Quantitative Gas Chromatographic Analysis of Lipids: Comparison of Gas Density Balance and Flame Ionization Detector[1]

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ABSTRACT

Accuracy of available detectors for gas chromatography is a subject of continuing research in analytical chemistry. The quantitative deficiency of the flame ionization detector, as well as of other detectors, has been widely recognized, and empirical correction factors have been required. By contrast, the gas density balance, the forgotten ideal detector, should not require calibration. A gas density balance, now available in a commercial chromatograph, and a flame ionization detector were compared for quantitative analyses of lipids. Wt percents of known methyl ester mixtures were determined, as well as mole percents of aldehyde fragments from certain ozonized octadecenoate isomers. Percentages were calculated from area response without correction factors for the gas density balance and with correction factors, based upon the number of ionizable carbon-atoms, for the flame ionization detector. Accuracy, as measured by percentage deviation from either known or theoretical values, was better for gas density balance data than for flame ionization detector data. Aldehyde and aldehydic ester fragments formed by reductive ozonolysis of octadecenoate isomers from partially hydrogenated methyl linolenate also were determined with each detector. Theoretically, ozonolysis of these monoenes should yield an aldehyde and an aldehydic ester in equal mole percents. Experimentally, the average of the ratios of aldehyde to aldehydic ester from each of the Δ5-Δ13 monoenes was 1.29 for the FID data (corrected) and 1.01 for GDB data (uncorrected). This difference in averages approaches significance at the 95% confidence level. For the Δ14 and Δ15 monoenes from which C_4 and C_3 aldehydes are formed, ionizable carbon-atom corrections proved even less adequate.

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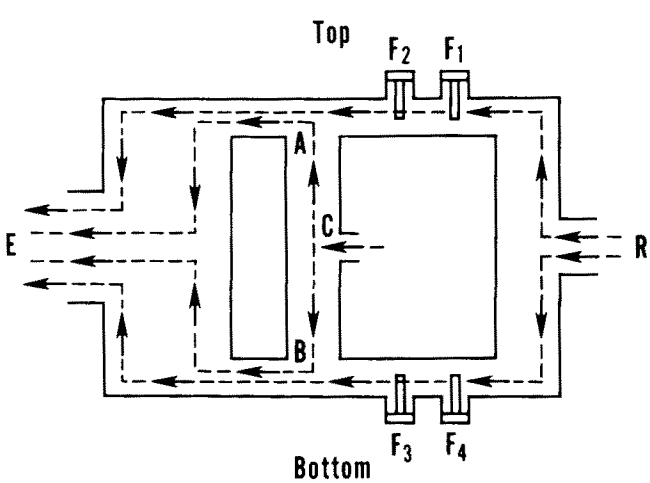

FIG. 1. Schematic of a gas density balance.

INTRODUCTION

Most detection systems of commercial gas chromatographs do not respond simply on a wt basis, on a molecular basis, or on some other stoichiometric basis. Consequently, surveys have been made to determine the nature of response of detectors to compounds with varying chain lengths and functional groups (1-3). Such a study with a flame ionization detector (FID) has established that different functional groups within organic molecules respond differently. Quantitative analyses involving the FID, therefore, have required difficult, tedious calibration by relating each component in a gas chromatographic run to a known component (usually benzene or heptane). However, this method provides neither a universal nor a permanent calibration.

Theoretical correction factors also have been proposed to allow quantitation of the more popular detectors. For example, Eastman (4) suggests that the square root of the mol wt of the component in question is feasible for thermal conductivity detectors, whereas effective (or ionizable) carbon-atom corrections often are used with the FID (1,5,6).

The exact mechanism of response of an FID is not understood entirely, but two prevalent theories have been suggested to account for its great sensitivity. The carbon aggregate hypothesis states that each detected material undergoes reactions which ultimately form carbon (7). This carbon condenses into particles of sufficiently low work function for thermal ionization. These ionizing particles bear a quantitative relationship to the carbon number of the original compound. The chemiionization theory (8) proposes that the exothermic energy released during chemical reactions in the flame first is retained by the molecular fragments formed and then redistributed by molecular collisions. This excess energy leads to the ionization. Whatever the cause of ionization, there appears to be a fundamental relationship between response and gram atoms of carbon. No one has established a universal method for calibrating a detector, and little more than theoretical corrections are available for quantitative results.

In an earlier communication (9), mass chromatography was studied as a gas chromatographic procedure for determining mol wt (3,10). Because a gas density balance (GDB) had been incorporated into the design, this instrument was well suited for direct quantitative analyses, as well as mol wt determinations.

The GDB is not a new detector. Invented by Martin and James (11) in 1956, it was widely acknowledged as an absolute detector. However, the GDB fell into disuse largely because of its complicated design and construction and was subsequently referred to as the forgotten ideal detector (12). Maintaining the operating principles of the original GDB, Nerheim (13) developed a less complex version, now available for the first time in a commercial gas chromatograph.

The schematic diagram (Fig. 1) shows a GDB in a mass chromatograph in its upright position. The same type gas is used as the reference and carrier gases. The reference gas enters at R, divides into two paths and passes over the sensing elements (F_{1-4}) which form the four arms of a

4/24/73
RUN 126 FILE 13 GD DETECTOR
MASS CHROMATOGRAPH N2
SP10006OV17(10%/W HP),6'X1/8";105MA
SAMPLE RATE 16:16:1; BUNCH, 4; 1MV
TOTAL MONDENE (RAO #50); OZO-TPP

SF1 2 SF2 3 CDC 8 NDC 5 DB 20 BC 4 HT 0 BO 1 STP 200

PK NO	TIME MIN	TEMP C	REL AREA	AREA %	IDENTITY	WEIGHT %	MOLE %	COMPOUND A	TYPE AE	MOLE %
1	0.3	34	46503.	0.68	C(3)-A	1.13	3.31	6.92		
2	1.8	46	45072.	0.66	C(4)-A	.93	2.18	4.57		
4	7.8	65	181290.	2.66	C(5)-A	3.37	6.66	13.95		
5	13.1	85	248362.	3.65	C(6)-A	4.33	7.36	15.39		
6	18.0	104	306759.	4.50	C(7)-A	5.11	7.61	15.92		
7	22.4	120	306643.	4.50	C(8)-A	4.93	6.54	13.68		
8	26.4	136	248482.	3.65	C(9)-A	3.89	4.65	9.73		
9	27.3	140	13164.	0.19	C(4)-AF	.22	.32		.61	
10	29.3	149	19459.	0.29	UNKNOWN					
11	30.0	152	197026.	2.89	C(10)-A	3.01	3.28	6.87		
12	30.7	155	61449.	0.90	C(5)-AF	.98	1.28		2.46	
13	32.6	162	38284.	0.56	UNKNOWN					
14	33.4	166	160470.	2.36	C(11)-A	2.41	2.41	5.04		
15	34.5	170	93781.	1.38	C(6)-AF	1.46	1.72		3.30	
16	35.6	175	37468.	0.55	UNKNOWN					
17	36.6	178	126192.	1.85	C(12)-A	1.87	1.72	3.61		
18	37.6	182	122610.	1.80	C(7)-AF	1.87	2.01		3.85	
19	38.6	186	36532.	0.54	UNKNOWN					
20	39.6	191	90874.	1.33	C(13)-A	1.33	1.14	2.39		
21	40.7	195	188490.	2.77	C(8)-AF	2.83	2.79		5.35	
22	41.4	197	28955.	0.43	UNKNOWN					
23	42.4	202	20168.	0.30	C(14)-A	.29	.23	.49		
24	43.5	208	346220.	5.08	C(9)-AF	5.12	4.67		8.95	
25	45.2	213	38430.	0.56	C(15)-A	.55	.41	.87		
26	46.3	216	485856.	7.13	C(10)-AE	7.09	6.02		11.54	
27	47.9	224	28150.	0.41	C(16)-A	.40	.28	.59		
28	48.9	231	656301.	9.63	C(11)-AE	9.48	7.52		14.41	
29	51.4	239	802198.	11.78	C(12)-AE	11.48	8.55		16.38	
30	53.7	248	749193.	11.00	C(13)-AE	10.64	7.47		14.30	
31	55.9	257	585342.	8.59	C(14)-AE	8.25	5.47		10.48	
32	58.0	270	377646.	5.54	C(15)-AE	5.29	3.33		6.37	
33	60.1	269	125105.	1.84	C(16)-AE	1.74	1.04		2.00	

FIG. 2. Computer output from chromatographic analysis of an ozonized monoene fraction isolated from partially hydrogenated methyl linolenate. A = aldehyde and AE = aldehydic ester.

Wheatstone bridge circuit. The two reference flows recombine and exit at E. At equilibrium, the reference flow rate across each pair of filaments is equal. Column carrier gas enters the GDB at C, splits into two paths which join the reference flows at A and B and exits at E. When sample vapor enters the GDB with carrier gas, the difference in densities of the sample vapor and reference gas changes flow rates. Sample vapor more dense than the carrier will fall preferentially, joining the reference stream at B, slowing the flow in arm RBE, and increasing the flow in arm RAE. Sample vapor less dense than the carrier gas will rise, reducing the flow rate in RAE and increasing it in RBE. These changes in flow rates create an imbalance in the bridge circuit and produce the electrical output.

Because the response of the GDB depends solely upon the amount of sample and density difference between the sample vapor and reference gas, no calibration should be necessary, and quantitative results should be obtained directly. Evaluating the GDB as a quantitative detector in the lipid field is one objective of our study; comparing accuracies of a GDB and an FID in quantitative gas chromatography is another.

MATERIALS AND METHODS

Procedures for quantitative analyses depend upon the nature of a sample. National Institutes of Health standard mixture E (even chain length C_{8-16} with known wt percentages) was injected directly into the gas chromatographs without further treatment. Monoene samples, including (A) methyl petroselenate ($\Delta 6$); (B) methyl oleate ($\Delta 9$); (C) a mixture of $\Delta 6$, $\Delta 9$, and $\Delta 12$; and (D) a monoene fraction isolated from a partial hydrogenation of methyl linolenate, served as sources of aldehydes and aldehydic esters.

Monoene samples were treated in the following manner: ozonolysis was carried out on a 20 μliter sample in a glass conical tube equipped with gas inlet and outlet tubes inserted through a silicon rubber septum. The microreactor apparatus (14) generated and monitored ozone. Ozonization, in 75 μliter solvent ($CHCl_3$ at 0 C), was followed by reduction with 40 μliter 50% triphenylphosphine in $CHCl_3$. The aldehydes and aldehydic esters were prepared just before analysis and were used in both FID and GDB chromatographs. At least three runs were made on each sample with each chromatograph, and averages of percentage composition were compared.

TABLE I

Accuracy of FID and GDB on NIH Standard Mixture E[a]

Component	Wt percentages Known	FID	GDB	Percent deviation from known FID	GDB
8E	6.3	4.7	6.1	25	3
10E	9.1	9.7	9.4	7	3
12E	12.1	13.3	12.0	10	1
14E	23.3	25.1	23.5	8	1
16E	49.2	47.3	49.0	4	0

[a]FID = flame ionization detector; GDB = gas density balance, and NIH = National Institutes of Health.

Gas chromatography with the FID was performed with a Beckman GC-5. Helium carrier gas flow was 38 cc/min; attenuation at 2 x 10^4; detector temperature, 350 C; temperature programed from -40-275 C at 4.4 C/min. The column was 2 ft x 1/8 in. stainless-steel packed with 10% OV-17 on 80/100 Chromosorb W HP, followed by 2 ft x 1/8 in. 10% OV-225 on 80/100 Chromosorb W HP (15). Acids were removed by a zinc oxide-on-sand precolumn (16).

The other instrument was an MC-2 mass chromatograph (Chromalytics Corp., Unionville, Pa.) equipped with a GDB. Nitrogen carrier flow was 10 cc/min, and reference flow was 166 cc/min; attenuation at x4; detector temperature 250 C; temperature programed from 30-280 C at 4 C/min. The chromatographic column was a 50:50 mixture of OV-17 and OV-225 (both 10% on 80/100 Chromosorb W HP). On-column injections bypassed the splitter and trapping-valving system used for mol wt determinations.

GDB response was sent directly to an IBM 1800 computer for integration. FID response was recorded on digital tape with subsequent playback to an Infotronics integrator (CRS 12/40) and to the 1800 computer for similar area determination. Since the digital tape-electronic integration of the FID response was not significantly different from the on-line computer integration of the same data, the same method of area integration was suitable for both sets of data.

For FID data, computer integrated areas were divided by the number of ionizable carbon-atoms in the molecule to give a mole percent. Investigations (1,6) have shown that carbonyl carbon-atoms do not contribute to the effective carbon content of a molecule. For methyl esters and aldehydes, therefore, the ionizable carbon-atoms are one less than the total number of carbons, and for aldehydic esters, two less. For GDB data, areas divided by the difference in mol wt between the component (MW_x) and carrier gas (MW_{cg}), i.e. [MW_x-MW_{cg}], yielded mole percent. Individual wt percentages were obtained by multiplying the mole percent by the mol wt of the component.

An assessment of FID and GDB responses was made by comparing the wt or mole percentages found with each detector to known or theoretical values. The uncorrected area is stored in the computer until the chromatographer identifies the individual peaks. The generated printout (Fig. 2) includes the time and temperature of elution, the relative area and area percent, the designated identity of each peak, and the wt and mole percentages.

RESULTS AND DISCUSSION

The accuracies for both detectors as obtained with National Institutes of Health standard mixture E are compared in Table I. The largest percentage deviation of

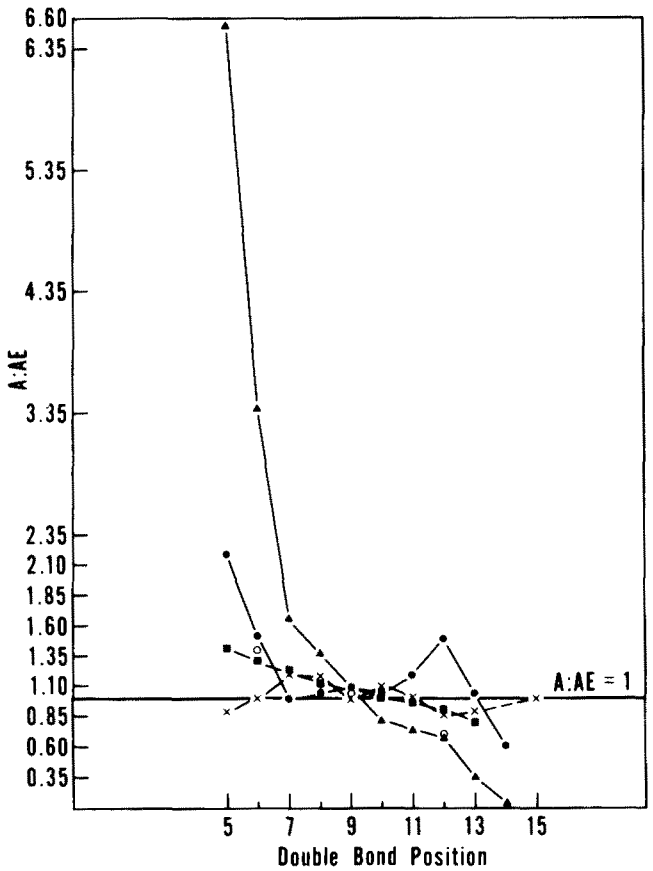

FIG. 3. Ratios of aldehyde (A):aldehydic ester (AE) vs double bond position obtained by using (a) area percents (flame ionization detector [FID], Δ); (b) mole percents corrected for ionizable carbon-atoms (FID, •); (c) mole percents calculated by Van der Plank's method (FID, ▪); (d) mole percents uncorrected (gas density balance, x); and (e) mole percents corrected for ionizable carbon atoms for a Δ6, Δ9, Δ12 mixture (FID, ○).

the averages of 3 runs from the known wt percents is 3% for the GDB and 25% for the FID. Each detector showed the largest error on the compounds with the shortest chain length; however, the relative standard deviation for the FID was 5.5% and for the GDB, 1.1%.

To demonstrate the expected equal molar yield (50%) of aldehyde and aldehydic ester fragments from an oxidative degradation of monoenoic methyl esters, methyl petroselenate (Δ6), and methyl oleate (Δ9) were ozonized individually. Reductive ozonolysis of methyl petroselenate gave average mole percentages for 12 aldehyde of 55.5% and 6 aldehyde ester of 44.5% for the FID. Corresponding values were 50.7 and 49.3% for the GDB. Only aldehydic mole percentages will be discussed subsequently since the aldehydic ester mole percentage is given as: % aldehyde ester = 100–% aldehyde. For the methyl oleate sample, both FID and GDB results (51.1 and 51.4 mole %, respectively) are within experimental error of theory.

The agreement with theory of FID results for methyl oleate is explicable if one considers the equal ionizable carbon-atoms of the fragments–eight. One would expect better agreement when fragment ionizable carbon-atoms are equal or ca. equal. As chain lengths shorten and the difference in ionizable carbon-atoms of complementary fragments increases, corrections become less adequate. This trend is evident with the mixture of Δ6, Δ9, and Δ12 octadecenoates. Table II shows the relative magnitudes of the aldehyde and aldehydic ester fragments from each monoene. Columns 1 and 2 give the area percents from the FID which, when divided by the corresponding mol wt, give uncorrected mole percent in columns 3 and 4. Columns 5 and 6 are FID mole percents corrected for ionizable carbon-atoms and columns 7 and 8, the mole percents from the GDB.

Data in Table II confirm that FID area percents cannot be equated directly to mole percents and that, although approaching the theoretically equal aldehyde and aldehydic ester percentages, uncorrected mole percents are not sufficient. The corrections for ionizable carbon-atoms bring the molar percentages into better agreement with theory. GDB mole percentages are much closer to theoretical expectation, i.e. more accurate, than FID data. The precision of FID data (relative standard deviation = 3.96) is less than that of GDB data (relative standard deviation = 2.14).

Octadecenoate isomers isolated from partially hydrogenated methyl linolenate supplied a good example for study of widely ranging double bond distribution–fragments from Δ5-Δ13. Area and corrected mole percentages for FID data and mole percentages for GDB data are given in Table III for the aldehyde and aldehydic ester from each isomer, where available. Ratios of mole percent aldehyde to mole percent aldehydic ester are calculated for data available with both detectors.

The average FID mole percents for double bond positions from 5-13 are 5.07 and 4.28 for aldehyde and aldehydic ester, respectively. Corresponding GDB values are 4.59 and 4.67. Statistics show that the difference between the two FID means is significant at the 95% level. The difference for the GDB, however, is not significant. Based upon nine values, the standard error of an FID mean was 0.21 and of a GDB mean, 0.14. The average ratio for FID data was 1.29 (standard error = 0.13) and for GDB data, 1.01 (standard error = 0.04). Statistical analysis of these ratios suggests that the difference between the FID and GDB is also significant at the 95% level.

Closer examination of Table III again shows the trend mentioned previously. Especially noticeable for area percent data, aldehyde values are much larger than aldehydic

TABLE II

Average Percentages of Aldehyde (A) and Aldehydic Ester (AE) from Reductive Ozonolysis of a Δ6, Δ9, and Δ12 Octadecenoate Mixture

Double bond position	Flame ionization detector						Gas density balance Mole %	
	Area %		Mole % uncorrected		Mole % corrected for ICA[a]			
	A	AE	A	AE	A	AE	A	AE
6	23.62	7.49	21.46	8.99	17.73	12.78	16.24	14.85
9	18.75	18.09	22.22	16.36	19.48	18.78	18.68	17.92
12	7.79	24.25	13.09	17.87	12.93	18.31	15.20	17.12
					S^b = 3.96		S = 2.14	

[a]ICA = ionizable carbon-atoms.

[b]S = standard deviation.

TABLE III

Comparison of Aldehyde (A) and Aldehydic Ester (AE) from Reductive Ozonolysis of Monoenes Isolated from Partially Hydrogenated Methyl Linolenate[a]

Double bond position	Area % FID		Mole % FID corrected for ICA		Mole % GDB		Molar ratios A:AE	
	A	AE	A	AE	A	AE	FID	GDB
5	2.09	0.32	1.48	0.68	1.14	1.28	2.18	0.89
6	3.37	1.00	2.60	1.71	1.72	1.72	1.52	1.00
7	3.07	1.85	2.60	2.62	2.41	2.01	0.99	1.20
8	3.84	2.81	3.62	3.41	3.28	2.79	1.06	1.18
9	5.05	4.67	5.35	4.95	4.56	4.67	1.08	0.98
10	5.52	6.75	6.69	6.36	6.54	6.02	1.05	1.09
11	5.84	7.88	8.26	6.69	7.61	7.52	1.23	1.01
12	4.91	7.25	8.33	5.59	7.36	8.55	1.49	0.86
13	3.17	9.16	6.73	6.48	6.66	7.47	1.04	0.89
14[b]	1.18	8.48	3.33	5.54	---	---		
15[b]	---	---	---	---	3.31	3.33		
	Average:		5.07	4.28	4.59	4.67	1.29	1.01

[a]FID = flame ionization detector and GDB = gas density balance.

[b]Double bond position 14 and 15 not used in calculations.

ester values for long chain aldehydes and much smaller for short chain aldehydes. The ionizable carbon-atom corrections bring the mole percentages closer to equality, but, as the double bond approaches either end of the molecule, e.g. Δ5, Δ6, Δ14, the inadequacy of ionizable carbon-atom corrections becomes increasingly evident. For GDB data, however, mole percentages display no apparent trend.

A better way to visualize this trend is to plot the ratios of aldehyde:aldehydic ester vs double bond position (Fig. 3). The divergence of ratios at the extremes of double bond positions is apparent with FID data. Ionizable carbon-atom corrections seem to flatten out the ratios for Δ7-Δ13 positions. There is reason to question the experimental validity of the Δ12 ratio of this run, because the aldehyde:aldehydic ester ratio for this compound in the Δ6, Δ9, Δ12 series lies closer to a predicted value (open circle).

Although the ionizable carbon-atom corrections greatly improve mole percent calculations, especially when fragment molecules are formed from Δ7-Δ13 monoenes, still a correction factor is needed that accurately quantitates gas chromatography data over a wide range of molecular sizes. Van der Plank (17) used 9 aldehyde and 9 aldehydic ester as internal standards. By relating each compound created by oxidative degradation to its corresponding standard, Van der Plank developed a relative sensitivity factor that he used to calculate the molar percentages. Then, choosing a few well resolved degradation products to establish a correction constant, a theoretical value of corresponding peak area ratios can be obtained. Thus, a theoretical, or corrected, area for an unreliable or unresolved peak can be calculated. Corrected areas then can lead to better agreement between aldehyde and aldehydic ester in quantitative analyses.

Using the somewhat complex Van der Plank method, double bond distributions (or mole percents) were recalculated for our FID data. Ratios of aldehyde:aldehydic ester from this corrected data are plotted in Figure 3. Visual inspection shows that Van der Plank's method reduces the divergence of ratios as the double bond shifts toward the ends of the molecule. Though smaller, the trend remains for theoretical corrections to fail in these terminal regions for FID data.

Revival of the GDB may well provide a simple and accurate quantitative detector for gas chromatographic analysis of lipid compounds.

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