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ANALYSIS OF GAS-LIQUID CHROMATOGRAMS  
BY A PUNCHED-CARD TECHNIQUE

Robert K. Tandy, Frank T. Lindgren,  
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ABSTRACT

A method is described whereby gas-liquid chromatograms may be analyzed using a punched-card technique. Although the application presented involves analysis of fatty acid methyl esters in which a beta particle ionization detector has been used, this method, with minor revisions, has potential applications to all gas-liquid chromatographic work. The advantages of this technique are (a) elimination of nearly all manual arithmetic calculations, (b) accuracy equivalent to or greater than that of existing manual techniques, and (c) ease of data manipulation and storage.

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INTRODUCTION

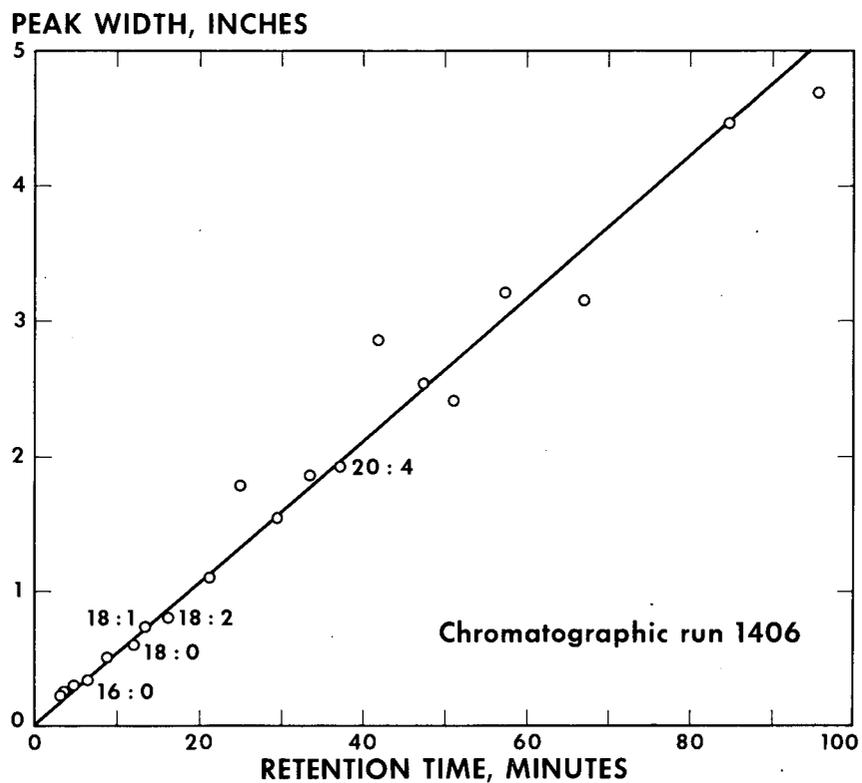
During the last decade one of the most promising and exciting developments for the analysis of volatile organic compounds of biologic interest has been the technique of gas-liquid chromatography.<sup>1</sup> An important application of this technique is in the study of long-chain fatty acids and their relationship to both normal and abnormal lipid metabolism.

However, one of the technical difficulties encountered in this and other applications of gas-liquid chromatography (GLC) has been the complicated nature of the data. In the fatty-acid studies, for example, there are the order of a hundred known biologically occurring fatty acids. In a typical GLC analysis, approximately 30 or more of these fatty acids are usually resolved on each chromatogram. Tabulation and comparison of such extensive data is a very tedious manual task. Usually quantitation of the amount of each fatty-acid component has been made by measuring the area under each chromatographic peak. This has been done by integration, planimetry, and triangulation.<sup>2</sup> If carried out manually, these methods are laborious and unfortunately subject to frequent human error.

To avoid the above-mentioned technical difficulties, we have developed a technique for analysis of gas-liquid chromatograms, using punched cards together with an appropriate computer (IBM 650). In essence, each chromatogram consisting of a complicated sequence of peaks is reduced to a small "deck" of IBM punched cards (one for each chromatographic component). The basic data placed on these "input" IBM punched cards consists of the elution time and peak height of each chromatographic component. From this data, a measure of the area under each peak on the chromatogram is calculated by multiplying the product of the elution time and peak height by a "first-order" correction function based upon the peak height. Thus, for a given chromatogram, the mass of each component is calculated together with the total mass of all chromatographic components present. For convenience, the weight percent of each component as well as its retention time (relative to methyl stearate) is also calculated. An additional calculation correcting each chromatographic component on the basis of its relative retention time completes the program.

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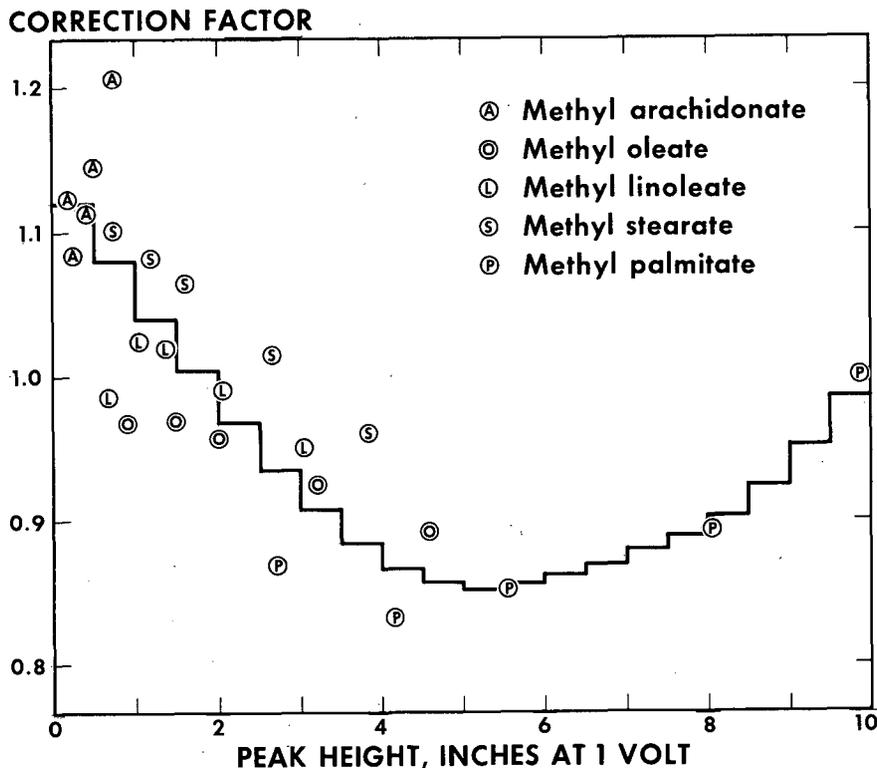
Fig. 1. Relationship between triangulated peak width and retention time. The data are from a duplicate analysis of the chromatographic run shown in Fig. 6.

## PROCEDURE AND EXPERIMENT

For a given component on a gas-liquid chromatogram, the peak width (at the baseline as calculated by triangulation) is roughly proportional to the elution time. Figure 1 shows this relationship for chromatographic run No. 1406. In addition, for a given component, the peak height is approximately 85% of the triangulated height. Thus, for any component, a measure of the triangulated area (which is approximately 95% of the absolute peak area) can be obtained from the product of the peak height and the elution time. This value is, of course, only a first approximation to component mass, because detection of a given component using our beta-particle ionization detector, <sup>3</sup> peak area (or peak height) is not actually linear with component mass. For a standard calibration mixture, Fig. 2 shows the first-order correction factors based only on peak height that are necessary to correct each component. Data from several calibration runs of from 10 to 100  $\mu\text{g}$  total sample mass have been plotted to provide, for each principal chromatographic component, ranges of peak height that are normally encountered in routine GLC analysis.

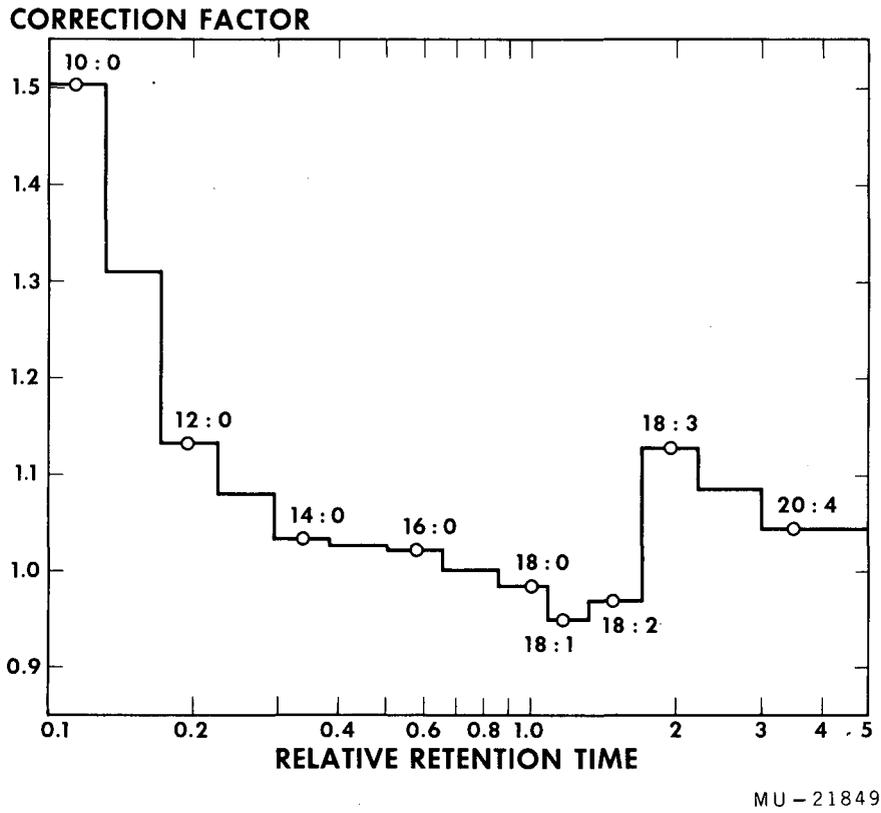
If desired an additional second-order correction can be made. This correction function is illustrated in Fig. 3. Here the first-order determination of the mass of each component is corrected on the basis of its retention time relative to methyl stearate. Thus, this second function corrects primarily for different detector responses to each chromatographic component. The actual manual work necessary to read a chromatogram such as run 1406 (shown in Fig. 4) consists of placing an appropriate base line on each chromatogram, noting the recorder input amplification factor used and measuring the retention time and peak height for each component. The base line, obtained, from a solvent injection of the same volume, recorded at the same amplification is conveniently traced on the chromatogram over a translucent fluorescent light box. This input data is transcribed to standard 80-column 7-3/8-in. by 3-1/4-in. IBM cards using a Model-026 card-punch machine and a Model-056 verifier. Figure 5 illustrates the usage of each column for both input and output data in the "reading" procedure of run No. 1406. For each component, the input data is punched in columns 1 through 19 of a single IBM card. Columns 1 through 5 contains the chromatographic number, 6 through 10 the elution time of the component, 11 through 14 the peak height, and 15 through 19 the amplification factor with reference to a 1-v signal giving a 10-in. (full scale) recorder deflection. Before the computer operation, each input deck of IBM cards is arranged numerically by set (run) number and each card within a deck is arranged in sequence by retention time. In this order, the cards are ready for the program. Although we use an IBM-650 electronic computer, any standard digital computer may be used for this operation with slight revisions in the actual program details.

The programmed computations proceed in the following manner for each set. First, the peak height is divided by the amplification factor. Then this normalized peak height is multiplied by the first correction function (see Fig. 2). This product is stored, and as each card is processed, this first-order component mass is summed. Also, the retention time of each component is divided by the stearate retention time, which is identified by



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Fig. 2. First correction function involving peak height. This function is a "stepped-interval" approximation using calibration data of the five principal fatty-acid methyl-ester components. The sample masses for each component cover the range usually encountered in GLC of the blood lipids and are the order of 5 to 50  $\mu$ g. This "first-order" function primarily corrects for the nonlinearity of all calibration curves, irrespective of differences in detector response for different fatty-acid methyl-ester components.



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Fig. 3. Second correction function involving relative elution time. This function is obtained from calibration data of methyl-ester components of from 10:0 to 20:4. Each point shown is the mean value of three correction factors determined over the usual mass range (1 to 50  $\mu$ g) encountered for each of these components. The first correction interval starts at zero time, and the last one (which includes 20:4) ends at 99:9 (stearate time units). Additional calibration data, particularly of components of the C-22 series, will allow further refinement in this correction function.

Fig. 4. Duplicate analysis of chromatographic run No. 1406. The methyl-ester sample is a total serum-phospholipid fraction from a 21-yr-old normal female. Details of the GLC technique and apparatus used for all gas-chromatographic work presented here are described in detail in reference 3. The liquid phase is polydiethylene glycol succinate. The column temperature is 195°C, the flow rate of the argon carrier gas is 80 ml/min, and the detector temperature approximately 195°C. For this run, two recorders are used--one operating continuously at IOX, the other at IX until the 20:4 component has been resolved, and thereafter at 33.3X. The chart speed is 15 in./hr, and retention time for methyl stearate is 12.07 min.

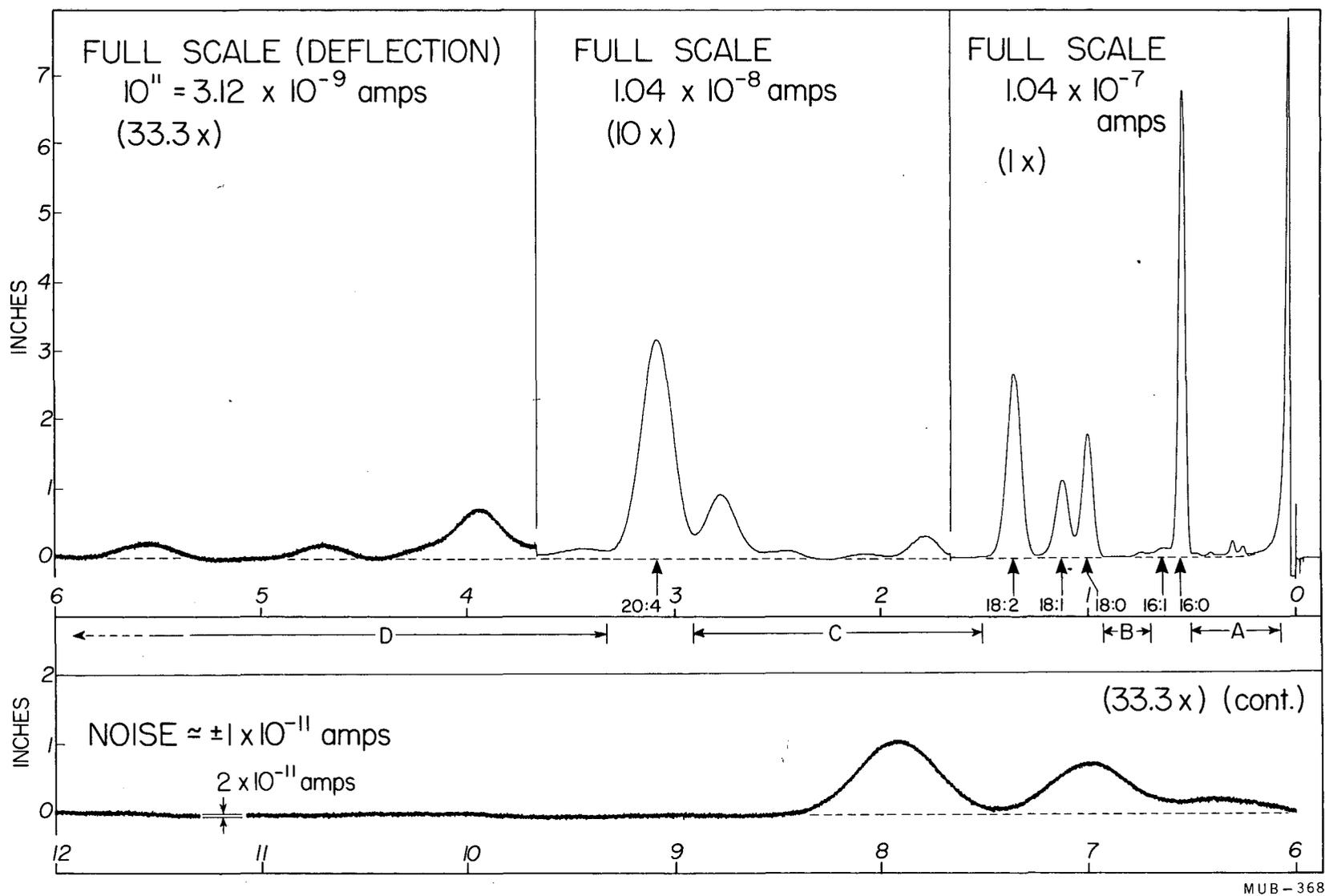


Fig. 4

Fig. 5. Input and output deck for set No. 1406. Each IBM card is arranged in sequence by component time. From left to right on columns 1 through 19 of the input-deck data entries (left) are set number, component time (min.), peak height (in.) and amplification factor relative to 1-v signal giving a 10-in. (full-scale) recorder deflection. For each input card there is a corresponding output card which in addition to the input data lists first-order corrected mass, retention time (relative to methyl stearate), first-order mass percent, second-order mass percent, and second-order corrected mass. A final output card contains the set number as well as the sums of both the first-and second-order masses.

INPUT DECK				OUTPUT DECK								
SET #	T	PH	AMPL	SET #	T	PH	AMPL	M1	REL T	M1 %	M2 %	M2
01406	00211	0007	01000	01406	00211	0007	01000	00002	00175	00001	00001	00002
01406	00307	0138	01000	01406	00307	0138	01000	00047	00274	00039	00039	00047
01406	00364	0214	01000	01406	00364	0214	01000	00067	00302	00062	00064	00067
01406	00425	0026	01000	01406	00425	0026	01000	00012	00352	00009	00005	00012
01406	00489	0071	01000	01406	00489	0071	01000	00039	00405	00028	00028	00040
01406	00573	0067	01000	01406	00573	0067	01000	00043	00475	00031	00031	00044
01406	00657	0645	00100	01406	00657	0645	00100	00653	00544	02603	02660	03733
01406	00761	0133	01000	01406	00761	0133	01000	00113	00630	00081	00072	00113
01406	00898	0093	01000	01406	00898	0093	01000	00063	00736	00059	00055	00063
01406	01003	0024	01000	01406	01003	0024	01000	00027	00831	00019	00015	00027
01406	01207	0164	00100	01406	01207	0164	00100	01987	01000	01416	01353	01988
01406	01350	0104	00100	01406	01350	0104	00100	01466	01118	01040	00968	01386
01406	01628	0249	00100	01406	01628	0249	00100	03574	01345	02796	02710	03802
01406	01868	0009	01000	01406	01868	0009	01000	00019	01548	00014	00014	00019
01406	02130	0138	05000	01406	02130	0138	05000	00067	01765	00048	00054	00076
01406	02493	0040	05000	01406	02493	0040	05000	00022	02065	00016	00016	00025
01406	02935	0066	05000	01406	02935	0066	05000	00043	02432	00031	00033	00047
01406	03333	0440	05000	01406	03333	0440	05000	00328	02761	00234	00254	00357
01406	03705	0284	01000	01406	03705	0284	01000	01160	03073	00641	00676	01232
01406	04131	0075	05000	01406	04131	0075	05000	00070	03464	00050	00052	00073
01406	04740	0108	05000	01406	04740	0108	05000	00117	03927	00063	00063	00122
01406	05101	0031	05000	01406	05101	0031	05000	00034	04276	00024	00025	00035
01406	05727	0033	05000	01406	05727	0033	05000	00045	04745	00032	00033	00047
01406	06694	0031	05000	01406	06694	0031	05000	00045	05546	00032	00033	00047
01406	07488	0018	05000	01406	07488	0018	05000	00034	06224	00024	00025	00035
01406	07866	0026	05000	01406	07866	0026	05000	00044	06517	00031	00033	00046
01406	08454	0106	05000	01406	08454	0106	05000	00155	07005	00142	00148	00206
01406	09540	0143	05000	01406	09540	0143	05000	00310	07994	00221	00231	00324
01406				01406				14035				14032

MUB-511

Fig. 5

SET #	T	PH	AMPL	M1	REL T	M1 %	M2 %	M2
1406	2.11	.07	10.00	.02	.175	.01	.01	.02
1406	3.07	1.38	10.00	.47	.254	.33	.36	.51
1406	3.64	2.14	10.00	.87	.302	.62	.64	.90
1406	4.25	.76	10.00	.12	.352	.09	.09	.12
1406	4.89	.71	10.00	.39	.405	.28	.29	.40
1406	5.73	.67	10.00	.43	.475	.31	.31	.44
1406	6.57	6.45	1.00	36.53	.544	26.03	26.60	37.33
1406	7.61	1.33	10.00	1.13	.630	.81	.82	1.15
1406	8.88	.83	10.00	.83	.736	.59	.59	.83
1406	10.03	.24	10.00	.27	.831	.19	.19	.27
1406	12.07-	1.64	1.00	19.87	1.000	14.16	13.93	19.55
1406	13.50	1.04	1.00	14.60	1.118	10.40	9.88	13.86
1406	16.28	2.49	1.00	39.24	1.349	27.96	27.10	38.02
1406	18.68	.09	10.00	.19	1.548	.14	.13	.18
1406	21.30	1.38	50.00	.67	1.765	.48	.54	.76
1406	24.93	.40	50.00	.22	2.065	.16	.18	.25
1406	29.35	.66	50.00	.43	2.432	.31	.33	.47
1406	33.33	4.40	50.00	3.29	2.761	2.34	2.54	3.57
1406	37.09	2.84	10.00	11.80	3.073	8.41	8.78	12.32
1406	41.81	.75	50.00	.70	3.464	.50	.52	.73
1406	47.40	1.08	50.00	1.17	3.927	.83	.87	1.22
1406	51.01	.31	50.00	.34	4.226	.24	.25	.35
1406	57.27	.33	50.00	.45	4.745	.32	.33	.47
1406	66.94	.31	50.00	.45	5.546	.32	.33	.47
1406	74.88	.18	50.00	.34	6.204	.24	.25	.35
1406	78.66	.26	50.00	.44	6.517	.31	.33	.46
1406	84.55	1.06	50.00	1.99	7.005	1.42	1.48	2.08
1406	95.40	1.43	50.00	3.10	7.904	2.21	2.31	3.24
1406				140.35				140.32

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Fig. 6. IBM printout sheet for gas-chromatographic set No. 1406. For this type of data printout, each output deck (obtained from the computer) is processed through a model-407 printer.

an "X" punched card. The first-order calculation is completed by dividing each component mass by the total mass of each set, giving a first-order mass-percent value for each component. The accuracy of this first-order determination of the masses of each principal chromatographic component and its mass percent of the total mass resolved is approximately 5 to 10%. This accuracy is limited primarily because of differences in response to different fatty acids by our beta-particle ionization detector.

For some applications a second-order correction may be desired or needed. The program for the second-order correction multiplies each first-order component mass by a correction factor based upon relative elution time. Thus, this function (see Fig. 3) essentially corrects for differences in detector response to different fatty-acid methyl esters. This is accomplished by a table "look-up" program similar to the one used to determine the appropriate factors for a given peak height (used in the first-order correction). Finally, after the mass of each component has been corrected and the total mass for each set has been summed, the second-order mass-percent value for each component is calculated. For set 1406, Fig. 6 shows both the first-and second-order corrected output data. The total computer time necessary to process in this manner a single chromatographic run consisting of 28 IBM cards (such as set 1406) is approximately 30 sec.

Table I shows the results of both these first-and second order corrections for a standard calibration mixture over the sample mass range of 0.9 to 232  $\mu\text{g}$ . Although quantitation of trace components as well as inadequately separated components still present difficulties, all principal GLC components are quantitated to within approximately 5%. To attempt further corrections would probably not yield additional accuracy since resistors used for different amplifications, recorder response, and over-all calibration are not easily controlled much beyond this level of accuracy. Also, the slide-wire width of the recorder imposes an ultimate limitation on accuracy. However, the relative accuracy of comparing the composition of two methyl-ester samples is considerably greater--approximately 2% for the principal components.

## DISCUSSION AND CONCLUSIONS

The principal advantage of this punched-card technique is that it takes most of the manual drudgery out of chromatographic data processing. However, base-line placement and limited manual work is still required. Although, further "automation" in the "reading" of gas-liquid chromatograms is possible, certain considerations at this time do not favor such extension of this technique. For instance, a principal difficulty in determining the necessary input data is that of placing an appropriate base line on each chromatogram. This is particularly true of the early base line contour if solvent injection is employed. Judgement and experience are necessary to do this, because uncontrollable drifts in the base line frequently occur. Reasons for these drifts include amplifier instability, intermittent gas leaks, alterations in gas flow rates and liquid-phase bleed, as well as temperature fluctuations of apparatus components. Most of these factors operate independently of each other, however, factors such as temperature and liquid-phase bleed are closely related.

Table I. Comparison of first-and second-order mass-percent determinations of a standard methyl-ester test mixture over the range of 0.9 to 232  $\mu\text{g}$  (total sample). The test mixture containing 11.6 mg/ml of methyl esters in normal hexane was diluted successively (by a factor of two). A 0.010-ml aliquot of these samples was analyzed in each case except the 232  $\mu\text{g}$  sample, for which 0.020 ml of the undiluted 11.6 mg/ml test mixture was used.

Com- ponent	Mass percent																		Mean values		Actual mass
	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1%	M2%	Percent
10:0	1.27	1.89	0.68	1.01	0.66	0.98	0.68	1.01	0.65	0.97	0.60	0.91	0.60	0.90	0.63	0.94	0.53	0.79	0.70	1.04	0.95%
12:0	1.27	1.26	1.36	1.69	1.48	1.64	1.10	1.27	1.50	1.70	1.49	1.69	1.50	1.69	1.51	1.70	1.41	1.60	1.40	1.58	1.87
14:0	1.90	1.89	2.04	2.03	1.97	1.97	1.86	1.94	1.95	2.02	1.99	2.04	1.98	2.03	1.96	2.02	1.84	1.89	1.94	1.98	2.49
16:0	22.15	22.64	23.47	23.99	23.03	23.44	23.12	23.56	22.45	22.82	22.69	23.08	22.70	23.10	23.36	23.77	25.36	25.82	23.15	23.58	23.04
18:0	15.82	15.72	15.65	15.20	16.28	15.90	16.34	16.05	15.96	15.63	16.12	15.80	16.19	15.86	15.97	15.65	15.80	15.48	16.01	15.70	15.59
18:1	19.62	18.24	20.75	19.59	20.39	19.34	21.00	19.85	20.14	19.02	20.41	19.29	20.64	19.50	20.58	19.45	19.90	18.81	20.38	19.23	19.19
18:2	17.72	16.98	17.69	16.89	17.93	17.38	17.95	17.31	17.05	16.44	17.01	16.42	17.04	16.44	16.69	16.10	16.90	16.31	17.33	16.70	16.14
18:3	2.53	3.14	4.08	4.73	4.11	4.59	4.15	4.65	3.94	4.40	3.96	4.43	4.08	4.58	3.83	4.30	3.68	4.13	3.82	4.33	4.39
20:4	17.72	18.24	14.29	14.86	14.14	14.75	13.80	14.36	13.84	14.38	13.80	14.34	13.56	14.11	13.78	14.32	13.05	13.57	14.22	14.77	14.07
Minor	-	-	-	-	-	-	-	-	2.52	2.63	1.92	2.00	1.70	1.79	1.69	1.77	1.54	1.62	-	-	2.27%
Components																					
Total mass analyzed ( $\mu\text{g}$ )	0.93		1.74		3.58		6.95		14.54		28.47		56.91		115.97		232.26				

For analysis of gas-liquid chromatograms in general, as well as the specific determinations of the input data for our punched-card reading technique, we suggest the use of blank chart paper. In our experinece, we have found that graphed chart paper has several disadvantages. First, the critical placement of the baseline on the chromatogram is frequently made difficult by the presence of adjacent horizontal lines. Similar difficulty is encountered when measuring each peak height because both peak extremity and base line may be close to distracting horizontal lines. Also, the vertical lines tend to interfere with the measurement of the retention time of each chromatograph component. On the other hand, a definite advantage in using blank chart paper is that the actual chromatograms may be photographed easily for reproduction.

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