



TEST METHOD

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Supersedes: New

TEL-001-E25A
WDN 2721

Epoxidized Soybean Oil in 1,3-Dichloropropene by Infrared Spectroscopy

1. Scope

- 1.1 This method is applicable to the determination of epoxidized soybean oil (ESO) in 1,3-dichloropropene over the range of 0.5-3.0% (w/w).

Analyte	Applicable Range
	(%, w/w)
Epoxidized Soybean Oil (ESO)	0.5-3.0

2. Principle

- 2.1 A sample of ESO in 1,3-dichloropropene is analyzed by transmission Fourier-transform Infrared (FTIR) spectroscopy. Quantitation is based on the absorbance peak height from ESO at 1741 cm^{-1} compared to external standards in the range from 0.5 to 3.0% (w/w) ESO. The analysis is performed in transmission mode using a CaF_2 liquid cell.

3. Summary

- 3.1 A sample of ESO in 1,3-dichloropropene is analyzed by transmission FTIR spectroscopy. Quantitation is by external standard based on baseline-corrected peak height.
- 3.2 This method is applicable to the determination of ESO in 1,3-dichloropropene.
- 3.3 The low end of applicable range lies at 0.5% (w/w). The upper end of applicable range lies at 3.0% (w/w).
- 3.4 The average recovery for ESO was 100.8%.
- 3.5 Replicate analyses of a sample of ESO on two separate days gave a relative standard deviation of 0.7% and average concentration of 1.4% (w/w).

4. Safety

- 4.1 Each analyst must be acquainted with the potential hazards of the equipment, reagents, products, solvents, and procedures before beginning laboratory work. SOURCES OF INFORMATION INCLUDE: OPERATION MANUALS, SAFETY DATA SHEETS,

LITERATURE, AND OTHER RELATED DATA. Safety information should be requested from the supplier. Disposal of waste materials, reagents, reactants, and solvents must be in compliance with applicable governmental and company requirements.

- 4.2 Acetone is a highly flammable liquid and vapor. Keep away from flames or sources of ignition. Refer to the current SDS for the most up-to-date safety guidelines (Section 4.1).
- 4.3 1,3-dichloropropene is toxic and may be fatal if swallowed or inhaled. It is suspected of causing cancer and may cause skin and eye irritation/damage. It is a flammable liquid with a flash point of 28 °C. Refer to the current SDS for the most up-to-date safety guidelines (Section 4.1).

5. Interferences

- 5.1 No direct interferences have been observed in the use of this method. If results are suspected based on the analytical history of the product, the data should be confirmed by an alternate method.
- 5.2 Absorbance at 1741 cm⁻¹ is observed in 1,3-dichloropropene with no ESO present. Baseline correction is applied to spectra prior to extracting peak height.

6. Apparatus

- 6.1 Analytical balance: Mettler Toledo capable of weighing to 0.0001 g, Mettler model AE200, available from Mettler-Toledo, Inc., or equivalent.
- 6.2 Fourier-Transform Infrared Spectrophotometer: Perkin-Elmer Frontier, available from Perkin-Elmer Corporation, Chalfont Road, Seer Green, Beaconsfield Bucks HP9 2FX United Kingdom, or equivalent.
- 6.3 Liquid Infrared Cell: Calcium fluoride (CaF₂), 0.1-mm pathlength, calibrated, demountable, available from Perkin-Elmer Corporation, or equivalent.
- 6.4 Transmission Liquid Infrared Cell Holder: Universal holder from Perkin-Elmer Corporation, Chalfont Road, Seer Green, Beaconsfield Bucks HP9 2FX United Kingdom, or equivalent.

7. Reagents

- 7.1 Acetone, Laboratory Reagent grade, ≥99.5%, available from Sigma Aldrich, or equivalent.
- 7.2 1,3-dichloropropene, available from The Dow Chemical Company.
- 7.3 ESO determined 99.1% by quantitative carbon NMR, available from The Dow Chemical Company.

Note: The purity of this standard should either be provided by the supplier or determined by the analyst for use in calculation of the standard solution concentrations.

8. Reagent Solutions

- 8.1 Calibration Standard:
 - 8.1.1 Prepare each Calibration Standard, according to the table below, by weighing ESO into 20-mL glass vials and adding 1,3-dichloropropene to obtain the total weight. Record each weight to the nearest 0.0001 g.

Calibration Standard	ESO	Total Weight
	(g)	(g)
ESO1	0.07 ± 0.01	14.0
ESO2	0.14 ± 0.01	14.0
ESO3	0.21 ± 0.01	14.0
ESO4	0.28 ± 0.01	14.0
ESO5	0.35 ± 0.01	14.0
ESO6	0.42 ± 0.01	14.0

8.1.2 Cap and mix well.

8.2 Determine the concentration of ESO in each calibration solution as follows:

$$C_{\text{ESO},i} = \frac{W_{\text{ESO},i} \times P_{\text{ESO}}}{W_{\text{total},i}}$$

where:

- $C_{\text{ESO},i}$ = concentration (% w/w) of ESO in the i^{th} calibration standard
 $W_{\text{ESO},i}$ = weight (g) of ESO added to the i^{th} calibration standard
 P_{ESO} = purity (% w/w) of ESO in its original standard
 $W_{\text{total},i}$ = Total weight (g) of the i^{th} calibration standard

9. Analysis Conditions

Note: The parameters summarized below were used in the validation of the method. Fourier Transform Infrared Spectrophotometer instruments and liquid cell pathlengths vary and may differ from those stated below.

Instrument:	Perkin Elmer Frontier FTIR spectrometer with Transmittance accessory
Liquid infrared cell:	Calcium fluoride (CaF ₂), nominal 0.10-mm pathlength
Cell holder:	Universal holder
Detector:	Deuterated triglycine sulfate (DTGS)
Spectral acquisition:	
Scan number:	32
Scan range:	4000 to 1100 cm ⁻¹
Resolution:	4 cm ⁻¹
Background:	Liquid Infrared Cell, empty
Software:	Spectrum Quant 1.0.0
Peak position	1741.18 cm ⁻¹
Baseline correction:	1771.68 to 1719.38 cm ⁻¹

9.1 Representative FTIR spectra of the 1,3-dichloropropene and Calibration Standards are shown in Figure 1.

10. Calibration

- 10.1 Calibrate the instrument according to the manufacturer's instructions.
- 10.2 Clean the transmission cell accessory with acetone and then thoroughly dry with a 10 s flush of nitrogen or air.
- 10.3 Obtain a background spectrum for a clean, empty liquid cell using the conditions in Section 9.
- 10.4 Review the background spectrum to ensure it is free of interfering peaks and acetone. If not, then repeat Section 10.2.

- 10.5 Using a plastic syringe, fill the liquid cell avoiding bubbles, then close the cell accessory with appropriate holder.
- 10.6 Collect the Calibration Standard spectrum using the conditions in Section 9.
- 10.7 Remove the sample from the liquid cell then clean following Section 10.2.
- 10.8 Repeat Sections 10.3 to 10.6 until all the Calibration Standards are measured.
- 10.9 Load the obtained spectra into the Spectrum Quant. software and initialize the quantification using Beers law calibration with calculation of maximum peak height for each Calibration Standard.
- 10.10 If manual calculations are used, perform a linear regression analysis on the data in a spreadsheet program such as Excel to calculate the slope and intercept of the calibration curve of each analyte.

$$y_i = mx_i + b$$

where:

- y_i = peak height (AU) of ESO in the calibration standard i
- x_i = concentration (% w/w) of ESO in the calibration standard i
- m = slope (AU / % w/w) of the linear regression line for ESO
- b = intercept (AU) of the linear regression line for ESO
- i = calibration standard of interest

11. Procedure

- 11.1 Clean the transmission cell accessory with acetone and then thoroughly dry with a 10 s flush of nitrogen or air..
- 11.2 Obtain a background spectrum from the instrument from 4000 cm^{-1} to 1100 cm^{-1}
- 11.3 Using a plastic syringe, fill the CaF_2 transmission cell then close the cell accessory with appropriate holder. Avoid introducing bubbles to the cell contents.
- 11.4 Collect the sample spectrum using the parameters in Section 9.
- 11.5 Empty the sample from the transmission cell, then clean the transmission cell accessory with acetone.
- 11.6 Load the obtained FTIR spectra into the Spectrum Quant. software and initialize the quantification based on the calibration in the instrument software or by manual calculation.

12. Calculations

- 12.1 Calibrate the FTIR software for an external standard using baseline correction as described in Section 10 (if manual calculations are used, proceed to the next section).
- 12.2 If manual calculations are used, calculate the concentration of the analyte of interest in the original sample as follows:

$$C = \frac{(y - b)}{m}$$

where:

- C = concentration (% , w/w) of ESO in the sample
 y = peak height (AU) for ESO
 b = intercept (AU) of the linear regression line for ESO
 m = slope (AU / % , w/w) of the linear regression line for ESO

12.3 Report results to two significant figures.

13. Precision

- 13.1 Precision data determined from multiple analyses [n=10] of ESO in 1,3-dichloropropene are given below. The analyses were performed over a 2-day period.
- 13.2 The Horwitz ratio [H_r] was determined, where $H_r = RSD/RSD_r = RSD/(0.67 \cdot 2^{(1-0.5 \cdot \log C_{mf})})$, where C_{mf} is the average concentration in mass fraction, RSD is the relative standard deviation, and RSD_r is the RSD under reproducibility conditions. A Horwitz ratio of $H_r \leq 1$ is acceptable. The values are shown below.
- 13.3 At the 95% confidence level, individual measurements on similar samples may vary from the long-term average by the values shown below [$\pm t_{(n-1)} \times s$; where $t_{(n-1)}$ = t-value at n-1 degrees of freedom, and s = standard deviation of the validation data].
- 13.4 This assumes a normal distribution of results. The validity of this assumption has been verified using the Shapiro-Wilk test for normality. The test confirmed that the results could originate from normal distributions.

Analyte	n	$t_{(n-1)}$	Average	Relative Standard Deviation	At the 95% confidence level, individual measurements may vary from the long-term average by:	RSD_r	H_r
			(%, w/w)	(%)	(\pm %)	(%)	
ESO	10	2.26	1.4	0.7	1.6	2.5	0.28

14. Accuracy

- 14.1 Recoveries from the analysis of ESO in 1,3-dichloropropene [n=6] based on the calculated concentration of the analytes in synthetic mixtures are given below:

Analyte	Concentration Range	Average Recovery	Range of Recoveries	Standard Deviation of Recoveries
	(%, w/w)	(%)	(%)	(%)
ESO	0.5 – 3.0	100.8	98.4 – 104.0	2.0

15. Linearity

- 15.1 Method response was found to be linear over the concentration ranges given below:

Analyte	Linear Concentration Range in Sample
	(%, w/w)
ESO	0.5 – 3.0

16. Limit of Detection/Limit of Quantitation

- 16.1 The limit of detection (LOD), defined as three times the standard deviation of a low concentration sample, and the limit of quantitation (LOQ), defined as ten times the standard deviation of a low concentration sample, are given below:

Analyte	LOD	LOQ
	(%, w/w)	(%, w/w)
ESO	0.04	0.13

17. Notes

- 17.1 Analytical method performance can be affected by minor differences in instrumentation, reagents, and laboratory technique. Consequently, the method should be qualified in the performing laboratory to confirm its performance and suitability. In addition, analytical instruments should be calibrated at appropriate frequencies.
- 17.2 All data were recorded by Dow internally and did not comply with GLP criteria.

18. References

- 18.1 Technical Active Substance and Plant protection products: Guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex (Section 4) of Regulation (EU) No 283/2013 and Annex (Section 5) of Regulation (EU) No 284/2013.”, SANCO/3030/99 rev.5, European Commission, (22-MAR-2019).

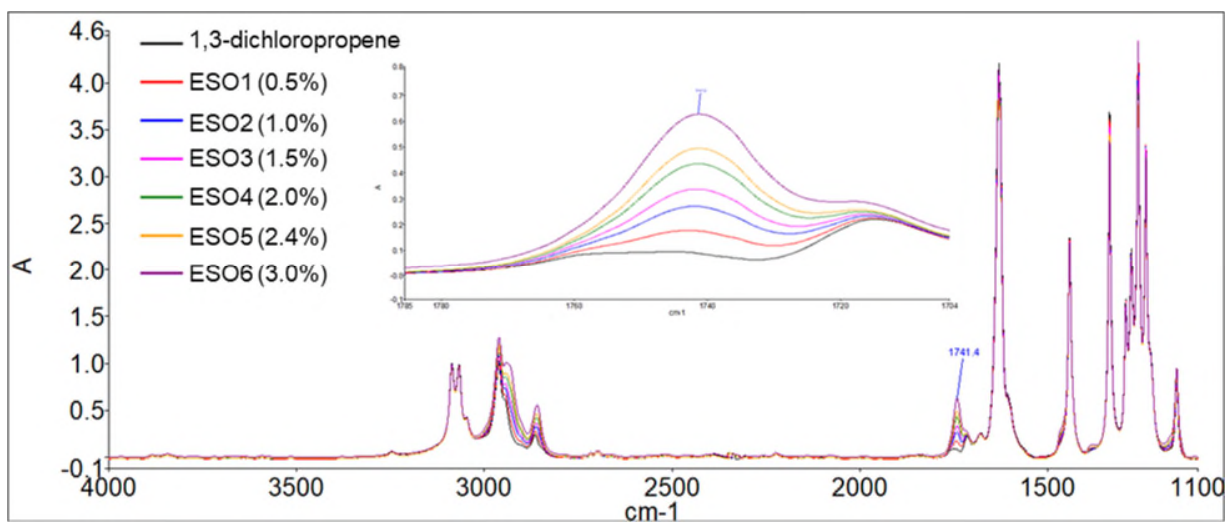


Figure 1. Representative FTIR spectra of neat 1,3-dichloropropene (black) and Calibration Standards (ESO1 – ESO6) ranging from 0.5-3.0% (w/w) ESO in 1,3-dichloropropene.