A sensitive method for quantitative microdetermination of lipids

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» Several detectors used for gas-liquid chromatography have sufficient sensitivity to measure quantitatively microgram quantities of organic material. In the work described, a method has been developed for adapting this capability to the quantitative microdetermination of lipids dissolved in solvents. It points the way to automatic, nonspecific, sensitive detection methods that should be applicable to liquid-liquid chromatography.

The method is based on the difference in volatility between lipid and solvent. Aliquots of the solution are injected into a miniature gas chromatographic column operated at room temperature but containing only uncoated, solid refractory material. Carrier gas is passed through this column to a hydrogen flame ionization detector. When the response of the detector indicates that the solvent has completely evaporated, the column is heated rapidly to 600°, pyrolyzing the remaining material. The products of pyrolysis are

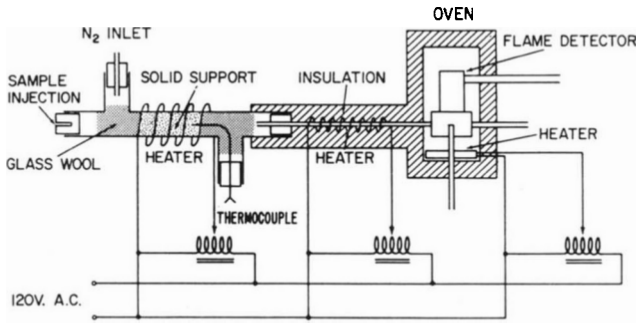

FIG. 1. Schematic drawing of column and hydrogen flame ionization detector.

delivered to the flame ionization detector. When the detector response again returns to baseline, the heat to the column is turned off and the system is ready for another injection.

The magnitude of the electric charge carried during the passage of the pyrolytic products through the detector has been found to be a function of the amount of total lipid injected.

Weighed amounts of corn oil, coconut oil, and cholesteryl oleate from commercial sources were dissolved in isooctane or benzene. Samples of lecithin (California Corp. for Biochemical Research, Los Angeles, Calif.), ganglioside, cephalin (Sigma Chemicals Corp., Cleveland, Ohio), and cerebroside (courtesy of Dr. Eberhard Trams), were dissolved in chloroform–methanol 2:1 (v/v).

Aliquots of the lipid solutions were taken up in a microliter syringe provided with a mechanical stop to assure reproducible delivery of 2.4 μl. These aliquots were injected through a silicone rubber stopper onto the surface of uncoated Chromosorb W (Johns-Manville Corp., NYC) contained in a Vycor (Corning Glass Co., Corning, N.Y.) tube, 10 x 1 cm, provided with a gas inlet and an additional sidearm for a thermocouple (Fig. 1).

The hydrogen flame ionization detector (1) was connected as shown in Fig. 2. This shows the usual method of operating this detector except that a capacitor was placed in the circuit in series with the source of current. The current flowing through the flame was amplified by a microvolt-ammeter (Model 425*A*, Hewlett-Packard Co., Inc., Palo Alto, Calif.) and recorded. In addition, the voltage developed across the capacitor was monitored with a Cary Model 31-31-V vibrating reed electrometer (Applied Physics Corp., Monrovia, Calif.). This voltage provided a signal proportional to the integrated area under the curve, or to the total charge produced.

The hydrogen flame detector was housed in an oven kept at 300°. The gas line from the end of the "miniature gas chromatography column" to the detector was also electrically heated to 300° throughout its length (Fig. 1).

The temperature needed to convert the lipid to volatile products was determined by injecting a sample of corn oil and raising the temperature of the pyrolysis tube stepwise to 100, 200, 300, 400, and 500°. The temperature was held at each value for approximately 3 min. Peaks were obtained at 200, 300, and 400°. When the temperature was raised at a rate of approximately 10°/sec, the pyrolytic products of corn oil were eluted as a single peak. When the temperature was raised more slowly, the pyrolytic products were eluted in several peaks. Since it was not the purpose of this work to attempt to fractionate the materials, it was decided to raise the temperature to 600° as quickly as possible.

The sensitivity of the method with various lipids was determined by injecting individual 2.4-μl samples of cholesteryl oleate in benzene; coconut and corn oils in isooctane; and lecithin, cerebroside, ganglioside, and cephalin in a mixture of methanol and chloroform. The response to these substances is shown in Table 1.

The reproducibility was tested by injecting five 2.4-μl samples of corn oil. The response varied between 396 and 370 mv on the capacitor with a standard deviation of 8.6 or 2.25%. The linearity of the response was measured by injecting samples of corn oil ranging between 5 and 100 μg. No departure from linearity was observed over this range of sample sizes.

The responses at each of the several dilutions of corn oil solution used for this test were then compared with the responses to identical dilutions of a solution of methyl stearate. The responses of the detector to corn oil and methyl stearate corresponded point for point.

This method of detection is more specific than detection by weight, since it depends on two additional

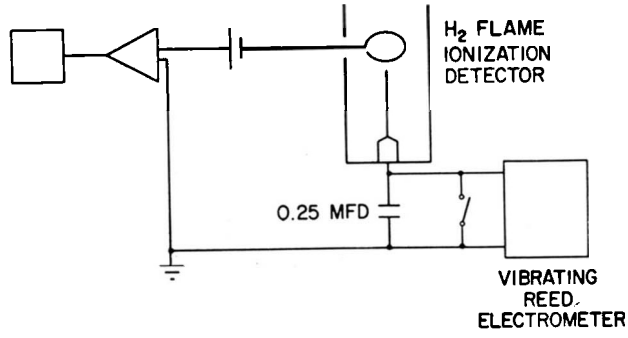

FIG. 2. Electrical circuit used for simultaneous monitoring of current flowing through the flame and the integral of the current with respect to time (electrical charge).

TABLE 1. RESPONSE OF DETECTOR TO VARIOUS SUBSTANCES

Substance	Amount Injected	Average Charge Sensitivity	
	μg	*μcoul*	*mcoul/g*
Cholesteryl oleate	72	0.102	1.42
Corn oil	72	0.095	1.32
Coconut oil	72	0.085	1.18
Lecithin	72	0.059	0.83
Cerebroside	48	0.048	1.00
Ganglioside	36	0.017	0.46
Cephlain	24	0.016	0.65

factors: quantitative conversion of the material to volatile compounds by heat, and detection of these volatile compounds by the hydrogen flame detector.

The conversion of materials such as triglycerides to volatile products was studied by comparing the response of the system to corn oil with the response to methyl stearate. It is known that methyl stearate can easily be made volatile without decomposing it, since it may be subjected to analysis and even purification by gas-liquid chromatography. The same may be said for such lipids as cholesterol, free fatty acids, and hydrocarbons in the same molecular weight range. It has not been shown conclusively, however, that compounds with higher molecular weight, such as triglycerides, can be made volatile without decomposing them. In the experiments reported, the responses with corn oil and methyl stearate were found to be identical over a range of sizes of samples. It is assumed that the conversion of the triglycerides to volatile compounds was probably quantitative whether or not simultaneous decomposition occurred. Since the response to cholesteryl oleate was in the same range, the same reasoning applies. The responses of the system to several phospholipids, however, were smaller. This may have been due to incomplete volatilization.

The response of the hydrogen flame detector itself varies from one compound to another, depending on the functional groups present. Sternberg, Gallaway, and Jones (2) reported a comprehensive study of the response to various kinds of compounds in which they showed that the response to a given molecule could be predicted from the number of carbon atoms in the molecule bound to oxygen or nitrogen, compared to the number bound to hydrogen. The small difference in the sensitivity of this system to corn oil and coconut oil may be explained on this basis since the portion of the total number of carbon atoms bound to oxygen is greater in the average triglyceride of coconut oil than in the average triglyceride of corn oil.

Because of the dependence of the response of the hydrogen flame detector on the nature of the compounds being detected, it may not have been the ideal choice for this procedure. If responses directly proportional to the weights of different materials delivered to the detector are required, a detector of the gas density balance type might be better. The factors governing the choice are the same as those governing the choice of a detector for performing any analysis by gas chromatography. Ionization detectors are simple, offer high sensitivity, and are relatively insensitive to changes in temperature and gas flow rate. They respond proportionally to the weight of organic material when the materials are the same or closely related compounds. Their use to perform precise quantification of different kinds of compounds requires that their response be calibrated for each kind of compound tested. This is somewhat more complicated if mixtures of several compounds are to be delivered simultaneously.

The sensitivity of the particular hydrogen flame detector used in these experiments was somewhat low compared with that reported elsewhere and that obtained using similar detectors in our laboratory. We attributed this to our failure to obtain an optimal gas mixture in the particular flame detector used.

The compounds injected in these experiments emerged as a peak with a base width of 1 min. This time, of course, was a function of the slow rate at which the pyrolyzer was heated. In other experiments in our laboratory, the hydrogen flame ionization detector has been used to detect and quantify, with good accuracy, 0.1-μg samples of ester of fatty acids emerging within 1 min from a gas-liquid chromatographic column, and there is no reason to believe that the procedure described could not be used for samples of that size.

One cycle takes almost 15 min, using the equipment and procedure described. The steps involving evaporation of the solvent and cooling of the pyrolysis tube can be separated from the pyrolysis and detection procedure by using more than one column, reducing the time required for each individual determination.

The procedure could be made suitable for detection in liquid-liquid chromatography. An automatic fraction collector could be used to collect the effluent of the liquid-liquid column, and the individual fractions could then be treated as described. Alternatively, the procedure could be coupled with an automatic stream-sampling valve, such as is used in connection with industrial process control gas chromatographs. Since conversion of the material to be detected to volatile products occurs at only moderately high temperature (600°), the method may be useful for detecting organic

materials eluted from columns in solvents containing inorganic buffers.

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