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Note

Determination of trimer in *Z*-11-hexadecenal pheromone by high-performance liquid chromatography with a rotating disc flame ionization detector

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Production of batches of the pheromone *Z*-11-hexadecenal sometimes results in the formation of an unwanted trimer of the aldehyde [1,3,5-trioxane-2,4,6-tris(*Z*-10-pentadecene)]. An accurate determination of the trimer content was required firstly to meet U.S. Environmental Protection Agency (EPA) requirements and secondly to meet customer specifications.

During the development of the method it was found that the refractive index detector lacked adequate sensitivity. The trimer absorbed only in the low UV making the UV detector unsuitable in the chosen mobile phase. A relatively new, commercially available detector, the Tracor Model 945 liquid chromatograph–flame ionization detector was used instead. The advantages of the flame ionization detector are well known. It is a mass detector and has a uniform response for many hydrocarbon compounds. Previously reported flame detectors for high-performance liquid chromatography (HPLC) have used moving wires[1–4], belts[5] and chains[6–10] to transport the solute to the detector while the mobile phase was being evaporated. These systems suffered problems with noise and reproducibility. Rotating disc flame detectors have been reported[11,12] and the Tracor detector is an improved version of one of these[12], combining the use of a quartz belt with a rotating disc.

EXPERIMENTAL

Apparatus

A Waters 6000A pump, Waters WISP 710B autosampler, Waters 720 system controller and a Tracor 945 liquid chromatograph–flame ionization detector were used for all separations. Data were collected using a Hewlett-Packard 3390A recording integrator. The column was 30.0 cm × 3.9 mm I.D. packed with Waters 10-μm, μBondapak C_{18}.

Reagents

Acetonitrile and methyl *tert.*-butyl ether were Burdick and Jackson UV grade. The trimer used as an external standard was synthesized in house. Its structure was confirmed by ^{1}H- and ^{13}C NMR and infrared analysis. Its purity was 95% as determined by HPLC.

Procedure

Tracor detector. The following settings were used for the fuel gases. Detector: hydrogen at 140 ml/min; air at 0.4 l/min. Cleaning flame: hydrogen at 300 ml/min; oxygen at 200 ml/min.

The block temperature controller was adjusted to give the following thermocouple readings (see Fig. 1 for location of thermocouples) 1, 73°C; 2, 75°C; 3, 80°C; 4, 77°C.

The mobile phase was acetonitrile–methyl *tert.*-butyl ether (55:45) at a flow-rate of 1 ml/min. Sample size used throughout was 200 μl. Standards of trimer in mobile phase at 0.05, 0.10, 0.20, 0.30 and 0.40 mg/ml concentrations were prepared. Samples were diluted by weighing 0.1 g into a 50-ml volumetric flask and diluting to volume with mobile phase.

Spiked samples were prepared by adding known amounts of trimer to a sample of hexadecenal which had been previously analyzed and contained less than 0.2% of trimer.

RESULTS AND DISCUSSION

The Tracor detector delivers the eluent from the HPLC system onto a quartz belt in a V-shaped groove on a rotating disc. The disc rotates inside a heated block and a low vacuum pulls a flow of ambient air over the belt. The combination of air flow and temperature combines to remove the mobile phase. The eluate remains on the quartz belt and travels around the block ultimately passing through a flame ionization detector. The belt then passes through a larger flame (cleaning flame) which removes any remaining organic matter and returns to the point at which the eluent is applied.

The detector as originally supplied had a two position switch which gave nominal block temperatures of 150°C and 180°C. We found that these temperatures would evaporate some compounds of interest off the belt (*i.e.* *n*-alkanes up to *n*-C_{32}). In a subsequent upgrade by Tracor a variable temperature control was added and safety

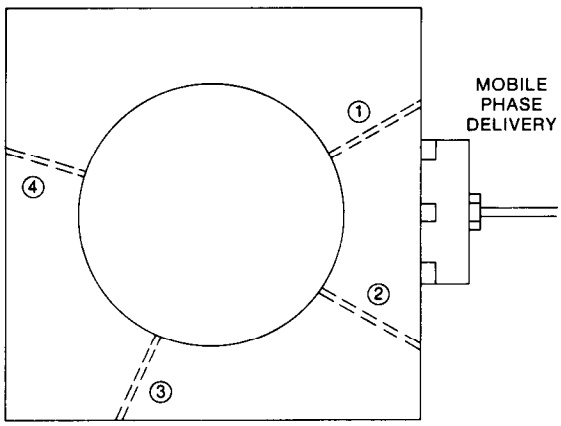

Fig. 1. Location of thermocouples installed in Tracor 945 liquid chromatograph–flame ionization detector block.

circuits were modified to permit much lower block temperatures. No temperature indicator or calibration was provided.

A series of four thermocouples were installed in the block at locations shown in Fig. 1. 1.6-mm I.D. holes were drilled in the rear half of the block and the thermocouples mounted flush with the inner surface so that they did not protrude into the belt space. These gave a measurement of the block temperature at the edge of the space in which the belt rotates. Belt temperatures may be higher, however, because the cleaning flame directly heats the belt.

The choice of block temperature is a compromise between the volatility of the mobile phase, the flow-rate and the volatility of the analyte. With the second thermocouple at 75°C and a flow-rate of 1 ml/min the noise level was satisfactory for this analysis and all data reported here were obtained at these settings.

Applicator

A choice of different applicators is provided with the detector. The applicator is a small piece of tubing that delivers the solvent onto the belt. The correct diameter applicator must be chosen for the required flow-rate and it must be positioned correctly otherwise much splashing of the solvent off the belt can occur. For this work the 0.2-mm I.D. applicator was used and its tip was located approximately 3 mm from the surface of the belt.

Detector response

Our experience has shown that the response of this detector depends on the volatility as well as the molecular structure of the analyte. A determination of the unit response for both the hexadecenal and the trimer indicated that belt losses were either negligible for both molecules or very similar as the trimer response was only 9% greater than the hexadecenal (hexadecenal, $110 \cdot 10^3$ area/μg; trimer, $120 \cdot 10^3$ area/μg). The molecular weight of the trimer is 714 and it is unlikely that it would volatilize off the belt.

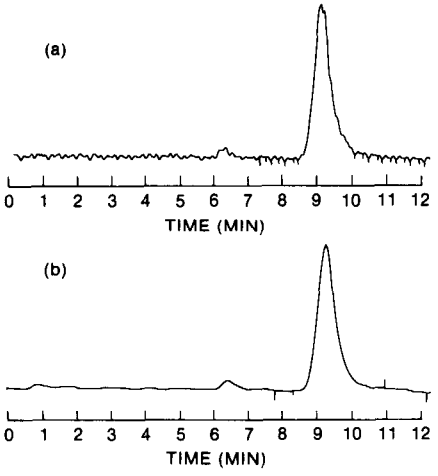

Fig. 2. Chromatograms of trimer standard at two HP 3390A peak width settings: (a) 0.16 peak width and (b) 0.64 peak width. Operating conditions: column, 30 cm × 3.9 mm I.D., C_{18}, 10 μm; mobile phase, 1 ml/min of acetonitrile–methyl *tert.*-butyl ether (55:45); detector, Tracor 945.

TABLE I

TRIMER CONTENT OF EIGHT BATCHES OF HEXADECENAL

Batch number	*Trimer content (%, w/w)**
1	0.72
2	1.13
3	<0.2
4	<0.2
5	2.43
6	<0.2
7	0.21
8	2.13

* Mean of three determinations.

The output from the detector shows cyclical variations. Although these can be reduced by the baseline correction system[13], which cancels out cyclical variations in the baseline, the envelope of a peak passing through the detector shows unacceptable fluctuations (see Fig. 2a). These are overcome by using a peak width setting of 0.64 on the HP 3390A integrator (see Fig. 2b) which applies more digital filtering to the detector output. The shape and area of narrow peaks could be distorted at this setting but the trimer peak is the correct width (area/height = 0.62) and is correctly integrated.

Analysis of samples and standards

A number of different batches of *Z*-11-hexadecenal were analyzed for trimer content. Standards were interspersed with the samples and one sample was injected ten times to determine the precision of the technique. Each of the standards and the remaining samples were injected three times and the mean area calculated. The standards gave a linear response with a correlation coefficient of 0.9996 over a range of concentrations from 0.05 mg/ml to 0.4 mg/ml (equivalent to trimer concentrations of 0.5 to 4% in the hexadecenal). The trimer content of eight different batches of pheromone is given in Table I. A typical chromatogram of *Z*-11-hexadecenal is given in Fig. 3. The retention volume of the trimer is approximately 9 ml.

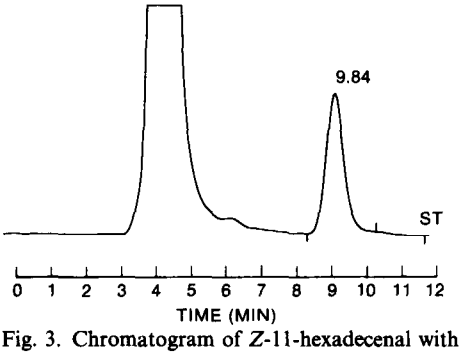

Fig. 3. Chromatogram of *Z*-11-hexadecenal with 2% trimer (0.2 mg/ml trimer; 10 mg/ml hexadecenal). Operating conditions as in Fig. 2.

TABLE II

PRECISION OF MEASUREMENT OF TRIMER IN HEXADECENAL

Degrees of freedom	*Mean (%)*	*S.D. (%)*	*Confidence limits (95% level) (%)*
9	1.13	0.0312	1.13 ± 0.02

TABLE III

RECOVERY OBTAINED BY SPIKING A HEXADECENAL SAMPLE WITH TRIMER

Spiked level (%)	*Found* (%)*	*Recovery (%)*
0.42	0.42	100
0.84	0.82	97
2.09	2.06	98

* Mean of three determinations.

Precision. Ten injections of one sample were made and the trimer content calculated from standards data taken concurrently. This data is given in Table II and indicates good precision of approximately 3% relative standard deviation.

The accuracy of the method was verified by adding known quantities of trimer to a sample of hexadecenal that had been analyzed and did not contain trimer. Spiked samples at levels equal to 0.42, 0.82 and 2.06% trimer were prepared and analyzed. Another series of standards was prepared and analyzed at the same time. Again standards were interspersed with samples and the complete series was injected three times. The mean of each area was used for calculation. Table III gives the added, found and percent recovery values. The largest deviation from the calculated values was 3% and this is within the precision of the method.

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