CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. Eutectic mixtures of diphenyl oxide and biphenyl

4.1.1. Analytical assessment

The mixture is traded as Therminol VP1™ or Dowtherm A™ and it is available in the ratio of 73.5% diphenyl oxide and 26.5% biphenyl. The chemical structures of these two compounds are illustrated in Figure 4.1. Dowtherm is a registered trademark of Dow Chemical Inc., USA, whereas Therminol is that of Solutia Inc., USA. This heating medium has excellent thermal stability at the operating temperature range of 12-260°C in liquid phase and 260-400°C in vapor phase[122].

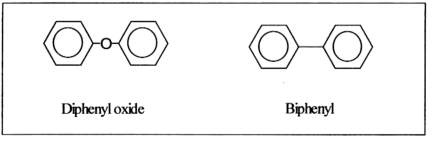

Figure 4.1. Chemical structures of biphenyl and diphenyl oxide molecules.

4.1.1.2. Spectrofluorometric analysis

The fluorescence excitation and emission spectra of biphenyl, diphenyl oxide, and Dowtherm A™ eutectic mixtures are shown in Figure 4.2. The biphenyl sample gives 5 distinct fluorescence excitation bands at 227, 236, 247, 261, and 268 nm and a maximum emission band at 314 nm. Diphenyl oxide, on the other hand, shows maximum excitation band at 272 nm and doublet maximum emission bands at 293 and 295 nm. In addition to the triplet excitation bands at 247, 253, and 259 nm as well as the doublet emission bands at 305 and 314, the characteristic band of benzene at 227 nm is also observed in Dowtherm A™.

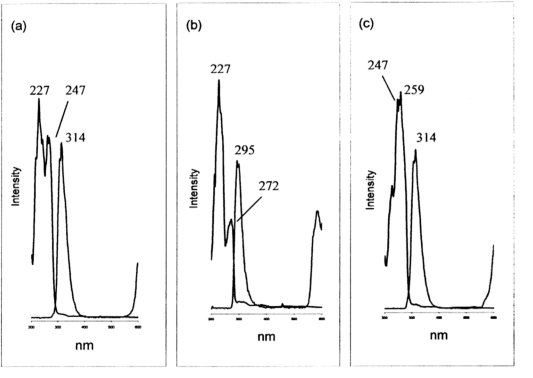

Figure 4.2. Fluorescence excitation and emission spectra of (a) biphenyl, (b) diphenyl oxide and (c) Dowtherm A™ solutions in hexane. All solutions are 1 μg/mL concentration.

4.1.1.2. HPLC-UV analysis

Figure 4.3a illustrates the HPLC chromatogram of biphenyl and diphenyl oxide (100 μg/mL each) obtained by using UV detection (254 nm). Due to its high conjugation, the biphenyl peak was calculated to be about 15.2 times more intense compared to the diphenyl oxide peak. By injecting various concentrations of biphenyl and diphenyl oxide solutions, the LOD of these 2 compounds were determined to be 0.02 and 0.23 μg, respectively. The UV detector was found to be linear (r^2>0.979) over the range of 0.005 to 10 μg/g as tested.

The HPLC-UV detection of the eutectic mixture is shown in Figure 4.3b. The first peak is ascribed to diphenyl oxide and the other is biphenyl as confirmed by comparing with the reference standards. The calibration graph plotted over 3 different concentrations of Dowtherm A™ is illustrated in Figure 4.4. The UV detector was found to be able to detect as low as 0.04 μg biphenyl and 0.07 μg diphenyl oxide of Dowtherm A™.

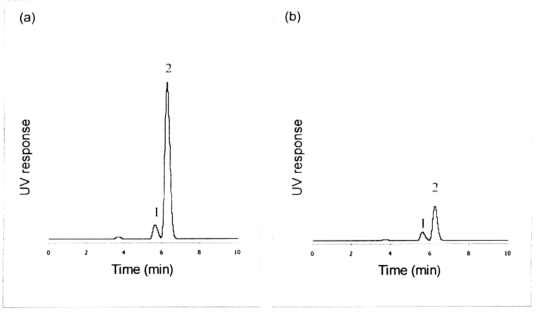

Figure 4.3. HPLC chromatograms of (a) diphenyl oxide and biphenyl standard solution of the same concentration (100 μg/mL each) and (b) Dowtherm A™ solution (100 μg/mL) plotted on similar scale. Peak identification: *1*, diphenyl oxide; and *2*, biphenyl. HPLC conditions: *column*, GL C_{18} 250 x 4.6 mm i.d.; *mobile phase*, methanol and water (90:10, vol/vol); *oven temperature*, 40°C; and *UV detection*, 254 nm.

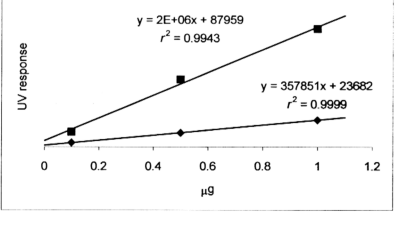

Figure 4.4. Calibration graph of the eutectic mixture with different concentrations plotted against the peak areas of diphenyl oxide (◆) and biphenyl (■).

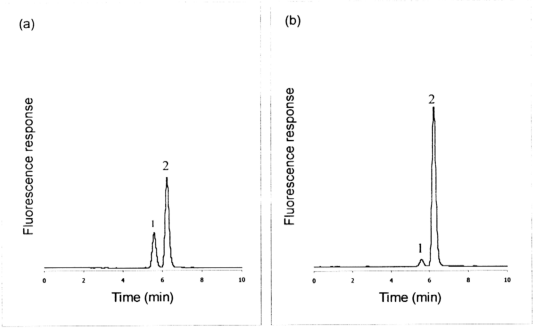

Figure 4.5. HPLC fluorescence chromatograms of Dowtherm A™ (10 μg/mL) plotted on the same scale. The wavelengths used were (a) 247 nm (excitation) and 310 nm (emission), and (b) 272 nm (excitation) and 300 nm (emission). Peak identification: *1*, diphenyl oxide; and *2*, biphenyl. For HPLC conditions see Figure 4.3.

4.1.1.3. HPLC-fluorescence analysis

The fluorescence excitation and emission wavelengths of biphenyl and diphenyl oxide were also assessed by an HPLC coupled to a fluorescence detector (Table 4.1). The detector setting at 260 nm (excitation) and 310 nm (emission) showed the strongest sensitivity, whereas setting the wavelength at 272 nm (excitation) and 300 nm (emission) produced the weakest sensitivity. The fluorescence chromatograms of these 2 settings used for the analysis of Dowtherm A™ are depicted in Figure 4.5. Although it consisted of only 26.5% of the total weight, the biphenyl peak was very much stronger than the diphenyl oxide peak.

The fluorescence excitation and emission at 260 and 310 nm for both biphenyl and diphenyl oxide were found to be about 11 and 18% higher, respectively, compared to those observed at 247 nm (excitation) and 310 nm (emission). However, when the fluorescence detector was tested for its sensitivity using the working solutions prepared from Dowtherm A™ (0.005-10.0 μg/mL), the lowest LOD obtained for these two settings was similar. Biphenyl has a lower LOD (0.34 ng) compared to diphenyl oxide (1.04 ng). The fluorescence detector also gives a good linearity (r^2 = 1).

Table 4.1. Optimization of fluorescence wavelengths for quantitation of biphenyl and diphenyl oxide

Wavelengths (nm)		Area under peaks, arbitrary units		Response factor	
Excitation	Emission	BP	DPO	BP	DPO
247	310	614682	36392	1.00	1.00
260	310	684011	64643	1.11	1.78
260	314	646675	39821	1.05	1.09
272	300	270792	117692	0.44	3.23

BP, biphenyl; DPO, diphenyl oxide.

4.1.1.4. GC-MS analysis

The total ion chromatogram of Dowtherm A™ gave 2 distinct peaks and they correlate well with the reference standards. The mass spectrum of biphenyl was represented by a strong molecular ion at 254 m/z, while diphenyl oxide shows a molecular ion peak at 170 m/z with other medium intensity fragment ions at 141 and 115 m/z.

4.1.2. Determination of the eutectic mixtures in spiked matrices

4.1.2.1. Optimization of HPLC-fluorescence detection

Chromatographic studies have previously showed that the fluorescence detector is more sensitive when compared to the UV detector for the determination of diphenyl oxide and biphenyl. In addition, the fluorescence wavelength setting at 247 (excitation) and 310 nm (emission) produced the highest sensitivity for detecting the eutectic mixtures. Thus, these basic parameters could be used for the quantitative analysis of Dowtherm A™ in various spiked samples in this study.

Another obstacle to be considered was the possible presence of other fluorescent components in the matrices. For example, highly conjugated non-aromatic, aromatic, and heterocyclic compounds can also fluoresce. Thus, the presence of tocopherols, tocotrienols or conjugated fatty entities in edible oil or oleochemical products will produce a certain degree of fluorescence. Oxidative products such as peroxides and aldehydes can also possess fluorescence properties. Hydrocarbons containing conjugated double bonds are known to fluoresce as well[123,124].

As a result, if these compounds co-eluted with Dowtherm A™, they may interfere with the quantitative results. Many possible sample pre-treatment or cleanup procedures have been studied to resolve the various interfering matrices prior to HPLC analyses. For example, Mackay *et al.*[125] have reported a solid phase extraction procedure followed by a simple derivatization step for the determination of nonylphenol and octylphenol ethoxylates in effluent samples from sewerage

plants. Saponification followed by oxidative UV photolysis steps were described by Buidini *et al.*[126] prior to the analysis of the inorganic matters in edible oils using HPLC.

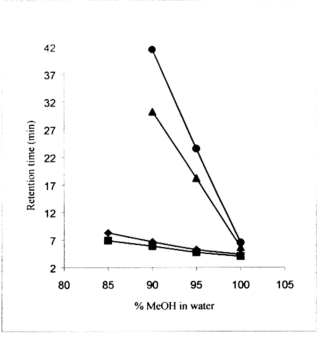

Figure 4.6. Changes in retention times of diphenyl oxide (■) and biphenyl (◆) with respect to ratios of methanol and water mixture. Interfering fluorescent components labeled A (▲) and B (●) are indicated in Figure 4.7.

In this study, different compositions of mobile phase were evaluated for the optimal separation of interfering components from Dowtherm A™ (Figure 4.6). By using methanol solely as the mobile phase, the interfering components (labeled A and B) eluted very close to the Dowtherm A™ peaks. Under such circumstances, a good baseline resolution for accurate quantitation was impossible to achieve. Further experiments showed that by decreasing the polarity of the mobile phase by adding acetonitrile, resulted in the overlapping of both Dowtherm A™ and other

fluorescent component peaks. By increasing the polarity of the mobile phase it helped to resolve the interfering components from Dowtherm A™. As excessive water in the system leads to long analysis time, the optimal mobile phase of methanol and water mixture in the ratio of 90:10 (vol/vol) was used. This condition was further used to analyze some of the blank matrices, namely the palm olein, corn oil, sunflower oil, fatty alcohol and fatty acid, and the chromatograms are given in Figure 4.7. The results showed that all the samples have equally smooth baselines with no major interfering components that co-eluted with the Dowtherm A™ peaks. Thus, this condition was found to be suitable for the quantitative analysis of Dowtherm A™ in these sample matrices.

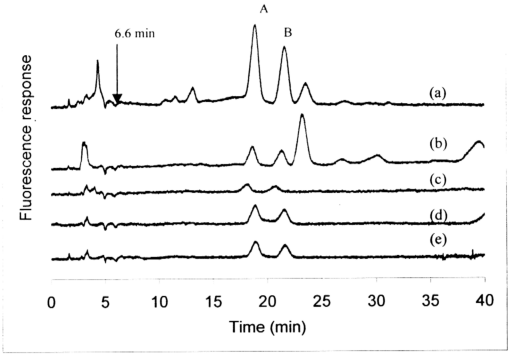

Figure 4.7. Stacked chromatograms of control blank samples of (a) fatty acid, (b) palm olein, (c) corn oil, (d) sunflower oils, and (e) fatty alcohol. No fluorescent components were observed at 6.6 min as indicated by an arrow that might interfere with the quantitation of biphenyl for the determination of Dowtherm A™. Labels A and B are interfering components present naturally in matrices. HPLC conditions: *column*, GL C_{18} 250 x 4.6 mm i.d.; *mobile phase*, methanol and water (90:10, vol/vol); *oven temperature*, 40°C; and *fluorescence detector*, 247 nm (emission) and 310 nm (emission).

4.1.2.2. Quantitative analysis of the eutectic mixtures in vegetable oils and oleochemicals

From a comparison made between the fresh and used Dowtherm A™ using fluorescence detection, it was found that the biphenyl peak in the fresh Dowtherm A™ fluoresces with relatively higher intensity compared to that in the used fluid. This could be due to the degradation of the fluid after prolonged usage at high

This could be due to the degradation of the fluid after prolonged usage at high temperatures. Thus, in this study, the used Dowtherm A™ was selected for constructing the calibration graph as it was more likely to be present in a contaminated sample in the event of a leak. In addition, since the biphenyl peak gave a lower LOD as compared to the diphenyl oxide as shown previously, the former peak would be used as a marker for the analysis of Dowtherm A™.

The fluorescence peak areas of biphenyl present in Dowtherm A™ obtained from the various calibration standards are given in Table 4.2. A plot of the peak areas against the concentration of Dowtherm A™ revealed that the calibration graph is linear (correlation coefficient, r^2 of 0.999) throughout the range analyzed, and with a regression equation of $y = 585685x + 12598$ (where y is the peak areas of biphenyl and x is the concentration of Dowtherm A™). The calibration was then validated using various spiked glycerin samples containing 0.11-7.5 μg/g of Dowtherm A™. The results (Table 4.3) showed that the highest recovery was observed in samples spiked with 7.5 μg/g of Dowtherm A™. The accuracy of the method decreased slightly as the spiked concentration decreased. For instance, the recovery obtained from the sample spiked with 0.1 μg/g of Dowtherm A™ was about 6-7% lower compared to that at 2.5 and 5.0 μg/g levels, respectively. However, the overall recovery of >90% was still satisfactory, even at LOQ of 0.1 μg/g level. The high recovery in this study could be attributed to the use of minimal sample preparation steps—sample matrices were diluted in suitable organic solvents and analyzed directly. The results on the precision of the method developed for the analysis of Dowtherm A™ in spiked glycerin samples are also given in Table 4.3. The coefficient of variations (CVs) obtained from the higher

spiked concentrations were generally small (<2%) except at the concentration of 0.1 μg/g of Dowtherm A™. The bigger variation in terms of CV could have been a result of a higher baseline noise at such a low level of detection. However, the CV of 12.0% was still within the acceptable limit of 20.0% for LOQ.

In view of the fact that there was no co-eluting components present in fatty acid, fatty alcohol, and vegetable oils analyzed (Figure 4.7), the suitability of the method for the determination of Dowtherm A™ in these matrices was also evaluated. The statistical results obtained from these samples spiked with various concentrations of Dowtherm A™ are summarized in Table 4.4. The overall recovery of the spiked samples with 0.15 to 10.7 μg/g Dowtherm A™ was generally good, ranging from 91.3 to 108.7%. The CV observed from these spiked concentrations ranged from 0.18 to 1.14%. A plot of the mean of triplicate analyses against spiked concentrations yielded a slope close to unity, r^2 of 0.9891. Separate analyses of 5 spiked basic oleochemicals at 0.1 μg/g level (Figure 4.8) showed the accuracy of the method was between 87.8 to 95.2% whereas the precision was 5.4 to 9.7%. It showed that the method was generally good for detecting Dowtherm A™ to as low as 0.1 μg/g.

Table 4.2. Calibration data obtained from Dowtherm A™ solutions in methanol

Conc., μg/mL	Mean of area counts (3 readings)	SD	CV., %
0.005	15694	66.06	0.420
0.01	43901	43.10	0.098
0.1	87581	8.50	0.009
1.08	601882	13566.51	1.760
5.4	3166802	1297.89	0.041
10.8	6345365	3120.69	0.050

SD; standard deviation

Table 4.3. Recovery studies of Dowtherm A™ from spiked glycerin samples

Spiked conc.,	Calculated results[a]			
µg/mL	Conc., µg/g	SD	CV., %	Rec., %
7.5	8.24	0.064	0.78	109.9
5.0	4.88	0.087	1.78	97.6
2.5	2.42	0.035	1.44	96.8
0.11	0.1	0.012	12	90.9

[a]Mean of 3 readings.
Rec.; recovery

Table 4.4. Recoveries of Dowtherm A™ from various spiked samples based on 3 reading each

Sample matrix	Spiked conc., µg/g	Calc. Conc., µg/g	SD	CV., %	Rec., %
$C_{12:0}$-$C_{14:0}$ fatty alcohols	10.70	9.77	0.05	0.51	91.3
$C_{8:0}$-$C_{10:0}$ methyl esters	5.12	5.36	0.05	0.93	104.7
Oleic acid	2.42	2.63	0.03	1.14	108.7
Palm olein	1.50	1.82	0.02	0.18	103
$C_{8:0}$-$C_{10:0}$ fatty acids	0.15	0.16	0.006	0.75	106.7
Slope	0.9547				
Intercept	0.2392				
r^2	0.9891				

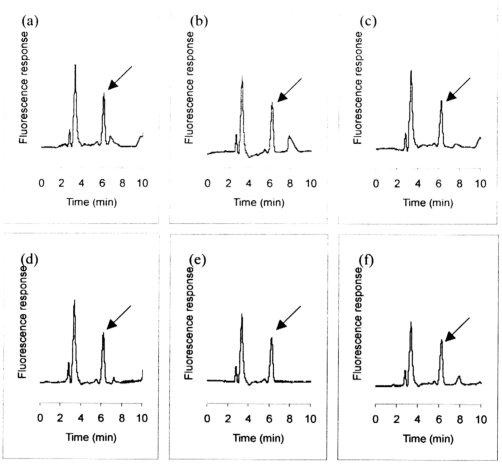

Figure 4.8. HPLC chromatograms showing samples spiked with 0.1 μg/g Dowtherm A™. (a) $C_{12:0}$ fatty acid, (b) $C_{12:0}$ fatty alcohol, (c) $C_{12:0}$ methyl ester, (d) $C_{14:0}$ fatty acid, (e) $C_{14:0}$ fatty alcohol, and (f) $C_{14:0}$ methyl ester. Diphenyl oxide eluted at 5.5 min and biphenyl, as indicated by an arrow, at 6.6 min. For HPLC conditions see Figure 4.7.

4.1.2.3. Inter-laboratory study of the HPLC method for the determination of the eutectic mixtures

In recent years[30], there is only 1 instrumental method that has been accepted as an official method for determining the eutectic mixtures of diphenyl oxide and biphenyl in vegetable oils and their by-products. The analytes are separated from the matrices using liquid-liquid extraction prior to GC analysis. The disadvantage of the method is that it involves a long and tedious sample preparation step, and it also

requires a very skillful technician to carry out the extraction step. In addition to this, the errors resulting from replicate studies are generally unsatisfactory especially at the lower concentration range.

In contrast, the HPLC method described in this study is rather straightforward; where sample matrices are dissolved in suitable organic solvents and analyzed directly with a HPLC coupled to a fluorescence detector. A communication with the AOCS showed that the method indeed has the potential to be accepted as an official method for the determination of Dowtherm A™ with the proviso that a short inter-laboratory study (at least 3 laboratories) be carried out to demonstrate the transferability of the method.

The 3 laboratories involved in this interlaboratory study were located at (1) the Advanced Oleochemical Technology Center and Product Development, and (2) the Quality Unit of the MPOB and (3) the Department of Food Technology of the University Putra Malaysia. The samples were prepared using the single-blind approach where one analyst prepares the spiked samples at varying levels and sends them to a second analyst, who would carry out the analyses. The standard solutions and spiked samples of known concentrations were prepared by a central laboratory and distributed to all the three laboratories to be analyzed by the respective analysts. During this period, the laboratories are in constant communication so that the goals of the method optimization and validation can be achieved in an efficient manner. At the end of the study, the final results are compiled by the central laboratory and submitted to a third party, a statistician from the Techno-Economic Services Unit of MPOB, for evaluation. This was to prevent any additional bias to the study. The results obtained are discussed in the subsequent sections.

The linearity of the analytical procedure was tested by using spiked palm olein, ranging from 0.1 to 2.0 μg/g Dowtherm A™, in 6 replicates for each concentration level. All the 3 laboratories produced straight regression lines with r^2 close to unity. The LOQ for the Dowtherm A™ were determined to be 0.1 μg/g for all samples and 0.2 μg/g for stearic acid. The LOD for the Dowtherm A™ was estimated to be 0.01 μg/g for palm olein, fatty alcohol, corn oil, and sunflower oil, but slightly higher for stearic acid of 0.08 μg/g.

The mean recoveries (Table 4.4) for all three laboratories were satisfactory for residues at 0.1-1.0 μg/g. The highest average intra-laboratory recovery was 116% for corn oil at a concentration of 0.2 μg/g, and the lowest recovery was 93% for sunflower oil at a concentration of 0.1 μg/g. The intra-laboratory repeatabilities ranged from 0.23 to 0.42% for fatty alcohol samples, 1.25 to 4.33% for stearic acid samples, 0.97 to 8.21% for corn oil samples, and 0.81 to 4.58% for sunflower samples. The results also showed that the inter-laboratory reproducibilities varied from 1.29 to 3.84%. The data obtained from all 3 laboratories showed good linearity between the actual spiked concentrations and the predicted concentrations, with r^2 exceeding 0.998 (Table 4.4).

Table 4.5. Inter-laboratory study on the recoveries of Dowtherm A™ from various spiked samples (n=6)

Laboratory	Rec. (μg/g) (CV., %)[a] Fatty alcohol	Stearic acid	Corn oil	Sunflower oil	Slope	Intercept
1[b]	0.956 (0.42)	0.515 (1.86)	0.246 (2.85)	0.098 (0.82)	1.07	-0.035
2[c]	0.975 (1.12)	0.505 (1.25)	0.238 (0.97)	0.081 (4.58)	1.031	-0.015
3[d]	0.982 (0.23)	0.483 (4.33)	0.212 (8.21)	0.100 (4.10)	1.028	-0.007
Amt. Added	1.0	0.5	0.2	0.1		
Ave. found	0.971	0.501	0.232	0.093		
Rec., %	97.1	100.2	116.0	93.0		
Within-lab CV., %	0.589	2.479	1.006	3.165		
Between-lab CV., %	1.287	2.435	3.836	3.118		

[a]Values in parentheses are CVs.
[b]$r^2 = 0.998$
[c]$r^2 = 0.998$
[d]$r^2 = 0.999$

4.2. Partially hydrogenated terphenyls

4.2.1. Analytical assessment

Trademarked as Therminol 66™ or Dowtherm HT™, the manufacturers claim 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. They consist of a complex mixture of terphenyls (3-8%), partially hydrogenated terphenyls (74-87%, quaterphenyls, higher polyphenyls and their hydrogenated products (18%)[122].

A preliminary GC analysis showed that the fluid gave a complex mixture of peaks. However, only *o*- *m*-, *p*-terphenyls and 1,4-dicyclohexylbenzene were assigned by comparing them to the standards. The identification of other major peaks, which could be attributed to the hydrogenation products of the terphenyls, posed some difficulty due to the unavailability of reference standards. Therefore, the hydrogenated products from the terphenyls were prepared based on the procedure described by Scola *et al.*[120].

According to the study, partial hydrogenation of the terphenyls proceeds stepwise by saturating the aromatic rings one at a time (Figure 4.9). For instance, *o*-terphenyl is hydrogenated primarily to 1,2-diphenylcyclohexane and 2-cyclohexylbiphenyl. Further hydrogenation of *o*-terphenyl gives 2-phenylbicyclohexyl, 1,2-dicyclohexylbenzene and finally *o*-tercyclohexyl. On the other hand, the major product from the hydrogenation of *m*-terphenyl is 3-cyclohexylbiphenyl together with small amounts of 1,3-diphenylcyclohexane, 1,3-dicyclohexylbenzene, 3-phenylbicyclohexyl and *m*-tercyclohexyl. The hydrogenation of *p*-terphenyl also followed the same pattern—4-cyclohexylbiphenyl is the major component while 1,4-diphenylcyclohexyl and 1,4-dicyclohexylbenzene are obtained in smaller quantities.

Such information on the reduction of terphenyls is important as it could assist in the process of identification of the hydrogenated products obtained in this study. In addition, since the final products of the hydrogenation of terphenyls consist of mixtures, HPLC and GC techniques are used to resolve the individual components for the purpose of identification.

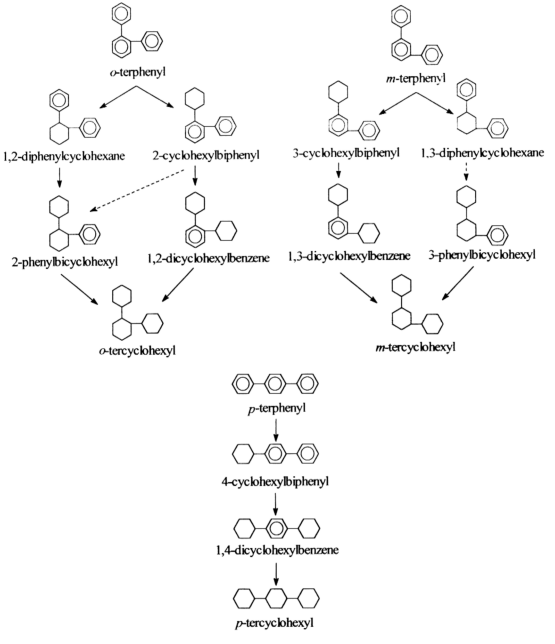

Figure 3.9. Partial hydrogenation pathways of terphenyls proposed by Scola *et al.*[120].

4.2.1.1. Spectrofluorometric analysis

The fluorescence excitation and emission spectra of the Therminol 66™ are illustrated in Figure 4.10a. Therminol 66™ showed a maximum excitation band at 257 nm and a maximum emission band at 320 nm. Figures 4.10b, 4.10c, 4.10d, and 4.10e illustrate the fluorescence spectra of *o*-, *m*-, *p*-terphenyl and 1,4-dicyclohexylbenzene solutions, respectively. *o*-Terphenyl gave similar excitation and emission at 314 nm. *m*-Terphenyl, on the other hand, shows two distinct and strong bands at 291 (excitation) and 343 (emission) nm. *p*-Terphenyl possesses two strong excitation bands at 207 and 271 nm, whereas the maximum emission band is observed at 341 nm. 1,4-Dicyclohexylbenzene shows doublet excitation bands at 228 and 266 nm and a singlet emission band at 293 nm.4.2.1.2. HPLC analysis

The linearity of calibration plot *via* the use of the UV detector for the analysis of terphenyl standard solutions is illustrated in Figure 4.11. The r^2 obtained were 0.997, 0.997 and 0.998 with instrumental limits of 19.8, 5.5 and 21.8 ng for *o*-, *m*- and *p*-terphenyl, respectively. The HPLC-UV chromatogram in Figure 4.12a also showed that each of these gave a different response factor even though it was of the same concentration (0.8 μg). The *m*-terphenyl was the most sensitive whereas the 1,4-dicyclohexylbenzene, due to the 2 cyclohexane rings, showed the least UV-absorbing chromophore. Thus the latter gave a higher LOD of 99.7 ng.

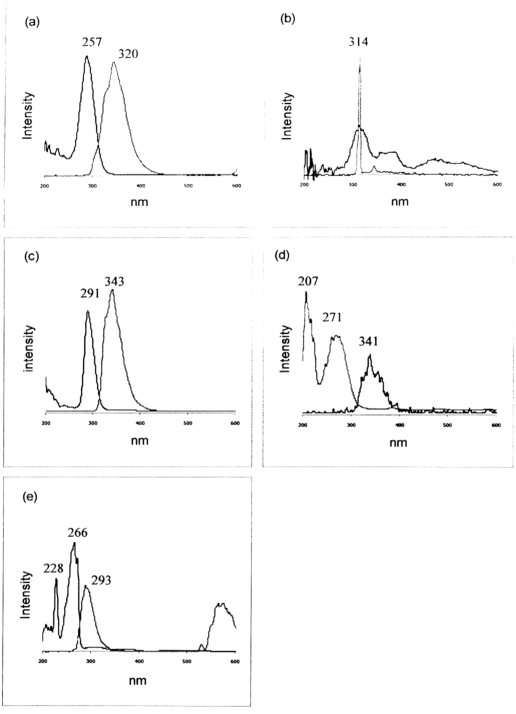

Figure 4.10. Fluorescence excitation and emission spectra of (a) Therminol 66™, (b) *o*-, (c) *m*-, (d) *p*-terphenyl and (e) 1,4-dicyclohexylbenzene in tetrahydrofuran-methanol (50:50, vol/vol).

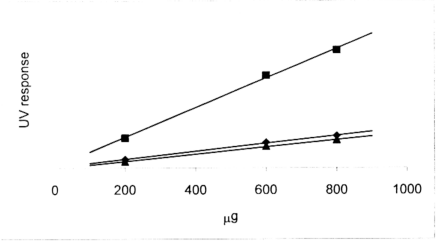

Figure 4.11. Calibration graphs derived from *o*- (◆), *m*- (■) and *p*-terphenyl (▲) solutions showing the linearity of the UV detector. r^2 was > 0.997. HPLC conditions: *column*, GL C_{18} 250 x 4.6 mm i.d.; *mobile phase*, methanol and water (90:10, vol/vol); *oven temperature*, 40°C; and *UV detector*, 254 nm.

As Therminol 66™ is made up of the hydrogenation products of various terphenyls and polyphenyl, the HPLC-UV chromatogram also showed a complex mixture of peaks (Figure 4.12b). By comparing the retention times of the reference standards available, only 4 peaks were readily identified, at this point, as *o*-, *m*-, *p*-terphenyl and 1,4-dicylohexylbenzene at 7.01, 9.63, 10.7 and 29.7 min, respectively. The identification of the other major peaks were attempted based on their absorption characteristics and by comparing them to those obtained by Scola *et al.*[120] as given in Table 4.6.

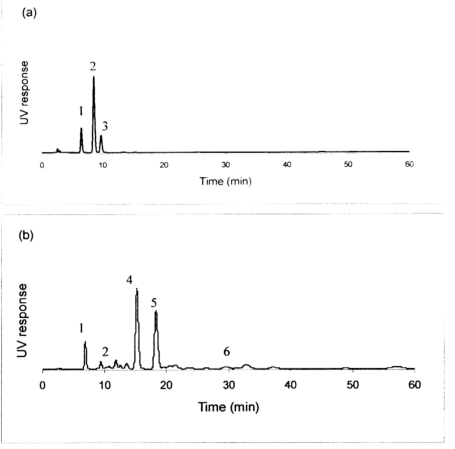

Figure 4.12. HPLC chromatograms of (a) terphenyls and (b) Therminol 66™ solution. Peak identification: *1*, *o*-; *2*, *m*-; *3*, *p*-terphenyl and *4*, 3-; *5*, 4-cyclohexylbiphenyl; and *6*, 1,4-dicyclohexylbenzene. For HPLC conditions see Figure 4.11.

Table 4.6. Absorption characteristics for the hydrogenated products of terphenyls reported by Scola *et al*.[120]

Hydrogenated compounds	Absorption wavelengths (nm)
o-terphenyl	
1,2-Diphenylcyclohexane	253, 259 and 265
2-Cyclohexylbiphenyl	233
2-Phenylbicyclohexyl	254, 260 and 266
m-terphenyl	
1,3-Diphenylcyclohexane	254, 259 and 268
3-Cyclohexylbiphenyl	248
p-terphenyl	
1,4-Diphenylcyclohexane	254, 260 and 268
4-Phenylbicyclohexyl	250, 254 to 260 and 268
1,4-Dicyclohexylbenzene	258, 263 and 272

The fluorescence chromatograms of hydrogenation products of the terphenyls prepared according to the procedure reported by Scola *et al.*[120] are illustrated in Figure 4.13. *o*-Terphenyl does not show any fluorescence property due to the 'Rayleigh effect' (*i.e.* having the same absorption and emission wavelengths). However, its retention time was confirmed by using the ELSD that was serially connected to the HPLC. In Figure 4.13a, the two most intense fluorescent peaks from the hydrogenation of *o*-terphenyl were identified as 1,2-diphenylcyclohexane and 2-cyclohexylbiphenyl. The maximum absorption wavelengths of these 2 peaks were found to be 253 and 233 nm, showing the characteristics of monoalkyl-substituted benzene and biphenyl absorption, respectively[120]. In terms of the hydrogenated product obtained from *m*-terphenyl, 3-cyclohexylbiphenyl showed the strongest fluorescence intensity (Figure 4.13b) and its maximum absorption band is obtained at 247 nm. However, more peaks were detected in the hydrogenated products of *p*-terphenyl (Figure 4.13c). 2 peaks were readily identified as 4-

cyclohexylbiphenyl and 1,4-dicyclohexylbenzene based on their absorption wavelengths of 247 and 254 nm, respectively. All the tercyclohexyls do not show any fluorescent property, but were detected by the ELSD.

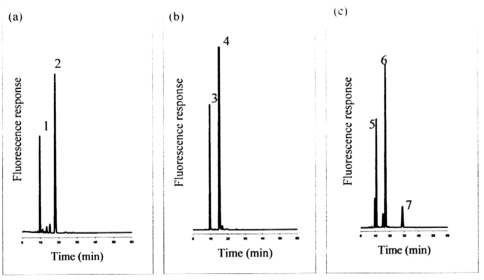

Figure 4.13. HPLC fluorescence chromatograms of hydrogenation products of (a) *o*-, (b) *m*- and (c) *p*-terphenyl. The hydrogenation was carried out according to the procedure reported by Scola *et. al.*[120]. Peak identification: *1*, 1,2-diphenylcyclohexane; *2*, 2-cyclohexylbiphenyl; *3*, *m*-terphenyl; *4*, 3-cyclohexylbiphenyl; *5*, *p*-terphenyl; *6*, 4-cyclohexylbiphenyl; and *7*, 1,4-dicyclohexylbenzene. HPLC conditions: *column*, GL C_{18} 250 x 4.6 mm i.d.; *mobile phase*, methanol and water (90:10, vol/vol); *oven temperature*, 40°C; and *fluorescence detector*, 257 nm (excitation) and 320 nm (emission).

In terms of the instrumental limits of terphenyl and 1,4-dicyclohexylbenzene standard solutions, the minimum detectable level *via* the fluorescence detector for *p*-terphenyl is 0.052 ng (52 pg), *m*-terphenyl is 0.15 ng (150 pg) and 1,4-dicyclohexylbenzene is 44.6 ng. Good linearity was also obtained with $r^2 > 0.9944$. *o*-Terphenyl was not detected at all within the analyzed range of 0-1000 ng. The

results show that the fluorescence detector is still more sensitive than the UV detector for the analysis of *m*-, *p*-terphenyl as well as 1,4-dicyclohexylbenzene.

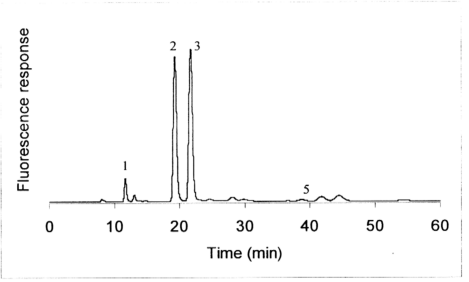

Figure 4.14. HPLC fluorescence detection of Therminol 66™. Peak identification: *1*, *m*-; *2*, 3-cyclohexylbiphenyl; *3*, 4-cyclohexylbiphneyl; and *4*, 1,4-dicyclohexylbenzene. HPLC conditions: *column*, GL C_{18} 250 x 4.6 mm i.d.; *mobile phase*, methanol and water (88:12, vol/vol); *oven temperature*, 40°C; and *fluorescence detector*, 257 nm (excitation) and 320 nm (emission).

Figure 4.14 shows the HPLC chromatogram of Therminol 66™. The elution pattern on a reversed-phase C_{18} column using methanol and water (88:12, vol/vol) as eluent, can be assigned as follows:- terphenyls eluting first (10-15 min) followed by cyclohexylbiphenyls (18-23 min), dicyclohexylbenzenes (38-45 min), and tercyclohexyls (55-60 min). The 2 strongest fluorescent peaks which have an absorption band at 247 nm, due to the characteristic biphenyl absorption, were identified as 3- and 4-cyclohexylbiphenyl peaks.

4.2.1.3. GC-MS analysis

Further confirmatory study of the compositions of Therminol 66™ was attempted by using the GC-MS. A typical total ion chromatogram of this heating fluid from the GC-MS instrument is illustrated in Figure 4.15. At least 35 peaks were detectable and separable into 2 groups. The first group can be assigned to the terphenyls and their hydrogenated products, and the second to those of polyphenyls and their hydrogenated products. Phenanthrene and triphenylene compounds are present in small quantities in Therminol 66™ and their mass spectra gave >99% matches with the library spectra. Further assignment of the retention times for the terphenyls and their hydrogenated products present in Therminol 66™ were achieved using the hydrogenated terphenyl compounds which have been synthesized earlier. The molecular ion for terphenyls is at m/z 230, cyclohexylbiphenyls or diphenylcyclohexane is at m/z 236, dicyclohexylbenzenes is at m/z 242, and tercyclohexyls is at m/z 248. The details of the fragmentation patterns of terphenyl and its hydrogenated products based on individual 'group' are given in Table 4.7. The total ion chromatogram of the 3- and 4-cyclohexylbiphenyl of Therminol 66™ which were collected after the HPLC-fluorescence detector is illustrated in Figure 4.16, and their mass spectra are given in Figure 4.17 and 4.18.

Table 4.7. Typical MS fragmentation patterns of terphenyl and its hydrogenation products

Compound	Fragmentation ions, m/z							
Terphenyl	215	202	113	107	101			
Diphenylcyclohexane	145	131	117	104	91	91	78	65
Cyclohexylbiphenyl	193	179	165	152	115			
Tercyclohexyl	165	109	95	83	67	55		

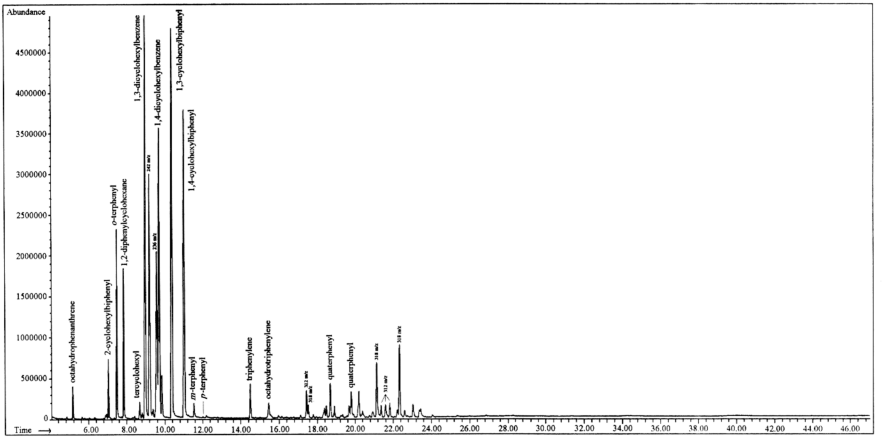

Figure 4.15. Total ion chromatogram of Therminol 66™ from GC-MS. GC conditions: oven temperature, 150°C to 280°C at the rate of 10°C/min, and hold at 280°C for 20 min; injector, 280°C; detector, 280°C; capillary column, 5% phenyl methylsiloxane 30 m x 0.32 mm i.d.; and helium flow, 0.8 mL/min.

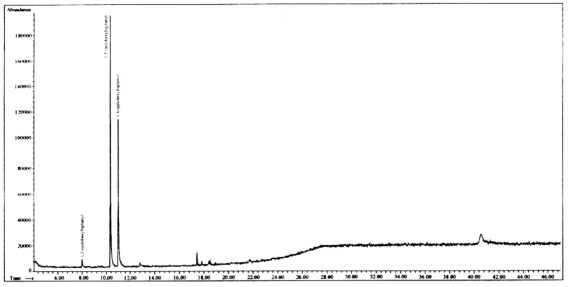

Figure 4.16. Total ion chromatogram of 3- and 4-cyclohexylbiphenyl collected from HPLC. For GC conditions see Figure 4.15.

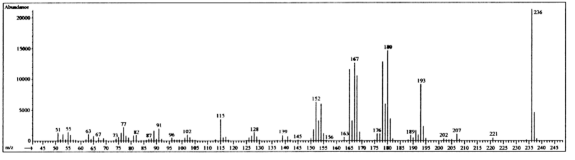

Figure 4.17. Mass spectra of 3-cyclohexylbiphenyl.

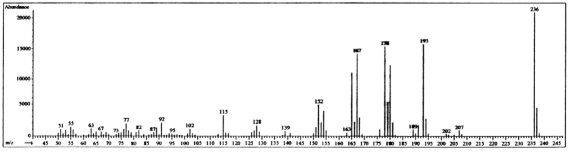

Figure 4.18. Mass spectra of 4-cyclohexylbiphenyl.

4.2.2. Quantitation of the partially hydrogenated terphenyls in spiked matrices

Since 3- and 4-cyclohexylbiphenyl showed the strongest peaks, they were used as markers for the determination of Therminol 66™. The areas of the peaks plotted against the concentrations of Therminol 66™ over the range of 0.01-10 μg/mL gave a linear regression graph with r^2 of 0.9999 (Figure 4.19). The detector was also found to be able to detect the 3- and 4-cyclohexylbiphenyl peaks of Therminol 66™ to as low as 0.01 μg/mL.

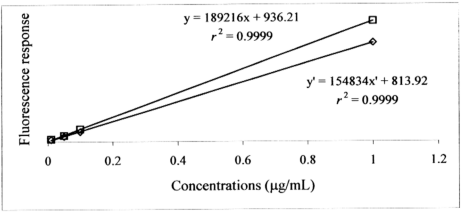

Figure 4.19. Calibration graphs derived by plotting peak areas of 3- (◇) and 4-cyclohexylbiphenyl (□) against the concentrations of the Therminol 6™ over the range of 0.01-1 μg/mL. For HPLC conditions see Figure 4.14.

Similar to the method developed for Dowtherm A™, the major obstacle encountered during the development of the fluorescence detection technique for the determination of Therminol 66™ in oleochemicals and edible oils was the co-elution of the interfering matrix components which are present naturally with the heating fluid. For example, by using only methanol as the mobile phase on a

reversed-phase column, the 3- and 4-cyclohexylbiphenyl peaks of Therminol 66™, which eluted at about 5-6 min, were co-eluted with other fluorescent components that are present in some of the fatty acids. When the polarity of the mobile phase was increased by introducing water into the system, the 3- and 4-cyclohexylbiphenyl peaks of Therminol 66™ eluted much later at about 10-12 min. However, a mobile phase mixture of methanol and water at 90:10 (vol/vol) was not sufficient enough to resolve the 3- and 4-cyclohexylbiphenyl peaks of Therminol 66™ from those fluorescent peaks found in the fatty acids. (Figures 4.20a and 4.20c). On the other hand, the mobile phase composition of methanol and water at 88:12 (vol/vol) gave the best resolution of the Therminol 66™ from the background fluorescent peaks of $C_{8:0}$ and $C_{10:0}$ fatty acids as depicted in Figures 4.20b and 4.20d, respectively. Other fatty acids such as $C_{12:0}$, $C_{14:0}$, $C_{16:0}$ and $C_{18:0}$, and glycerin samples examined also showed a very smooth background. Therefore, a methanol and water composition of 88:12 (vol/vol) was found to be optimum for the quantitative analysis of Therminol 66™ in glycerin and fatty acids.

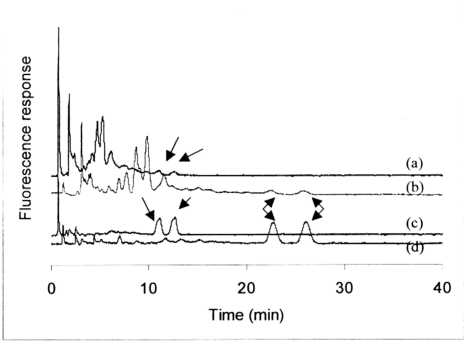

Figure 4.20. Stacked fluorescence chromatograms of spiked (a and b) $C_{8:0}$ and (c and d) $C_{10:0}$ with Therminol 66™ at 0.1 and 1.0 μg/g levels, respectively, showing the changes of elution times of 3- and 4-cyclohexylbiphenyls as indicated by arrows on different rations of mobile phase Chromatograms (a) and (c) were obtained with 90:10 (vol/vol) of methanol and water whereas chromatograms (b) and (d) were obtained with 88:12 (vol/vol) of methanol and water. For other HPLC conditions see Figure 4.14.

Similarly, palm olein which also possess some fluorescent peaks, labeled 1 to 4 as shown in Figure 4.21a, that are co-eluted with the 3- and 4-cyclohexylbiphenyl peaks of the Therminol 66™. Even though different mixtures of mobile phases were examined, from the less polar phases to the more polar phases, separation of the background peaks (labeled 1 and 2) whose retention times were very close to that of 3- and 4-cyclohexylbiphenyl peaks was not achieved. Analyses of the palm olein solutions after derivatization with N,O-Bis(trimethylsilyl)trifluoroacetamide or N,N-dimethylformamide was still not able

to resolve the background peaks from the Therminol 66™ either. However, it was observed that following a simple saponification of the palm olein with ethanolic solution, the interfering peaks that co-eluted with the Therminol 66™ disappeared (Figure 4.21b). In addition, saponification does not affect the hydrocarbons of the Therminol 66™. This was confirmed by the spiked palm olein sample as illustrated in Figure 4.21c.

On the other hand, blank runs of sunflower oil, soybean oil, and canola oil solutions did not show any co-eluting fluorescent components with Therminol 66™ as observed in the palm olein samples. However, in order to use the same procedure as that of palm olein samples, these oils were also subjected to the saponification step as well. This is because if non-polar sample matrices are injected continuously onto a reversed-phase system that employed polar mobile phase such as methanol and/or water, the non-polar matrices could be retained in the column for long periods of time. Subsequently it would require a solvent of lower polarity to flush them out. Therefore, by converting the oil sample to soap *via* saponification, the resulting polar matrix is more suitable to be analyzed on a reversed-phase system. In addition, the saponified sunflower oil, soybean oil, and canola oil did not show any fluorescent peaks that might interfere with the quantitative results. Thus, the mobile phase composition of methanol and water at 88:12 (vol/vol) used for the determination of Therminol 66™ in glycerin and fatty acid samples was also suitable for the edible oil samples.

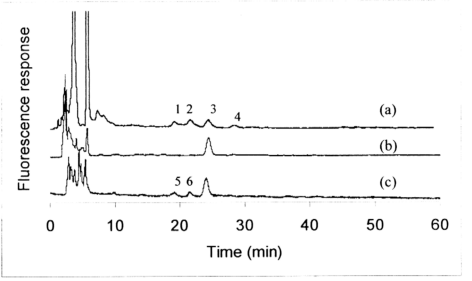

Figure 4.21. Fluorescence chromatograms of blank palm olein (a) before, (b) after saponification and (c) spiked palm olein with Therminol 66™ (1.0 μg/g) analyzed after saponification. Peaks labeled *1* to *4* were the fluorescent components present naturally in palm olein, and peaks labeled *5* and *6* were 3- and 4-cyclohexylbiphenyl, respectively. For HPLC conditions see Figure 4.14.

4.2.2.1. Spiked fatty acid and glycerin samples

The calibration graph developed from Therminol 66™ solutions was validated by the analysis of glycerin samples spiked at the levels of 0.1, 0.5 and 1.0 μg/g with Therminol 66™ and 3 replicates were analyzed for each spiked level. Data for the recovery of Therminol 66™ from these spiked samples are given in Table 4.8. The LOQ for the method was found to be 0.1 μg/g at which the size of the 4-cyclohexylbiphenyl peak was at least 10 times bigger than the baseline noise (S/N≥10). In addition, at this low concentration of 0.1 μg/g level, the method was accurate to about 82%. The reproducibility of the method as demonstrated by the CV was 2.56%. The satisfactory results, which were obtained, as a result of the smooth background of the glycerin sample (Figure 4.22), would meet the required

permissible level of 20% for the accuracy and reproducibility tests in order to set a LOQ.

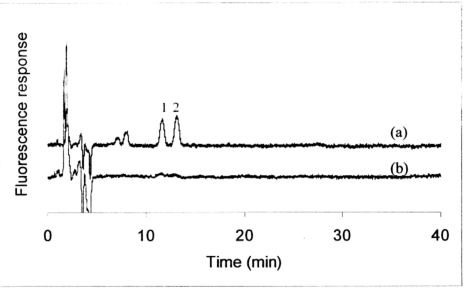

Figure 4.22. Typical fluorescence chromatograms of (a) spiked glycerin with Therminol 66™ at 1.0 μg/g level, and (b) blank glycerin. Peak identification: *1*, 3- and *2*, 4-cyclohexylbiphenyl. For HPLC conditions see Figure 4.14.

In the case of fatty acid samples spiked with Therminol 66™, the statistical results based on the spiked samples at the level of LOQ of 0.1 μg/g are given in Table 4.9. As the sample solution was analyzed directly without any further pre-treatment step such as saponification, distillation *etc*, the absolute recoveries of Therminol 66™ added at this concentration are quite acceptable, with a mean recovery of >92%. In addition, there are no other major fluorescent components present in the matrices that would interfere with the quantitation of Therminol 66™ to any significant extent (Figure 4.23). The intra-day variability of repeated injections of the same fatty acid sample ranged from 1.80-6.51% (3 determinations). The inter-day variability however was slightly higher, ranging

from 6.54 to 9.03% (6 determinations). The inter-day assays were conducted over a 2-week period, during which the stability of Therminol 66^{TM} was acceptable as indicated by the high mean inter-day recovery of 87.6±5.2%.

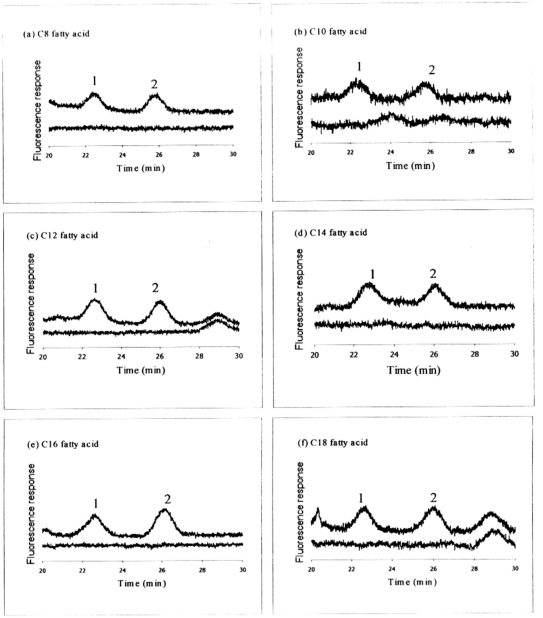

Figure 4.23. Enlarged fluorescence chromatograms of blank and spiked (a) $C_{8:0}$, (b) $C_{10:0}$, (c) $C_{12:0}$, (d) $C_{14:0}$, (e) $C_{16:0}$ and (f) $C_{18:0}$ fatty acid with 0.1 µg/g Therminol 66^{TM}. Peak identifications: *1*, 3- and *2*, 4-cyclohexylbiphenyl. For HPLC conditions see Figure 4.14.

Table 4.8. Linearity and recovery results of Therminol 66™ in spiked glycerin obtained by measuring the 4-cyclohexylbiphenyl peak

Spiked conc. of Therminol 66™	Peak areas (arbitrary units)	[a]Calculated conc. μg/g	SD	CV., %	Rec., %
0.1	4694.9	0.08	120.16	2.56	82
0.5	14349.9	0.53	685.62	4.78	106
1.0	24163.1	0.98	878.22	1.71	98

[a]Mean of 3 readings

Table 4.9. Precision and accuracy of the HPLC method for the determination of Therminol 66™ in spiked fatty acids

Fatty acids	Conc. Added (μg/g)	[a]Conc. Found (μg/g)	[b]Precision	Inter-day accuracy
$C_{8:0}$	0.1	0.095±0.003	2.66 (8.81)	94.7
$C_{10:0}$	0.1	0.092±0.031	3.33 (7.47)	91.7
$C_{12:0}$	0.1	0.102±0.007	6.51 (9.03)	102.3
$C_{14:0}$	0.1	0.097±0.004	4.36 (5.71)	92.7
$C_{16:0}$	0.1	0.101±0.003	2.84 (8.47)	101.7
$C_{18:0}$	0.1	0.096±0.00	1.80 (6.54)	96.0

[a]Determined by measuring the 4-cyclohexylbiphenyl peak.

[b]Intra- (n=3) and inter- (n=6) assay variations. Inter-assay variations appear in parentheses.

4.2.2.2. Spiked edible oil samples

The LOQ for the vegetable oil samples was found to be 0.2 μg/g of Therminol 66™, which is slightly higher when compared to that observed in the glycerin and fatty acid sample. It could be that during the saponification process, there was a slight loss of Therminol 66™ through evaporation or adhesion onto the walls of the glassware. However, this overall limit could still be acceptable for quantification purposes.

The performance of the method for the determination of Therminol 66™ in spiked palm olein samples are given in Tables 4.10 and 4.11. The intra-day precision was calculated over 6 replicate injections for each sample at 3 spiked concentrations. At the high and medium concentrations of 1.0 and 0.5 μg/g levels, the accuracy of the method was good with a mean recovery of >95% whereas the CV was <1.84%. However, at the low concentration of 0.2 μg/g level, the accuracy of the method was slightly lower at about 87%, and with slightly higher CV of 6.43%. This was because the quantitation was achieved at the level of LOQ, thus, the background signal has a significant effect and also causes a bigger variation (Table 4.10).

The mean recoveries of Therminol 66™ obtained from the inter-day analyses were 93.1% for the spiked concentration at 1.0 μg/g, 94.1% for 0.5 μg/g, and 88.1% for 0.2 μg/g at the level of LOQ. The variability of 6 repeated injections over a period of a week was 3.77% for the spiked concentration at 1.0 μg/g, 4.23% for 0.5 μg/g, and 10.4% for 0.2 μg/g, which were all slightly higher compared to the intra-day variations.

The applicability of the method for the analysis of Therminol 66™ in other vegetable oils was also examined. The results on the recovery studies obtained from spiked soybean oil, sunflower oil, and canola oil at 0.2 μg/g level each are given in Table 4.12. The mean recoveries of these samples ranged from 88.7 to 92.3%, with small CVs of 2.01-3.26%. The fluorescence chromatograms of these oils are illustrated in Figure 4.24, showing that no interfering fluorescent components co-eluted with the heating fluid. In addition, the LOD of Therminol 66™ by the fluorescence detector was found to be as low as 0.1 μg/g (Figure 4.24a). However, at this level the CV (16.8±9.2%) and the recovery (64.7-73.6%) were found to be slightly poorer; and apart from the losses occurred during the saponification process, the high background noise also affected the quantitation.

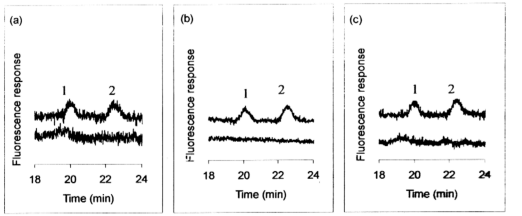

Figure 4.24. Fluorescence chromatograms showing blank and spiked vegetable oils with Therminol 66™. (a) Sunflower oil was spiked at 0.1 μg/g level, (b) soybean oil and (c) canola oil were spiked at 0.2 μg/g level. Peak identification: *1*, 3- and *2*, 4-cyclohexylbiphenyl. For HPLC conditions see Figure 4.14.

Table 4.10. Intra-day precision obtained from the analysis of spiked palm olein with Therminol 66™

No. of readings	High conc. at 1.0 μg/g	Medium conc. at 0.5 μg/g	Low conc. at 0.2 μg/g
1	0.932	0.501	0.157
2	0.952	0.484	0.179
3	0.977	0.475	0.172
4	0.957	0.485	0.168
5	0.945	0.494	0.178
6	0.972	0.486	0.190
Mean	0.956	0.49	0.174
SD	0.0167	0.0089	0.0112
CV., %	1.76	1.84	6.43
Rec., %	95.6	97.5	87.0

Table 4.11. Inter-day precision obtained from the analysis of spiked palm olein with Therminol 66™

No. of days	High conc. at 1.0 μg/g	Medium conc. at 0.5 μg/g	Low conc. at 0.2 μg/g
1	0.915	0.488	0.198
2	0.978	0.473	0.157
3	0.924	0.451	0.168
4	0.937	0.493	0.186
5	0.958	0.476	0.156
6	0.877	0.443	0.192
Mean	0.93	0.47	0.18
SD	0.0351	0.0199	0.0183
CV., %	3.77	4.23	10.4
Rec., %	93.1	94.1	88.1

Table 4.12. Recovery studies of Therminol 66™ at 0.2 μg/g obtained from various spiked vegetable oils (n=3)

Oil matrix	Rec., %	CV., %
Sunflower	92.3	2.35
Soybean	88.7	3.26
Canola	90.2	2.01

4.3. Synthetic oil

4.3.1. Instrumental assessment

Therminol 55™ is a synthetic thermal heating fluid used in moderate temperature applications (-25 to 290°C). It possesses several important performance attributes such as prolonged usage, more inert to oxidation (10 times better than mineral oils), and is pumpable even at –25°C[122].

The fluorescence excitation and emission spectra of Therminol 55™ is depicted in Figure 4.25, showing 3 distinct excitation bands at 238, 262 and 270 nm. The fluorescence emission spectrum is quite broad and less intense when compared to that of the fluorescence excitation spectrum. Maximum bands in the fluorescence spectrum were observed at 312 and 320 nm.

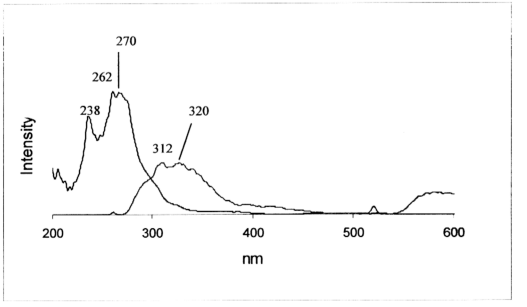

Figure 4.25. Fluorescence excitation and emission spectra of Therminol 55™.

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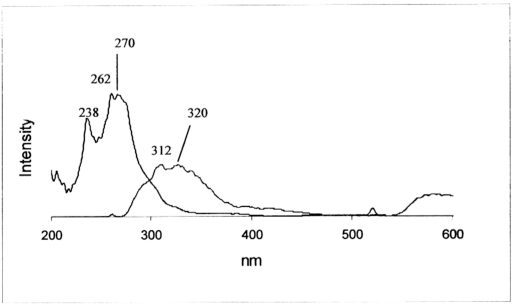

Figure 4.25. Fluorescence excitation and emission spectra of Therminol 55™.

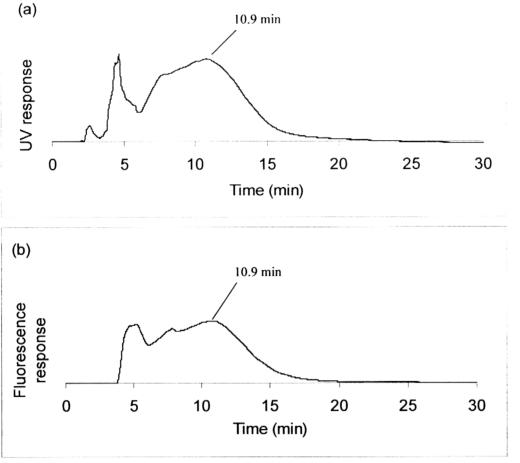

Figure 4.26. Typical HPLC chromatograms of Therminol 55™ analyzed using (a) UV detection at 262 nm, and (b) fluorescence detection at 270 nm (excitation) and 320 nm (emission). HPLC conditions: *column*, GL C_{18} 250 x 4.6 mm i.d.; *mobile phase*, ethanol and water (95:5, vol/vol); *oven temperature*, 40°C.

The reversed-phase HPLC analysis of Therminol 55™ is characterized by the presence of a distinct broad peak which cannot be resolved into individual peaks (Figure 4.26). The linearity of both the UV and fluorescence detectors was demonstrated by analyzing various concentrations of Therminol 55™ solutions (0-1000 μg/mL). It was shown to be very good with the r^2 of >0.9945 (Figure 4.27). The LOD for the analysis of Therminol 55™ obtained from the UV detection was found to be 7 μg while the fluorescence detection showed slightly better sensitivity of 3 μg level. However, by comparing it to the LOD of the eutectic mixture of

diphenyl oxide and biphenyl as well as the partially hydrogenated terphenyls, which are in regions of ng, the sensitivity of both UV and fluorescence detection for the analysis of Therminol 55™ appear much lower.

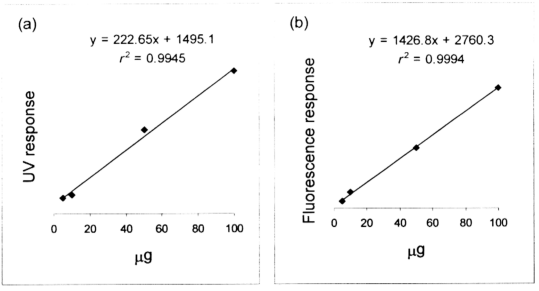

Figure 4.27. Linearity of the calibration graphs derived from Therminol 55™ using (a) UV detection at 262 nm and (b) fluorescence detection at 270 nm (excitation) and 320 nm (emission). For HPLC conditions see Figure 4.26.

Therefore, in order to improve the sensitivity of the detectors to enable it to detect Therminol 55™ in sample matrices, a pre-concentration step is needed. However, the concentrating process can only be carried out after the fluid has been extracted from the sample matrix. Since Therminol 55™ consists of hydrocarbons, solvent-solvent extraction[30], distillation[101] or column chromatography[28,29,108] reported in other studies can be considered. However, the major drawback in using the solvent extraction method is that it usually involves a saponification process. This could result in a lower recovery mainly due to the loss of the hydrocarbon content during saponification and extraction process, and further during the

evaporation of the solvent. Therefore, this technique is not considered in this study for recovering Therminol 55™ from sample matrices.

In contrast, column chromatography is more widely used nowadays for the extraction of hydrocarbons from vegetable oil matrices not only because it is more convenient and simpler, but it also enables scaling up for a larger recovery once the procedure is established. Secondly, by monitoring the polarity of the mobile phase carefully, compounds of different polarities can also be recovered after they have been separated in the column. There are many types of packing materials (*i.e.* silica gel, alumina, bonded phase silica *etc.*) which are readily available for specific purposes. In view of these advantages, a column chromatographic approach for the extraction of Therminol 55™ from the sample matrices is selected in this study.

4.3.2. Column chromatography

Alumina was selected as the sorbent for normal-phase column chromatography mainly because it is more stable to moisture compared to silica gel. A non-polar solvent (hexane) was used as a mobile phase because the presence of more polar solvents (*e.g.*, isopropanol or dichloromethane) would reduce the efficiency of alumina to retain the undesirable components such as tri- and partial glycerides, and free fatty acids.

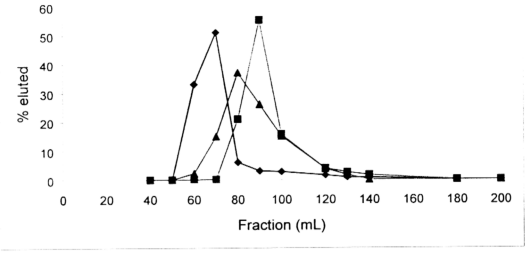

Figure 4.28. Elution profile of *n*-eicosane (◆), *o*-terphenyl (■) and Therminol 55™ (▲) by column chromatography with 50 g alumina. Hexane was used as the mobile phase.

The elution behavior of hydrocarbons on an alumina column is largely dependent on the adsorbent and mobile phase. For instance, if a larger amount of alumina is used to pack the column, then a larger volume of solvent is needed to elute the hydrocarbons. This was demonstrated by eluting a known quantity of *n*-eicosane through a number of columns packed with 10, 30, and 50 g of alumina, respectively. By collecting each eluent fraction, the GC-FID analysis showed that *n*-eicosane was completely eluted within the 160-mL fraction on a column packed with 50 g alumina (Figure 4.28). However, for the 10 and 30 g packing, the *n*-eicosane was eluted completely with less than 100 mL of the solvent.

While *n*-eicosane is chosen to represent the *n*-alkane hydrocarbons, the aromatic hydrocarbons as demonstrated by *o*-terphenyl and Therminol 55™ were eluted slightly later than *n*-eicosane but are still within the 160-mL fraction on the 50-g alumina (Figure 4.28).

Furthermore, the amount of loading of the sample matrix on the alumina column is also an important parameter because it affects the efficiency of the adsorbent. The amount loaded was examined from 1 to 10 g, and the results showed that at least 15 g of alumina was required to retain 1 g of vegetable oil. Even though alumina was found to be able to retain greater amounts of fatty acids, the flow rate was reduced dramatically or to the extent that it stopped flowing as more fatty acids were adsorbed.

Since the glass columns used in this study were small (250 mm x 2.0 mm i.d.), a loading size of 3-g was found to be optimal for a column packed with 50 g alumina, and the flow rate found to give the best results was 3-4 drops per sec.

4.3.3. HPLC analysis of glycerin and fatty acids

After the extracts of the blank and spiked oleochemical samples were concentrated to 1mL each, a 100-μL of the aliquot was injected onto the reversed-phase C_{18} column to be analyzed by both UV and fluorescence detections.

By comparing the blank and spiked samples, it was found that the UV detector was able to detect Therminol 55™ in glycerin and $C_{12:0}$ to $C_{16:0}$ fatty acids (Figure 4.29). However, the determination of Therminol 55™ in $C_{18:0}$ fatty acid was not possible due to the presence of a strong interfering background (Figure 4.29).

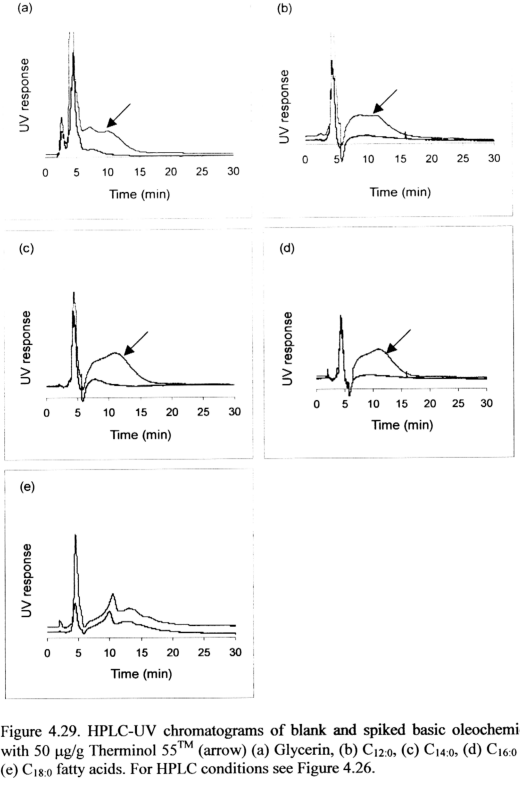

Figure 4.29. HPLC-UV chromatograms of blank and spiked basic oleochemicals with 50 μg/g Therminol 55™ (arrow) (a) Glycerin, (b) $C_{12:0}$, (c) $C_{14:0}$, (d) $C_{16:0}$ and (e) $C_{18:0}$ fatty acids. For HPLC conditions see Figure 4.26.

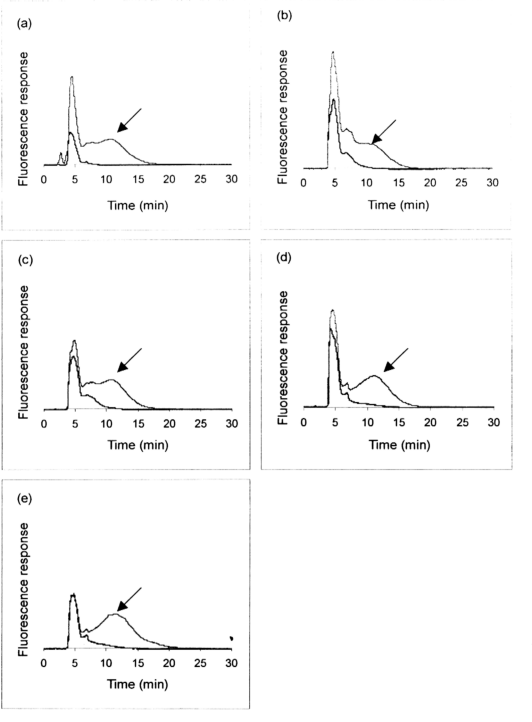

Figure 4.30. HPLC-fluorescence chromatograms of blank and spiked basic oleochemicals with 50 μg/g Therminol 55™ (arrow). (a) Glycerin, (b) $C_{12:0}$, (c) $C_{14:0}$, (d) $C_{16:0}$ and (e) $C_{18:0}$ fatty acids. For HPLC conditions see Figure 4.26.

A similar HPLC analysis using fluorescence detection is illustrated in Figure 4.30. Unlike the UV detection, the fluorescence chromatograms of the blank oleochemical samples gave smoother baselines in the region where Therminol 55™ would elute. The interfering background signal that was observed in the UV chromatogram for $C_{18:0}$ fatty acid was absent in the fluorescence chromatogram. This enabled the determination of Therminol 55™ in $C_{18:0}$ fatty acid using the fluorescence detector.

The LOD for both UV and fluorescence detection of Therminol 55™, in the analyses of oleochemical samples containing 0 to 500 μg/g Therminol 55™, was found to be 20 μg/g level (S/N=3). The LOQ by both detectors was also found to be quite similar, that was at 30 μg/g level (S/N=10). In addition, by plotting the peak heights against the concentrations of Therminol 55™ from the spiked samples, the correlation coefficients obtained were higher than 0.989, which therefore confirmed the linearity for the analysis within the range of tested concentrations.

The recoveries of Therminol 55™ using UV and fluorescence detection at 3 different concentrations, namely high (100 μg/g), medium (50 μg/g) and low (30 μg/g), are summarized in Table 4.13. For the UV detection, the mean recovery of Therminol 55™ in the oleochemicals ranged from 79.3 to 83.6% at the high concentration level of 100 μg/g, whereas at the medium concentration level of 50 μg/g, the recoveries ranged from 74.8-87.1%, and at low concentration level of 30 μg/g it was from 72.8-84.6%. Though the CVs varied among the analyses, the values were lower than 10%, and the results were based on 6 replicate determinations each. The determination of Therminol 55™ using the fluorescence detection yielded a mean recovery which was quite close to that of UV detection

(Table 4.13). The mean values for all 3 concentrations also ranged from 70-80%, and the CV of less than 10% was obtained. However, the main advantage of using the fluorescence detection over UV detection was that the $C_{18:0}$ fatty does not show any fluorescent properties that would interfere with the Therminol 55™ peak during quantitation.

4.3.4. Analysis of vegetable oils

4.3.4.1. HPLC

The applicability of the UV and fluorescence detectors for the analysis of Therminol 55™ was also demonstrated by comparing the HPLC chromatograms of the blank and spiked vegetable oils as previously reported for oleochemicals. The UV chromatograms obtained from 4 different vegetable oils are shown in Figure 4.31. All blank oil samples showed a high background noise caused by the UV absorbing materials that are present in the extract. By overlaying the spiked samples against the blank, it was found that the quantitation of Therminol 55™ by UV detection was not possible at the low concentration in most vegetable oil samples, except for sunflower oil where the peak of Therminol 55™ was still distinguishable at the 50 μg/g level. In view of this limitation, the quantitative analysis of Therminol 55™ in vegetable oils by using the UV detection was not further investigated in this study.

Table 4.13. Recovery of Therminol 55™ in basic oleochemicals using HPLC-UV and -fluorescence detections (n=6)

Sample	UV detection			Fluorescence detection		
	High conc., 100 μg/g	Med. conc., 50 μg/g	Low conc., 30 μg/g	High conc., 100 μg/g	Med. conc., 50 μg/g	Low conc., 30 μg/g
Glycerin	83.6 (4.78)	73.6 (6.33)	83.2 (4.88)	86.5 (6.35)	75.6 (5.69)	79.4 (5.64)
$C_{12:0}$ fatty acid	79.3 (6.83)	87.1 (4.32)	84.6 (7.25)	84.6 (4.65)	78.7 (5.36)	89.3 (3.56)
$C_{14:0}$ fatty acid	87.5 (5.46)	74.8 (9.26)	72.8 (6.11)	83.8 (5.22)	73.5 (8.65)	76.3 (7.22)
$C_{16:0}$ fatty acid	81.1 (5.38)	85.1 (7.22)	74.3 (6.58)	73.6 (4.98)	82.5 (3.56)	74.2 (4.44)
$C_{18:0}$ fatty acid	na	na	na	86.3 (7.55)	87.7 (5.69)	79.6 (8.88)

na, not applicable for quantitation due to interfering background.
The results are reported as percentage of recovery whereas the CV (%) are in parentheses.

The chromatograms of the same vegetable oil samples as analyzed by HPLC-fluorescence detection are illustrated in Figure 4.32. The overlaid chromatograms showed that the detection of Therminol 55™ at 50 μg/g level in palm olein sample was not possible due to the presence of a strong fluorescence background in the same retention time region of the fluid sample (Figure 4.32a). This indicated that the HPLC-fluorescence detection is not suitable for the analysis of Therminol 55™ in palm olein. In contrast to this, in the case of other vegetable oils such as soybean oil, sunflower oil and canola oil, they did not show any interfering fluorescence peaks that could affect the quantitative analysis of Therminol 55™ (Figures 4.32b to d). Hence, the HPLC-fluorescence detection of Therminol 55™ in these vegetable oils was carried out.

The LOQ of the HPLC-fluorescence detection for the determination of Therminol 55™ in soybean oil, sunflower oil and canola oil was found to be 30 μg/g which is similar to that of the glycerin and fatty acid samples. The plots of

peak heights against the concentrations of the fluid also showed linear relationships with r^2 better than 0.925. The recovery results for the 3 concentrations studied are given in Table 4.14. At the high spiked concentration of 100 μg/g Therminol 55™, the mean recovery for soybean oil was 81.4%, whereas sunflower oil and canola oil yielded only 87.3 and 86.1% recoveries, respectively. The CV at this concentration was also within acceptable limits ranging from 3.26 to 7.26%. At the medium and low concentration levels, the mean recovery of Therminol 55™ were also within the 70-80% range. However, at the low concentration level, the CVs of the replicate studies as observed in sunflower oil and canola was slightly higher, with the CVs of 12.3 and 82.6%, respectively. This was because the analysis was performed at near the LOQ level, at which not only the background noise would affect the quantitation, but also the instrumental noise could be a major problem.

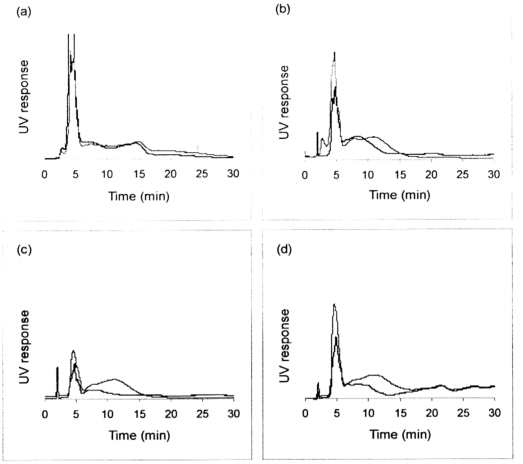

Figure 4.31. HPLC-UV chromatograms of blank and spiked vegetable oils with 50 μg/g Therminol 55™. (a) Palm olein, (b) soybean oil, (c) sunflower oil, and (d) canola oil. For HPLC conditions see Figure 4.26.

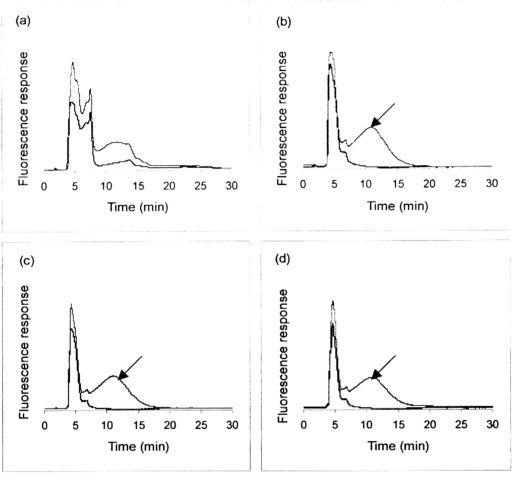

Figure 4.32. HPLC-fluorescence chromatograms of blank and spiked vegetable oils with 50 μg/g Therminol 55™ (arrow). (a) Palm olein, (b) soybean oil, (c) sunflower oil, and (d) canola oil. For HPLC conditions see Figure 4.26.

Table 4.14. Recovery of Therminol 55™ in vegetable oils using HPLC-fluorescence detection (n=6)

Sample	High conc., 100 μg/g	Med. Conc., 50 μg/g	Low conc., 30 μg/g
Soybean oil	81.4 (7.26)	73.2 (6.88)	77.6 (8.55)
Sunflower oil	87.3 (3.26)	82.4 (6.31)	81.3 (12.8)
Canola oil	86.1 (5.41)	85.7 (7.33)	82.6 (13.2)

The results are reported as percentage of recovery and the CV (%) are in parentheses.

4.3.4.2. GC-FID

Since Therminol 55™ in palm olein could not be determined by HPLC using either the UV (Figure 4.31a) or fluorescence (Figure 4.32a) detection due to the presence of strong background peaks, the sample extract was subjected to GC-FID analysis.

The GC chromatogram of Therminol 55™ is illustrated in Figure 4.33. Similar to the UV (4.26a) and fluorescence (Figure 4.26b) profiles, Therminol 55™ is characterized by a broad band of unresolved GC peaks eluted at about 32 to 44 min. Based on the analysis of a series of Therminol 55™ solutions (10-1000 μg/mL), the sensitivity of the GC instrument was found to be able to detect the fluid down to as low as 15 ng. This result was based on the use of 1 μL splitless injection volume into the GC-FID, and the quantitation of Therminol 55™ was based on the peak area counts in the region of 32-41 min.

The GC chromatogram of the extract of blank palm olein is illustrated in Figure 4.34. A close examination of the GC-FID trace showed that the blank extract contains a host of minor peaks which are mainly hydrocarbons and esters of the non-polar materials originating from the oil itself[129,130] (Figure 4.35a). Even though the background peaks were also present in the region where Therminol 55™ eluted, the former peaks were well resolved. Therefore, in the case of contamination, the presence of Therminol 55™ can still be recognized provided the concentration is high enough to be detected.

Based on a series of spiked palm olein samples with Therminol 55™ (10-500 μg/g), the LOD of the GC-FID instrument was found to be 20 μg/g level,

whereas the LOQ was 30 μg/g level (Figures 4.35b to d). The results obtained were similar to that of the HPLC detection. The recovery results obtained from palm olein samples spiked with Therminol 55™ at 3 concentrations are summarized in Table 4.15. The mean recoveries of Therminol 55™ obtained from the intra-day analyses (3 determinations) were higher than 79.9% for samples spiked with the high (100 μg/g) and medium (50 μg/g) concentrations. However, at the LOQ level (30 μg/g), the mean recovery was slightly lower at 68.0%. At this low concentration level, the Therminol 55™ peak tends to flatten out, thus making it difficult to obtain the peak areas. In addition, the difficulty in determining the peak areas was also caused by the presence of other background peaks. The inter-day variability of the GC-FID instrument when tested for the analysis of Therminol 55™ conducted over a period of one week gave a CV of 8.15-9.75%, which was very close to that of the intra-day analysis (6.54-8.98%). The overall recovery was within acceptable limits ranging from 76.2 to 85.7%.

The recovery of Therminol 55™ in other vegetable oils at 2 concentration levels are given in Table 4.16. The GC traces are shown in Figure 4.37. The mean recoveries ranged from 72.5-76.3% at the LOQ level, and from 77.3-81.2% at the 100 μg/g level. These are very similar to that estimated by using the HPLC-fluorescence detection. Even though the CV was slightly higher at the low concentrations, the values are still within the acceptable limit of 20%. These results have demonstrated that Therminol 55™ contamination in vegetable oils could be analyzed by using the GC-FID method.

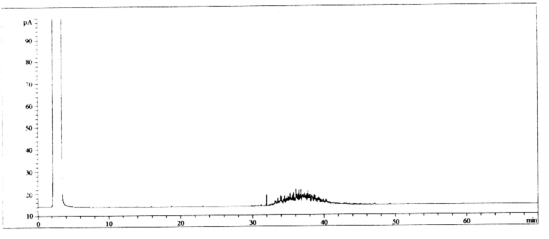

Figure 4.33. GC-FID chromatogram of Therminol 55^{TM}.

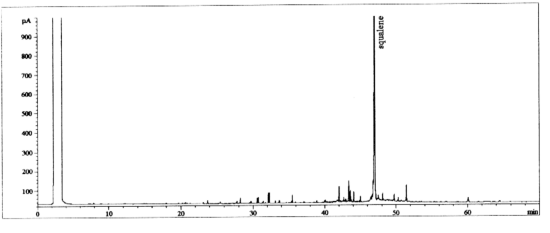

Figure 4.34. GC-FID chromatogram of blank palm olein.

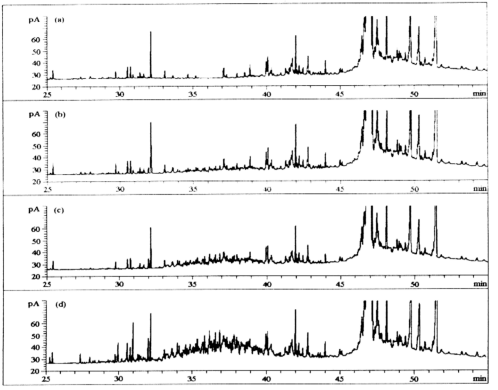

Figure 4.35. Enlarged GC-FID chromatograms of (a) blank and (b-d) spiked palm oleins with 20, 50 and 100 μg/g Therminol 55™, respectively.

Table 4.15. Reproducibility of GC analysis of Therminol 55™ in spiked palm olein (n=3)

	Mean calc., μg/g	CV., %	Rec., %
Intra-day study			
30	20.4	6.54	68.0
50	43	7.21	86.0
100	79.7	8.98	79.9
Inter-day study			
30	22.9	9.75	76.2
50	42.8	8.76	85.7
100	80.5	8.15	80.5

Table 4.16. Recovery of Therminol 55™ in vegetable oils (n=3)

Sample	Spiked conc., 30 μg/g		Spiked conc., 100 μg/g	
	Rec., %	CV., %	Rec., %	CV., %
Soybean oil	72.5	13.5	81.2	9.1
Sunflower oil	76.3	8.5	79.6	10.9
Canola oil	76.2	12.9	77.3	6.5

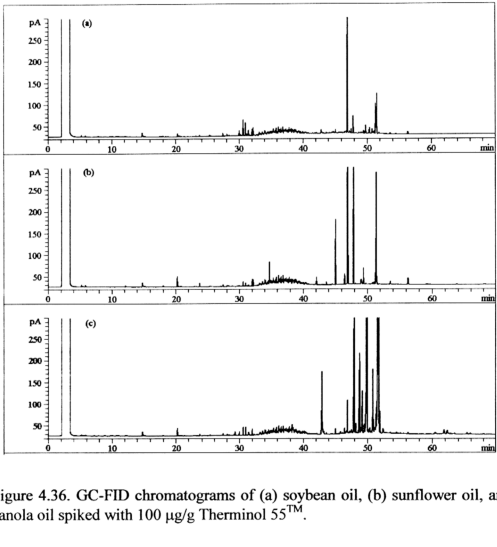

Figure 4.36. GC-FID chromatograms of (a) soybean oil, (b) sunflower oil, and (c) canola oil spiked with 100 μg/g Therminol 55™.

4.4. Diesel fuel

4.4.1. Spectrofluorometric analysis

Figure 4.37 illustrates the fluorescence excitation and emission spectra of 5 different brands of diesel obtained from local petrol stations. These brands namely, Petronas, British Petroleum (BP), Esso, Mobil and Shell, showed a maximum excitation band at about 309 nm. Fluorescence excitation bands at 245, 325 and 360 nm were also common in all the brands analyzed. The maximum fluorescence emission band was observed at about 344 nm for all brands.

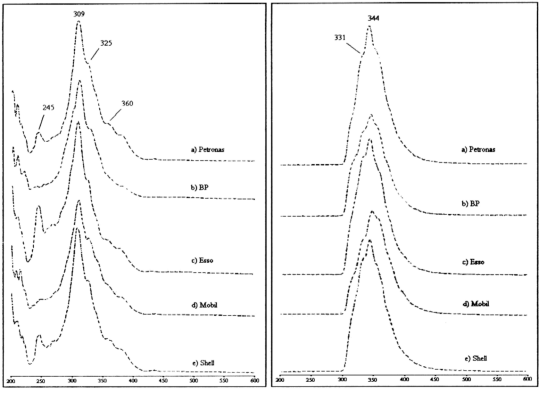

Figure 4.37. Fluorescence excitation and emission spectra of diesel solution (1000 μg/mL) as supplied from local petrol stations.

4.4.2. GC-mass spectroscopic analysis

A typical total ion chromatogram of diesel is illustrated in Figure 4.38, revealing that a majority of the constituents are *n*-alkanes ($C_{11}H_{24}$ to $C_{30}H_{62}$). The mass spectra of *n*-heptadecane, *n*-octadecane and *n*-nanodecane are given in Figure 4.39. In addition, a number of naphthalene-based derivatives (1,2,3,4-tetrahydro-5-methylnaphthalene, 1,4-dimethyl-1,2,3,4-tetrahydronaphthalene, 2-methylnaphthalene, 1-methylnaphthalene, and 1,7-dimethylnaphthalene) were also identified in which a majority of them matched the findings reported by Xiang *et al.*[127]. The mass spectra obtained from some of these components are illustrated in Figure 4.40. The presence of these aromatics could contribute to the fluorescence properties of the diesel.

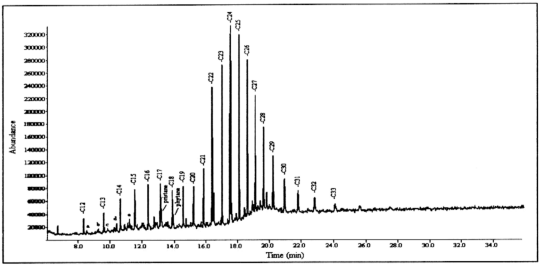

Figure 4.38. A typical total ion chromatogram of *n*-alkane (-C) in diesel. Peak identification: *a*, 1,2,3,4-tetrahydo-5-methylnaphthalene; *b*, 1,4-dimethyl-1,2,3,4-tetrahydronaphthalene; *c*, 2-methylnaphthalene; *d*, 1-methylnaphthalene; and *e*, 1,7-dimethylnaphthalene. GC conditions: *oven temperature*, 70°C for first 5 min, then programmed at 15°C/min to 280°C and hold at 280°C for 20 min; *injector*, 250°C; *detector*, 250°C; *capillary column*, 5% phenyl methylsiloxane 30 m x 0.32 mm i.d.; and *helium flow*, 0.8 mL/min.

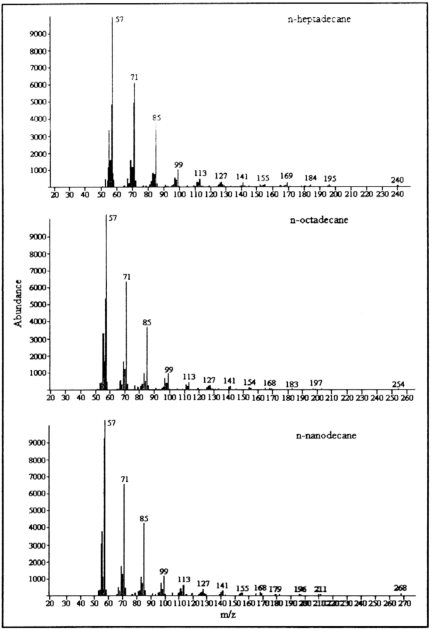

Figure 4.39. Mass spectra of *n*-heptadecane, *n*-octadecane and *n*-nanodecane of diesel. For GC conditions see Figure 4.38.

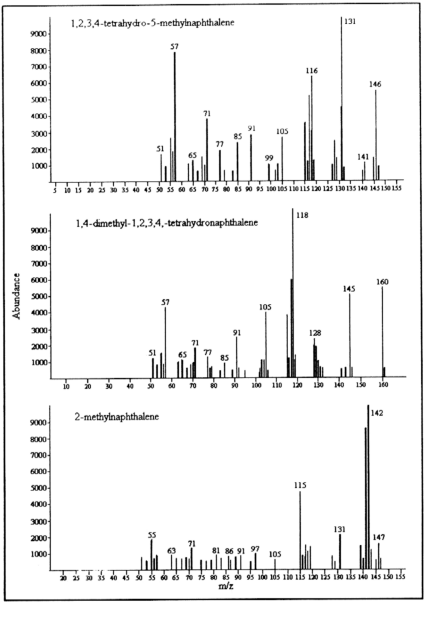

Figure 4.40. Mass spectra of 1,2,3,4-tetrahydo-5-methylnaphthalene, 1,4-dimethyl-1,2,3,4-tetrahydronaphthalene and 2-methylnaphthalene of diesel. For GC conditions see Figure 4.38.

4.4.3. Reversed-phase HPLC-fluorescence detection

Diesel solutions were chromatographed on a reversed-phase C_{18} column using different compositions of mobile phases such as dichloromethane in acetonitrile, water in acetonitrile, and water in methanol. The profiles of the diesel solutions analyzed using various acetonitrile mixtures are depicted in Figure 4.41. In the presence of dichloromethane in the mobile phase, the diesel components did not show strong retention on the reversed-phase column (Figures 4.41a and b). However, the diesel components started to resolve into several fluorescence peaks as the polarity of the mobile phase was increased, *i.e.* by introducing water into the system. The chromatogram shows that the diesel contains a lot of fluorescent components as illustrated in Figure 4.41d.

Mobile phases consisting of different compositions of methanol and water were also examined. A typical fluorescence chromatogram of diesel solution using 90:10 (vol/vol) of methanol and water is illustrated in Figure 4.42a. The chromatogram showed a complex mixture of fluorescent components detectable in diesel. A series of spiked crude palm oil with diesel was also analyzed after saponification, and the chromatograms of the spiked samples with 50 and 100 μg/g diesel are depicted in Figures 4.42c and 4.42d, respectively. The minimum measurable level of diesel was 35 μg/g, due to the presence of other fluorescent components after saponification of crude palm oil as illustrated in Figure 4.42b. Even though other fluorescence wavelengths such as those for benzene, naphthalene, and anthracene[128] were also tested, it was still not possible to remove the interfering fluorescent components present in the matrices. Thus, due to the high

background noise level that co-eluted with the diesel, the reversed-phase HPLC method was not suitable for the analysis of diesel in crude palm oil.

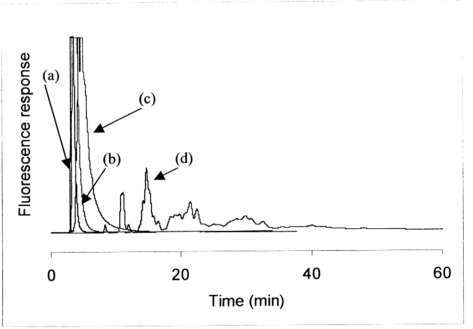

Figure 4.41. Overlaid fluorescence chromatograms of diesel solutions analyzed using different ratios of acetonitrile (ACN), dichloromethane (DCM) and water (H_2O) mixture as mobile phase. (a) ACN:DCM; 50:50, (b) ACN:DCM; 70:30, (c) ACN; 100, and (d) ACN:H_2O; 70:30 (vol/vol). HPLC conditions: *column*, GL C_{18} 250 x 4.6 mm i.d.; *oven temperature*, 30°C; and *fluorescence detector*, 309 nm (excitation) and 344 nm (emission).

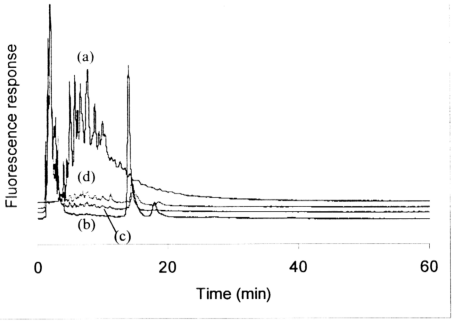

Figure 4.42. Overlaid fluorescence chromatograms of (a) diesel solution, (b) blank and spiked saponified crude palm oil with (c) 50 μg/g and (d) 100 μg/g diesel. HPLC conditions: *column*, GL C_{18} 250 x 4.6 mm i.d.; *mobile phase*; methanol and water (90:10, vol/vol); *oven temperature*, 30°C; and *fluorescence detector*, 309 nm (excitation) and 344 nm (emission).

4.4.4. Normal-phase HPLC fluorescence chromatogram

4.4.4.1. Optimization of wavelengths

When these 2 wavelengths—309 nm excitation and 344 nm emission—were applied for detecting diesel on a normal-phase column and the use of heptane and isopropanol (94:6, vol/vol) as the mobile phase, a single peak was obtained as shown in Figure 4.43b. In addition, diesel was further analyzed using the excitation and emission wavelengths of naphthalene, anthracene, and benzene[128], and the chromatograms obtained are depicted in Figures 4.43a, 4.43c and 4.43d,

respectively. A comparison showed that diesel fuel exhibited the strongest fluorescence intensity at the naphthalene wavelengths of 286 nm excitation and 321 nm emission, about 2 times stronger than that of the diesel wavelengths obtained from spectrofluorometry. As identified by the GC-MS, diesel fuel contains a relatively high composition of naphthalene-based derivatives.

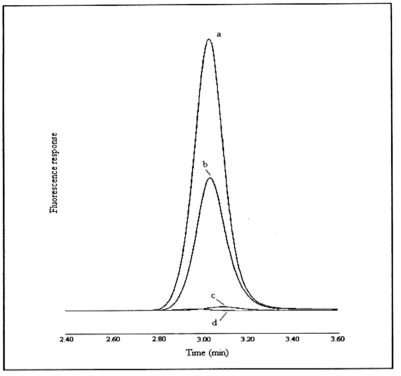

Figure 4.43. HPLC chromatograms of diesel solutions analyzed using excitation and emission wavelengths of (a) naphthalene; 268 nm and 321 nm, (b) diesel; 309 nm and 344 nm, (c) anthracene; 365 nm and 400 nm, and (d) benzene; 205 nm and 278 nm. Twenty μL diesel solution (1000 μg/mL) was injected. HPLC conditions: *column*, DioL II 250 x 4.6 mm i.d.; *mobile phase*, heptane and isopropanol (94:6, vol/vol); and *oven temperature*, 30°C.

The relationship between the fluorescence response using the naphthalene wavelengths against the concentration of diesel solutions, over a range of 1.0-50 μg/mL, showed good linearity, with r^2 of 0.9998. In addition, the suitability of this

analytical condition when applied to the 5 different local brands of diesel at a level of the LOD (1 μg/mL) did not show any significant difference (P>0.05) in terms of fluorescent response. Thus, the naphthalene wavelengths of 286 nm (excitation) and 321 nm (emission) were sensitive enough for the detection of diesel fuel.

4.4.4.2. Analysis of spiked vegetable oils

The selectivity of the normal-phase HPLC system for the determination of diesel fuel in vegetable oils is discussed in the following section. Figure 4.44a illustrates the ELSD chromatogram of blank crude palm oil using the mobile phase composition of heptane and isopropanol (94:6, vol/vol). The large peak is attributed to the tri- and di-glycerides, and free fatty acids based on reference standards. The ELSD chromatogram also indicates that crude palm oil was eluted completely within 15.0 min with mono-glyceride at 12.0 min. Figure 4.44b, on the other hand, shows the typical fluorescence chromatogram of crude palm oil. The peaks labeled (b), (c), (d), and (f) were identified as vitamin E isomers, namely α-tocopherol, α-, γ- and δ-tocotrienols, respectively. Peaks labeled (e) and (g) were unknown fluorescent components present.

The peak labeled (a) in Figure 4.44b is attributed to the ester components present in the crude palm oil. It is eluted earlier than the tri-glycerides on the normal-phase column, showing that it is less polar. The esters did not show on the ELSD chromatogram (Figure 4.44a) as they have co-eluted with the tri-glycerides, unless a less polar mobile phase system was employed (*i.e.* 100% heptane) for the elution. As noted (Figure 4.44b), these esters do possess some fluorescence

properties, and was found to elute close to the diesel peak. Therefore, different mixtures of heptane and isopropanol were employed to resolve the diesel peak from the ester peak of the oil. The resulting tests showed that when the polarity of the mobile phase was increased by raising the concentration of isopropanol in the mobile phase system, the ester peak overlapped with the diesel peak. Decreasing the polarity of the mobile phase (*e.g.* 100% heptane), on the other hand, resulted in a broadening of the diesel peak without any improvement in the resolution.

As a result, even though the baseline resolution of the ester peak and the diesel peak was not achieved completely, the best resolution was achieved by employing the mobile phase consisting of heptane and isopropanol at 94:6 (vol/vol). For this reason, blank crude palm oils, free from contamination with diesel fuel, were used to establish the background (Figure 4.45). These blank chromatograms were then overlaid with the spiked chromatograms so that the retention time of the diesel peak could be assigned as illustrated in Figure 4.45. Subsequently, the baseline was established by drawing a line parallel to the x-axis from the retention time of 2.5 to 3.5 min; starting at the front end of the ester peak until the tail end of the diesel peaks, and the quantitation of diesel was based on the peak height counts at 3.10±0.02 min.

The calibration results obtained from various spiked crude palm oil samples are summarized in Table 4.17. A typical linear calibration curve over the analyzed range of 5-1000 μg/g of diesel produced a regression of $y = 0.0042x - 27.11$ with an r^2 of 0.9984 (where y is the concentration of diesel and x is the peak height counts). The repeatability of the method gave satisfactory CV values of 0.233-2.071%, mainly because the diesel was analyzed directly from the crude palm oil solutions

without being subjected to further pre-treatment steps. The LOQ of the method was found to be 5 μg/g diesel in crude palm oil.

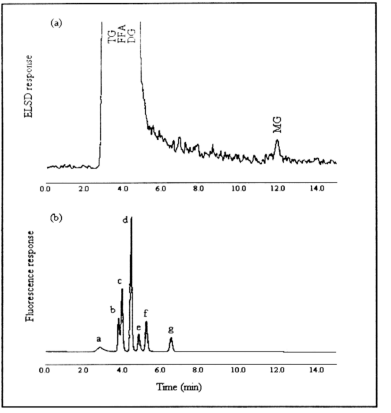

Figure 4.44. HPLC of blank crude palm oil analyzed using (a) evaporative light scattering detection (ELSD) showing that the oil is eluted completely; tri- (TG), di- (DG) and mono-glyceride (MG), and free fatty acid (FFA), and (b) fluorescence detection showing the presence of other fluorescent components in the oil. Peaks identification: *a*, ester fraction, *b-d* and *f*, vitamin E isomers, and *e* and *g*, unknowns. HPLC conditions: ***column***, DioL II 250 x 4.6 mm i.d.; *mobile phase*, heptane and isopropanol (94:6, vol/vol); and *oven temperature*, 30°C.

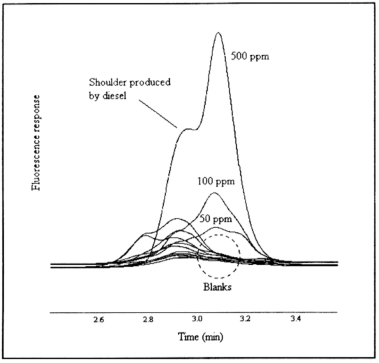

Figure 4.45. HPLC showing the presence of background fluorescent components in blank crude palm oil. Diesel was eluted slightly after the fluorescent components as demonstrated using spiked samples (50, 100, and 500 μg/g diesel). For HPLC conditions see Figure 4.44.

Table 4.17. Calibration data obtained from spiked crude palm oil solutions

Conc. of diesel (μg/g)	Mean of peak height (arbitrary units)[a]	SD	CV., %
1000	250,194	2525.27	1.009
500	120,097	451.59	0.376
100	33,403	20174	0.603
50	18,364	42.77	0.233
20	14,666	186.49	1.271
10	7,106	23.62	0.332
5	6,554	135.72	2.071

[a]Mean of 3 readings

The applicability of the method for the analysis of diesel in other vegetable oils was also evaluated. The chromatograms of the oils spiked with various concentrations of diesel are illustrated Figure 4.46. The LOQ estimated for sunflower oil, palm olein, and groundnut oil was 5 μg/g. However, the LOQ found for corn oil was 40 μg/g which is higher than that for groundnut oil. This was because the fluorescence chromatogram of the corn oil, which contains a higher percentage of esters[129], gave a bigger ester peak which lowers the estimation of the LOQ. The mean recoveries obtained from these oils were generally good, ranging from 94.4 to 101.3%, indicating that the method was also suitable for the analysis of diesel contamination in other vegetable oils. The CV of the intra-day assay based on 3 determinations was within a good range of normal acceptable limit of 20%, ranging from 4.56-10.5%. On the other hand, the inter-day assays, which were conducted in a 2-week period, gave a CV in the range of 12.5-17.6%. It was found that other than the instrumental factors, evaporation of diesel from the sample during storage was another major contributing factor that could result in the high variability of the method. Therefore, it was important that the oil samples be heated at as low a temperature as is possible if melting was required such as in a case of crude palm oil.

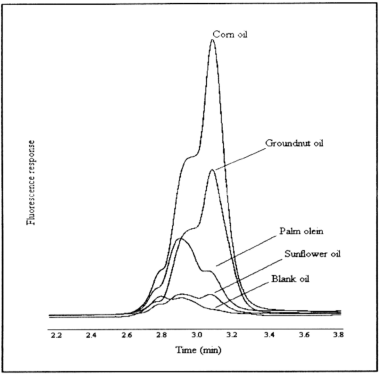

Figure 4.46. HPLC with fluorescence detection of spiked corn oil (1000 μg/g), groundnut oil (500 μg/g), palm olein (100 μg/g), and sunflower oil (50 μg/g). Crude palm oil was used as blank. For HPLC conditions see Figure 4.44.

Table 4.18. Recovery results of diesel from various spiked samples (n=3)

Oil sample	Mean peak height (arbitrary units)	Spiked amt. (μg/g)	Calc. amt. (μg/g)	Rec., %
Sunflower	15,310	50	47.2	94.43
Palm olein	32,964	100	101.34	101.34
Groundnut	117,390	500	465.93	93.19
Corn	222,146	1000	948.91	94.89

$y = 0.9943x + 1.3152$

$r^2 = 0.9998$

4.4.4.3. Analysis of commercial crude palm oil

The HPLC method developed was also used to check the levels of diesel in some samples of the commercial shipments of crude palm oil from another country. As shown by the fluorescence peaks that are similar to that exhibited by diesel, some of the samples were determined to be contaminated with 17.97 to 75.96 μg/g of diesel (Figure 4.47).

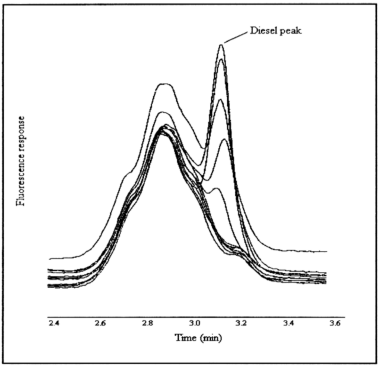

Figure 4.47. HPLC with fluorescence detection of some commercial crude palm oil samples suspected to be contaminated with diesel. Diesel was observed at 3.10 min. For HPLC conditions see Figure 4.44.

In order to confirm this contamination, a few blank and contaminated samples were subjected to normal-phase column chromatography and GC-MS analysis as described in the *Experimental* sections 3.3.4.5 and 3.3.4.7. The principal

advantage of the normal-phase column chromatography was the possibility of a complete separation of non-polar components (hydrocarbons and esters) from the other more polar components present in the crude palm oil when a non-polar solvent such as hexane is used as the eluant. Secondly, the eluate can be further concentrated via evaporation prior to GC-MS analysis.

In this study, a bigger glass column chromatography (800 mm x 40 mm i.d.) was used so that a larger amount of oil sample could be loaded each time. This in turn enabled a higher concentration of the non-polar materials to be isolated from the oil sample for it to be analyzed by the GC-MS.

Figure 4.47 is the total ion chromatogram of the hydrocarbon fraction of a blank crude palm oil. It shows the presence of mainly even-chain *n*-alkanes ($C_{12}H_{26}$ to $C_{20}H_{42}$), *n*-alkenes ($C_{12}H_{24}$ to $C_{30}H_{60}$), squalene and relatively lesser amounts of odd-chain *n*-alkanes ($C_{13}H_{28}$ to $C_{31}H_{64}$). These observations were similar to those reported by Goh and Gee[130], and Lerker[131] where both considered these hydrocarbons to be naturally present in the crude palm oil. In contrast, the corresponding total ion chromatogram of the contaminated crude palm oil (Figure 4.48) reveals crucial differences when compared to that of the uncontaminated crude palm oil. In this case, another group of peaks was identified by the GC-MS as $C_{21}H_{44}$ to $C_{31}H_{64}$, with both even- and odd-chained hydrocarbons, and the appearance of a bell-shaped distribution characteristic of hydrocarbons of diesel as shown in Figure 4.38. The analysis results from GC-MS provide confirmation that samples identified by the HPLC method were contaminated with diesel.

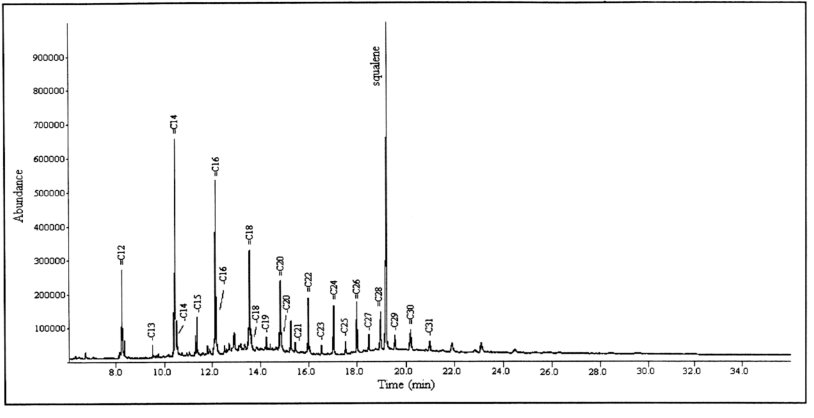

Figure 4.48. A typical total ion chromatogram of blank crude palm oil. *n*-alkane (-C), and *n*-alkene (=C). For GC conditions see Figure 4.38.

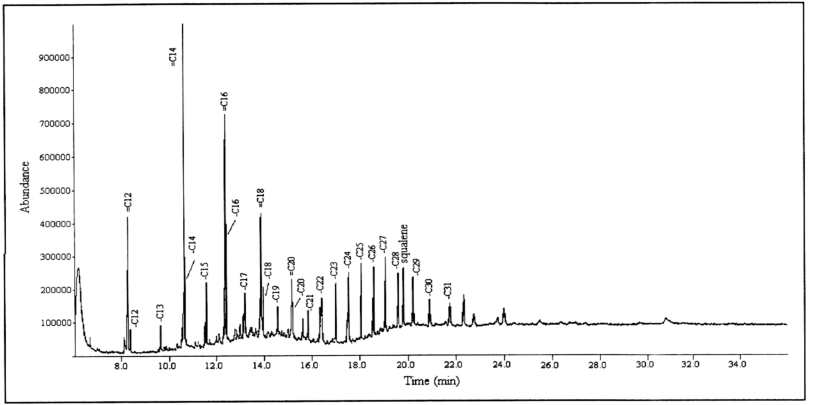

Figure 4.49. A typical total ion chromatogram of crude palm oil suspected to be contaminated with diesel. *n*-alkane (-C), and *n*-alkene (=C). For GC conditions see Figure 4.38.