

Quantitative FTIR Method for Determination of Mineral Oil Deposited on Plant Leaves

Keywords: Petroleum; spray; oil; plant; foliar

INTRODUCTION

The quantification of mineral oil deposited on foliar tissues of plants is an integral part of bioassay work concerned with the use of petroleum spray oils in horticulture. In this paper we describe a method that is both rapid and accurate.

Previously reported methods have suffered from problems with analysis speed or accuracy. Early attempts used ether extracts of leaves followed by sulfonation. Final readings were made in Babcock skim milk bottles by volume (English, 1930). Changes in the solvent system (Swain and Green, 1933; Rohnbaugh, 1933; Tikenko and Hensill, 1937) or purification system (Dawsey and Hass, 1933; Gunther and LaDue, 1944) did not alleviate the tediousness of the original method. Accuracy of low rate applications was also limiting.

An alternative strategy resulted in the addition of oil-soluble red dyes to the spray oils. These dyes were then extracted and measured colorimetrically (Riehl *et al.*, 1953; Avens *et al.*, 1962). It was assumed that the dye was extracted at the same rate as the oil. Later work identified a negative bias in these methods due to the photodegradation of the dye (Dean *et al.*, 1964). An alternative, less photoactive red dye was substituted, but the general method has not been widely accepted. This may be due to concerns that the oil and dye may not coextract.

Other techniques such as fluorescence spectroscopy have been used successfully to measure concentrations of an oil-soluble fluorescent dye *in situ* (Furness and Newton, 1988), but the instrumentation required is not commonplace in most laboratories.

Most recent work involving the assay of oil deposits has used the technique of gas chromatography. Early work achieved good recoveries (Furness *et al.*, 1979) but suffered from slow analysis times. Although both detection limit and analysis time of GLC methods have been much improved (Allender and Beattie, 1990), some problems still exist. Sample preparation by extraction of oil with refluxing followed by activated floursil cleanup is still very time-consuming, and while the use of external standards is reported to cause no bias, it is recognized that use of an internal standard would be an improvement.

The work presented here describes the development of a rapid, accurate, and sensitive method for measuring oil deposit concentrations. The method makes use of an instrument that is commonly available (FTIR) and a nonlaborious extraction procedure. Slight methodology changes from those given here would allow the method to be used in other applications such as assessment of crude oil spills on plant tissue.

MATERIALS AND METHODS

Extraction. Treated leaves of sour orange plants were excised after a known time post spraying with petroleum spray oil (Ampol D-C-Tron NR). Leaves taken soon after spraying represent the applied dose, whereas leaves sampled at a later date yield information of oil disappearance. Leaf disks (10 × 1 cm²) were punched out from the leaves and placed in a

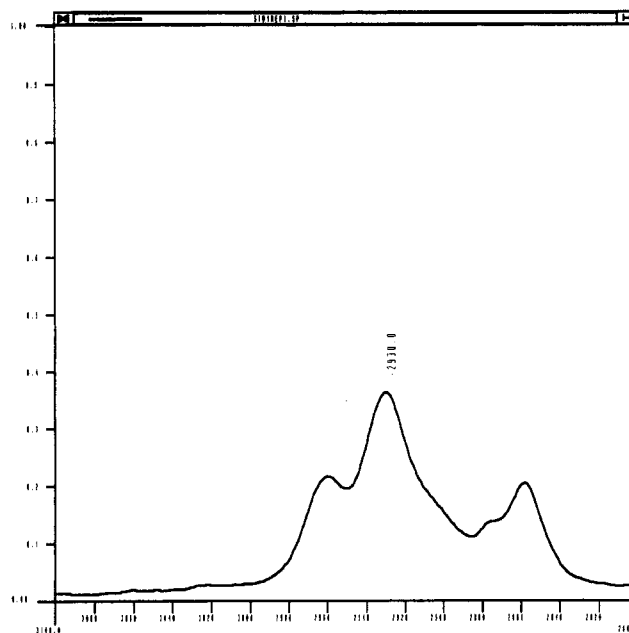


Figure 1. FTIR spectrum of leaf extract showing the position of the analytical wavenumber (2930 cm⁻¹) used in the analysis.

Table 1. FTIR Instrument Conditions

scan range and number	3100–2800 cm ⁻¹ , 16 scans at 1 cm ⁻¹
peak height measurement	2930.0 cm ⁻¹ on base line 3100–2800 cm ⁻¹
beam split	KBr
detector	MCT B
apodisation	strong
smoothing	none
sample cell	ZnSe, 1.0 mm path length

Teflon-capped vial with 10.0 mL of freon (C₂Cl₃F₃, AR grade or redistilled). Vials were then placed in a sonicating bath for 2 h.

Analysis. Quantification was achieved by constructing a standard curve. The standard chosen was an oil used as a base for a petroleum spray oil (70 Neutral; CAS Registry No. 64742-55-8) and was diluted in freon to give concentrations ranging from 10 to 1000 mg L⁻¹. This allowed for the optimum analysis range (Compton *et al.*, 1987) to be between 0.2 and 0.9 absorbance unit, which represents oil deposit concentrations of 50–300 μg cm⁻² when the above extraction method is used. This is the likely concentration range to be found after the application of petroleum spray oils. Fourier transform infrared spectroscopy (FTIR) was then used to measure the peak height of the C–H absorption band at 2930.0 wavenumbers (Figure 1).

RESULTS

Optimum FTIR instrument conditions are given in Table 1, while the calibration procedure is given in Table 2.

The reproducibility of analysis was determined by adding 1000 μg of oil to 3 × 10 mL vials of freon. These standards were then analyzed in triplicate. Measurement of samples was not consecutive since they were separated by analysis of freon blanks. Variation of readings for a single vial was assigned to analytical variance (s²_A) while over all variation was assigned to

Table 2. Linear Regression Analysis for the Calibration Curve of Absorbance vs Concentration of Deposited Oil

parameter	value
slope \pm 95% C. I.	$3.79 \times 10^{-3} \pm 5 \times 10^{-5}$
Y intercept	3.12×10^{-3}
mean extinction coeff	$3.79 \times 10^{-2} \text{ L cm}^{-1} \text{ mg}^{-1}$
correlation coeff ($n = 7$)	0.9999

Table 3. Analytical Performance of the Method with Respect to Precision at 100 mg L⁻¹

	precision (total)	analytical precision	sampling precision
% RSD ^a ($n = 9$)	5.9	1.6	5.4
variance	4.68×10^{-4}	3.6×10^{-5}	4.32×10^{-4}

^a RSD, relative standard deviation ($s \times 100/\text{mean}$).

Table 4. Analytical Performance of the Method with Respect to Accuracy at 100 mg L⁻¹ as a Function of Time between Sampling and Analysis

time between treatment and analysis	amt deposited ($\mu\text{g cm}^{-2}$)	amt determined ($\mu\text{g cm}^{-2}$)	amt after elimination of biogenic hydrocarbons ($\mu\text{g cm}^{-2}$)
1 h	100	89	89
1 Day	100	95	95
3 Days	100	92	92
5 Days	100	95	95
untreated	0	<10	<10

total variance (s^2_{total}) (Table 3). Sampling variance (s^2_{s}) was calculated by applying the following equation:

$$s^2_{\text{total}} = s^2_{\text{A}} + s^2_{\text{S}}$$

Freon blanks ($n = 9$) were used to calculate a detection limit (DT). Calculation yielded a value of 8 mg L⁻¹, which equates to about 10 $\mu\text{g cm}^{-2}$ when the above extraction method is used (DT = blank concentration + 6 standard deviations of blank readings) (Massart *et al.*, 1988).

Method accuracy was determined by applying 100 μg of oil to each of 90 1 cm² leaf disks. Blank extractions were made by using untreated leaf disks. The recovery was found to be between 92 and 97% and was not significantly affected by the time taken between sampling and analysis for up to 5 days post-treatment (Table 4). Analysis of leaves sampled at different times post-treatment can be used to assess the disappearance rate of oil in plants.

Bias due to codetection of biogenic hydrocarbons was assessed in control and treated leaf extractions or by the addition of activated alumina (about 1 g) after a prior FTIR analysis. Activated alumina is known to adsorb the mostly polar biogenic hydrocarbons. The vial contents were mixed, allowed to stand, and then reanalyzed. We found that freon did not extract any detectable amount of hydrocarbons from untreated sour orange leaves and that the addition of alumina did not change the results significantly.

The method presented here is a rapid alternative to previously published methods for quantification of oil deposits. Sampling and preparation of the extraction take under 2 min, while FTIR analysis and quantification require about another 3 min.

CONCLUSIONS

Method validation has yielded performance data that are acceptable for the routine quantification of petroleum spray oil deposits on plants. In a wider view, it is reasonable to assume that the method can find application in other situations such as the determination of the concentration of accidentally spilled oil on marine or tidal plants.

LITERATURE CITED

- Allender, W. J.; Beattie, G. A. C. Determination of petroleum oil deposits on *Citrus sinensis* (L.) Osbeck leaves. *Pestic. Sci.* **1990**, *31*, 133–139.
- Avens, A. W.; Chapman, P. J.; Gibbs, S. D. A spectrophotometric method for determining petroleum oil deposits on plant surfaces with oil red O dye. *J. Econ. Entomol.* **1962**, *55* (5), 819–821.
- Compton, D. A.; Grasselli, J. G.; Kollar, R. G.; Mooney, J. R.; Young, J. R. Some applications of computer-assisted quantitative infrared spectroscopy. In *Computerized Quantitative Infrared Analysis*; McClure, G. L., Ed.; ASTM: Philadelphia, 1987; STP934, pp 36–58.
- Dawsey, L. H.; Hass, A. J. A method for determining the quantity of mineral oil retained by leaf surfaces after spraying. *J. Agric. Res.* **1933**, *48*, 41–49.
- Dean, H. A.; Bailey, J. C.; Riehl, L. A.; White, R. W.; Wilson, E. L. A field technique for oil deposit determination on citrus through colorimetric analysis. *J. Econ. Entomol.* **1964**, *57* (4), 459–461.
- English, L. L. A method for determining the quantity of oil retained by citrus foliage after spraying. *J. Agric. Res.* **1930**, *41*, 131–133.
- Furness, G. O.; Newton, M. R. A leaf surface scanning technique using a fluorescence spectrometer for measurement of spray deposits. *Pestic. Sci.* **1988**, *24*, 123–137.
- Furness, G. O.; Howard, R. T.; Smith, P. J. Measurement of petroleum oil deposits on citrus leaves by gas-liquid chromatography. *Pestic. Sci.* **1979**, *10*, 478–484.
- Gunter, F. A.; LaDue, J. P. Determination of oil deposit on citrus leaves by the steam distillation method. *J. Econ. Entomol.* **1994**, *37* (1), 52–56.
- Massart, D. L.; Deming, S. N.; Kaufman, L.; Michottery; Vandeginst, B. G. M. *Chemometrics: A Textbook*; Elsevier: Amsterdam, 1988.
- Riehl, L. A.; Beier, R. L.; Gunther, F. A. Application of precision photoelectric colorimetry to the determination of oil deposit on laboratory-sprayed grapefruit. *J. Econ. Entomol.* **1953**, *46* (5), 743–750.
- Rohraugh, P. W. Penetration and accumulation of petroleum spray oils in leaves, twigs and fruit of citrus trees. *Plant Physiol.* **1933**, *9*, 699–730.
- Swain, A. F.; Green D. Detection and determination of surface oil on citrus following spraying. *J. Econ. Entomol.* **1933**, *26* (6), 1021.
- Tikenko, V. J.; Hensill, G. S. Method for determining the amount of mineral oil deposited on orange leaves immediately after spraying. *J. Econ. Entomol.* **1937**, *30* (2), 355–360.

Received for review May 31, 1995. Accepted September 21, 1995.

Mark C. Hodgkinson,* Dalius S. Sagatys,
A. Peter Mackey, and Graham Smith

Ampol Research and Development Laboratory, P.O.
Box 40, Wynnum Central, Brisbane, Australia 4178

JF950329H