SFC interface with MS. The SFC–(APCI)MS apparatus was applied successfully to the analysis of natural triacylglycerol mixtures of berry oils. Mass-spectrum data provided information not only on the molecular weight species, but also on the molecular species composition of triacylglycerols. In addition, the formation of $[M - RCOO]^+$ and $[M + H]^+$ ions was affected by the regiospecific distribution of fatty acyl residues; this

will be a topic for future studies. In general, SFC–(APCI)MS may offer some advantages compared with LC–(APCI)MS. Several LC applications require a gradient elution, that results in continuous change in the reactant ion composition in the ionization chamber, whereas the ionization conditions will be nearly constant during the density/pressure programming in capillary SFC. This may be important, for example, in quantitative analysis. In addition to berry oils, the SFC interface with MS could easily be applied for the analysis of other components suitable for capillary SFC.

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