# Determination of Partially Hydrogenated Terphenyls-Based Thermal Heating Fluid in Vegetable Oils by HPLC with Fluorescence Detection

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**ABSTRACT:** An HPLC method for the determination of partially hydrogenated terphenyls-based thermal heating fluid, Therminol 66™, in various vegetable oils is described. Direct analysis of palm olein showed that the 3- and 4-cyclohexylbiphenyl peaks of the Therminol 66™ used in quantitative analysis co-eluted with other fluorescent peaks present naturally in the oil. However, those interfering peaks were readily removed after saponification of palm olein. The concentrations of the 3- and 4-cyclohexylbiphenyls of Therminol 66™ were monitored by fluorescence detection at 257 (excitation) and 320 nm (emission). The calibration graph obtained by using the peak areas of the 3- and 4-cyclohexylbiphenyls against the concentrations of Therminol 66™ was linear, with a correlation coefficient of 0.994. The limit of quantitation, using spiked palm olein, was as low as 0.2 μg/g. The coefficients of variation obtained from the intra- and inter-day studies obtained by using three spiked concentrations (0.2, 0.5, and 1.0 μg/g) were 1.76–6.43 and 3.77–10.4%, respectively. The mean recovery value obtained from sunflower, soybean, and canola oils was more than 88.7%.

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**KEY WORDS:** Hydrogenated terphenyl, thermal heating fluid, Therminol 66™.

The physical refining of crude vegetable oil involves several stages. Different temperatures are employed at different stages for different purposes. For instances, the crude oil is preheated to 40°C in the degumming process to remove the phosphatidic materials, whereas the bleaching process is usually carried out at about 105°C. The degummed and bleached oil then undergoes the final deodorization process where temperatures up to 270°C are required to strip off the FFA under vacuum distillation to obtain refined, bleached, and deodorized oil (1). These temperatures are commonly achieved by indirectly heating the oil in heat exchangers with a suitable heating medium whose temperature may reach 300°C or more. High-pressure steam is preferred nowadays, although thermal heating fluids (THF) are still used in many older plants. Most of these heating fluids are petroleum based and are non-food grade, namely a eutectic mixture of biphenyl and diphenyl oxide, partially hydrogenated terphenyls, and mineral oils. Therefore, there are concerns related to possible contamination resulting from pinhole leaks or faulty joints in the heating coils (2–4).

Many publications have appeared regarding GC procedures for the determination of biphenyl and diphenyl oxide mixtures. However, these require tedious extraction or distillation steps (5–8). Moh *et al*. (9,10) have described an HPLC method for the determination of the mixture using fluorescence detection. The advantages of the HPLC method are its simplicity and that it requires no pretreatment of the sample matrix prior to analysis.

In this study, an HPLC method for the determination of the partially hydrogenated terphenyls-based THF in vegetable oils using fluorescence detection is proposed.

## MATERIALS AND METHODS

All solvents used were of HPLC grade, and double-distilled water was used throughout the study. Vegetable oils were obtained from reliable sources that use high-pressure steam in their processes.

*Chemical and reference standards. o*-, *m*-, And *p*-terphenyls were purchased from Fluka (Buchs, Switzerland), and 1,4-dicyclohexylbenzene was from Sigma (St. Louis, MO). THF (Therminol 66™) was a gift from Solutia (St. Louis, MO).

*Analytical HPLC*. The system consisted of a pump (Jasco PU-980; Jasco International Co., Ltd., Tokyo, Japan), a 3-inline degasser (Jasco DG-980-50), a ternary gradient unit (LG-980-02S), an autosampler (Jasco 851-AS), and a column oven (Jasco CO-965). The Jasco programmable fluorescence detector (FP-970) and the multi-wavelength UV detector (MD-1510) were controlled by Borwin 1.21 (JMBS Developpements, Le Fontanil, France) chromatographic software. The system was run isocratically with a mobile phase of methanol and water (88:12, vol/vol). The flow rate was set at 1 mL/min, and a reversed-phase column (5 μm Lichrospher C18, 250 × 4.6 mm i.d.; GL Sciences Inc., Tokyo, Japan) was maintained at 40°C. The detector was optimized at an excitation wavelength of 257 nm and an emission wavelength of 320 nm.

*GC–mass selective detection (MSD)*. The system was a Hewlett-Packard (Palo Alto, CA) HP-5890 GC equipped with an HP-5970 MSD and a split/splitless injection port. The GC column was an HP-5 cross-linked with 5% phenyl methylsiloxane capillary column, 30 m × 0.32 mm i.d. Carrier gas was helium at 0.8 mL/min. The column temperature was programmed

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from 150 to 280°C at the rate of 10°C/min, and then held at 280°C for 20 min until all peaks had eluted. The injector and detector temperatures were set at 200°C. Data were acquired using a HP-59970 MS Chemstation.

*Working solutions*. A 100-µg/mL stock solution was prepared by dissolving 10 mg Therminol 66™ with ethanol to the mark in a 100-mL volumetric flask. The appropriate aliquots of the stock solution in ethanol were diluted to produce working standards with 10, 5, 1, 0.5, 0.1, and 0.01 µg/mL. Linearity of the detector was checked from this set of six working standards. Calibration graphs were prepared by plotting the peak area of 3- and 4-cyclohexylbiphenyl against the concentrations of Therminol 66™.

*Sample preparation*. The blank sample (10% wt/vol) was prepared by mixing 5 g of oil with 45 mL ethanol (95%) and 5 mL of aqueous KOH (50%, wt/vol) in a 250-mL round-bottomed flask. The mixture was then saponified at about 80°C for 1 h under reflux (11). A 20-µL aliquot of the cooled sample solution was analyzed directly.

To obtain a spiked oil sample with 1.0 µg/g Therminol 66™ for recovery study, 5 mL of the working solution (1 µg/mL) was added to a preweighed oil sample (5 g) in a 250-mL round-bottomed flask. The solution was further mixed with 40 mL ethanol and 5 mL aqueous KOH (50% wt/vol). The spiked solutions containing 0.5 and 0.1 µg/g Therminol 66™ were prepared in a similar manner except that 0.5 and 0.1 µg/mL of working solutions were used, respectively. The final concentration of oil mixture was 10% (wt/vol), and 20 µL of each of these solutions was analyzed by HPLC. Recoveries were calculated by interpolation from the calibration curve established earlier.

## RESULTS AND DISCUSSION

Therminol 66™ THF is a complex mixture of terphenyls (3–8%), partially hydrogenated terphenyls (74–87%), quaterphenyls, higher polyphenyls, and their hydrogenated products (18%) (12). The manufacturer claims that this clear, 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 pumpability to 0°C. Details of the hydrogenation of terphenyls have been described by Scola *et al.* (13). On the basis of this study, the partial hydrogenation of terphenyls proceeds stepwise by saturating the aromatic rings one at a time. For example, *o*-terphenyl is hydrogenated mainly to 1,2-diphenylcyclohexane. Further hydrogenation gives 1,2-dicyclohexylbenzene and tercyclohexyl. The partial hydrogenation of *m*- and *p*-terphenyls also follows the same patterns. The catalytic hydrogenation of polyphenyls has been reported by Hoijtink (14).

Figure 1 is an HPLC chromatogram of Therminol 66™ using fluorescence detection. The elution pattern of terphenyls and their hydrogenated products also has been studied (15). The terphenyl isomers are eluted first (7–12 min) followed by cyclohexylbiphenyls (15–23 min), dicyclohexylbenzenes (23–30 min), and tercyclohexanes (45–52 min). The retention

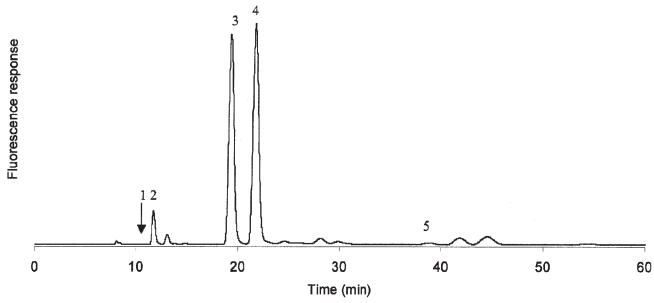


**FIG. 1**. A typical HPLC chromatogram of a Therminol 66™ solution using fluorescence detection. The mobile phase was methanol and water (88:12, vol/vol), and the detector was set at 257 nm (excitation) and 320 nm (emission). Peak identifications: 1, *o*-terphenyl; 2, *m*-terphenyl; 3, 3-cyclohexylbiphenyl; 4, 4-cyclohexylbiphenyl; 5, 1,4-dicyclohexylbenzene.

times for *o*-, *m*-, *p*-terphenyls, and 1,4-dicyclohexylbenzene peaks are readily assigned using reference standards. However, owing to the Rayleigh effect or to having similar excitation and emission wavelengths (16), *o*-terphenyl does not show up in the fluorescence chromatogram but is observed by using UV detection. Similarly, the tercyclohexyl isomers do not fluoresce or possess any chromophore. The peaks can be detected by using ELSD (15). Thus, the fluorescence peaks observed at 50–60 min (Fig. 1) are likely due to the hydrogenated products of polyphenyls in the Therminol 66™.

The two strongest fluorescence peaks of Therminol 66™, labeled 3 and 4 (Fig. 1) at 19.1 and 21.6 min, respectively, were used as markers, but only the latter peak was used for quantitative analysis. The two peaks were subjected to multi-wavelength UV detection, and the absorption spectra are illustrated in Figure 2. The maximal absorption band at 247 nm was the characteristic of biphenyl absorption of 3- and 4-cyclohexylbiphenyl (13). Additional confirmation of the peaks collected from the HPLC was performed using GC–MSD. The total ion chromatogram of the fraction is illustrated in Figure 3A, and the fragmentation patterns of 3- and 4-cyclohexylbiphenyl with molecular ion at *m/z* 236 are shown in Figures 3B and 3C, respectively. 2-Cyclohexylbiphenyl, co-eluted

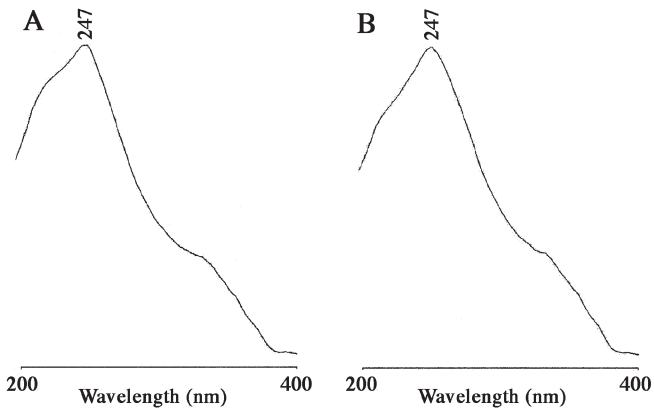


**FIG. 2**. Absorption spectra of (A) 3- and (B) 4-cyclohexylbiphenyl obtained using multi-wavelength UV detection.

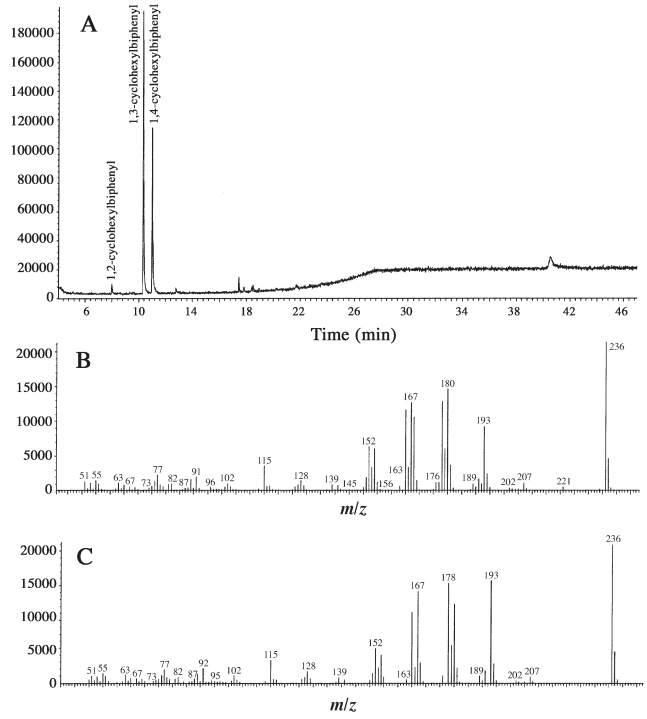


**FIG. 3**. (A) A total ion chromatogram of the two strongest fluorescence peaks collected from HPLC; mass spectra of (B) 3- and (C) 4-cyclohexylbiphenyls.

with 3- and 4-cyclohexylbiphenyl on the reversed-phase column, was also detected by GC–MSD (Fig. 3A).

The linearity of the fluorescence detection of the Therminol 66™ was tested by plotting peak areas of 4-cyclohexylbiphenyl against concentrations over the range of 0.01–10 µg/mL. A typical straight regression line of $y = 361{,}753x + 5{,}792$ (where $y$ is the peak area and $x$ is the concentration of Therminol 66™) with a correlation coefficient $r^2$ of 0.9985 was obtained. The sensitivity of the fluorescence detector was such that the 4-cyclohexylbiphenyl peak of Therminol 66™ could be detected to as low as 0.2 ng.

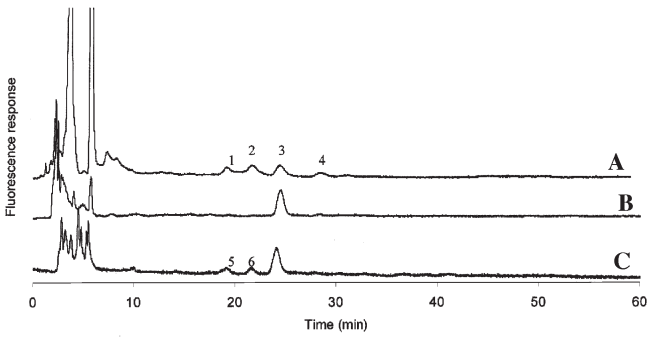


**FIG. 4**. Fluorescence chromatograms of blank palm olein (A) before and (B) after saponification and (C) palm olein spiked with Therminol 66™ (1.0 µg/g), analyzed after saponification. Peaks labeled 1 to 4 were the fluorescent components present naturally in palm olein. Peaks labeled 5 and 6 were 3- and 4-cyclohexylbiphenyl, respectively.

Figure 4, lines A and B, illustrate the HPLC chromatograms of the palm olein blank before and after saponification, respectively. Without the saponification step, there were about four fluorescent peaks for palm olein between 18 and 30 min (labeled 1 to 4, line A). Peaks 1 and 2 possessed retention times similar to those for 3- and 4-cyclohexylbiphenyls. Attempts to increase water concentration in the mobile phase, thus increasing its polarity, could not resolve the cyclohexylbiphenyls from the interfering peaks. In addition, greater water content meant fewer polar glyceridic components of palm olein were bound too strongly onto the reversed-phase column. As a result, flushing of the column with a less polar solvent, such as dichloromethane or THF, was needed after several injections. However, by simply saponifying the palm olein, the interfering peaks (labeled 1 and 2) were removed from the HPLC chromatogram of the soap solution (Fig. 4, line B).

The limit of detection of Therminol 66™, based on spiked palm olein, was found to be 0.1 µg/g (signal-to-noise, S/N = 3), whereas the limit of quantitation was 0.2 µg/g (S/N = 10). The reproducibility of the HPLC method examined at three spiked concentrations (0.2, 0.5, and 1.0 µg/g) in palm olein is given in Table 1. The mean recovery of Therminol 66™ from the saponification process was satisfactory, 87.0% at a level of 0.2 µg/g and 95.6% at 1.0 µg/g, based on six replicate analyses each. The CV obtained from the intra-day study were small, ranging between 1.76 and 6.43%, whereas the inter-day study showed slightly higher values in terms of the CV, ranging from 3.77 to 10.4%. The applicability of the HPLC method using sunflower, soybean, and canola oils was also examined. Mean recovery was more than 88.7%, with acceptable CV of less than 3.3% (Table 2). The HPLC chromatograms of the vegetable oils spiked with different concentrations of Therminol 66™ are illustrated in Figure 5. The

**TABLE 1**
**Reproducibility of the HPLC Analysis of Therminol 66™ in Spiked Palm Olein**

| | Mean calculated[a] (µg/g) | SD | CV (%) |
|---|---|---|---|
| Intra-day study[b] | | | |
| 0.2 | 0.174 | 0.011 | 6.43 |
| 0.5 | 0.488 | 0.009 | 1.84 |
| 1.0 | 0.956 | 0.017 | 1.76 |
| Inter-day study[b] | | | |
| 0.2 | 0.176 | 0.018 | 10.4 |
| 0.5 | 0.471 | 0.020 | 4.23 |
| 1.0 | 0.931 | 0.035 | 3.77 |

[a]Means of six readings.
[b]Examined at three different concentrations (µg/g).

**TABLE 2**
**Recovery Studies of Therminol 66™ at 0.2 µg/g Obtained from Various Spiked Oils ($n$ = 3)**

| Oil matrices | Recovery (%) | CV (%) |
|---|---|---|
| Sunflower | 92.3 | 2.35 |
| Soybean | 88.7 | 3.26 |
| Canola | 90.2 | 2.01 |

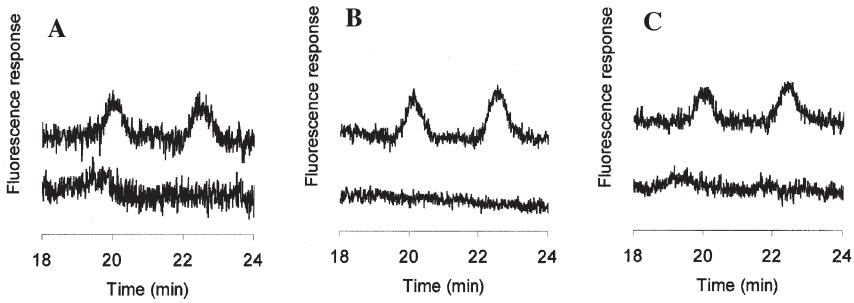


**FIG. 5**. Fluorescence chromatograms showing blanks and vegetable oils spiked with Therminol 66™. (A) Sunflower oil was spiked at a 0.1 µg/g level; (B) soybean oil and (C) canola oil were spiked at a 0.2 µg/g level.

chromatograms of these blank oils illustrated the absence of any background fluorescent components that would interfere with the quantitative results.

The HPLC method described in this study offers a sensitive procedure for the determination of Therminol 66™ contamination in vegetable oils. Saponification of the vegetable oils effectively removes the undesirable fluorescent components that originally interfered with the quantitative analysis. This method is ideal for process control and quality monitoring purposes in the refining of the vegetable oils.

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