Quantitative Determination of Diesel Oil in Contaminated Edible Oils Using High-Performance Liquid Chromatography

M.H. Moh[a,*], T.S. Tang[a], and G.H. Tan[b]

[a]Advanced Oleochemical Technology Center, Malaysian Palm Oil Board, Selangor, Malaysia, and [b]Department of Chemistry, University of Malaya, 50603 Kuala Lumpur, Malaysia

ABSTRACT: A simple and reliable high-performance liquid chromatography method for the analysis of diesel oil in contaminated edible oils is described. Analysis is performed using a diol column with a mobile phase of heptane and isopropanol (94:6, vol/vol). Although baseline separation between diesel and other background fluorescent components was not achieved, quantitation was still possible using baseline integration. The method is linear over the range of 5–1000 μg/g with a correlation coefficient (r^2) of 0.9984. Average recoveries from spiked edible oils were 94.4–101.3%, with a limit of quantitation (LOQ) of 5 μg/g for sunflower oil, palm olein, and groundnut oil. Corn oil has a higher content of ester components, thus, LOQ was slightly worse (40 μg/g). The applicability of the method was confirmed by gas chromatography–mass spectroscopic detection to show the presence of diesel hydrocarbons in the suspected contaminated crude palm oil. This procedure provides a simple and sensitive method for determining diesel oil concentration in contaminated edible oils without prior sample cleanup or extraction.

Paper no. J9730 in *JAOCS 78,* 519–525 (May 2001).

KEY WORDS: Contamination, diesel oil, edible oils, high-performance liquid chromatography.

In the modern bulk handling system, where edible oils of different qualities must be pumped through common valves and pipelines, higher-grade edible oils risk being contaminated by lower-grade oils (1,2). The problem becomes more serious when edible oils are contaminated with inedible ones such as castor oil, argemone oil, and petroleum oil (3), because such cases pose serious health implications to consumers. Accidental contamination by lubricating oil or diesel oil could also occur through the use of second-hand drums for storage or inadequately cleaned road transport tankage for transportation of edible oils.

Many publications report tedious and time-consuming gas chromatographic (GC) procedures for the determination of various mineral hydrocarbons in vegetable oils after saponification (4,5) or column chromatography (6–8). However, none of them addresses diesel oil contamination. Our preliminary testing showed that neither saponification nor column chromatography was suitable for recovering diesel oil from edible oils. Due to the high volatility of the oil, serious losses occur in both pretreatment processes.

In the petroleum industry, high-performance liquid chromatography (HPLC) analysis of diesel and other petroleum products has been reported (9,10). In most cases, these products are separable into three hydrocarbon groups—saturates, olefins, and aromatics (11). Further separation of the aromatics into one-, two-, and three-ring compounds is also possible (10). A refractive index detector is ideal for measuring saturates and olefins at high concentrations. Owing to the strong absorbing nature of chromophores, a ultraviolet (UV) detector is usually used for measuring aromatics (12,13). A fluorescence detector, on the other hand, offers greater sensitivity and selectivity than the UV detector for aromatic molecules and conjugated polyolefinic compounds (14).

Bhati *et al*. (15) described an HPLC technique for the separation of hydrocarbons and triglycerides in edible oil. The hydrocarbons eluted at 3 min were detected by a refractometer, and triglycerides eluted at 22 min were detected by infrared absorption set at 1740 cm^{-1} for measuring the C=C stretching. Constante *et al*. (16) reported another HPLC technique with UV detection to determine mineral oil contamination in edible oils. However, the procedure required hydrocarbons to be separated by thin-layer chromatography prior to the analysis.

Recent reports (17,18) indicated that up to 85,000 metric tons of crude palm oil (CPO) shipped to Europe from Indonesia were contaminated with variable amounts of diesel. However, the actual cause is still uncertain. It was speculated that the contamination could have occurred in storage tanks or during handling or transportation. It would be of great benefit to the industry if a rapid method could be developed to detect diesel contamination in edible oils.

In this paper, we propose HPLC coupled with a fluorescence detector for the quantitation of diesel in edible oils. The technique is simple, rapid, and requires no sample preparation.

MATERIALS AND METHODS

Reference chemicals. Tri-, di-, and monoglycerides, palmitic acid, and α-tocopherol were purchased from Sigma Chemical Company (St. Louis, MO), and diesels were obtained from local petrol stations. All solvents used were of HPLC grades.

Standard solutions. Stock solution of diesel (1000 μg/mL) in heptane was prepared. Appropriate volumes of stock solu-

*To whom correspondence should be addressed at Advanced Oleochemical Technology Center, Malaysian Palm Oil Board, Lot 9 & 11, Jalan P/14, Seksyen 10, 43650 Bandar Baru Bangi, Selangor, Malaysia.
E-mail: felixmoh@mpob.gov.my

tions were diluted in heptane to give working standards of 5, 10, 20, 50, 100, and 500 μg/mL. One-gram CPO samples were accurately weighed into six 10-mL volumetric flasks, then 1.0 mL of each working standard was added to one of the six samples, and it was diluted to 10 mL with heptane. This provided spiked solutions containing 5 to 1000 μg diesel oil in 1.0 g edible oil. Twenty microliters of each solution was injected into the HPLC system.

Test samples. Ten CPO samples from different local palm oil mills were used as blank references. Another 20 CPO samples from overseas, suspected to be contaminated by diesel oil, were used in the analysis. All samples were stored in a cold room until analysis. Prior to analysis, they were thoroughly mixed after warming.

Validation. Linearity of the method was demonstrated with different diesel oil standards at seven concentrations over the range of 5–1000 μg/mL. Diesel oil concentrations were plotted against the peak height at 3.10 min and analyzed using simple linear regression. One gram of each edible oil sample was weighed into a 10-mL volumetric flask and diluted with heptane to the mark. Triplicate injections of 20 μL each were performed, and the peak heights at 3.10 min were obtained. The recoveries were calculated by interpolation from the calibration curve established earlier.

Luminescence spectroscopy. Excitation and emission spectra were collected with a PerkinElmer Model LS 50B spectrofluorometer (PerkinElmer Corp., Norwalk, CT) using the FL Winlab software (PerkinElmer). The instrument was set at a slit width of 3.0 nm, a scanning speed of 1000 nm/min, and a scanning range from 200 to 600 nm.

High-performance liquid chromatography (HPLC). The HPLC system used 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). A programmable fluorescence detector (Jasco FP-970) operated at 286 (excitation) and 321 nm (emission) was connected in series with an evaporative light-scattering detector (ELSD: Sedex 55; Sedere, Alfortville, France). Separation was performed at 30°C on an Apex Diol II (25 × 4.6 cm i.d., 5 μm particle size; Jones Chromatography Ltd., Mid Glamorgan, England). The isocratic mobile phase consisted of heptane and isopropanol (94:6, vol/vol) with a flow rate of 1.0 mL/min. Data were recorded and analyzed using Borwin Version 1.21 chromatographic software (JMBS Développements, Le Fontanil, France).

Glass chromatographic column (80 × 4 cm, i.d.). The column was packed with about one-third of premixed silica gel slurry in hexane. Approximately 30 g CPO, both blank and suspected samples, was loaded onto the adsorbent and eluted with an additional 500 mL of hexane. The eluate was collected in a 500-mL flask, and the solvent was evaporated in a gentle stream of nitrogen with the flask at 60°C (water bath). The residue was redissolved with 1 mL hexane, and 1 μL was injected into the gas chromatograph with mass selective detector.

Gas chromatography-mass selective detection (GC–MSD). The system used was a Hewlett-Packard (Palo Alto, CA) model 5890 GC connected to 5970 MSD and operated by a 59970 MS Chemstation. Conditions were as follows: HP-5 cross-linked with 5% phenyl methylsiloxane capillary column, 30 m × 0.32 mm i.d.; injector and detector temperatures set at 250°C; and oven temperature at 70°C for first 5 min, then programmed at 15°C/min from 70 to 280°C and held until all peaks eluted.

RESULTS AND DISCUSSION

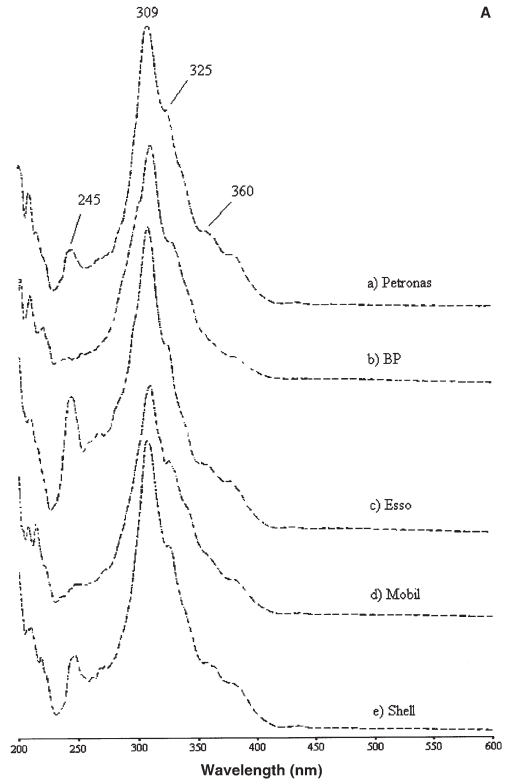
Stock solutions of diesel at a concentration of 1000 μg/mL were prepared in hexane, and spectra between 200 and 600 nm were recorded. As shown in Figures 1A and 1B, excitation and emission wavelengths for diesels are maximal at 309 and 344 nm, respectively. Thus, spectroscopically, the local diesels checked were similar.

When these two wavelengths (309 nm excitation and 344 nm emission) were applied for detecting diesel by using HPLC, a single fluorescent peak was obtained as shown in Figure 2B. However, the GC–MSD chromatogram (Fig. 3) revealed that the majority of constituents of diesel are *n*-alkanes ($C_{11}H_{24}$ to $C_{30}H_{62}$). 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; the majority of them matched those reported by Xiang *et al*. (19). The presence of these aromatics would contribute to the fluorescent properties of the diesel.

The fluorescence detector was further assessed using the excitation and emission wavelengths of naphthalene, anthracene, and benzene as reported by Wehry (20), and chromatograms are depicted in Figures 2A, 2C, and 2D, respectively. The comparison showed that diesel oil exhibited the strongest fluorescence intensity using naphthalene's wavelengths (286 nm excitation and 321 nm emission). Measurements using diesel oil solutions of 5–50 μg/mL at 286 nm excitation and 321 nm emission showed that the detector was linear with correlation coefficient (r^2) = 0.9998. The suitability of the analytical condition applied on the five different local brands of diesel at a level of 1 μg/mL showed no significant difference ($P > 0.05$) in terms of fluorescent response.

Figure 4A illustrates the ELSD chromatogram obtained from the analysis of blank CPO using the same conditions used for diesel oil mentioned in the previous section. The large peak was attributed to triglycerides, diglycerides, and free fatty acids based on the reference standards. The ELSD chromatogram also indicates that CPO eluted completely within 15.0 min with monoglycerides at 12.0 min. Figure 4B shows the typical fluorescent chromatogram of CPO. Peaks labeled *b*, *c*, *d*, and *f* were identified as the vitamin E isomers, namely α-tocopherol, and α-, γ-, and δ-tocotrienols, respectively. Peaks labeled *e* and *g* were unknown fluorescent components present in the CPO.

Worthington and Hitchcock (21) and Trost (22) reported that hydrocarbons and ester components such as steryl esters, and triterphenyl esters were less polar than triglycerides.



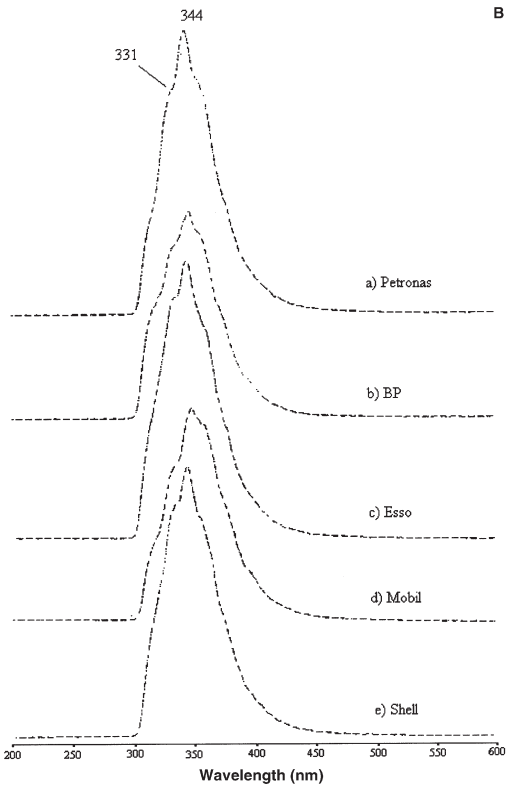


FIG. 1. (A) Excitation and (B) emission spectra of diesel solutions (1000 µg/mL) as supplied from local petrol stations.

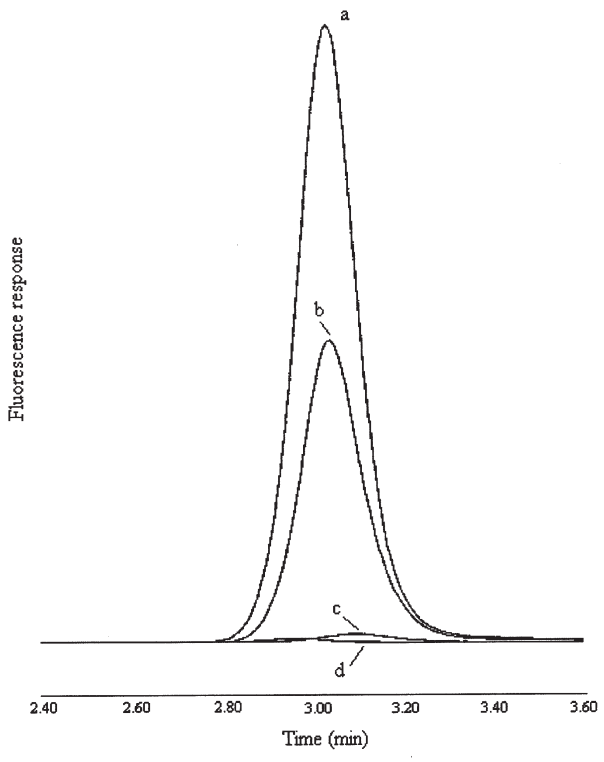


FIG. 2. High-performance liquid chromatograms of diesel solutions analyzed using excitation and emission wavelengths of (A) naphthalene, (B) diesel, (C) anthracene, and (D) benzene. Two microliters of diesel solution (1000 µg/mL) was injected.

Therefore, they eluted slightly earlier than triglycerides on normal-phase HPLC. Saturated hydrocarbons do not fluoresce. However, ester components as shown in Figure 4B (peak labeled *a*) do possess some fluorescent properties. Previous tests showed that the aromatic component in diesel (Fig. 2) also eluted at this region. Therefore, different mixtures of heptane and isopropanol were employed to resolve the diesel peak from the less polar peak of oil. Experiments showed that increasing the polarity of the mobile phase and raising the concentration of isopropanol in the mobile phase system resulted in overlapping of both diesel and fluorescent peaks of the ester region. Decreasing the polarity of the mobile phase (e.g., 100% heptane) broadened the diesel peak without improving the resolution. Figure 5 shows fluorescence chromatograms of CPO spiked with 50, 100, and 500 µg diesel, respectively, overlaid with those of unspiked samples using the optimal mobile phase consisting of a mixture of heptane and isopropanol (94:6, vol/vol). Baseline resolution of ester and diesel was still not achieved. For this reason, 10 blank CPO of different origins were used to establish the background. Quantitation of diesel was based on the peak height counts at 3.10 ± 0.02 min and baseline was accomplished by drawing a line parallel to the x-axis from the retention time of 2.5 to 3.5 min.

Table 1 shows the calibration data determined from the measurement of diesel peak in CPO samples spiked between

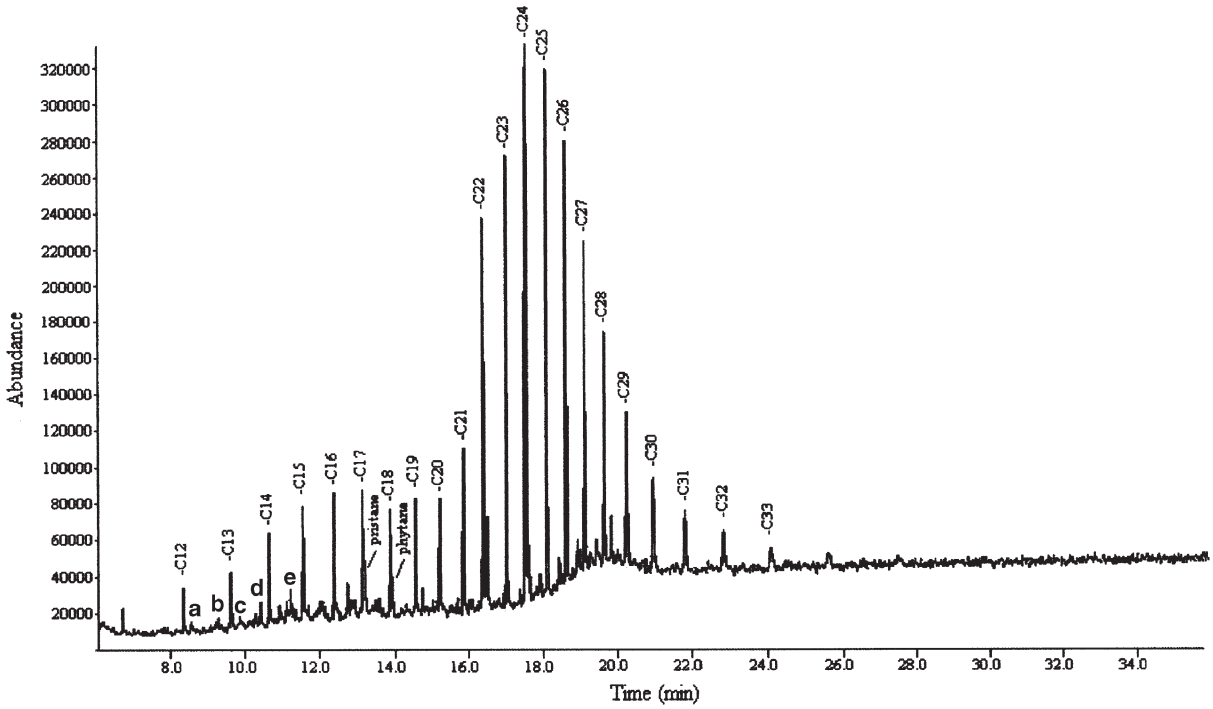


FIG. 3. A typical total ion chromatogram of a diesel. *n*-Alkane (-C) and *n*-alkene peaks: *a*, 1,2,3,4-tetrahydro-5-methylnaphthalene; *b*, 1,4-dimethyl-1,2,3,4-tetrahydronaphthalene; *c*, 2-methylnaphthalene; *d*, 1-methylnaphthalene; and *e*, 1,7-dimethylnaphthalene.

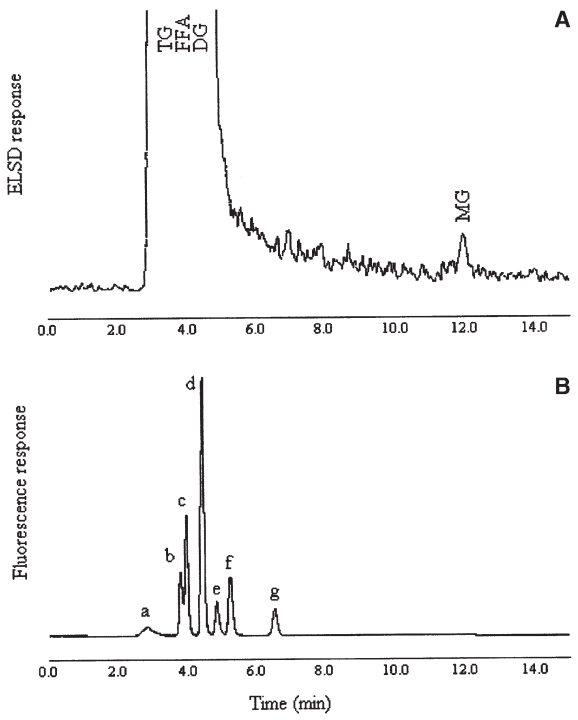


FIG. 4. High-performance liquid chromatograms of blank crude palm oil (CPO) analyzed using (A) evaporative light-scattering-detection (ELSD) showing that the oil is eluted completely; tri- (TG), di- (DG), and monoglyceride (MG) and free fatty acid (FFA), and (B) fluorescence detection showing the presence of other fluorescent components in the oil. Peak *a* is ester fraction, peaks *b–d* and *f* are vitamin E isomers, and *e* and *g* are unknowns.

5 and 1000 µg/g. A typical calibration curve over this range 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 suitability of the method was evaluated with other spiked oil samples (Fig. 6). The results are

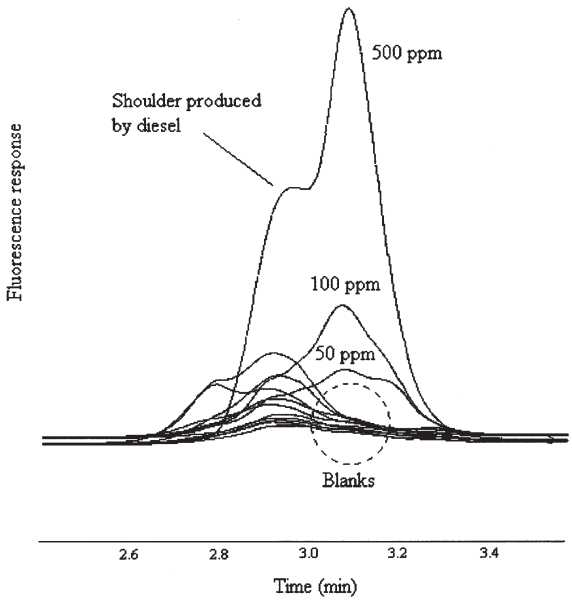


FIG. 5. High-performance liquid chromatograms showing the presence of background fluorescence components in blank CPO. Diesel was eluted slightly after these fluorescence components as demonstrated using spiked samples (50, 100, and 500 µg/g diesel). For abbreviation see Figure 4.

TABLE 1
Calibration Data Obtained from Spiked Crude Palm Oil[a]

Concentration diesel oil (μg/g)	Mean peak height (arbitrary units)[b]	SD[c]	CV[d] (%)
1000	250,194	2525.27	1.009
500	120,097	451.59	0.376
100	33,403	201.74	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
$y = 0.0042x - 27.11$, $r^2 = 0.9984$			

[a]Crude palm oil solutions.
[b]Mean of three readings.
[c]Standard deviation.
[d]Coefficients of variation.

TABLE 2
Recovery Results of Diesel Oil from Spiked Samples ($n = 3$)

Sample	Mean peak height (arbitrary units)	Spiked amount (μg/g)	Calculated amount (μg/g)	Recovery (%)
Sunflower	15,310	50	47.20	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
$y = 0.9443x + 1.3152$				
$r^2 = 0.9998$				

given in Table 2 with recoveries from 94.4 to 101.3%. A plot of the means of triplicate analyses plotted against spiked concentrations yielded a slope close to unity with $r^2 = 0.9998$. The limit of quantitation (LOQ) estimated from representative blank samples was 5 μg/g for sunflower oil, palm olein, and ground nut oil. However, the corresponding LOQ for corn oil, containing a high percentage of esters (22), was 40 μg/g. The HPLC method was also applied to study the levels of diesel in some commercial CPO originated from Indonesia, as illustrated in Figure 7. Some of the samples were estimated to be contaminated with 17.97 to 75.96 μg/g of diesel.

The HPLC procedure described so far prescribes a method of determining contamination of vegetable oils with diesel by exploiting the presence of fluorescent components in the contaminant. In order to confirm the contamination, a few blank and contaminated samples analyzed as described were subjected to column chromatographic separation using silica gel to isolate the hydrocarbon fraction from the oil matrices. The eluants of the blank and contaminated samples were then concentrated and analyzed by GC–MSD.

Figure 8 is the total ion chromatogram of the hydrocarbon fraction of a blank CPO. It shows the presence of mainly even-chain *n*-alkane ($C_{12}H_{26}$ to $C_{20}H_{42}$), *n*-alkene ($C_{12}H_{24}$ to $C_{30}H_{60}$), and 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 (6) and Lerker (23), who both considered these hydrocarbons as naturally present in CPO. In contrast, the corresponding total ion chromatogram of the contaminated CPO (Fig. 9) reveals crucial differences compared to that of the uncontaminated CPO. Here, an-

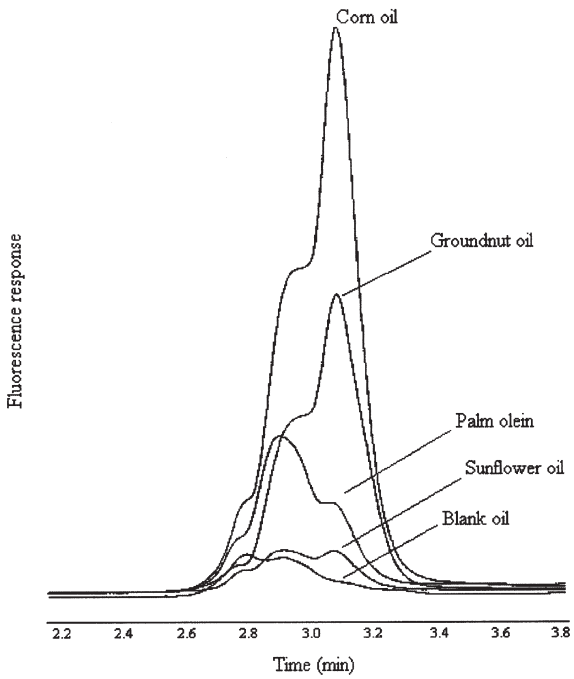


FIG. 6. High-performance liquid chromatograms with fluorescence detection of spiked corn oil (1000 μg/g), groundnut oil (500 μg/g), palm olein (100 μg/g), and sunflower oil (μg/g). CPO was used as blank. For abbreviation see Figure 4.

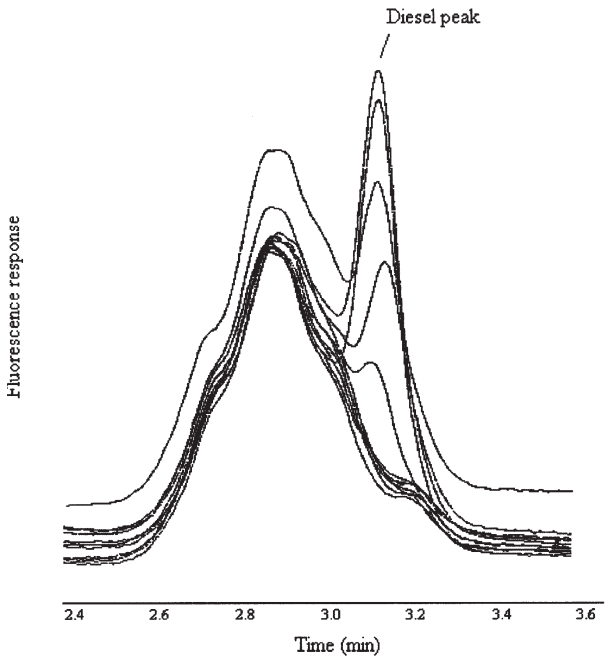


FIG. 7. High-performance liquid chromatograms with fluorescence detection of some commercial CPO samples. Fluorescent peaks of diesel are obvious at 3.10 min. For abbreviation see Figure 4.

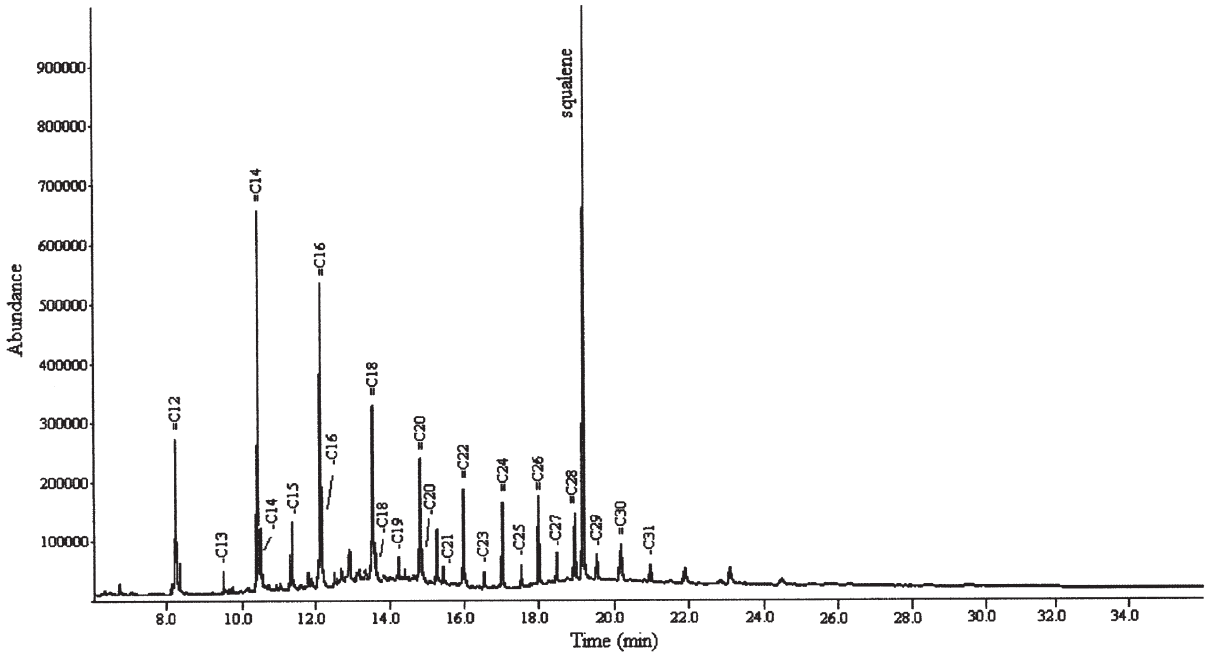


FIG. 8. A typical total ion chromatogram of blank CPO. For abbreviations see Figures 3 and 4.

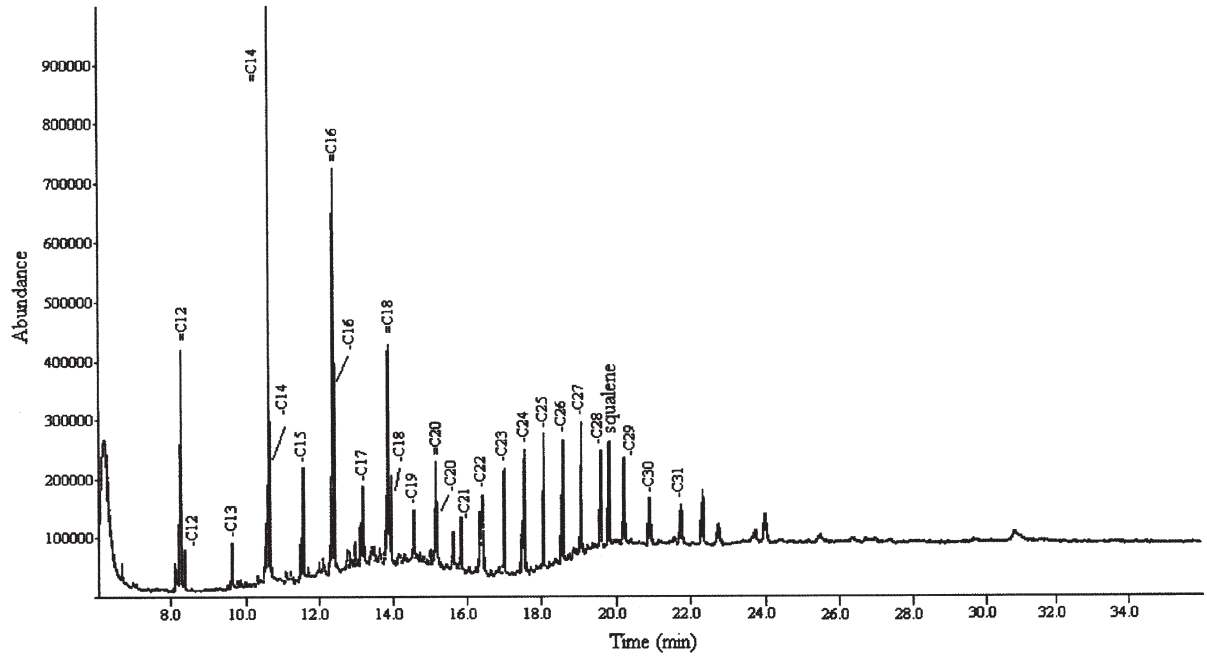


FIG. 9. A total ion chromatogram of CPO suspected to be contaminated with diesel. For abbreviations see Figures 3 and 4.

other array of peaks was identified by MSD as $C_{21}H_{44}$ to $C_{31}H_{64}$, with both even- and odd-chained hydrocarbons and the appearance of bell-shaped distribution characteristic of hydrocarbons of diesel (Fig. 3).

Thus, the analysis result from GC–MSD provides confirmation that samples identified by the HPLC method were indeed contaminated with diesel. Because of its relative simplicity, the HPLC method with fluorescence detection can serve as a rapid method for the determination of diesel contamination in vegetable oils.

ACKNOWLEDGMENT

We wish to thank the Director-General of the Malaysian Palm Oil Board for his permission to publish this paper.

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[Received August 8, 2000; accepted February 7, 2001]