A Direct High-Performance Liquid Chromatographic Method for the Determination of Therminol 66 Contamination in Commercial Glycerin and Fatty Acids

M.H. Moh[1,*], T.S. Tang[1], and **G.H. Tan[2]**

[1]Advanced Oleochemical Technology Center, Malaysian Palm Oil Board, No. 6 Persiaran Institusi, Bandar Baru Bangi, 43650 Kajang, Selangor, Malaysia and [2]Department of Chemistry, University of Malaya, Lembah Pantai, 50603 Kuala Lumpur, Malaysia

Abstract

A simple and sensitive high-performance liquid chromatographic method for the determination of Therminol 66 thermal heating fluid in glycerin and fatty acids is developed. Sample solutions dissolved in methanol–tetrahydrofuran (50:50, v/v) are injected directly into a reversed-phase C18 column and eluted with a methanol and water mixture (88:12, v/v). The concentration of the thermal heating fluid is monitored by fluorescence detection at 257 nm (excitation) and 320 nm (emission). The calibration graph obtained from various concentrations of the thermal heating fluid in the methanol and tetrahydrofuran mixture is linear (correlation coefficient = 0.999), and the limit of detection is 0.01 µg/mL. Spiked glycerin containing 0.1 to 1.0 µg/g of the thermal heating fluid also gives good linearity with a mean recovery of 95.3%. The mean intra- and interassay precision are 1.80–6.51% and 5.71–9.03%, respectively, at the 0.1-µg/g level. The method is simple and does not require any pretreatment step, thus it is ideal for quality assurance purposes.

Introduction

In the hydrolytic splitting process of glyceridic oil to produce fatty acids, "sweet water" (which contains approximately 20% glycerin in water) is a byproduct. Sweet water is converted to yield various grades of glycerin via several processes such as removal of impurities, evaporation, distillation, and treatment with an activated carbon. However, the crude fatty acid obtained from the splitting process invariably contains small amounts of colored components, unsplit glycerides, oxidized materials, unsaponifiable material, and other products. As a result, its color is usually yellow to dark brown. In order to produce higher-grade fatty acids suitable for cosmetic and pharmaceutical uses, crude fatty acids are purified by straight or fractional distillation (1).

*Author to whom correspondence should be addressed: email felixmoh@mpob.gov.my.

In newer plants, steam is the heating medium in the distillation towers. However, in older plants, heating systems using thermal heating fluids are commonly installed. The thermal heating fluids commonly used are usually petroleum-based including mineral oil, hydrogenated terphenyl, and a eutectic mixture of biphenyl and diphenyl oxide (2). As such, there are concerns about contamination resulting from pinhole leaks or faulty joints in the heating coils (2,3). Although there were attempts to ban the use of thermal heating fluid in edible oil processing plants (4), the Codex Committee on Fats and Oils had resolved to allow the use of heating media other than high-pressure steam on the basis of safety and risk evaluation and inspection procedures (5). Similarly, because the glycerin and fatty acids are important ingredients in both pharmaceutical and food products, accidental contamination of thermal heating fluid with these basic oleochemicals is also a major concern.

Several gas chromatographic (GC) methods have been described for the determination of Dowtherm A (a eutectic mixture of biphenyl and diphenyl oxide) in fats and oils (6–8). They require pretreatment of the samples prior to analysis via either a thin-layer chromatographic cleanup or a distillation step, whereas the American Oil Chemists' Society procedure (9) uses a solvent extraction method instead. Although these GC techniques are sensitive (with detection limits of 0.2 µg/g), the procedures are tedious and time-consuming.

Because the molecular structures of biphenyl and diphenyl oxide consist of two aromatic rings each and exhibit fluorescent properties, a high-performance liquid chromatographic (HPLC) method with fluorescence detection has been previously developed by us (10,11). This reported HPLC method was sensitive with quantitation limits ranging from 0.1 to 0.2 µg/g for all edible oils and oleochemicals evaluated. The main advantage of this HPLC technique is that no tedious sample pretreatment is required.

Therminol 66 (T66) thermal heating fluid is a complex mixture of terphenyl (3–8%), partial hydrogenated terphenyls (74–87%), quaterphenyls, and higher polyphenyls (18%) (12).

The manufacturer claims that this clear and pale yellow fluid is the most popular high-temperature liquid-phase heating fluid in the world with a maximum operational temperature of 345°C and pumpable to 0°C (12,13). A survey conducted by Tang et al. (14) showed that T66 is also widely used as a heating medium in the edible oil and oleochemical industries. Thus, we find that it is important to develop a fast method for quality control purposes.

In this study we report an HPLC method coupled with fluorescence detection for the determination of T66 in spiked glycerin and fatty acids.

Experimental

Chemicals

o-, *m*-, and *p*-Terphenyls were purchased from Fluka (Bellefonte, PA), and 1,4-dicyclohexylbenzene was from Sigma (St. Louis, MO). Palm-based glycerin (99.5%) and fatty acids (> 99%) such as caprylic acid ($C_{8:0}$), capric acid ($C_{10:0}$), lauric acid ($C_{12:0}$), myristic acid ($C_{14:0}$), palmitic acid ($C_{16:0}$), and stearic acid ($C_{18:0}$) were all provided by the local oleochemical industries. Methanol and tetrahydrofuran were purchased from Merck (Darmstadt, Germany), and deionized distilled water was used throughout the experiment.

The thermal heating fluid (T66) was a gift from Solutia, Inc. (St. Louis, MO).

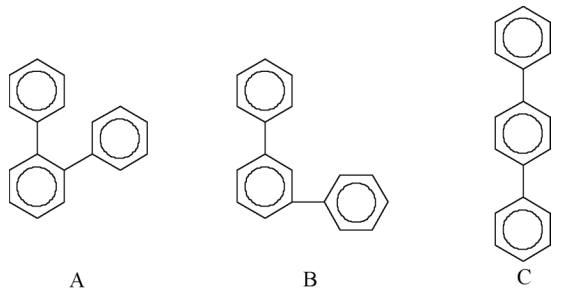

Figure 1. Chemical structures of (A) *o*-, (B) *m*-, and (C) *p*-terphenyl.

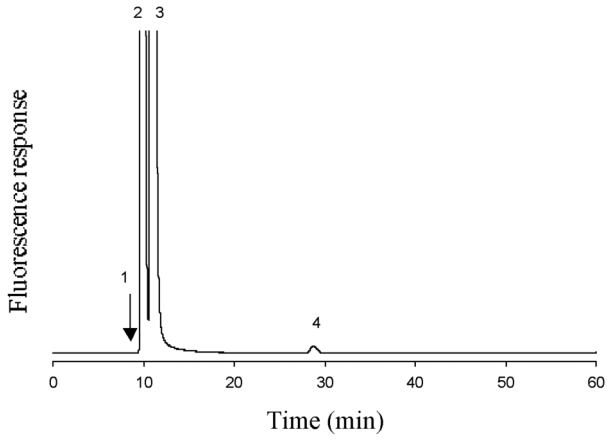

Figure 2. Fluorescence chromatogram showing the separation of o-, *m*-, and *p*-terphenyl and 1,4-dicyclohexylbenzene. Peak identification: *o*-terphenyl, 1; *m*-terphenyl, 2; *p*-terphenyl, 3; and 1,4-dicyclohexylbenzene, 4.

HPLC instrument

The HPLC system consisted of a pump (Jasco PU-980, Jasco International Co., Ltd., Tokyo, Japan), a 3-line degasser (Jasco DG-980-50), a ternary gradient unit (LG-980-02S), an autosampler (Jasco 851-AS), and a column oven (Jasco CO-965). A Jasco programmable fluorescence detector (FP-970) that was connected in series with a SEDEX 55 evaporative light-scattering detector (ELSD) (SEDERE, Alfortville, France) was controlled by Borwin 1.21 (JMBS Developpements, Le Fontanil, France) chromatographic software. A reversed-phase column (150- × 4.6-mm i.d.) packed with 5 µm Lichrosphere RP-C18 (Supelco Inc., Bellefonte, PA) was used with a 50- × 4.6-mm-i.d. guard column packed with the same material. The system was run isocratically

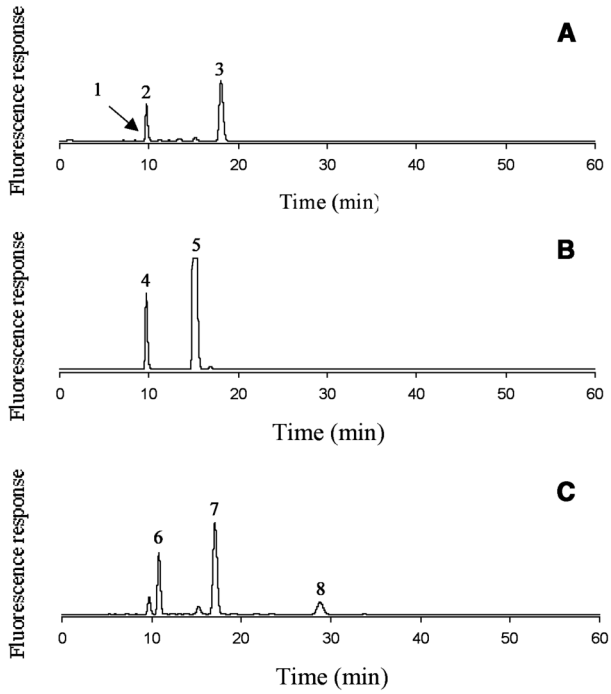

Figure 3. HPLC analysis of the hydrogenated products of (A) *o*-, (B) *m*-, and (C) *p*-terphenyl. Peak identification: *o*-terphenyl, 1; 2-CHBP, 2; 1,2-diphenylcyclohexane, 3; *m*-terphenyl, 4; 3-CHBP, 5; *p*-terphenyl, 6; 4-CHBP, 7; and 1,4-dicyclohexylbenzene, 8.

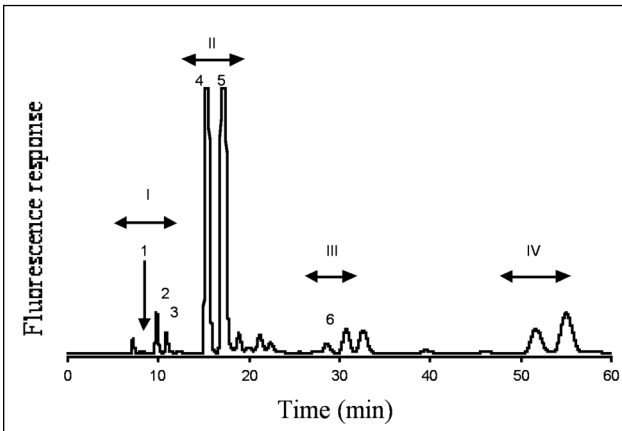

Figure 4. Fluorescence chromatograms showing the separation of T66. Peak identification: *o*-terphenyl, 1; *m*-terphenyl, 2; *p*-terphenyl, 3; 3-CHBP, 4; 4-CHBP, 5; and 1,4-dicyclohexylbenzene, 6.

with a mobile phase of a methanol and water mixture. The flow rate was set at 1 mL/min, and the column was maintained at 40°C. The fluorescence detector was optimized at an excitation wavelength of 257 nm and an emission wavelength of 320 nm.

Table I. Calibration Data of T66 Solutions Obtained from the Peak Areas of 3-CHBP and 4-CHBP

T66 concentration (µg/mL)	Mean (n = 3)		Standard deviation	
	3-CHBP	4-CHBP	3-CHBP	4-CHBP
0.01	3200.0	3924.9	178.21	28.88
0.05	8234.1	9503.5	136.92	132.39
0.1	15715.3	19595.1	225.55	63.11
1.0	155714.0	190213.0	3345.9	835.7

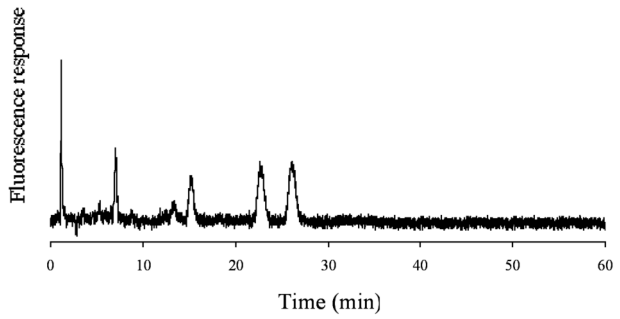

Figure 5. A typical fluorescence chromatogram of glycerin spiked with 0.1 µg/g of T66.

Table II. Linearity and Recovery Results of T66 in Spiked Glycerin Obtained by Measuring the 4-CHBP Peak

Spiked concentration of T66 (µg/mL)	Peak areas (arbitrary units)	Standard deviation	Coefficient of variation (%)	Recovery (%)
0.1	4694.9	120.16	2.56	82
0.5	14349.9	685.62	4.78	106
1.0	24163.1	878.22	1.71	98

Table III. Accuracy and Precision of the HPLC Method for the Determination of T66 in Spiked Fatty Acids

Fatty acids of different carbon-chain lengths	Concentration added (µg/g)	Concentration found* (µg/g)	Accuracy (%)	Precision (%coefficient of variation)†
$C_{8:0}$	0.1	0.095 ± 0.0025	94.67	2.66 (8.81)
$C_{10:0}$	0.1	0.092 ± 0.0306	91.67	3.33 (7.47)
$C_{12:0}$	0.1	0.102 ± 0.0067	102.33	6.51 (9.03)
$C_{14:0}$	0.1	0.097 ± 0.0041	92.67	4.36 (5.71)
$C_{16:0}$	0.1	0.101 ± 0.0029	101.67	2.84 (8.47)
$C_{18:0}$	0.1	0.096 ± 0.0017	96.00	1.80 (6.54)

* Determined by measuring the 4-CHBP peak.
† Intra-assay (n = 3) and interassay (n = 6) variations. The interassay variations appear in parentheses.

Quantitation

Calibration solutions of T66 (0.01 to 1.0 µg/mL) were prepared gravimetrically in methanol–tetrahydrofuran (50:50, v/v), and 20 µL each was injected into the HPLC system in triplicates. A calibration graph was then obtained by plotting the peak areas against the concentrations of the thermal heating fluid.

The recovery study was carried out on spiked samples because contaminated samples were not available. A 1-g sample was accurately weighed into each of three 10-mL volumetric flasks, then 1, 0.5, and 0.1 mL of the working solution (1 µg/mL) were added to the samples, and diluted to 10 mL with methanol–tetrahydrofuran in order to provide spiked solutions containing 1, 0.5, and 0.1 µg/g T66. A total of three injections of 20 µL each were carried out for each sample, and respective peak areas of the thermal heating fluid were obtained. Recoveries were calculated by interpolation from the calibration curve established earlier. Sample blanks that were dissolved in methanol–tetrahydrofuran were also analyzed as control.

Detection limits were examined by injecting standard solutions of progressively lower concentrations into the HPLC system until the signal was no longer detected. Accuracy was determined as the difference between calculated and actual concentrations of the spiked samples, and precision was assessed in terms of the coefficient of variation of the calculated concentrations in a replicate set.

Hydrogenation of terphenyls

The hydrogenation was conducted according to the procedure reported by Scola et al. (15). In each experiment, 50 g of terphenyl and 3.0 g of a powdered Harshaw 5256P nickel catalyst (Engelhard De Meern B.V., De Meern, The Netherlands) were introduced into the reaction chamber of a high-pressure reactor (Yamazaki Keiki, Tokyo, Japan). The reactor was pressurized with H_2 to 20 kg/cm^3 without stirring. Once the desired temperature (approximately 200–250°C) was reached, stirring was started. The hydrogenation process was then carried out for 1–5 min at 50 kg/cm^3, and the progress of hydrogenation was monitored by following the pressure drop in the system. After the reaction mixture was cooled to room temperature, a small quantity of the product was dissolved in methanol–tetrahydrofuran (50:50, v/v). The solution thus obtained was filtered in order to remove the catalyst before analysis. Identity of the compounds was established by comparison with authentic standards, and also with data reported by Scola et al. (15).

Results and Discussion

The isomeric structures of the terphenyls are showed in Figure 1. Figure 2 illustrates the HPLC analysis of the authentic standard mixture of *o*-, *m*-, and *p*-terphenyl and 1,4-dicyclohexylbenzene using fluorescence detection. The mobile phase employed was a methanol and water mixture (90:10, v/v). Because of the Rayleigh effect or from having a similar excitation and emission wave-

length, *o*-terphenyl was not detected by the fluorescence detector. However, its peak was identified by ELSD. *m*- and *p*-Terphenyl were eluted slightly after the *o*-terphenyl peaks. It was also noticed that despite the lessening of conjugation in the molecule through hydrogenation, 1,4-dicyclohexylbenzene still showed weaker fluorescence compared with the similar levels of *m*- and *p*-terphenyl concentrations.

According to the study by Scola et al. (15), partial hydrogenation of the terphenyls proceeds stepwise by saturating the aromatic rings one at a time. For instance, *o*-terphenyl is hydrogenated primarily to 1,2-diphenylcyclohexane and 2-cyclohexylbiphenyl (2-CHBP) (Figure 3A). Further hydrogenation of *o*-terphenyl gives 2-phenylbicyclohexyl, 1,2-dicyclohexylbenzene, and finally *o*-tercyclohexyl. However, the major product from the hydrogenation of *m*-terphenyl is 3-CHBP together with small amounts of 1,3-diphenylcyclohexane, 1,3-dicyclohexylbenzene, 3-phenylbicyclohexyl, and *m*-tercyclohexyl. The 3-CHBP peak from the hydrogenation of *m*-terphenyl carried out in this study is shown in Figure 3B. A total of four fluorescent peaks were detected in the hydrogenation products of *p*-terphenyl (Figure 3C). The peaks labeled 7 and 8 were attributed to 4-CHBP and 1,4-dicyclohexylbenzene, respectively. Scola et al. (15) reported that *p*-tercyclohexyl was also present in an extremely small quantity, but no 1,4-diphenylcyclone was found. The catalytic hydrogenation of quaterphenyl has also been reported elsewhere (16).

Shown in Figure 4 is the fluorescence chromatogram of T66 (1000 µg) using methanol–water as the mobile phase (which is categorized into four main groups). The terphenyl isomers were eluted first, followed by diphenylcyclohexanes, dicyclohexylbenzenes, and tercyclohexyls. Because of the absence of unsaturation, the tercyclohexyl isomers did not fluoresce, but were detected by ELSD. Therefore, the fluorescence peaks observed at 50–60 min (Figure 4) were likely because of the hydrogenated products of other polyphenyl compounds. Because T66 still remains a trade secret, we have no intention of reviewing in great detail its composition in this study.

A preliminary quantitative study showed that some of the fluorescent components that are present naturally in fatty acids might coelute with the 3-CHBP and 4-CHBP peaks. Attempts proved that by increasing the polarity of the mobile phase slightly to 88:12 (v/v), 3-CHBP and 4-CHBP of T66 would not be interfered with by those fluorescent backgrounds present naturally in some of the fatty acid samples, thus it was suitable for quantitative analysis. Because these two peaks gave the strongest intensity, they were used as markers for the quantitative analysis of T66 by HPLC using fluorescence detection.

The calibration graph of the T66 solutions prepared over the

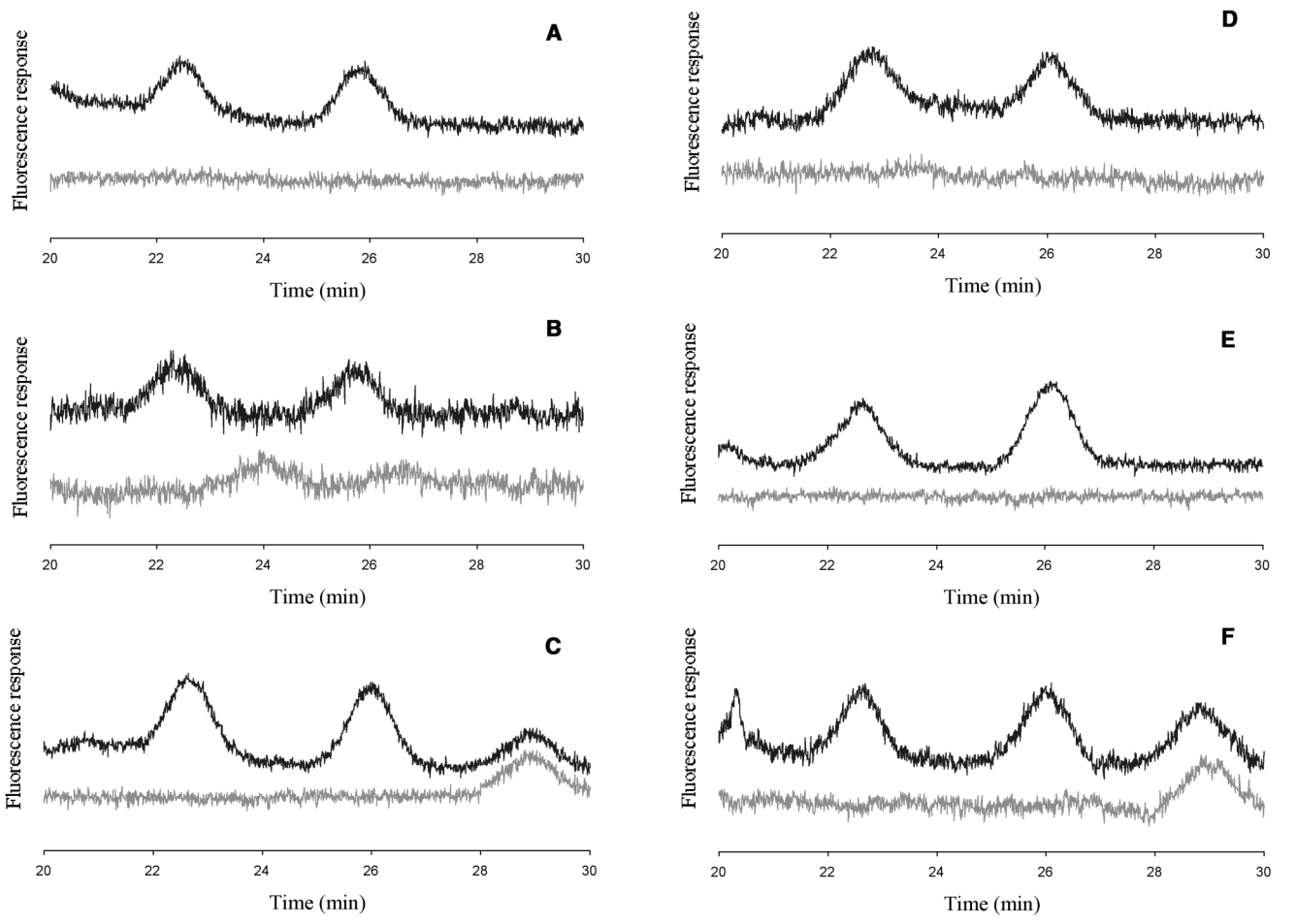

Figure 6. Enlarged fluorescence chromatograms of blank and spiked $C_{8:0}$ (caprylic), A; $C_{10:0}$ (capric), B; $C_{12:0}$ (lauric), C; $C_{14:0}$ (myristic), D; $C_{16:0}$ (palmitic), E; and $C_{18:0}$ (stearic) fatty acid, F, with 0.1 µg/g T66.

range of 0.01–1 µg/mL showed good linearity (correlation coefficient = 0.999) (Table I). The limit of detection (signal-to-noise ratio = 3) obtained from these working solutions was found to be 0.01 µg/mL. Evaluation of the calibration graph from the analysis of spiked glycerin at three concentrations gave mean recoveries of 82–106% (Table II). The limit of quantitation (signal-to-noise ratio = 10) was 0.1 µg/g (Figure 5). The suitability of the method was further assessed with different types of fatty acids. The accuracy and precision results for the method are summarized in Table III. The intra-assay precision ranged from 1.80% to 6.51%, whereas the overall analytical variability tested on interassay precision was slightly higher (5.71–9.03%). The fluorescent chromatograms of the spiked fatty acids are also shown in Figure 6.

Conclusion

A reliable HPLC method for the analysis of T66 in glycerin and fatty acids using fluorescence detection has been described. The method showed satisfactory recovery with good precision and accuracy. Because of its rapidity and simplicity, this method is useful and convenient for routine control.

References

1. A. Salmiah. *Selected Readings on Palm Oil and Its Uses*. Palm Oil Research Institute of Malaysia, Kuala Lumpur, Malaysia, 1994, pp. 160–82.
2. B. Rossell. Heat-transfer fluids in the oils and fats industry. 1. Specification, types and toxicity. *Lipid Technol.* **5:** 110–14 (1993).
3. R.T. Sleeter. Effects of processing on quality of soybean oil. *J. Am. Oil Chem. Soc.* **58:** 239–47 (1981).
4. C.J.M. Meershoek. It is time to end use of THFs. *INFORM* **9:** 542–48 (1999).
5. *Report of the 16th Session of the Committee of Fats and Oils*. Codex, London, U.K., March 8–12, 1999, p. 15.
6. T. Takagi. Detection and determination of biphenyl-diphenylether heat transfer medium in fatty oils. *Yukagaku* **18:** 235–39 (1969).
7. C. Imai, H. Watanabe, N. Haga, and T. Li. Detection of heat transfer media in edible oil. *J. Am. Oil Chem. Soc.* **51:** 495–98 (1974).
8. D.B. Min and J. Wen. Gas chromatographic determination of biphenyl and phenyl ether in oil. *J. Am. Oil Chem. Soc.* **59:** 278–79 (1982).
9. *Official and Recommended Practices of the American Oil Chemists' Society*, 5th ed. American Oil Chemists' Society, Champaign, IL, 1998.
10. M.H. Moh and T.S. Tang. Liquid chromatographic detection of Dowtherm A contamination in oleochemicals and edible oils. *J. AOAC Int.* **82:** 893–96 (1999).
11. M.H. Moh, T.S. Tang, and G.H. Tan. Optimization and validation of high-performance liquid chromatographic method for the determination of Dowtherm A in edible oils and oleochemicals. *J. Am. Oil Chem. Soc.* **77:** 1077–82 (2000).
12. "Material Safety Data Sheet". Reference Number 000000000198. Solutia, Inc., St. Louis, MO, 2000.
13. Z. Lu, G. Wu, Y. Gu, and Y. Guan. Development of hydroterphenyl for the use as high temperature organic heat carrier. *Shiyou Huagong* **5:** 305–309 (1986).
14. T.S. Tang, N. Sudin, and R. Baharuddin. "PORIM's Survey of Thermal Heating Oils Used by the Malaysian Palm Oil Refineries". In *PORIM Report PO 274*. Palm Oil Research Institute of Malaysia, Kuala Lumpur, Malaysia, 1996.
15. D.A. Scola, J.S. Adams, C.I. Tewksbury, and R.J. Wineman. Path of partial hydrogenation of *o*-, *m*-, and *p*-terphenyl. *J. Am. Chem. Soc.* **30:** 384–88 (1965).
16. G.T. Hoijtink. A general theoretical approach to the course of chemical reduction, electrochemical reduction and catalytic hydrogenation of hydrocarbons with conjugated double bonds. *Recueil* **76:** 885–906 (1957).

Manuscript accepted September 5, 2001.