

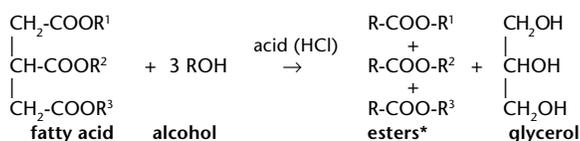
Derivatization of Corn Oil for Analysis by GC

In the derivatization of corn oil fatty acids with methanolic HCl, a general purpose transesterification reagent, removal of one of the reaction products drives the reaction toward completion. 2,2-Dimethoxypropane increases the methyl ester yield by reacting with glycerol as it is formed, but causes reaction byproducts to appear in the chromatogram. This effect can be avoided by adding dimethylsulfoxide to the reaction mixture.

Key Words:

- fatty acids • fatty acid methyl esters • esterification
- transesterification • methanolic HCl

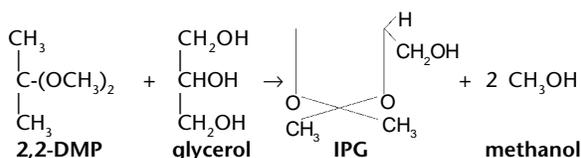
Before the fatty acid composition of a lipid can be analyzed by gas chromatography, the lipid must be converted to low molecular weight, volatile, nonpolar derivatives, such as fatty acid methyl esters. For triglycerides, which are esters, this conversion usually is through a transesterification – the glycerol (alcohol) portion of the molecule is displaced by another alcohol, in the presence of an acid. The reaction is represented by the general equation:*



The equation depicts the overall reaction; the process usually consists of a series of consecutive, reversible cleavage steps – triglyceride to diglyceride to monoglyceride – with 1 mole of methyl ester formed at each step.

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. Water in the reaction mixture also can prevent the reaction from going to completion (1).

Transesterification is best done in the presence of a volatile, acidic catalyst (e.g., hydrochloric acid/HCl^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 isopropylidene glycerol (IPG) (2). The reaction is:



To ensure a high reaction rate, methanol should be present in the initial reaction mixture in excess. To ensure complete conversion of the lipid, the amount of 2,2-DMP used should be a molar excess, relative to the total glycerol expected from the reaction.

Corn oil can contain five essential fatty acids (EFA): linoleic (C18:2, typically approximately 60% of the total triglyceride content of the oil), oleic (C18:1, 26%), palmitic (C16:0, 11%), stearic (C18:0, 2%), and linolenic (C18:3, 1%). Arachidic (C20:0) and other minor fatty acids represent less than 1% of the total triglyceride content. The corn oil used in this investigation contained C16:0, C18:1, and C18:2 acids. We used methanolic HCl, 3N, a general purpose transesterification reagent that methylates free fatty acids very rapidly, to derivatize the sample.

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. We capped the vials and heated the samples at 70°C for 10-15 minutes. The samples were allowed to cool, then we added 1mL water and 1mL hexane.^c The vials were vigorously shaken and the phases allowed to separate. The ester (upper) layers were sampled for analysis by GC.

The chromatograms in Figure A are representative derivatizations of corn oil, using methanolic HCl, 3N and various molar ratios of 2,2-DMP (Table 1). With 0-50µL of 2,2-DMP, transesterification is incomplete, hindered by the glycerol formed in the reaction. By removing glycerol from the reaction mixture, addition of 250µL of 2,2-DMP drove the reaction the right, but also caused the mixture to turn yellow,^d and reaction byproducts appeared in the chromatogram (Figure A). Levels of 500µL to 1000µL 2,2-DMP produced increasing amounts of byproducts in addition to increasing amounts of methyl esters.

Other investigators have suggested adding dimethylsulfoxide (DMSO) to the transesterification reaction mixture, to inhibit byproduct formation (4). DMSO dissolves certain organic and inorganic compounds that form in the hexane layer. Then, because DMSO is soluble in water and insoluble in hexane, the byproducts dissolved in DMSO are removed from the hexane layer into the water layer. A drawback to using DMSO, however, is that DMSO may interfere with the chromatography of early eluting fatty acid methyl esters (Figure A).

This study shows that using 2,2-DMP in preparing methanolic HCl-derived methyl esters of fatty acids increases the methyl ester yield. The use of 2,2-DMP is simple and convenient. Excess 2,2-DMP, however, produces byproducts that can interfere with the

chromatography of the derivatives. This unwanted effect can be avoided by adding a small quantity of DMSO to inhibit accumulation of byproducts. In any specific application, exact proportions of sample, 2,2-DMP, and DMSO should be determined by experiment.

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 corn oil derivatized with methanolic HCL +/- 2,2-DMP, split 100:1

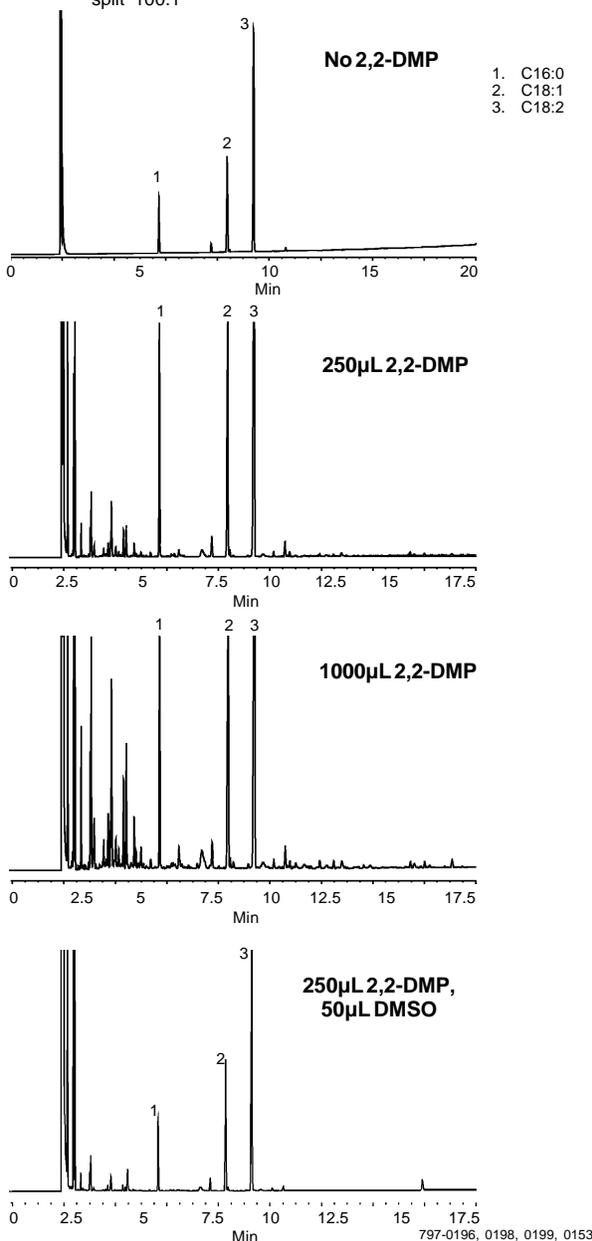


Table 1. 2,2-DMP to Glycerol Ratio Affects the Reaction

Volume 2,2-DMP (µL)	2,2-DMP: Glycerol Molar Ratio	Observations
50	37:1	incomplete reaction
100	74:1	incomplete reaction
250	185:1	complete reaction, solution yellow, extra peaks in chromatogram
500	370:1	complete reaction, solution orange/red, extra peaks in chromatogram
750	546:1	complete reaction, solution black, extra peaks in chromatogram
1000	739:1	complete reaction, solution black, extra peaks in chromatogram

Calculations:

Moles Linoleic Acid
 (mol. wt. linoleic acid) (3) + mol. wt. glycerol = moles linoleic acid
 (280.50) (3) + 92.09 = 933.59 moles linoleic acid

Moles 2,2-DMP
 (volume 2,2-DMP) (density 2,2-DMP) = grams 2,2-DMP/mol. wt. 2,2-DMP = moles 2,2-DMP
 (0.05mL) (0.847g/mL) = 4.07 x 10⁻⁴ moles 2,2-DMP

Molar Ratio 2,2-DMP:Glycerol
 moles 2,2-DMP/moles linoleic acid
 (4.07 x 10⁻⁴) (0.11 x 10⁻⁴) = 37, etc.

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).
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 4. Blau, K. and J.M. Halket (eds), *Handbook of Derivatives for Chromatography* (2nd ed.), John Wiley & Sons (1993) (Supelco Cat. No. Z24,622-0).
- References 1-3 not available from Supelco.
- *Adapted from (1). If the alcohol used is methanol, the products are methyl esters. *HCl is recommended for its high acid strength and ease of removal.
- †The concentration of methanolic HCl is significant. Higher concentrations of the reagent 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).
- ‡Solvent choice is important (e.g., cholesterol esters are not completely soluble in methanol). If the lipid is not completely dissolved, transesterification will be incomplete, yielding poor recovery rates and irreproducible quantification.
- §This color is produced by a polymer originating from condensation of 2,2-DMP (3). SP is a trademark of Sigma-Aldrich Co.

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