COSMOSIL

Fatty Acid Analysis by HPLC

Technical Note

Current interest in fatty acids and trans-fatty acids in food materials has been increasing. Fatty acid is an organic acid found in fats, oils, and lipids. It can be classified according to chaing length, short chain (2-4 carbons), middle chain (5-10 carbons), and long chain (more than 11 carbons). Although gas chromatography is the predominant technique used for fatty acid analysis, high-performance liquid chromatography (HPLC) plays an important role in applications such as the geometrical isomer separation. By using both HPLC and GC, a better fatty acid profile can be obtained.

		High-Performance Liquid Chromatography (HPLC)		Gas Chromatography (GC)
		COSMOSIL C_{18}	COSMOSIL Cholester	
Free fatty acids	Short fatty acids	Good	(HILIC)	Poor
	Middle and long fatty acids	Excellent		Poor
Fatty acid ester	Short fatty acids	Fair		Poor
	Middle and long fatty acids	Good		Excellent
Position or geometrical isomers of unsaturated fatty acids		Fair	Good	Excellent
High sensitive analysis		Fair [labeling] Excellent		Excellent
Preparative separation		Excellent		Poor
Lipids composed of complex fatty acid mixture		Using both HPLC and GC to achieve better fatty acid profiling (*1)		

*1: Wakako Tsuzuki and Kaori Ushida, "Preparative Separation of *cis*- and *trans*- Isomers of Unsaturated Fatty Acid Methyl Esters Contained in Edible Oils by Reversed-Phase High-Performance Liquid Chromatography" Lipids (2009) 44: 373-379

1. Unsaturated Fatty Acid Analyses by HPLC

(1) Geometrical Isomer Separations (*cis*/*trans*)

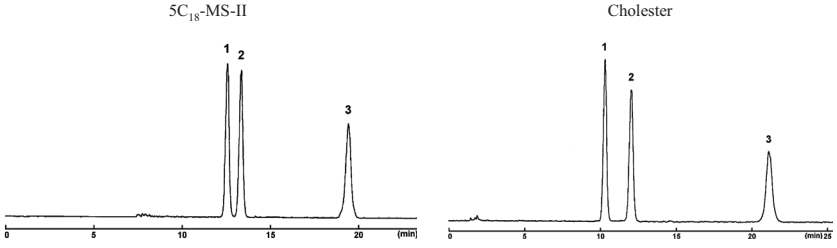
Geometrical isomers (*cis*/*trans*) are difficult to separate by a C_{18} column due to their similar hydrophobicity. COSMOSIL Cholester column can achieve better separation due to higher molecular-shape selectivity.

• 18 :1 *cis*-9 / *trans*-9 Fatty Acid

COSMOSIL Application Data

Column:
Column size: 4.6mmI.D.-150mm
Mobile phase: 0.05%TFA-90%Methanol
Flow rate: 1.0 ml/min
Temperature: 30°C
Detection: ELSD

Sample: 1; Oleic Acid (3.0 μg)
2; Elaidic Acid (3.0 μg)
3; Stearic Acid (3.0 μg)



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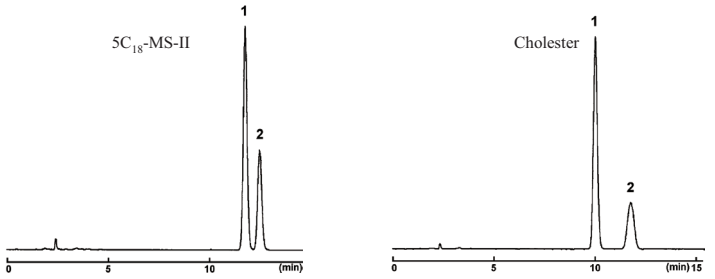
AP-1038

・**18 :1 *cis*-11 / *trans*-11 Fatty Acid**

COSMOSIL Application Data

Column:
Column size: 4.6mmI.D.-150mm
Mobile phase: 0.05%TFA-90%Methanol
Flow rate: 1.0 ml/min
Temperature: 30°C
Detection: ELSD

Sample: 1; *cis*-Vaccenic Acid (3.0 μg)
2; *trans*-Vaccenic Acid (3.0 μg)



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(2) Positional Isomers

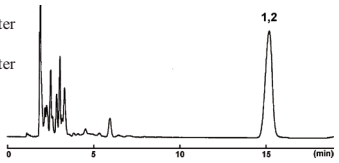
COSMOSIL Cholester column with high molecular-shape selectivity offers improved separation for positional isomers. Labeling can increase detection sensitivity.

・**Labeled 18 : 3 (n-3) / 18 : 3 (n-6) Fatty Acid Ester**

COSMOSIL Application Data

Column: $5C_{18}$-MS-II
Column size: 4.6mmI.D.-150mm
Mobile phase: Methanol/ H_2O = 90/10
Flow rate: 1.0 ml/min
Temperature: 30°C
Detection: UV254nm

Sample: 1; Linolenic Acid *p*-Bromophenacyl Ester
2; γ-Linolenic Acid *p*-Bromophenacyl Ester



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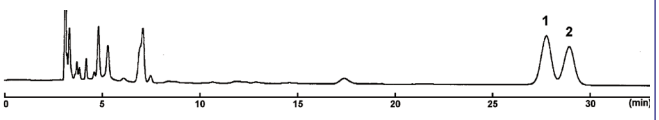
AP-0209

COSMOSIL Application Data

Column: Cholester
Column size: 4.6mmI.D.-250mm
Mobile phase: Methanol/ H_2O = 90/10
Flow rate: 1.0 ml/min
Temperature: 30°C
Detection: UV254nm

Sample: 1; Linolenic Acid *p*-Bromophenacyl Ester
2; γ-Linolenic Acid *p*-Bromophenacyl Ester



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AP-0211

2. Free Fatty Acid Analyses by HPLC

Methyl esterification of free fatty acids is required for GC analyses to obtain better peak shapes and lowered boiling points. Derivatization is not required for HPLC analyses.

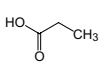
(1) Short-chain Free Fatty Acids

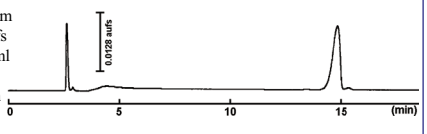
Short-chain free fatty acids have less retention on C_{18} columns. Therefore COSMOSIL C_{18}-PAQ column (100% water compatible) and COSMOSIL HILIC column are better for short-chain free fatty acids analyses.

・**Propanoic Acid (3:0)**

COSMOSIL Chromatogram Index

Sample: Propionic Acid
CAS No.: [79-09-4]
Molecular formula: $C_3H_6O_2$
Column: HILIC
Column size: 4.6mmI.D.-250mm
Mobile phase: Acetonitrile/ 10mmol/l Ammonium acetate =50/50
Flow rate: 1.0 ml/min
Temperature: 30°C
Detection: UV210 nm
Attenuation: 0.128 aufs
Sample conc.: 10.0mg/ml
Injection volume: 2.0μl
Retention time: 14.85min
Capacity factor: 4.24





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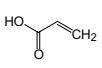
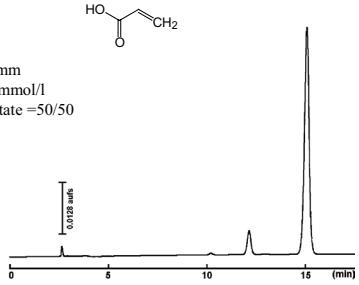
・**Acrylic Acid (3:1)**

COSMOSIL Chromatogram Index

Sample: Acrylic Acid
CAS No.: [79-10-7]
Molecular formula: $C_3H_4O_2$
Column: HILIC
Column size: 4.6mmI.D.-250mm
Mobile phase: Acetonitrile/ 10mmol/l Ammonium acetate =50/50
Flow rate: 1.0 ml/min
Temperature: 30°C
Detection: UV220 nm
Attenuation: 0.128 aufs
Sample conc.: 1.0mg/ml
Injection volume: 1.0μl
Retention time: 15.05min
Capacity factor: 4.28





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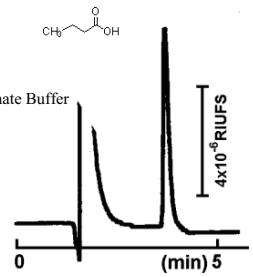
・**Butyric Acid (4:0)**

COSMOSIL Chromatogram Index

Sample:	*n*-Butyric Acid
CAS No.:	[107-92-6]
Molecular formula:	$C_4H_8O_2$
Column:	5C18-MS-II
Column size:	4.6mmI.D.-150mm
Mobile phase:	Methanol/20mmol/l Phosphate Buffer (pH2.5) =40/60
Flow rate:	1.0 ml/min
Temperature:	30°C
Detection:	RI
Attenuation:	4x10^{-5} RIU/FS
Sample conc.:	10.0mg/ml
Injection volume:	2.0µl
Retention time:	3.79min
Capacity factor:	1.24



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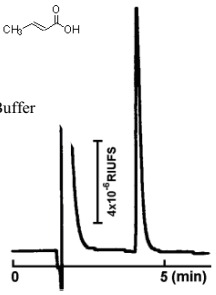
・**Crotonic Acid (4:1)**

COSMOSIL Chromatogram Index

Sample:	Crotonic Acid
CAS No.:	[3724-65-0]
Molecular formula:	$C_4H_6O_2$
Column:	5C18-MS-II
Column size:	4.6mmI.D.-150mm
Mobile phase:	Methanol/20mmol/l Phosphate Buffer (pH2.5) =30/70
Flow rate:	1.0 ml/min
Temperature:	30°C
Detection:	RI
Attenuation:	4x10^{-5} RIU/FS
Sample conc.:	10.0mg/ml
Injection volume:	2.0µl
Retention time:	4.21min
Capacity factor:	1.46



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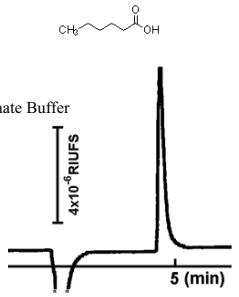
(2) Middle or Long-chain Free Fatty Acids

COSMOSIL C_{18}-MS-II column was used for non-derivatized middle or long-chain free fatty acid analyses.

・**Capronic Acid (6:0)**

COSMOSIL Chromatogram Index

Sample:	*n*-Capronic Acid
CAS No.:	[142-62-1]
Molecular formula:	$C_6H_{12}O_2$
Column:	5C18-MS-II
Column size:	4.6mmI.D.-150mm
Mobile phase:	Methanol/20mmol/l Phosphate Buffer (pH2.5) =60/40
Flow rate:	1.0 ml/min
Temperature:	30°C
Detection:	RI
Attenuation:	4x10^{-5} RIU/FS
Sample conc.:	10.0mg/ml
Injection volume:	2.0µl
Retention time:	4.63min
Capacity factor:	1.72



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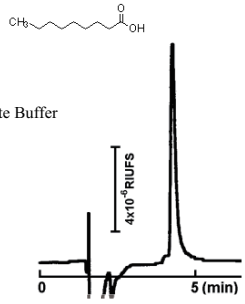
・**Pelargonic Acid (9:0)**

COSMOSIL Chromatogram Index

Sample:	Pelargonic Acid
CAS No.:	[112-05-0]
Molecular formula:	$C_9H_{18}O_2$
Column:	5C18-MS-II
Column size:	4.6mmI.D.-150mm
Mobile phase:	Methanol/20mmol/l Phosphate Buffer (pH2.5) =80/20
Flow rate:	1.0 ml/min
Temperature:	30°C
Detection:	RI
Attenuation:	4x10^{-5} RIU/FS
Sample conc.:	10.0mg/ml
Injection volume:	2.0µl
Retention time:	4.29min
Capacity factor:	1.56



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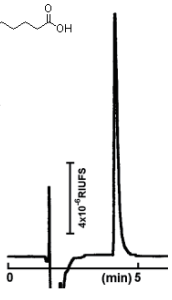
・**Lauric Acid (12:0)**

COSMOSIL Chromatogram Index

Sample:	Lauric Acid
CAS No.:	[143-07-7]
Molecular formula:	$C_{12}H_{24}O_2$
Column:	5C18-MS-II
Column size:	4.6mmI.D.-150mm
Mobile phase:	Methanol/20mmol/l Phosphate Buffer (pH2.5) =90/10
Flow rate:	1.0 ml/min
Temperature:	30°C
Detection:	RI
Attenuation:	4x10^{-5} RIU/FS
Sample conc.:	10.0mg/ml
Injection volume:	2.0µl
Retention time:	4.18min
Capacity factor:	1.51



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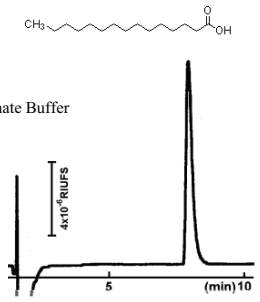
・**Pentadecanoic Acid (15:0)**

COSMOSIL Chromatogram Index

Sample:	*n*-Pentadecanoic Acid
CAS No.:	[1002-84-2]
Molecular formula:	$C_{15}H_{30}O_2$
Column:	5C18-MS-II
Column size:	4.6mmI.D.-150mm
Mobile phase:	Methanol/20mmol/l Phosphate Buffer (pH2.5) =90/10
Flow rate:	1.0 ml/min
Temperature:	30°C
Detection:	RI
Attenuation:	4x10^{-5} RIU/FS
Sample conc.:	10.0mg/ml
Injection volume:	2.5µl
Retention time:	7.97min
Capacity factor:	3.79



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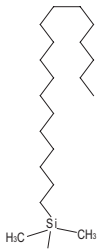
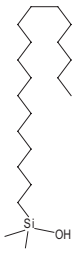
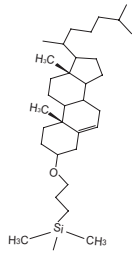
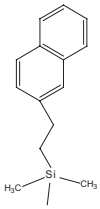
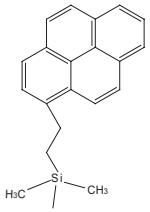
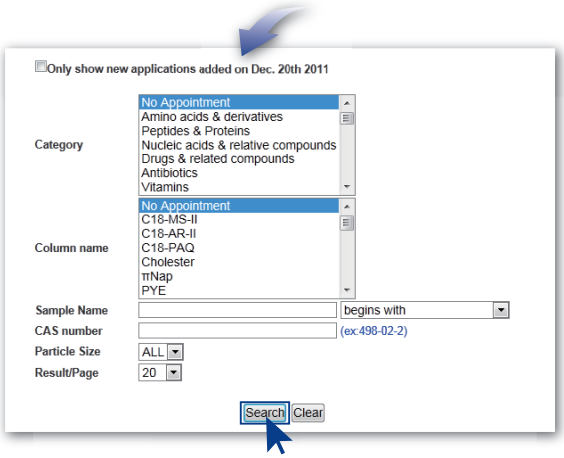
Specifications

Packing Material	C_{18}-MS-II	C_{18}-PAQ	Cholester	πNAP	PYE	HILIC
Bonded Phase Structure						
Bonded Phase	Octadecyl Group	Octadecyl Group	Cholesteryl Group	Naphtylethyl Group	Pyrenylethyl Group	Triazole
Main Interaction	Hydrophobic Interaction	Hydrophobic Interaction	Hydrophobic Interaction Molecular Shape Selectivity	Hydrophobic Interaction π-π Interaction	Hydrophobic Interaction π-π Interaction Stereoselectivity Charge-tansfer Interaction	Hydrophilic Interaction
Features	• Multi-purpose C_{18} column • for separation of the widest range of compounds	• Compatible with 100% water based mobile phase. • Suitable for hydrophilic com-pounds.	• Specialty for structual isomers • Usable under the same condition as C_{18}.	• Stronger π-π inter-action than phenyl column	• The most powerful π-π interaction	• Suitable for highly-polor compounds • Ion-pair reagent is not reqired
Product Code Column Size: 4.6 x 150	38019-81	02486-71	05976-61	08085-41	37837-91	07056-51

COSMOSIL Application

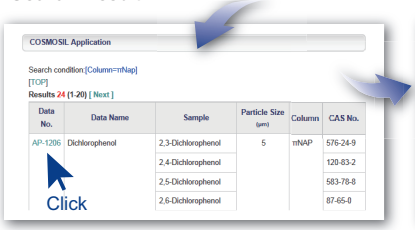
COSMOSIL Application has more than 7,000 applications using COSMOSIL columns. Setting optimal HPLC experimental parameters is the one of the most important processes that requires experience and time. COSMOSIL Application provides you with sample analysis conditions with widely used ODS columns and other specialty columns.

Visit our web site at http://www.nacalai.co.jp/cosmosil/data/csmosrchtop.cfm?lc=E
or type “COSMSOIL Application” .



Applications are search by
1. Sample Category
2. Sample Name
3. CAS No.,
4. Column Name
5. Particle Size

Search Result



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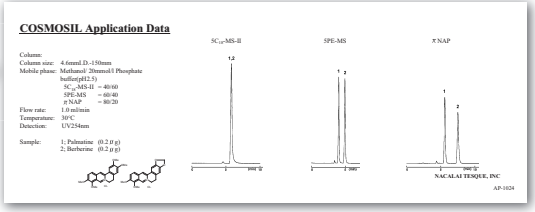

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The quality for certainty.

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