クロマトグラフィーを中心とする機器分析の実際

（株）日東技術情報センター　掘　正典

1. はじめに

クロマトグラフィーは化学に関するあらゆる分野で利用されている分離・分析技術です。機器の取り扱いに不慣れな人や、この手法に習熟していない分析者が試みても、何らかの有益な情報を得ることができる簡便さとパワフルさの両方を備えています。しかし一見、表面的には簡単そうに見えるこの技術も目的に合った情報を得るためのプロセスは複雑な場合が多く、クロマトグラフィーと別の機器を組み合わせ複合技術として扱う場合も少なくありません.

今回はクロマトグラフィーとその周辺の技術を使って得られた分析結果について、て(1）GC を用いる発生ガス分析、（2）はによる天然油脂の分子種解明、を主題として実際の分析例を中心に紹介します。

2. GC を用いる発生ガス分析

ある環境下で試料から発生する化学物質を分析することは、それほど単純ではなく現在では種々の方法が考案、開発されています。その理由の一つは試料のおかれている環境すなわち温度、放置している時間、試料の回りの雰囲気（空気中不活性ガス中あるいは真空中等）が分析目的によって異なるため、一つの分析手法だけで包括してしまうことが事実上不可飴である事があげられます．我々は市販の装置や比較的安価に製作でさるサンプリング装置を製作しこれらに対処してきましたので以下に紹介します.

2．1 フィルム中の残存溶剤の定量

従来から樹脂やフィルム中の低沸点成分の分析にはスタティツクヘッドスペース法やパージアンドトラップを用いるダイナミックヘッドスペース法が用いられてきました。後者の方法は検出感度がスタテック法に比べ 10〜100 倍向上すると言われその代表的な装置が JHS－100 ですが、我々はキュリーポイントパイロライサーを用いてこの種の分析も試みてきました.

図 1 にフィルム中に残存している高沸点溶剤 NMP（N－メチルピロリドン）を、キュリーポイントパイロライサー（機種 JHS-3S 型）GC により分

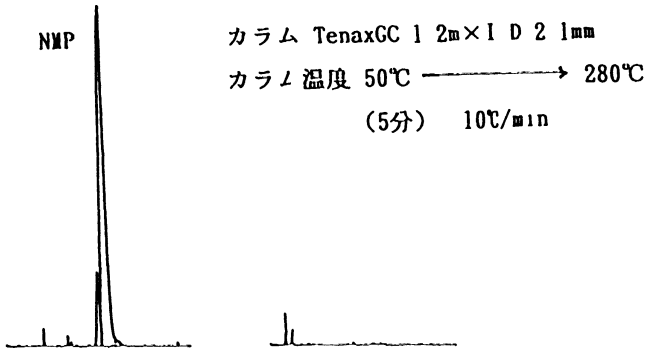


図1 a) 315℃ 5分加熱 b) a)と同一試料を再度加熱して測定

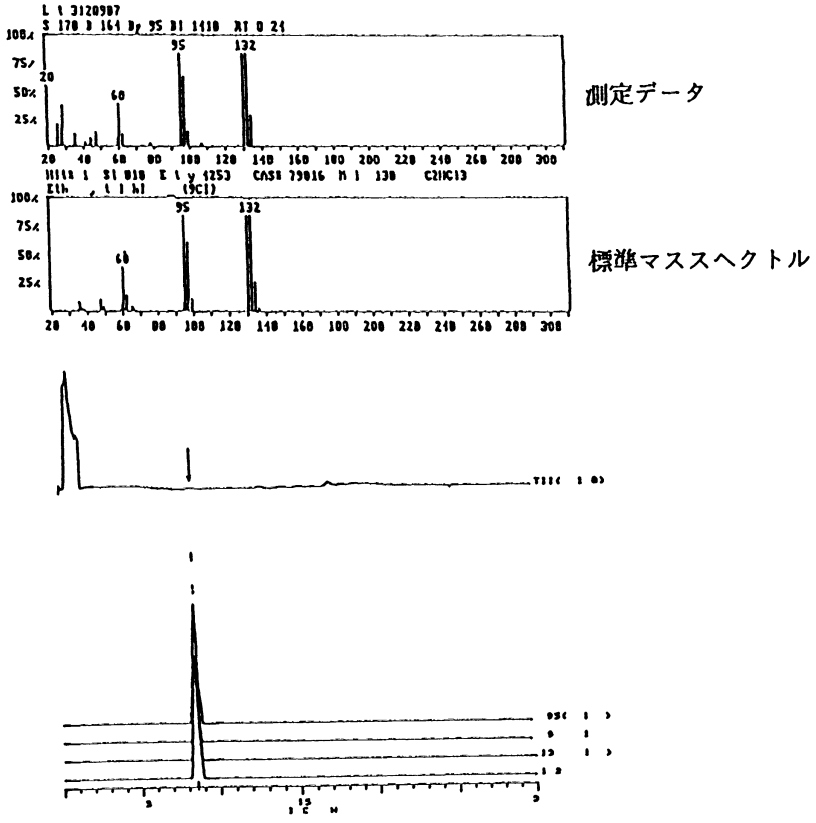


図2

析した時のクロマトグラムを示しました。試料は 315℃で 5 分間加熱され、その間カラムを 50℃に保って生成したガスをカラム内に吸着させ、その後カラム温度を昇温し NMP を溶出させています。分離カラムは TenaxGC を用いていますが、これは同時に吸着管としても作用します。試料の加熱温度は試料があまり分解しない温度でかつ効率よくガスを発生する条件を見いだす必要がありますが、一度条件が決まれば溶媒抽出と比べ簡便で高感度な方法です。本法の場合発生したガスを室温付近で吸着させるため沸点があまり低すぎたり、吸着能力の弱い物質の定量精度は悪くなりますが、この手法が適用できる物質は比較的多く存在しています。

図 2 には同様な方法で樹脂中のトリクロロエチレンを検出した例を示してあります。試料はパイロホイルに包まず直接石英の試料管に入れ 150℃で 10 分間加熱しました。検出器に質量分析計を用いていますので試料の同定がより確実にできマスクロマトグラムから定量も可能です。この試料の場合 ppb レベルでの検出が可能でした。

2.2 害虫防除フエロモンデイパイスからのフエロモン放出速度の測定

農薬による害虫駆除一辺倒から抜け出し作物の被害を許容し得る水準以下に抑えるように害虫の密度を管理する害虫防除が、最近積極的に進められています。その一端を担うものとして性フエロモンを用いる生物的駆除があります。これを実用化させるにはフエロモンを一定期間分解する事なくほぼ一定の量で放出させるフエロモンデイパイスが必要となりますが、デイパイスの評価としてフエロモンの揮散量すなわちフエロモン放出速度を測定することが必要です。

フエロモンは図 3 に示したように酢酸エステルやアルデヒド、アルコール、ケトン等の脂肪族化合物が多くまた高沸点を有するものがほとんどです。従って放出速度を正確に求めるには、これら高沸点物を効率よく捕集するシステムが必要となります。我々はフエロモンを温度や風速などが制御された環境下で吸着剤に捕集しそれを溶媒抽出して GC 分析することにより放出速度を直接的に測定する手法を、デイパイス評価の一つとして用いてきました（図 4 参照)。検出下限はチャノコカクモンハマキの性フエロモンの一種である Z－11－テトラデセニルアセテート（Z－11－TDA）で、捕集時間を 5 時間として 50ng／day と求まっています。図 5 には実際のデイパイスから得られたガスクロマトグラムの一例を示しましたが、

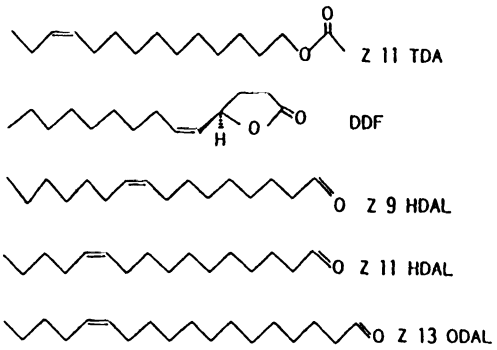
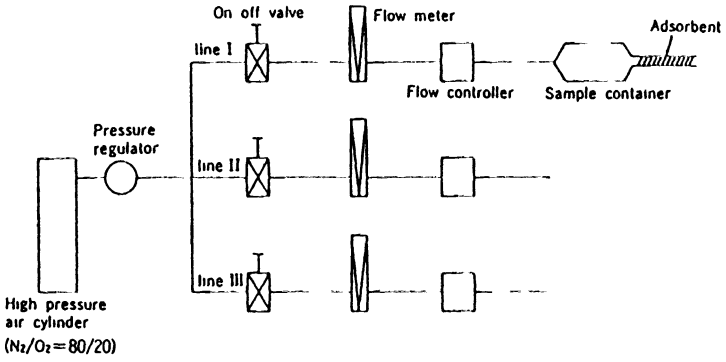
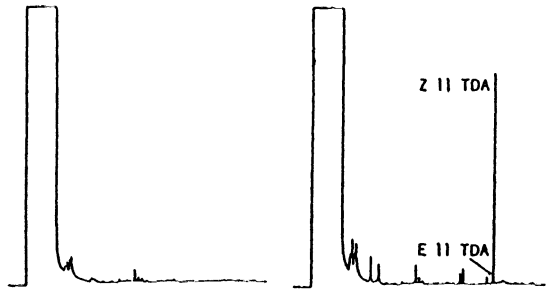


図3　フェロモンの化学構造
Chemical structure of pheromones



捕集装置の概略図
図4　Schematic d agram of air flow apparatus for vapor collection



(A) Blank　(B) Pheromone dispenser

捕集法によって得られたガスクロマトグラム

この手法を使えば野外の環境をシュミレーションしておおよそのフエロモン放出速度を見積もることも可能です。また吸着管を JHS－100 に装着し分析すれば感度は約 1000 倍は向上すると予想されるので、捕集環境を整えれば虫体一匹から放出されるフエロモンの定量も比較的容易にできるかもしれません.

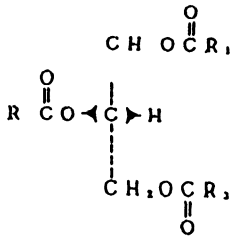
3. LC による天然油脂の分子種解明

トリグリセライド（TG）は天然油脂の主成分で動物の皮下脂肪や植物の種子などに多く存在しています。この分子構造は図 6 に示したように 3 個の脂肪酸が 1 個のグリセリンにエステル結合したものです。したがって理論上、油脂が n 種の脂肪酸で構成されているとき異性体を全く考慮しなくても（N^3+3n^2+2n）／6 個の分子種が考えられます．天然油脂は通常数種類の脂肪酸よりなっているので、その組み合わせよりなる分子種は非常に複雑になります.

これらの分子種解明に最近では逆相系の LC を使うことが多くなっています。これは脂肪酸の総炭素数と二重結合の総数から定義される Partition Number（PN）とよばれる分離因子と TG の保持時間の対数が経験的にはぼ linear な関係になることが発見されたからです（図 7）．この法則と LC/MS を組み合わせれば天然油脂の組成解明に非常に有効であることは既に示したことがあります（試料 1 参照)。しかし脂肪酸組成の複雑な海産油の解明には今のところそのままでは適用が困難であるのが現状です．図 8、図 9 に一例として高度不飽和脂肪酸（PUFA）を多く含む魚油の LC/MS のクロマトグラムと構成脂肪酸のガスクロマトグラムを示しました。魚油に含まれる個々の TG 分子種の存在と量的関係を明らかにするためには少なくとも理論段救が数十万以上のはカラムが必要と言われています．従って現在ではその分析が困難なことから分子種に関する知見がほとんどありません．図 8 のクロマトグラムの画分Ⅰ、Ⅱは油旨のなかでも最も極性の高いフラクションですが、同じ PN を多く含みかつ完全分離が不可億なため分析をさらに進めるうえでは LC/MS のデータの他に分取が必要です。図 10、図 11 に LC－908 を用いて得られたリサイクルはのクロマトグラムと分取した後の分析データを示しました．完全に単離できないまでも精製と分取が同時にできるリサイクルはのメリットは後の工程を考えれば大きいものがあります。

4. 結語

クロマトグラフィーは 1906 年、TsWett らが色素混合物を分離したことより始まりましたが、今現在でも分離、精製の中核をなしています. クロマトグラフィーの技術は今後さらに発達して行くものと思いますがその中でもパイロライサーやリサイクル LC により、今まで限界であったことが少しづつ克服されていくようです.



n^3 異性体を全て考慮

$\frac{n^3+n^2}{2}$ 光学異性体を除く

$\frac{n^3+3n^2+2n}{6}$ 異性体を全く考慮しない

図6 トリグリセライトの分子構造と分子種の数

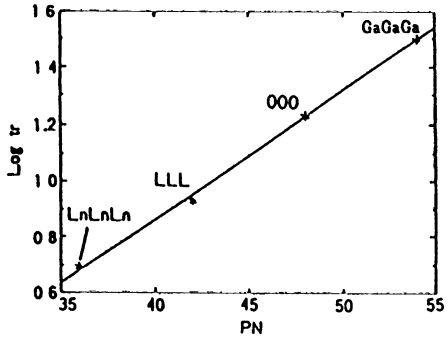


図7 Partition number とトリグリセリトの保持時間との関係
Correlation between partition number and retention time of triacylglycerols
Column ODS Hypersil 100mm × 2 1mm 5μm
Mobile phase CH CN/THF = 80/20
UV detector 210nm

Ln linolenic acid 18 3
L linoleic acid 18 2

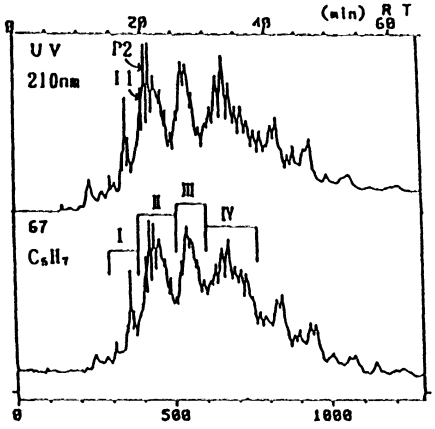


図8 UV and mass chromatograms of fish oil by LC/MS

Columun ODS Hypersil 2 1mm×200mm×2 2 1mm×100mm

Elution solvent (A)acetonitrile (B)acetone 40% ⟶ 60%

UV 210nm Flow rate 0 15ml/min

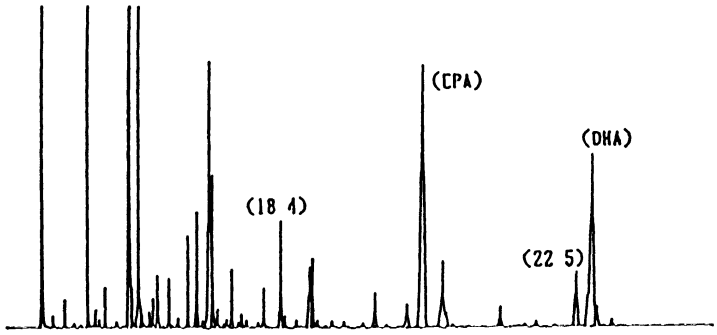


図9 Gas chromatogram of methyl esters of fatty acids from fish oil

Columun DB-23 30m×I D 0 25mm

Columun temp 170℃(2min) ⟶ 220℃

1 5℃/min

表1 Fatty acid composition of fish oil

PUFA	%	FA	%
18 4(n 3)	2 7	14 0	6 4
EPA 20 5(n 3)	14 8	16 0	17 7
DHA 22 6(n-3)	10 1	16 1(n 7)	6 1
22 5(n-3)	2 4	18 1(n 9)	9 3

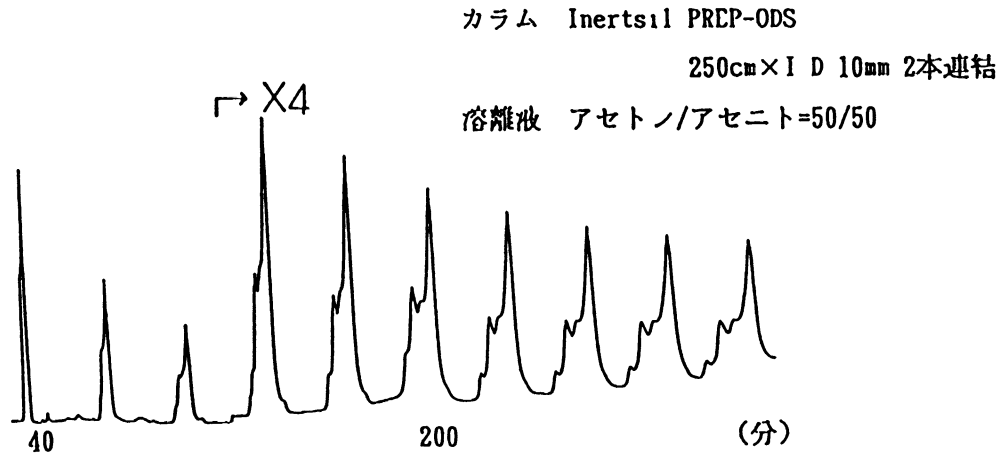


図10　P1のリサイクルLCクロマトグラム

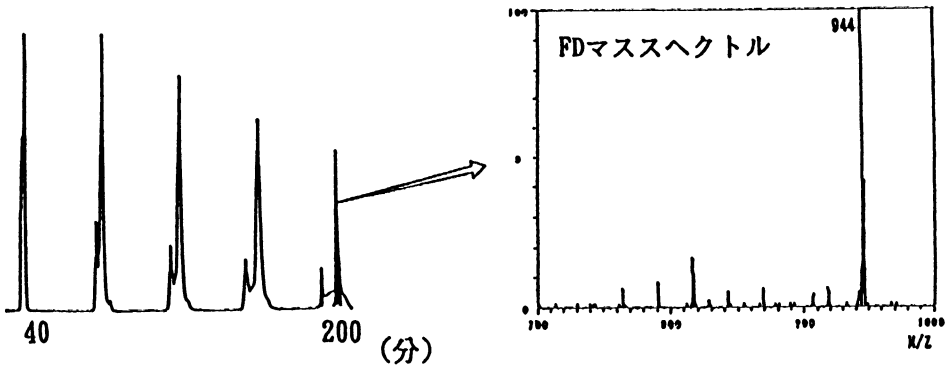


図11　P2のリサイクルLCクロマトグラムと分取物の分析データ

資料 1

Determination of Molecular Species of Triacylglycerols by Reversed Phase Liquid Chromatography/ Double Focussing Mass Spectrometry with a Frit-CI Interface

Masanori Hori. Kenji Sugiura.Yuko Sahashi,I, and Seiji Koike

[質量分析. voI. 39, No. 3, p, I33〜140(1991). 別冊]

MASS SPECTROSCOPY REGULAR PAPERS Vol. 39. No. 3.June 1991

Determination of Molecular Species of Triacylglycerols by Reversed Phase Liquid Chromatography/ Double Focussing Mass Spectrometry with a Frit-CI Interface

Masanori Hori. Kenji Sugiura.Yuko Sahashi,I, and Seiji Koike

(Received January 26, 199l)

This paper demonstrates the application of a combined system of reversed phase liquid chromatography and chemical ionization mass spectrometry with a frit interface (frit-CI LC/MS) for identification of molecular species of triacylglycerols in natural oils. From the studies of fundamental operating conditions of this LC/MS system. we found that the chemical ionization using ammonia as a reagent gas yielded readily detectable ammonium adduct and protonated ions as well as characteristic fragment ions. The intensities of these ions depended considerably on the ion source temperature. The ion source temperature operated from 350 to 400℃ was suitable for both the resolution of chromatograms obtained by the LC/MS and the relative intensities of the molecular ion species. This method enabled to get the identifiable mass spectrum of trieicosapentaenoin (20 : 5-20 : 5-20 : 5 MW=944). while it has been difficult to analyze by the GC/MS. This system was also applied to the compositional analysis of evening primrose oil composed of triacylglycerols having total carbon number of 52 and 54. Mass chromatographic technique as well as mass spectra provided detailed information on the structures of triacylglycerols in the oil.

1. Introduction

Triacylglycerols, main components in natural oils, are esters formed from one glycerol combined with three fatty acids and exist mainly in subcutaneous tissues of animals and seeds of plants. Bacause many kinds of fatty acids exists in nature,
it is expected that there are many molecular species of triacylglycerols in natural oils. Based on a theoretical calculation,[1] when the oil is constructed of *n* kinds of fatty acids, the number of molecular species of triacylglycerols is $(n^3+3n^2+2n)/6$ without thinking of isomers. Therefore the experimental determination of their detailed structure remains the most formidable problem in lipid chemistry. Gas chromatography/mass spectrometry (GC/MS) has been used to study the composition of these complicated oils.[2] However, it is generally not easy to analyze by GC/MS when they have the high boiling points. Reversed phase liquid chromatography/mass spcctrometry equipped with a direct liquid inlet[3~5] or a thermospray interfaces) has been recently used for the studies of lipids. The advantage of LC/MS of triacylglycerols is that it provides not only the mass spectra and mass

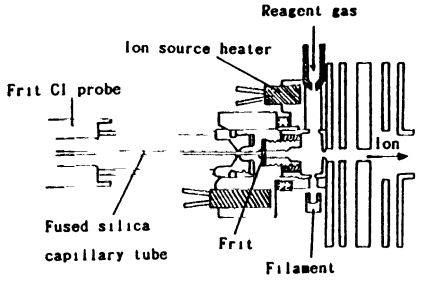


Fig 1 Schematic diagram of frit CI interface

chromatograms but also information of the partition number (PN)[7] defined by the number of double bonds (DB) and that of total carbons (TC) of a triacylglycerol (PN =TC-2DB).

In this paper some Preliminary results are reported on the frit-CI LC/MS of authentic triacylglycerols and evening primrose oil to get information on molecular species of triacylglycerols in natural oils.

2. Experimental

2.I Materials

Triolein and trilinolein from Gasukuro Kogyo lnc. were used without further purification. Evening primrose oil was purchased from a commercial source and it had a purity better than 95%. Trieicosapentaenoin (tri-EPA) was synthesized by lipase esterification of eicosapentaenoic acid and glycerol. Resulting tri-EPA was purified by a FIorisil column. All the samples were stored in closed containers at ～20cC. Tetrahydrofuran of HPLC grade was used within a day or two after openning the bottle. The other chemicals were of analytical reagent grade.

2.2 Apparatus

The HPLC analyses were performed on a Hewlett Packard model l090L liquid chromatograph with a reversed phase column, ODS-Hypersil(100 mmx2.1 mm). The mobile phase composed of acetonitrile and tetrahydrofuran was used in a helium atmosphere. The column was operated at a flow-rate of 0.l5 ml/min and a column temperature of 40℃. Triacylglycerols were dissolved in the mobile phase at 5～50mg/ml. and 1～3μl portions were injected into the column. The eluent *via* a UV detector was introduced into a JOEL JMS-AX505H double focussing mass spectrometer equipped with a firt-CI interface (shown in Fig. I). A pneumatic splitter was used to reduce the flow-rate of the eluent into the ion source to as little as a few A/min. While the reduced eluent permeated through the frit. triacylglycerols and the solvents were evaporated by the ion source heaters. Triacylglycerols were then chemically ionized with the solvent vapor or ammonia as the reagent gas.

All the positive ion mass spectra were obtained at 250 eV of the electron energy and at +3kV of the ion accelerating voltage. A post acceleration detector using a conversion dynode at -10kV was used in order to detect the high mass region sensitively. Full mass spectra (50～l500 mass unit) were recorded every 3 see over the entire elution profile, and the data were processed using a JEOL JMA-DA5000 data system. A Hewlett Packard model 5890A gas chromatograph with a columun DB-23 (30 mxO.25 mm df=0.25pm) was used for GC analysis. and a Hitachi model M-80A double focussing mass spectrometer was used for FD mass spectrometry.

3. Results and Discussion

3.I Fundamental operating conditions of LC/MS

The LC/MS equipped with a direct liquid inlet operated in chemical ioniza-

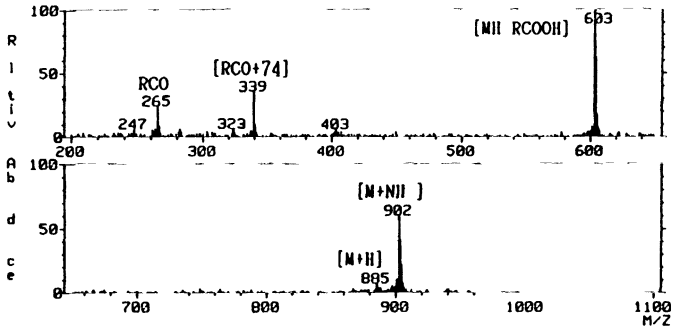


Fig 2 Ammonia CI mass spectrum of triolein obtained by frit-CI LC/MS
Ion source temerature 390℃
Mobile phase acetonitrile–tetrahydrofuran (70 30 isocratic)
Concentration 5 $\mu g/\mu l$ Injection volume 3 μl

tion mode using an elution solvent as the reagent gas have been demonstrated to be
suitable for the identification of triacylglycerols by Kuksis et al.[3] In the mass spectra obtained by this system. the $[MH-RCOOH]^+$ ion of the base pat was used to determine the fatty acid composition of triacylglycerols. However, the other characteristic ions including the RCO', $[RCO+74]^+$, and $[M+H]^+$ ions were observed with unsufficient intensity for analysis of the mass spectra. Murata[8] employed gas chromatography/chemical ionization mass spectrometry (GC/CIMS) with ammonia to determine the composition of triacylglycerols. By this method, the $[MH-RCOOH]^+$ and $[M+NH4]^+$ ions were recorded intensely enough to be used for the structural study. Therefore., it is expected that the liquid chromatography in combination with ammonia CI mass spectrometry can provide more information than use of an elution solvent as the reagent gas.

Figure 2 shows the ammonia CI mass spectrum of triolein measured by the frit-CI LC/MS at 390℃ of the ion source temperature. When ammonia was introduced into the ion source, the ammonium adduct ion $[M+NH4]^+$ and the protonated ion $[M+H]^+$ were observed as the molecular ion species. In the GC/CIMS using ammonia Murata[8] reported that only the $[MHNRCCN3H]^+$ ion was observed as the fragment ions, and no ions were recorded in the low mass region. However under the LC/MS conditions in Figure 2, the $[M+NH4]^+$ ion at m/I 902 and the fragment ions such as the RCO' at m/I 265 and the $[RCO+74]^+$ at m/I 339 were recorded intensely enough to analyze the mass spectrum. The base peak was the $[MH-RCOOH]^+$ ion at m/I 603 and the $[M+NH4]^+$ ion was recorded with relative intensity of 59% against the base peak as shown in Table I. On the other hand, in case of using the elution solvent as the reagent gas, the mass spectrum of triolein showed only the $[M+H]^+$ ion as molecular ion specis with relative intensity of ca. 3% against the $[MH-RCWH]^+$ ion of the base peak ion. The other characteristic ions observed were similar to those of the ammonia CI mass spectrum.

Figure 3 shows the ammonia CI mass

Table 1 Characteristic Ions in Mass Spectra of Triolein and Trilinolein Measured by frit LC/MS

Observed ion species	Relative intensity					
	Triolein			Trilinolein		
	Observed ion (*m/z*)	Frit CI* (%)	Frit CI (NH_3)** (%)	Observed ion (*m/z*)	Frit CI* (%)	Frit CI (NH_3)** (%)
RCO^+	265	14	25	263	22	44
$[RCO+74]^+$	339	18	36	337	9	39
$[MH-RCOOH]^+$	603	100	100	599	100	100
$[M+H]^+$	885	3	6	879	35	28
$[M+NH_4]^+$	902	—	59	896	—	32

Ion source temperature 390℃
* Elution solvent was used as reagent gas
** Ammonia was used as reagent gas

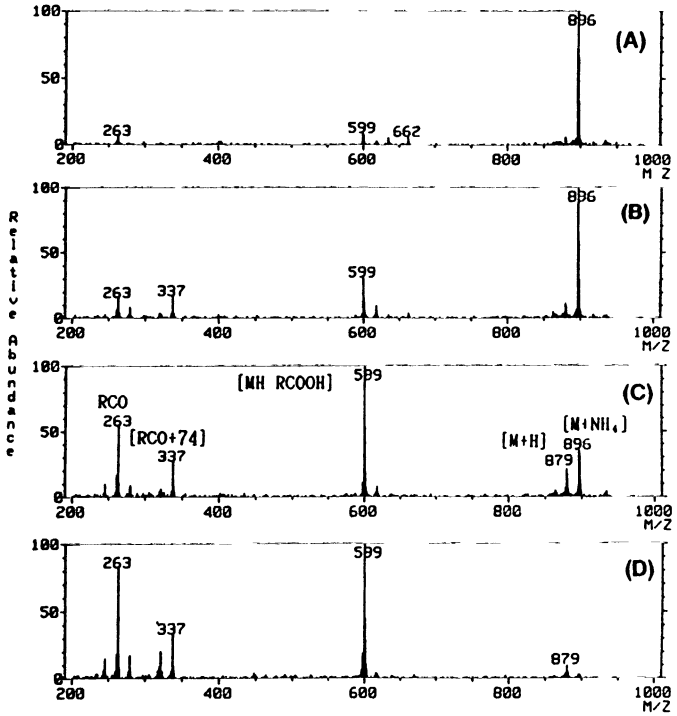


Fig 3 Ammonia CI mass spectra of trilinolein obtained by frit CI LC/MS
Ion source temperature (A) 300℃ (B) 350℃ (C) 400℃ (D) 450℃
LC conditions as in Fig 2

spectra of trilinolein measured by the frit-CI LC/MS at 300 to 450℃ of the ion source temperatures. When the ion source temperature was 300℃, the base peak was the $[M+NH4]^+$ ion at m/I 896. and the fragment ions were recorded with

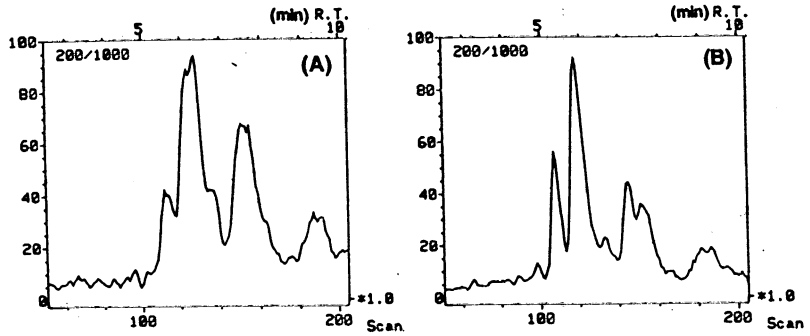


Fig. 4. Reconstructed ion chromatograms from *m/z* 200 to 1000 of evening primrose oil obtained by frit-CI LC/MS.
Ion source temperature: (A) 300℃, (B) 350℃.
Mobile phase: acetonitrile–tetrahydrofuran (75 : 25 isocratic).
Concenration: 50 μg/μl, Injection volume: 1 μl.

relative intensity of less than 10% against the base peak. The [M+H]' ion at m/I 879 was also observed more intensely compared with triolein and the intensity showed a maximum at 400T. An increase in the ion source temperature was accompanied with an increase in intensity of the fragment ions, and a decrease in intensity of the $[M+NH.]^+$ ion. These results indicate that pyrolysis of triacylglycerols on the frit and/or the cleavage of the $[M+NH.]^+$ ion are accelerated with an increase of the ion source temperature. The temperature more than 350T was necessary to get the fragment ions of intensity enough to determine the structure of trilinolein. In case of using the elution solvent as the reagent gas at an ion source temperature of 390°C, the $[M+H]^+$ ion was observed with relative intensity of 35% against the base pear at *m/z* 599 as shown in Table I. The intensity was also larger than that of triolein, and enough for the structural study. Above mentioned the intensity of the $[M+H]^+$ ion was affected by the number of double bonds of the triacylglycerol as well as the ion source temperature.

Figure 4 shows the reconstructed ion chromatograms (RIC). from *m/z* 200 to 1000 of evening primrose oil measured by the frit-CI LC/MS to investigate the dependence of the resolution of RIC on the ion source temperature. From the GC analysis after hydrolysis and methyl esterification, evening primrose oil was composed of palmitic acid (l6:0), stearic acid (18 : 0), oleic acid (l8 : 1 n-9), Iinolenic acid (l8: 3 n-6), and linoleic acid of main component (l8 : 2 n-6. 73% in fatty acids). The carbon number of the triacylglycerols in this oil were estimated to be 52 and 54 from the FD mass spectrum. The RICs shown in Fig. 4 indicate that the ion source temperature more than 350°C is necessary to get better resolution for this oil. The resolution of RIC at 350°C was similar to that of the chromatogram given by the UV detector.

The results mentioned above lead to an unambiguous conclusion that the firt-CI LC/MS using ammonia operated at an ion source temperature of 350 to 400°C is suitable for the structural study of triacylglycerols. The mass spectrum and the RIC of tri-EPA (20 : 5-20 : 5-20 : 5 MW

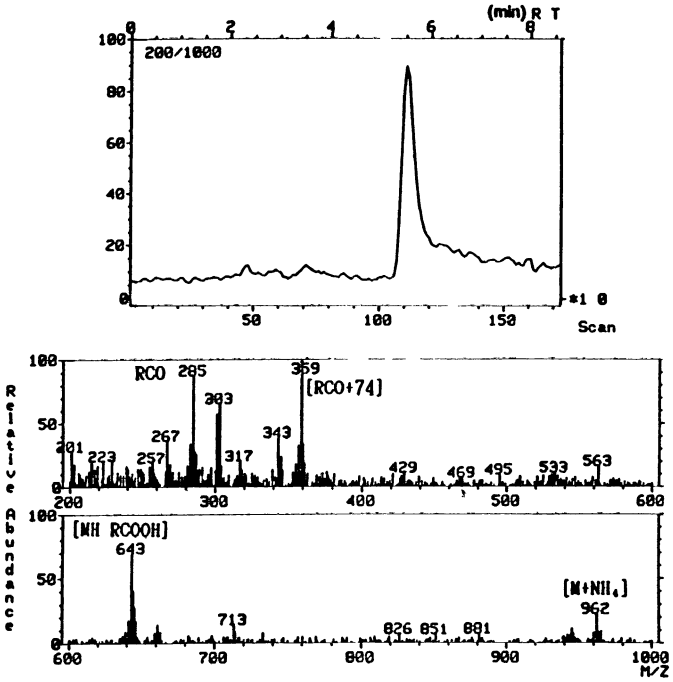


Fig 5 Reconstructed ion chromatogram from *m/z* 200 to 1000 and mass spectrum of tri EPA obtained by frit-CI LC/MS
Ion source temperature 390℃
Mobile phase acetonitrile–tetrahydrofuran (80 20 isocratic)
Concentration. 10 $\mu g/\mu l$ Injection volume 1 μl

=944) measured under the similar condition were shown in Fig. 5. Since the tri-EPA having fifteen's double bonds is not stable when exposed to high temperature for a long time. it is difficult to analyze by the GC/MS. The frit-CI LC/MS enabled to get identifiable mass spectrum of tri-EPA,
however the mass spectrum became more complicated than those of triolein and trilinolein.

3.2 Compositional analysis of evening primrose oil

This LC/MS system was applied to the identification of triacylglycerols in the evening primrose oil. A linear gradient of 0～30% (0～30 min) of tetrahydrofuran in acetonitrile was employed. The gradient analysis provided sharper peaks and more
complete detection of the low degree unsaturated species than any of the isocratic
solvent system. Furthermore. this gradient system apperas to be applicable to diacylglycerol and triacylglycerol mixtures.

The total ion chromatogram and mass chromatograms of the evening primrc6e oil recorded by the RCO+ ion at m/I 263. the $[RCO+74]^+$ ion at m/z 313 and $[MH-RCOOH]^+$ ions at m/z 575. 601. 599 are shown in Fig. 6. The peaks of m/z 575 and 60l in the mass chromatograms have slightly different retention time in the peak 5. This result indicates that the peak

5 contains triacylglycerols more than two species. The mass chromatograms and the LC/MS spectrum (shown in Fig. 7) of the peak 5 represent 18: I-18: 2-18: 2 of triacylglycerol as indicated by the following ions: the [MH-RCCX]H]$^+$ ions at m/I 599(l8:2-18:2)and *mz* 60l (18:1-18:2),the RCO$^+$ ions at m/z 263 (l8:2) and 265 (l8:I), the [M+H]$^+$ion at m/I 88landthe [M+NH4]$^+$ ion at m/I 898. The presence of l6:018:2-l8:2 of triacylglycerol is also indicated by the following ions: the [MH-RCCK]H]$^+$ ion at m/z 575 (16:0-l8:2), the[M+H]' ion at m/I 855 and [M+NH.]$^+$ ion at m/I 872.

Mass chromatograms and mass spectra given by the frit-CI Lt/MS provide usefull information on the possible combination of fatty acids in each triacylglycerol component. As shown in Fig. 6, the distribution of fatty acids in the oil could be also determined by recording mass chromatograms of the RCO$^+$ ion or [RCO +74]$^+$ ion. For example, the presence of linoleic acid was confirmed in the peaks of
2, 3, 5, and 6 by monitoring the RCO' ion at m/I 263. Since the saturated acid such as palmitic acid had a weak intensity of the RCO' ion, the [RCO+74]$^+$ ion was monitored to investigate the distribution.

Table 2 showed the fatty acid composition of each peak analyzed in a similar manner as described above. We could identify all the major triacylglycerols in the evening primrose oil by means of the frit-CI LC/MS. However. it was difficult to distinguish positional isomers such as POL, OLP, and LPO where P. O, and L

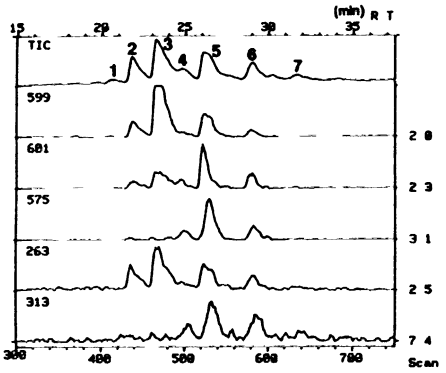


Fig 6 Mass chromatograms of evening prim rose oil
Ion source temperature 390℃
Mobile phase linear gradient of 0 ~ 30% (0~30 min) of tetrahydrofuran in acetonitrile
[MH−RCOOH]$^+$ *m/z* 599 (18 2) (18 2)
m/z 601 (18 1) (18 2)
m/z 575 (16 0) (18 2)
RCO$^+$ *m/z* 263 (18 2)
[RCO+74]$^+$ *m/z* 313 (16 0)

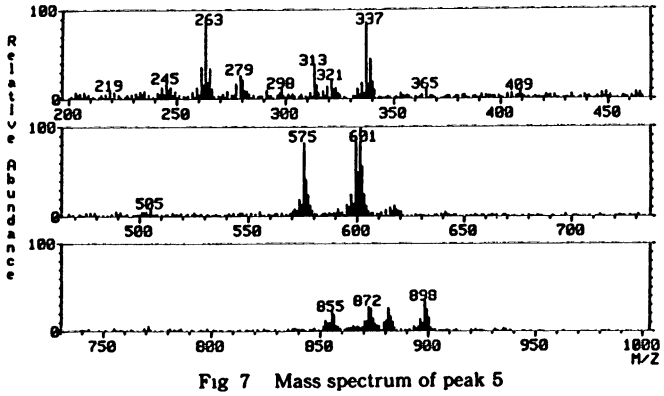


Fig 7 Mass spectrum of peak 5

Table 2 Molecular Species of Triacylglycerols of Evening Primrose Oil

Peak No	PN	MW	Molecular species	Ion sum* (area %)
1	38	874	18 2 18 3 18 3	2 5
2	40	876	18 2 18 2 18 3	16 4
3	42	878	18 2 18 2 18 2	36 1
4	42	852	16 0 18 2 18 3	2 9
5	44	880	18 1 18 2 18 2	13 2
	44	854	16 0 18 2 18 2	12 1
6	46	882	18 0 18 2 18 2	3 9
	46	856	16 0 18 1 18 2	8 5
7	48	884	18 0 18 1 18 2	1 8
	48	858	16 0 18 0 18 2	1 5

* Ion sum from *m/z* 200 to 1000 Overlapping species were calculated using the abundances of the appropriate [MH−RCOOH] or [RCO+74] ions

represent palmitic. oleic, and linoleic acid.
respectively. These results given by the frit-CI LC/MS also coincide with results from the partition number. The quantities
of individual species were estimated from the peak area of RIC from m/I 200 to 1000. However. better chromatographic separation and determination. of appropriate calibration factors are necessary for accurate quantitation since the yields of quasi-molecular and fragment ions were found to vary with the degree of unsaturation and the ion source temperature.

Acknowledgement

The authors are grateful to K. Matsuura of JEOL Ltd. for helpful advice.

References

1) B. F. Daubert, *J. Am. Chem. Soc.*, **26**, 556 (1949).
2) T. Murata and S. Takahashi, *Anal. Chem.*, **45**, 1816 (1973).
3) A. Kuksis, L. Marai, and J. J. Myher, *J. Chromatogr.*, **273**, 43 (1983).
4) J. J. Myher, A. Kuksis, L. Marai, and F. Manganaro, *J. Chromatogr.*, **283**, 289 (1984).
5) K. Matsumoto, S. Tsuge, and H. Hirose, *Mass Spectrosc.*, **35**, 240 (1987).
6) H. V. Kim and N. Salem, Jr., *Anal. Chem.*, **59**, 722 (1987).
7) S. Wada, *Yukagaku*, **36**, 105 (1987).
8) T. Murata, *Anal. Chem.*, **49**, 2209 (1977).

Keywords
Frit-CI LC/MS
Triacylglycerol
Evening primrose oil
Trieicosanopentaenoin