The Reporter

Reprinted from Volume 16, No. 3, 1997

T297053

For more information, or current prices, contact your nearest Supelco subsidiary listed below. To obtain further contact information, visit our website (www.sigma-aldrich.com), see the Supelco catalog, or contact Supelco, Bellefonte, PA 16823-0048 USA.

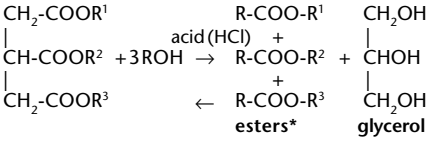
ARGENTINA • Sigma-Aldrich de Argentina, S.A. • Av. Pueyrredon 2446/50 • Piso 5-B • Buenos Aires 1119
AUSTRALIA • Sigma-Aldrich Pty. Ltd. • Unit #2, 14 Anella Avenue • Castle Hill NSW 2154
AUSTRIA • Sigma-Aldrich Handels GmbH • Hebbelplatz 7 • A-1110 Wien
BELGIUM • Sigma-Aldrich N.V./S.A. • K. Cardijplein 8 • B-2880 Bornem
BRAZIL • Sigma-Aldrich Quimica Brasil Ltda. • Rua Sabara, 566-Conj. 53 • 01239-010 São Paulo, SP
CANADA • Sigma-Aldrich Canada, Ltd. • 2149 Winston Park Dr., Oakville, ON L6H 6J8
CZECH REPUBLIC • Sigma-Aldrich s.r.o. • Pobrezni 46 • 186 21 Praha 8
DENMARK • Sigma-Aldrich Denmark A/S • Vejlegaardsvej 65B • DK-2665 Vallensbaek Strand
FINLAND • Sigma-Aldrich Finland/YA-Kemia Oy • Teerisuonkuja 4 • FIN-00700 Helsinki
FRANCE • Sigma-Aldrich Chimie • Chromatographie Supelco • L'Isle d'Abeau Chesnes - B.P. 701 • 38297 Saint-Quentin Fallavier Cedex
GERMANY • Sigma-Aldrich Chemie GmbH • Geschäftsbereich Supelco • Grünwalder Weg 30 • D-82041 Deisenhofen
GREECE • Sigma-Aldrich (o.m.) Ltd. • 72 Argonafton Str. • 16346 Ilioupoli, Athens
HUNGARY • Sigma-Aldrich Kft. • Nagy Diófa u. 7., IV fl. • H-1067 Budapest
INDIA • Sigma-Aldrich Co. • Survey No. 31/1, Sitharamapalaya • Mahadevapura P.O. • Bangalore 560 048
IRELAND • Sigma-Aldrich Ireland Ltd. • Airton Road • Tallaght • Dublin 24
ISRAEL • Sigma Israel Chemicals Ltd. • Park Rabin • Rohovot 76100
ITALY • Sigma-Aldrich s.r.l. • Via Gallarate, 154 • 20151 Milano
JAPAN • Sigma-Aldrich Japan K.K. • Division Supelco • JL. Nihonbashi Building • 1-10-15 Nihonbashi Horidome-cho, Chuo-ku • Tokyo 103
KOREA • Sigma-Aldrich Korea • Samhan Camus Annex, 10th Floor • 17-26 Yoido-dong, Yungdeungpo-ku • Seoul
MALAYSIA • Sigma-Aldrich (M) Sdn. Bhd. • 9-2, Jalan 2/128, Taman Gembira • Off Jalan Kuchai Lama • 58200 Kuala Lumpur • Selangor
MEXICO • Sigma-Aldrich Química S.A. de C.V. • Calle 6 North No. 107 • Parque Industrial Toluca 2000 • 50200 Toluca
NETHERLANDS • Sigma-Aldrich Chemie BV • Postbus 27 • 3330 AA Zwijndrecht
NORWAY • Sigma-Aldrich Norway • Sandakerveien 102 • N-0483 Oslo
POLAND • Sigma-Aldrich Sp. z o.o. • Szelagowska 30 • 61-626 Poznañ
PORTUGAL • Sigma-Aldrich Quimica, S.A. • P.O. Box 131 • Sintra 2710
RUSSIA • Sigma-Aldrich Russia • TOO Techmedbiochem • Makarenko Str. 2/21 • Building 1, Flat 22 • Moscow 103062
SINGAPORE • Sigma-Aldrich Pte. Ltd. • 102E Pasir Panjang Road • #08-01 Citilink Warehouse • Singapore 118529
SOUTH AFRICA • Sigma-Aldrich (pty) Ltd. • CNR Kelly & Ackerman Streets • Southern Life Industrial Park Unit • Unit 16/17 • Jet Park 1459
SPAIN • Sigma-Aldrich Quimica, S.A. • Apt. Correos 161 • 28100 Alcobendas, Madrid
SWEDEN • Sigma-Aldrich Sweden AB • Solkraftsvägen 14C •135 70 Stockholm
SWITZERLAND • Supelco Switzerland • Industriestrasse 25 • P.O. Box 260 • CH-9471 Buchs
UNITED KINGDOM • Sigma-Aldrich Company Ltd. • Supelco UK • Fancy Road, Poole • Dorset BH12 4QH
UNITED STATES • Supelco • Supelco Park • Bellefonte, PA 16823-0048 · Phone 800-247-6628 or 814-359-3441 · Fax 800-447-3044 or 814-359-3044 · email:supelco@sial.com

H

Derivatization of Corn Oil for Analysis by GC

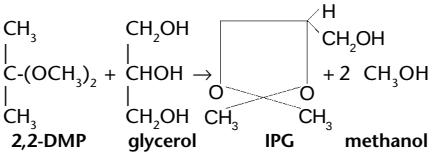
K. Kiefer, K. Herwehe, Chemical Standards, Supelco, Bellefonte, PA, USA

Before the fatty acid composition of a lipid can be analyzed by gas chromatography, the lipid must be converted to low molecular weight, volatile, nonpolar derivatives (e.g., fatty acid methyl esters). This conversion usually is through a transesterification – the glycerol (alcohol) portion of the triglyceride (ester) is displaced by another alcohol, in the presence of an acid. The reaction is represented by the general equation:*



Transesterification is an equilibrium reaction. The stoichiometry of the reaction dictates that for each mole of lipid to be completely derivatized there must be 3 moles of alcohol but, in fact, it is necessary to use an excess of alcohol in the reaction mixture, or to remove one of the reaction products, to drive the reaction to the right. When the second option is employed, the reaction can go to completion.

Transesterification is best done in the presence of a volatile, acidic catalyst[a] which can be removed, along with excess alcohol,when the reaction is completed. 2,2-Dimethoxypropane (dimethylacetal acetone/2,2-DMP) also helps drive triglyceride transesterification to completion by reacting with glycerol as it is formed. In the presence of acid, excess 2,2-DMP, and excess alcohol (methanol), glycerol from a lipid transesterification is converted largely to isopropylidine glycerol (IPG) (2). The reaction is:



To ensure complete conversion of the lipid, the amount of 2,2-DMP used should be a molar excess of the total glycerol expected from the reaction.

We used methanolic HCl, 3N, a general purpose transesterification reagent, to derivatize the triglycerides in a sample of corn oil. We weighed 10mg of corn oil into reaction vials and added 1mL methanolic HCl, 3N,[b] 1mL hexane, and various amounts of 2,2-DMP (0 to 1000µL) to each vial. The vials were capped and heated at 70°C for 10-15 minutes. The samples were allowed to cool, then we added 1mL water and 1mL hexane. The vials were vigorously shaken and the phases allowed to separate. The ester (upper) layer was sampled for analysis by GC.

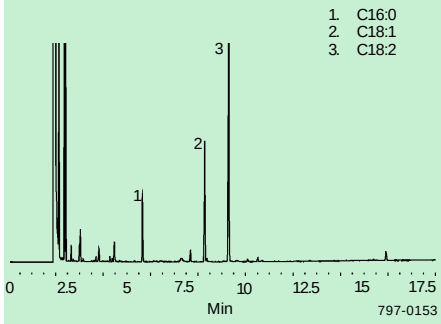
The chromatogram in Figure A is a representative derivatization of corn oil, using methanolic HCl, 3N, 250µL 2,2-DMP, and 50µL dimethylsulfoxide (DMSO). Without 2,2-DMP, transesterification is incomplete. On the other hand, addition of 250µL of 2,2-DMP causes the reaction mixture to turn yellow,[c] and reaction byproducts appear in the chromatogram. Higher levels of 2,2-DMP produce greater amounts of byproducts. Addition of DMSO to the transesterification reaction mixture inhibits byproduct formation (4). DMSO, however, may interfere with the chromatography of early eluting fatty acid methyl esters (Figure A).

Our study showed that using 2,2-DMP in preparing methyl esters of fatty acids increased the methyl ester yield.

Figure A. Fatty Acid Methyl Esters from Corn Oil

Column: **SP-2380, 30m x 0.25mm ID, 0.20µm film**
Cat. No.: **24110-U**
Oven: 150°C to 250°C at 4°C/min
Carrier: helium, 20cm/sec, set at 150°C
Det.: FID, 300°C
Inj.: 3µL, split 100:1

Derivatized with 1mL methanolic HCl, 3N, 250µL 2,2-DMP, 50µL DMSO



Byproducts that can interfere with the chromatography can be eliminated by adding a small quantity of DMSO to inhibit their accumulation.

Ordering Information:

Description	Cat. No.
Methanolic HCl	
0.5N, 20 x 1mL	**33354**
0.5N, 10 x 5mL	**33095**
3N, 20 x 1mL	**33355**
3N, 10 x 3mL	**33051**
3N, 400mL	**33050-U**
2,2-Dimethoxypropane	
25g	**33053**
Corn Oil	
1000mg	**47112-U**
SP™-2380 Capillary Column	
30m x 0.25mm ID, 0.20µm film	**24110-U**

References

1. *Bailey's Industrial Oil & Fat Products* John Wiley & Sons (1996).
2. Lorette, N.B., and J.H. Brown, Jr., *J. Org. Chem.*, **24:** 261 (1959).
3. Mason, M.E. and G.R. Walker, *Anal. Chem.*, **36:** 583 (1964).
4. Blau, K. and J.M. Halket (eds), *Handbook of Derivatives for Chromatography* (2nd ed.), John Wiley & Sons (1993) (Cat. No. Z24,622-0).

References 1-3 not available from Supelco.

[a]HCl is recommended for its high acid strength and because it can be removed easily.

[b]Higher concentrations of methanolic HCl greatly reduce the reaction time, but must be stored carefully or they will weaken rapidly. Lower concentrations can be used, but this prolongs the reaction time and requires a greater total amount of reagent (see 3).

[c]Produced by a polymer originating from condensation of 2,2-DMP (3).

*Adapted from (1). If alcohol used is methanol, products are methyl esters.

Reprinted from Volume 16, No. 3, 1997