Optimization and Validation of High-Performance Liquid Chromatographic Method for the Determination of Dowtherm A[TM] in Edible Oils and Oleochemicals

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ABSTRACT: A method using high-performance liquid chromatography and fluorescence detection is optimized and validated for the determination of Dowtherm A[TM] in spiked oleochemicals and edible oils. The samples are directly injected into a reversed-phase C18 column, and Dowtherm A is detected using a fluorescence detector set at 247 nm excitation and 310 nm emission wavelengths. The simple isocratic mobile phase used is a mixture of methanol and water (90:10, vol/vol) at a flow rate of 1 mL/min. The limits of quantitation are from 0.1 to 0.2 µg/g. Mean recoveries ranged from 93.0 to 116% with reproducibilities of 1.29–3.84%. The procedure provides a simple, reliable and sensitive method for determining Dowtherm A residue in oleochemicals and edible oils without prior sample cleanup or extraction.

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KEY WORDS: Biphenyl and diphenyl oxide, Dowtherm A, thermal heating fluid.

Deodorization, which is the final stage of the refining of an edible oil, requires temperatures up to 270°C. This is achieved by indirectly heating the oil in heat exchangers with a suitable heating medium whose temperature may reach 300°C. High-pressure steam is preferred nowadays, though thermal heating fluids (THF) are still used in many older plants. A commonly used THF is a eutectic mixture of biphenyl and diphenyl oxide, trademarked as Dowtherm A and Therminol VP-1. However, concern arises if contamination results from pinhole leaks or faulty joints of the coils (1,2). Although there were attempts to ban the use of THF in edible oil processing plants (3), the Codex Committee on Fats and Oils (CCFO) had resolved to allow the use of THF other than high-pressure steam on the basis of safety and risk evaluation and inspection procedures (4).

Gas chromatographic (GC) methods have been reported for the determination of Dowtherm A in fats and oils (5–7). They require pretreatment of the samples prior to analysis *via* either a thin-layer chromatographic clean-up or a distillation step, whereas the American Oil Chemists' Society procedure (8) uses a solvent extraction technique. Although these GC techniques are sensitive, with detection limits of 0.2 µg/g, the procedures are time-consuming and tedious.

A new high-performance liquid chromatographic (HPLC) method with fluorescence detection was developed by Moh and Tang (9) for the determination of Dowtherm A in spiked edible oils and oleochemicals. The limit of quantitation was found to be 0.1 µg/mL for all types of samples analyzed. This method is superior to those GC methods previously reported in that it requires no pretreatment. In order to ascertain the validity of the method developed, an interlaboratory study was undertaken to establish reproducibility data.

MATERIALS AND METHODS

All solvents were of HPLC grade.

Reference standards. Biphenyl and diphenyl oxide, 98% purity, were purchased from Fluka Chemika AG (Buchs, Switzerland). Dowtherm A was supplied by Dow Chemical Company (Midland, MI).

Working standards. A 100-µg/mL stock solution was prepared by dissolving 10 mg Dowtherm A with methanol to the mark in a 100-mL volumetric flask. The appropriate aliquots of the stock solution in methanol were diluted to produce working standards with 10, 5, 1, 0.1, 0.01, and 0.005 µg/mL. Linearity of the detector response was checked from this set of six working standards. Calibration graphs were prepared by plotting the peak area of biphenyl against the concentration.

Recovery studies. Since contaminated samples were not available, all analyses were performed on spiked samples. A 1 g sample was accurately weighed into each of five 10-mL volumetric flasks. Then 1, 0.5, 0.2, and 0.1 mL of working standard solution (1 µg/mL) were added to the samples, and diluted to 10 mL with tetrahydrofuran, to provide spiked solutions containing 1, 0.5, 0.2, and 0.1 µg/g Dowtherm A. A total of six injections of 20 µL each were carried out for each sample, and respective peak areas for biphenyl were obtained. Recoveries were calculated by interpolation from the calibra-

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tion curve established earlier.

The limit of detection in the HPLC system using the fluorescence detector was estimated from representative blank samples. It was equal to the minimum concentration of at least three times that of the noise (S:N ≥ 3) (10).

Collaborators. The laboratories that participated in this interlaboratory study were Advanced Oleochemical Technology Center, Palm Oil Research Institute of Malaysia (PORIM); Faculty of Food Technology, University Putra Malaysia; and Product Development and Quality Unit, PORIM.

HPLC instrument. An HPLC system consisting 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), a column oven (Jasco CO-965), and a Jasco FP-970 programmable fluorescence detector was controlled by Borwin 1.21 (JMBS Développpements, Le Fontanil, France) chromatographic software. A reversed-phase column (250 × 4.6 mm i.d.) packed with 5 μm LiChrospher C_{18} (GL Sciences Inc., Tokyo, Japan) was used with a 50 × 4.6 mm i.d. guard column packed with the same material. The system was run isocratically with a mobile phase of methanol/water (90:10, vol/vol). The flow rate was 1.0 mL/min, and the column was maintained at 35°C. The detector was optimized at an excitation wavelength of 247 nm and an emission wavelength of 310 nm.

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 slit-width was set at 3.0 nm with a scanning speed of 1000 nm/min ranging from 200 to 600 nm.

RESULTS AND DISCUSSION

Biphenyl is a highly conjugated molecule of two phenyl rings joined by a single bond. Diphenyl oxide, on the other hand, possesses an ether linkage in between and thus loses some of its conjugation and fluorescent intensity compared to biphenyl (11). The excitation and emission spectra are depicted in Figures 1A and 1B. The corresponding spectra of Dowtherm A, a eutectic mixture of biphenyl (26.5%) and diphenyl oxide (73.5%), is illustrated in Figure 1C. It shows triplet maximal excitation bands at 247, 253, and 259 nm and doublet maximal emission bands at 305 and 314 nm. As a result, the selection of the optimal pair of wavelengths is critical in order to obtain maximal sensitivity for the HPLC analysis.

An assessment of the analytical wavelengths is provided in Table 1. Based on area counts, the intensities of fluorescent excitation and emission at 260 and 310 nm for both biphenyl and diphenyl oxide were found to be highest, and were 11 and 78% higher, respectively, compared to those observed at 247 and 310 nm. However, when the fluorescence detector was tested for its sensitivity using the working standards (0.005 –10.0 μg/g), the lowest limit obtained was similar to that at 247 nm excitation and 310 nm emission. The blank sample also gave a similar profile. Furthermore, the biphenyl peak was stronger than diphenyl oxide (Figs. 2A and 2B), and this

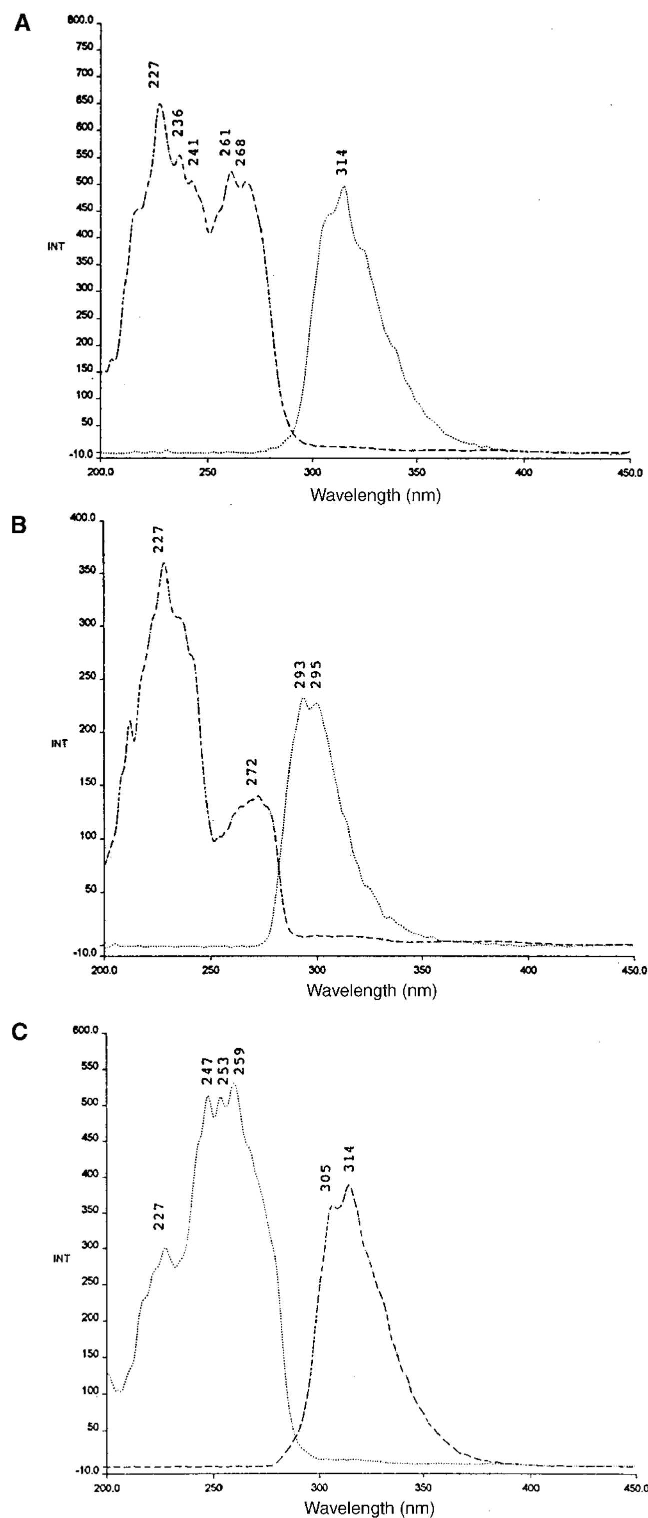

FIG. 1. Excitation and emission spectra of (A) biphenyl, (B) diphenyl oxide, and (C) Dowtherm A solutions in hexane. All are at 1 μg/mL concentration.

TABLE 1
Optimization of Fluorescent Wavelengths for Quantitation of BP and DPO[a]

Wavelength		Area under peaks[b]		Response factor	
Excitation (nm)	Emission (nm)	BP	DPO	BP	DPO
247	310	614,682	36,392	1.00	1.00
260	310	684,011	64,643	1.11	1.78
260	314	646,675	39,821	1.05	1.09
272	300	270,792	117,692	0.44	3.23

[a]Abbreviations: BP, biphenyl; DPO, diphenyl oxide.
[b]Expressed in arbitrary units.

is consistent with the relatively lower intensity observed for diphenyl oxide. Thus, the optimum wavelengths of 247 nm excitation and 310 nm emission were chosen for these studies.

Another problem considered was the possible presence of other fluorescent components in the matrices. If these compounds co-elute with the Dowtherm A, they will interfere with the quantitative results. Therefore, different mixtures of mobile phase were evaluated for the optimal separation of interfering components from Dowtherm A (Fig. 3). By using methanol as the mobile phase, the interfering components (labeled A and B) were very close to the Dowtherm A peaks.

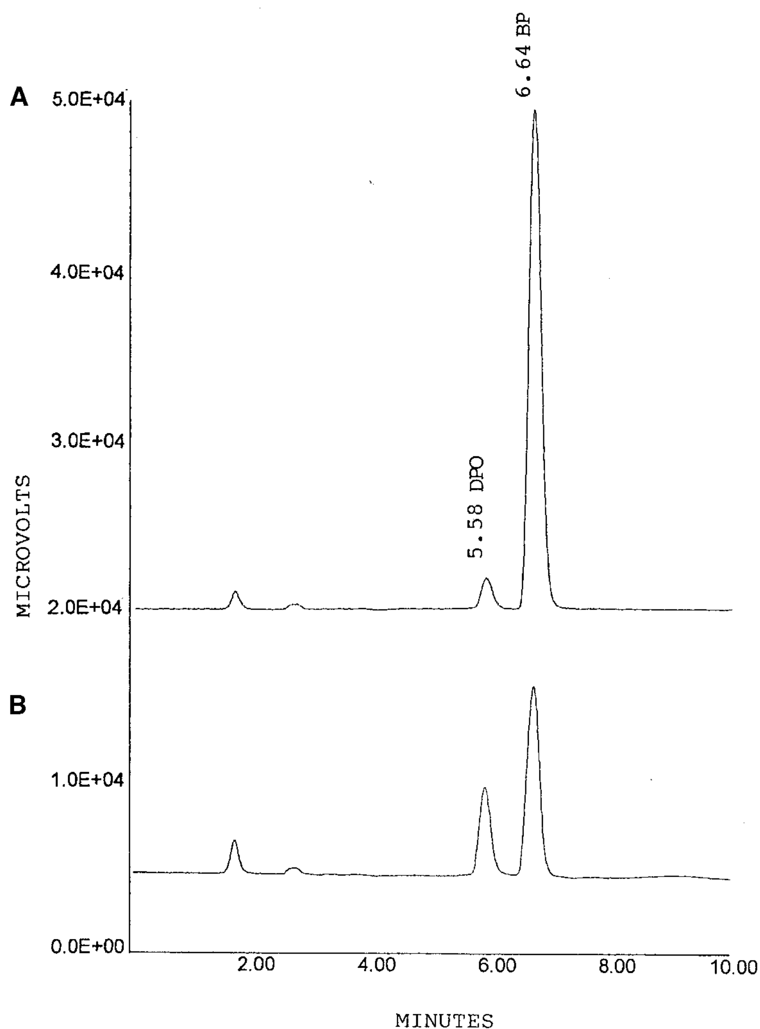

FIG. 2. Stacked high-performance liquid chromatography chromatograms of Dowtherm A analyzed using different wavelength settings: (A) 247 nm excitation and 310 nm emission, and (B) 272 nm excitation and 300 nm emission. The mobile phase used was a mixture of methanol and water (90:10, vol/vol). BP, biphenyl; DPO, diphenyl oxide.

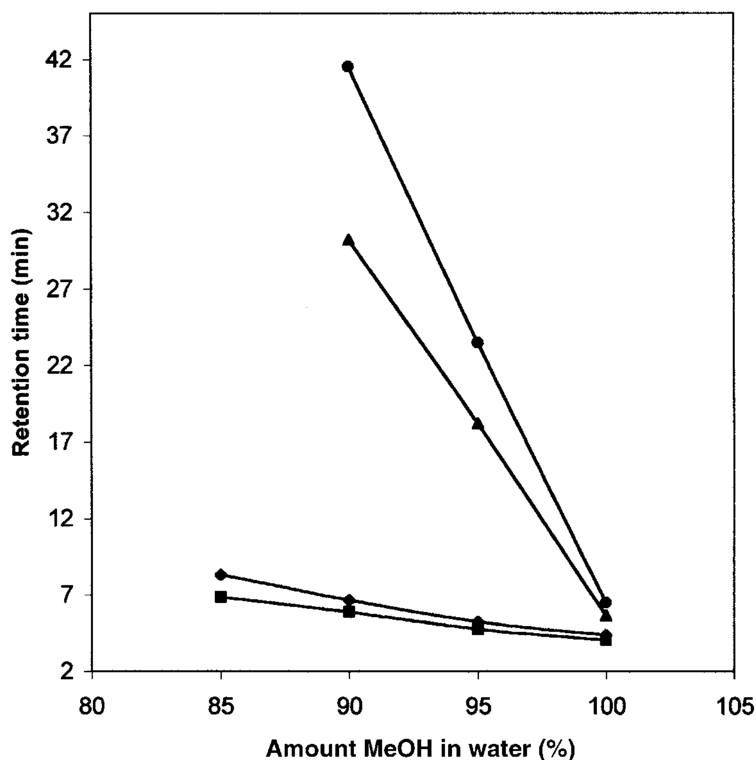

FIG. 3. Changes in retention times of BP (◆) and DPO (■) with respect to ratios of methanol and water mixture. Interfering fluorescent components labeled A (▲) and B (●) are indicated in Figure 5.

Under such circumstances, good baseline resolution for accurate quantitation was impossible to achieve. Experiments showed that by decreasing the polarity of the mobile phase by adding acetonitrile, overlapping of both Dowtherm A and other fluorescent component peaks resulted. Increasing the polarity of the mobile phase helped to resolve 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 in this study.

Chromatograms of blank samples are given in Figure 4. All samples have equally smooth baselines with no major interfering components that co-elute with the biphenyl peak.

The linearity of the analytical procedure was also tested by using spiked palm olein samples with known concentrations of Dowtherm A, ranging from 0.1 to 2.0 µg/g, in six replicates at each concentration (Fig. 5). The correlation coefficient (r^2) of the linear regression equation of the standards was close to 0.999. The suitability of the method was also validated with other oleochemical and edible oil products (9). The limits of quantitation were determined to be 0.1 µg/g for all samples and 0.2 µg/g for stearic acid. The limit of detection was estimated to be 0.01 µg/g for palm olein, fatty alcohol, corn oil, and sunflower oil, and 0.08 µg/g for stearic acid.

The mean recoveries (Table 2) for all three laboratories were satisfactory for residues at 0.1–1.0 µg/g. The highest av-

TABLE 2
Recovery Studies of Dowtherm A from Spiked Samples (n = 6)

	Recovery (µg/g) (CV, %)[a]						
Laboratory	Fatty alcohol	Stearic acid	Corn oil	Sunflower oil	r^2	Slope	Intercept
1	0.956 (0.42)	0.515 (1.86)	0.246 (2.85)	0.098 (0.82)	0.998	1.070	−0.035
2	0.975 (1.12)	0.505 (1.25)	0.238 (0.97)	0.081 (4.58)	0.998	1.031	−0.015
3	0.982 (0.23)	0.483 (4.33)	0.212 (8.21)	0.100 (4.10)	0.999	1.028	−0.007
Amount added	1.0	0.5	0.2	0.1			
Average found	0.971	0.501	0.232	0.093			
Recovery (%)	97.1	100.2	116.0	93.0			
Within-laboratory CV (%)	0.589	2.479	4.006	3.165			
Between-laboratory CV (%)	1.287	2.435	3.836	3.118			

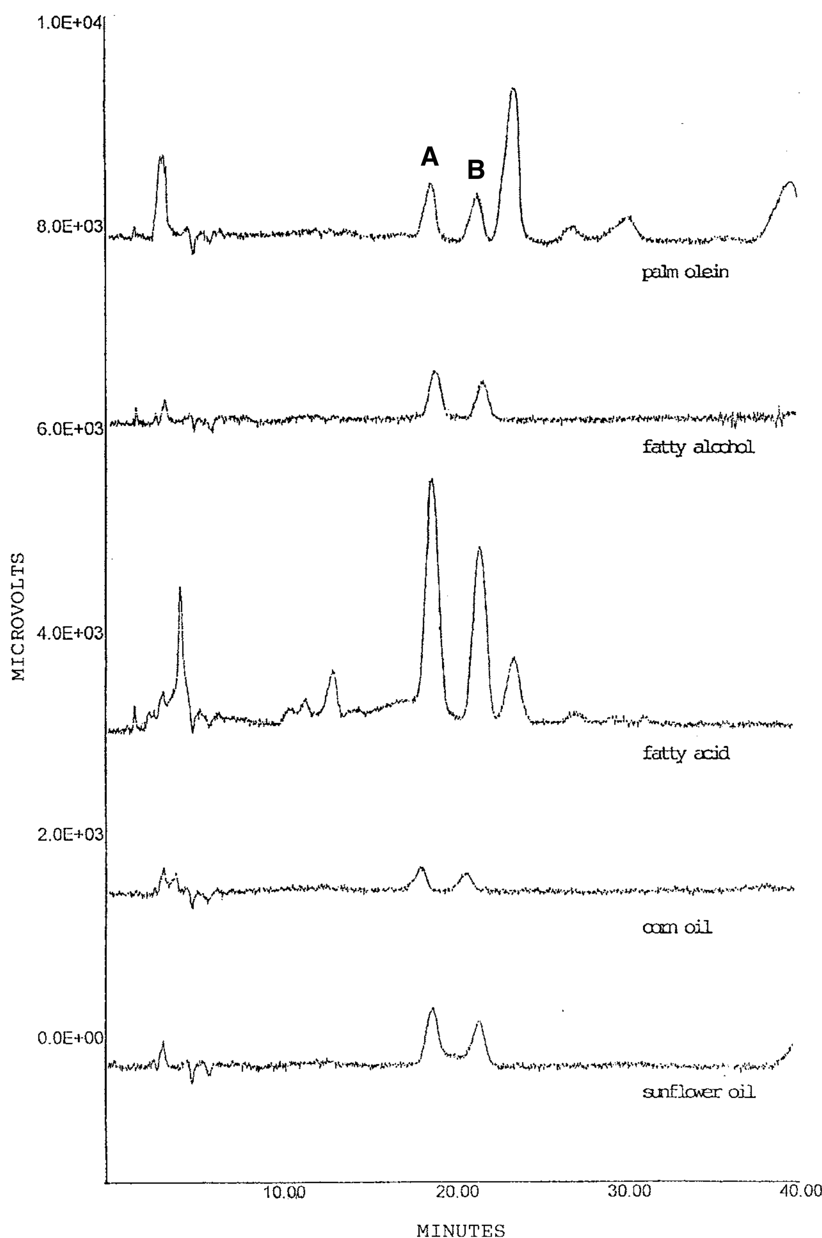

FIG. 4. Stacked chromatograms of control blank samples of palm olein, fatty alcohol, fatty acid, and corn and sunflower oils. No fluorescent components were observed at 6.6 min that might interfere with the quantitation of biphenyl for the determination of Dowtherm A. Labels A and B are interfering components present naturally in matrices.

erage within-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 within-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. Results also show that the between-laboratory reproducibilities varied from 1.29 to 3.84%. Data obtained from all three laboratories show good linearity between actual spiked concentrations and predicted concentrations, with r^2 exceeding 0.998 (Table 2).

Repeatability and reproducibility data obtained from the interlaboratory studies involving three Malaysian laboratories in different locations show that the proposed HPLC procedure developed is suitable for determining Dowtherm A in oleochemicals and edible oils. The method is applicable to these products as long as the blank run shows no interfering component that fluoresces in the same region as Dowtherm A.

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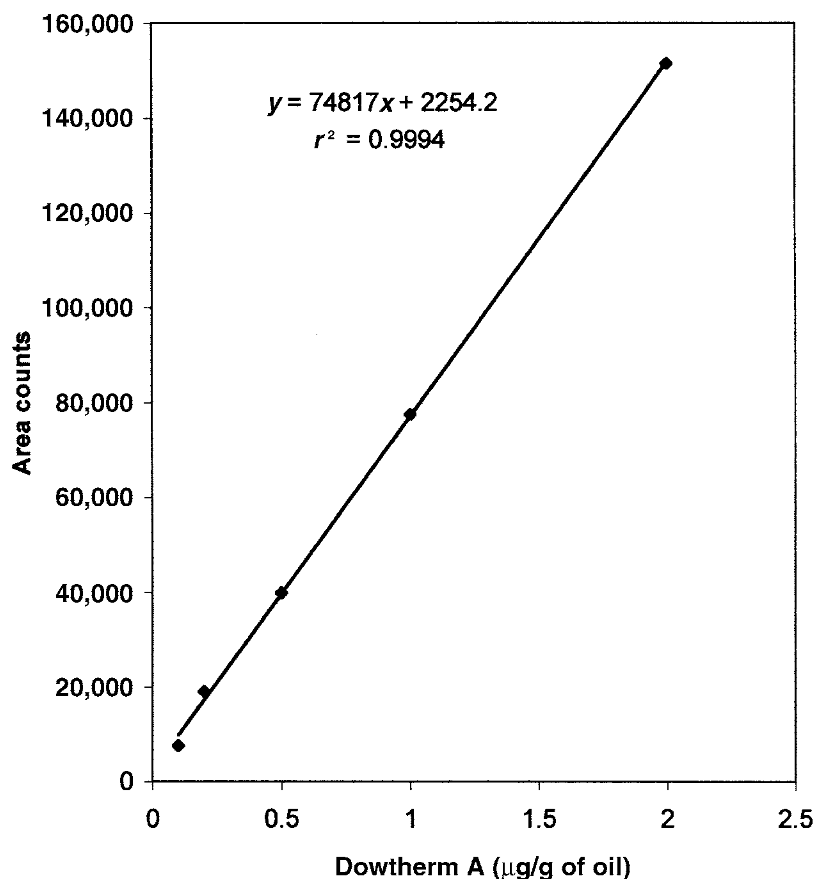

FIG. 5. Calibration graph of palm olein samples spiked with Dowtherm A™ (0.1–2.0 μg/g) plotted against areas calculated under biphenyl peaks.

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REFERENCES

1. Sleeter, R.T., Effects of Processing on Quality of Soybean Oil, *J. Am. Oil Chem. Soc. 58*:239–247 (1981).
2. Rossell, B., Heat-Transfer Fluids in the Oils and Fats Industry. 1. Specification, Types and Toxicity, *Lipid Technol. 5*:110–114 (1993).
3. Meershoek, C.J.M., It Is Time to End Use of THFs, *inform 9*:542–548 (1999).
4. Codex, Reports of the 16th Session of the Codex Committee of Fats and Oils, London, March 8–12, 1999 p. 15.
5. Takagi, T., Detection and Determination of Biphenyl-Diphenylether Heat Transfer Medium in Fatty Oils, *Yukagaku 18*:235–239 (1969).
6. Imai, C., H. Watanabe, N. Haga, and T. Li, Detection of Heat Transfer Media in Edible Oil, *J. Am. Oil Chem. Soc. 51*:495–498 (1974).
7. Min, D.B., and J. Wen, Gas Chromatographic Determination of Biphenyl and Phenyl Ether in Oil, *Ibid. 59*:278–279 (1982).
8. *Official and Recommended Practices of the American Oil Chemists' Society*, 5th Edn., American Oil Chemists' Society, Champaign, 1998.
9. Moh, M.H., and T.S. Tang, Determination of Dowtherm A Using High-Performance Liquid Chromatography Coupled with Fluorescence Detection in Edible Oil and Oleochemical, *J. AOAC Int. 82*:893–896 (1999).
10. Vial, J., and A. Jardy, Experimental Comparison of the Different Approaches to Estimate LOD and LOQ of an HPLC Method. *Anal. Chem. 71*:2672–2677 (1999).
11. Konstantinova-Shlesinger, M.A., *Fluorometric Analysis*, Kraner, N. (transl.) Davey, New York, 1965, p. 280.

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