
GC/IR/MS Analysis of C-18 Unsaturated Fatty Acid Esters

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IRD Application Brief

**HP 5965A IRD
HP 5970B MSD**

IRD89-3

IRD Productivity Profile

Industry
Consumer products

Chemicals
Unsaturated fatty acid esters
(oleic and elaidic acids)

Sample matrix
Animal fat

Analysis
Identification of fatty acid
esters and differentiation of cis-
and trans-isomers

The 18-carbon fatty acids have many important uses in the consumer products industry. For example, they are found in vegetable oils for cooking and are significant components in soaps, lotions, and beauty creams. In this work, a combined system consisting of the HP 5965A Infrared Detector (IRD) and the HP 5970B Mass Selective Detector (MSD) was used to analyze for C-18 fatty acid esters in their primary source, animal fat.

In order to provide optimum quality control for the consumer products containing the fatty acids, it is important not only to identify them, but also to determine the approximate cis/trans ratio of the unsaturated double-bond compounds. Two important isomers in the manufacture of soap are oleic acid and elaidic acid, which are the C-18 9-cis and 9-trans species, respectively. The cis/trans ratio in soaps is a factor in product shelf life where too much trans will

cause cracking and breakage. The combined system proved useful in determining the approximate ratio of these isomers.

Figure 1 shows the total response chromatogram (TRC) from the IRD and the total ion chromatogram (TIC) from the MSD when tallow (animal fat), a triglyceride, was hydrolyzed, methylated, extracted with petroleum ether, and injected into the combined system. As indicated by the arrows, the cis and trans C-18 esters coelute under the chromatographic conditions described at the end of this note. Figure 2 shows the infrared transmission spectra and the mass spectra for standards of the pure 9-cis and 9-trans C-18 esters. The mass spectral data are helpful in identifying these compounds as unsaturated C-18 esters and are nicely complemented by the infrared data which differentiate the two isomers. Figure 3 shows expanded infrared spectra in the C-H stretching region from 2800 to 3100 cm^{-1}

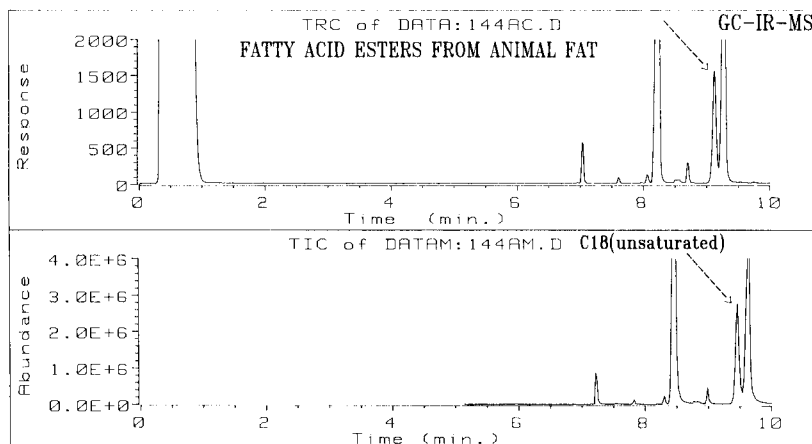


Figure 1. TRC and TIC for animal fat sample

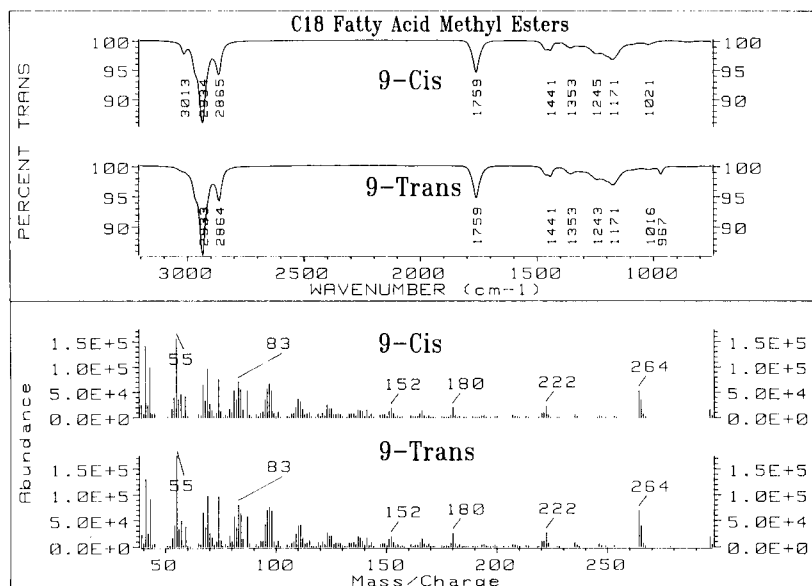


Figure 2. Infrared and mass spectra for standards of pure 9-cis and 9-trans C-18 fatty acids esters

revealing a unique band at 3012 cm^{-1} for the cis isomer and a C-H bending specific for the trans isomer at 967 cm^{-1} .

It was of interest to determine if the infrared data could be used to determine the approximate cis/trans ratio in the coeluting peak. To test this, a series of known cis/trans solutions was analyzed; the results are summarized in Table 1. To calculate the ratios, $1\ \mu\text{l}$ of known standard solutions of the pure cis and trans compounds were injected to obtain the absorptivities of each isomer at both 3012 cm^{-1} and 967 cm^{-1} . The results in Table 1 are also from $1\text{-}\mu\text{l}$ injections and were calculated by solving the two simultaneous equations shown below for the two unknown quantities, Q(C) and Q(T):

$$A'(C + T) = a'(C)b*Q(C) + a'(T)b*Q(T)$$

$$A''(C + T) = a''(C)b*Q(C) + a''(T)b*Q(T)$$

where:

$$A'(C + T) = \text{absorbance at } 3012\text{ cm}^{-1} \text{ for cis-trans mixture}$$

$$A''(C + T) = \text{absorbance at } 967\text{ cm}^{-1} \text{ for cis-trans mixture}$$

$$a'(C) = \text{absorptivity of cis at } 3012\text{ cm}^{-1} \text{ in mAU/ng}$$

$$a'(T) = \text{absorptivity of trans at } 3012\text{ cm}^{-1} \text{ in mAU/ng}$$

$$a''(T) = \text{absorptivity of trans at } 967\text{ cm}^{-1} \text{ in mAU/ng}$$

$$Q(C) = \text{quantity of cis in ng}$$

$$Q(T) = \text{quantity of trans in ng}$$

$$b = \text{path length (constant for all measurements)}$$

Table 1. Cis/trans ratio determination

Sample	Cis, ng		Trans, ng		Cis:Trans		mAU 2933 cm ⁻¹ (Cis + Trans)
	Calc.	Act.	Calc.	Act.	Calc.	Act.	
Known Mix 1	145	200	180	200	45:55	50:50	32.6
Known Mix 2	275	250	280	250	49:51	50:50	77.6
Known Mix 3	972	800	126	200	88:12	80:20	95.1
Animal Fat A	598	---	32	---	95:5	---	57.5
Animal Fat B	687	---	69	---	91:9	---	66.9

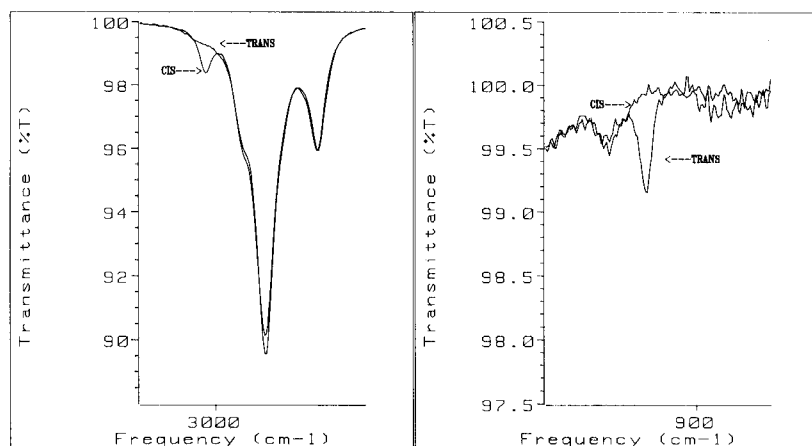


Figure 3. Infrared spectra expanded in the C-H stretching region (2800-3100 cm⁻¹) and C-H bending region (800-1100 cm⁻¹) for 9-cis and 9-trans isomers

The data suggest that this approach can provide a fairly good measure of the quantities of the cis and trans present and, therefore, the cis-to-trans ratio. Sample results for two actual fat samples are also shown in Table 1. The results show that the cis isomer predominates in each of these samples. Note that the absorbance at 2933 cm⁻¹ is a good approximation of the total amount of cis + trans present. This is to be expected since the molar absorptivity at this predominant C-H stretching vibration should be very similar for the two isomers. To determine the overall quantitative precision and accuracy of this technique, additional testing on a variety of samples would be required.

Conclusion

The combined system has been shown to be a useful tool for the analysis of unsaturated 18-carbon fatty acid esters. IR and MS data complement each other for qualitative identification in complex mixtures. In addition, the IR data are able to provide a semi-quantitative analysis of the cis/trans ratio from a coeluting gas chromatographic run.

Conditions

Sample preparation

The animal fat was heated under pressure to form fatty acids and glycerine. The fatty acids were then methylated and extracted with petroleum ether.

Column

25 m HP Ultra 2 (5% phenyl-methylsilicone), 0.32 mm id, 0.52 m film

Carrier gas

He at 24 psi

Injection

1 μ l splitless, port at 275°C

Oven

55°C to 240°C at 40°C/min hold for 6 min

Light pipe

250°C

IRD transfer lines

260°C

MS transfer line

280°C

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