FIGURE 4
GAS CHROMATOGRAPH OF URINARY ACID PROFILE
A. PATIENT ACIDOTIC (2-17-73)
B. PATIENT NORMAL (3-10-73)

```
mass 140, amp   46.44
mass 144, amp   89.93
Amino Acid ALA    Masses 140/144    Areas   11349.2/ 21382.5      RATIO =     0.5308   Location Error =   0
mass 168, amp    7.23
mass 176, amp   41.46
Amino Acid VAL    Masses 168/176    Areas    1938.8/ 10947.3      RATIO =     0.1771   Location Error =  -2
mass 126, amp   22.65
mass 128, amp   22.76
Amino Acid GLY    Masses 126/128    Areas    6222.4/  6054.7      RATIO =     1.0277   Location Error =   2
mass 182, amp    1.80
mass 192, amp   14.23
Amino Acid ILE    Masses 182/192    Areas     393.2/  3653.7      RATIO =     0.1076   Location Error =  -1
mass 182, amp    3.50
mass 192, amp   26.32
Amino Acid LEU    Masses 182/192    Areas     836.1/  6769.0      RATIO =     0.1235   Location Error =   2
mass 166, amp   26.54
mass 173, amp  100.00
Amino Acid PRO    Masses 166/173    Areas    7603.1/-33028.8      RATIO =    -0.2298   Location Error =   2
mass 153, amp    4.59
mass 158, amp   10.31
Amino Acid THR    Masses 153/158    Areas    1162.7/  2722.9      RATIO =     0.4270   Location Error =   0
mass 139, amp   13.10
mass 142, amp   12.32
Amino Acid SER    Masses 139/142    Areas    3293.4/  3104.7      RATIO =     1.0608   Location Error =   0
mass 148, amp    9.85
mass 155, amp   32.24
Amino Acid PHE    Masses 148/155    Areas    2920.1/  8971.8      RATIO =     0.3255   Location Error =  -2
mass 240, amp    8.61
mass 243, amp   20.71
Amino Acid ASP    Masses 240/243    Areas    2281.8/  5751.0      RATIO =     0.3968   Location Error =   0
mass 198, amp   58.15
mass 203, amp   67.56
Amino Acid GLU    Masses 198/203    Areas   16883.6/ 19353.8      RATIO =     0.8724   Location Error =  -1
Amino Acid # 12 location error
  FINAL ANALYSIS ( mg/100ml)
    AMINO ACID        FOUND
    ALA               1.38
    VAL               0.52
    GLY               3.51
    ILE               0.23
    LEU               0.48
    PRO              -0.50
    THR               0.76
    SER               2.79
    PHE               0.74
    ASP               1.81
    GLU               2.04
```

FIGURE 5

ANALYSIS OF 12 AMINO ACIDS IN URINE

USING MASS FRAGMENTOGRAPHY

P-52

FINAL

THE SIMULTANEOUS QUANTITATION OF TEN AMINO ACIDS IN SOIL EXTRACTS

BY MASS FRAGMENTOGRAPHY

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Running Title: Mass Fragmentography of Amino Acids

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The analysis of amino acids from terrestrial and extraterrestrial sources is becoming increasingly important (1-5). The need for a specific, sensitive and rapid method of quantitation is desirable. The methods currently employed for amino acid analysis involve ion exchange procedures (6,7) or gas chromatography (8-10). These techniques, although of immense value, are limited by their non-specificity for the absolute identification of any substance responsible for a gas chromatographic peak.

In the present communication we report an absolute, unambiguous method for the positive identification and quantitation of ten amino acids present in soil extracts using GLC-mass fragmentography. In mass fragmentography the mass spectrometer is used only to detect certain preselected ions known to be characteristic for each compound being quantitated, and the internal standard. The technique of mass fragmentography using sector mass spectrometers is usually restricted to the simultaneous monitoring of up to three integer mass values (11, 12), although with one instrument five ions were used (13). Using a quadrupole mass spectrometer up to eight ions have been selected and their respective analog signals monitored (14). We now wish to report the modification of the gas chromatography-quadrupole mass spectrometer-computer system previously described (15) for the simultaneous monitoring under computer control of the ion currents from 25 pre-selected integer mass values. These values can range between masses 0 and 750 in contrast to the limited range available for mass fragmentography using sector meters. If required this number could be increased by suitable alteration of the computer control programs. Specifically we wish to report the application of this system to the quantitation of ten of the amino acids present in soil extracts.

METHODS

Reagents: A deuterated amino acid mixture was supplied by Merck Laboratory Chemicals (New Jersey). 1.25N HCl in n-butanol, 25% (v/v) trifluoroacetic anhydride in methylene chloride and Tabsorb (EGA on Chromw) column packing were obtained from Regis Chemical Co., Illinois. A standard amino acid solution was purchased from Pierce Chemical Co., Illinois.

Equipment: A Varian model 1200 gas chromatograph was coupled by an all glass membrane separator (16) to a Finnigan 1015 Quadrupole mass spectrometer which was interfaced to the ACME computer system of the Stanford University Medical School (15). GLC separations were conducted using a 6 foot by 4 mm. (I.D.) coiled glass column packed with Tabsorb (Regis Chemical Co.). The flow rate of the carrier gas (helium) was 60 ml/minute.

The uniqueness of the mass spectrometer instrumentation lies in the modified computer software (program) used. The hardware is the system previously described (15) and assumes an operating cycle of:

(a) transmission of a control number, N, from the computer to an interface controller which sets the quadrupole mass analyser to a particular mass point in the m/e continuum.

(b) an integration of the ion signal for a pre-set period, T (integration time = 8 milliseconds in our work), and

(c) computer reading of the integration value with a twelve bit A → D conversion.

For the recording of normal mass spectra N is selected such that successive

cycles result in m/e values of 1,2, ...750. At the beginning of each

day the instrument is calibrated using a reference compound. Idiosyncracies

of the IBM 360/50 to IBM 1800 computer data paths dictate that the mass

values be buffered into groups of 250.

For normal g.c.-m.s. procedures the operator is allowed to select

a mass range of 1 to n x 250 (n = 1,2, or 3 buffers). For mass fragmento-

graphy n is set to zero and instead a "precision collect" buffer of 250

control-data acquisition cycles is employed. The operator must then enter

the pre-selected m/e values he wishes to scan. When the precision collect

buffer is constructed, 10 cycles are allocated to each m/e value selected.

The first of the 10 cycles sets N to N_m-4. The returned integrated ion

measurement is discarded; this cycle serves only to slew the quadrupole

electronics from anywhere in the m/e continuum to the mass region of

interest. The additional 9 cycles are used with $N = N_m-4....N_m.....N_m+4$.

The returned values represent a set of readings about the m/e value of

interest $\pm$ 0.5 amu. The center three points are then smoothed with a

five point qudratic function (17). The highest value of these three

smoothed points is then selected as the precision collect value. Thus

small drifts in calibration are corrected and a signal average obtained.

Finally, the abbreviated "spectrum" of 25 precision intensities for each

m/e are filed on disc.

Such a "spectrum" is recorded every 2 seconds and a summation of all

the ion intensities is used to construct the ion chromatogram shown in Fig.

2. Individual ion chromatograms can also be constructed if required

(Fig. 3). A threshhold is established from the ion currents before and

after each gas chromatographic peak and a computer program performs

integration of the ion currents under each peak.

Procedure

1 g of sieved, air-dried soil (Stanford University garden soil) was refluxed with 6N HCl (10 ml) for 20 hrs. The mixture was filtered and the residue washed with 1N HCl (5 ml). The combined filtrate and washings were extracted with chloroform (4 x 10 ml) and the aqueous phase evaporated to dryness. The residue is dissolved in water (5 ml) and passed through a column of "Ion Retardation Resin" AG 11-A8 (50-100 mesh, 1 x 21 cm). The amino acids were eluted with water (50 ml) and the eluate evaporated in vacuo to dryness. The residue is dissolved in water (5 ml) and placed on a column of

cation exchange resin (AG 50W-X12, 50-100 mesh, 1 x 21 cm) and washed

with water (50 ml) to remove neutral and anion contaminants. The amino

acids were eluted with 4N NH$_4$OH (80 ml) and the eluate evaporated to

dryness. The residue was dissolved in water and made up to a volume of

4 ml. A portion of this solution (1 ml) was used for the amino acid

analysis using an amino acid analyser. To another 2 ml of the processed

solution was added 2 ml of the deuterated amino acid standard solution

(100 mg in 100 ml of 0.1N HCl) and the mixture evaporated to dryness.

The residue was refluxed with 1.2 N HCl in n-butanol (1 ml) for 30 min.

and evaporated to dryness in vacuo. To the residue trifluoroacetic

anhydride in methylene chloride (0.7 ml) was added and refluxed for 10

min. The solution was evaporated to dryness at room temperature and the

residue dissolved in ethyl acetate (100 µl). An aliquot (1 µl) was

injected into the injector port of the gas chromatograph and the oven

kept at 100° for 1 min. when it was programmed at 4°/min. to 220°.

To each of 4 tubes containing 2 ml of the deuterated amino acid

standard solution (100 mg in 100 ml of 0.1N HCl) was added 150, 200,

250 and 300 µl respectively of a standard amino acid solution (2.5 µmoles

of each amino acid per ml). The solutions were mixed and evaporated to

dryness. Each residue was derivatized by the above method and an

aliquot of each (1 µl) injected into the gas chromatograph which

was operated under the conditions described above. This procedure

was used to construct a standard curve for the quantitation of each

amino acid. A typical standard curve is shown (Figure 1) for glutamic

acid.

The N-TFA, O-n-butyl derivative was chosen for the derivatization of amino acids for two reasons. Firstly, these derivatives have excellent glc separation characteristics (17) and secondly the selected characteristic fragment ions of the deuterated and non-deuterated derivatives do not interfere with each other, nor with other α-amino acids. Table I records the individual ions monitored for quantitation in the mass spectra of each of the deuterated and non-deuterated amino acids. The computer integrates the intensity of the deuterated and non-deuterated ion currents with time and quantitation is achieved by calculation of the ratio of their respective peak areas.

Our results of a typical soil analysis are compared with those from an amino acid analyser in Table II. The higher value obtained with lysine by the amino acid analyser is due to a ninhydrin positive substance in soil interfering with the quantitation of lysine. In this respect mass fragmentography is superior to the amino acid analyser in that using a mass spectrometer as detector only characteristic pre-selected ions are detected and quantitated and any impurity present under the same gas chromatographic peak is not measured. A summation of 20 such characteristic ions was plotted as an ion chromatogram of a derivatized soil sample and is shown in Fig. 2.

Preliminary experiments showed that when the deuterated amino acid mixture was added directly to the soil sample extensive hydrogen-deuterium exchange occurred during acid hydrolysis of the soil extract. The removal of the isotopic label was catalysed by the hot mineral acid in presence of excess mineral used in the soil hydrolysis step. Fox

and collaborators have reported (4) a similar finding concerning the decomposition of amino acids in soil upon direct acid hydrolysis. In the present work the deuterated amino acid mixture was added just before derivatization (i.e. after hydrolytic extraction of the soil) in order to avoid this problem. However, in cases where it is necessary to quantitate the free amino acid content of complex mixtures, such as in serum or urine samples, the deuterated amino acid mixture may be added directly to the sample before processing without any deleterious effects (18).

Although only ten amino acids present in soil were quantitated the method can be extended to all the normal amino acids found in protein. The deuterated analogs of arginine, histidine, serine, threonine and tyrosine are commercially available. Appropriate deuterated analogs of methionine, tryptophane, cysteine and cystine would have to be chemically synthesized from the appropriate precursors. In these instances at least two deuterium atoms should be incorporated in non-exchangeable positions so that for the characteristic ion chosen the P + 2 peak is separate from the ^{13}C isotope contribution of the unlabeled amino acid. Furthermore, the deuterium substitution need not be quantitative (>90%) provided the same characteristic ion of that deuterated analog is used for the construction of a standard curve such as Figure 1.

In our experience, the use of a single mass value for each amino acid together with the g.c. retention time is sufficient for accurate identification and quantitation. The chemical work-up specifically yields a basic fraction thereby eliminating acid and neutral compounds which could possibly co-elute and interfere with the determination.

Instrument analysis time is approximately one hour and with our system we have been able to achieve accurate quantitation with samples containing as little as 10 nanograms of an amino acid.

SUMMARY

A specific and sensitive method for the identification and simultaneous quantitation by mass fragmentography of ten of the amino acids present in soil has been developed. The technique uses a computer driven quadrupole mass spectrometer and a commercial preparation of deuterated amino acids is used as internal standards for purposes of quantitation. The results obtained are comparable with those from an amino acid analyser. In the quadrupole mass spectrometer-computer system used up to 25 pre-selected ions may be monitored sequentially. This allows a maximum of 12 different amino acids (one specific ion in each of the undeuterated and deuterated amino acid spectra) to be quantitated. The method is relatively rapid (analysis time of approximately one hour) and is capable of the quantitation of nanogram quantities of amino acids.

ACKNOWLEDGMENTS

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Table I. CHARACTERISTIC FRAGMENT IONS SELECTED FOR MASS FRAGMENTOGRAPHY OF UNDEUTERATED AND DEUTERATED N-TFA-O-$\underline{n}$-BUTYL-AMINO ACIDS.

Amino Acids	Fragment Ion	Deuterated Amino Acids	Fragment Ion
ALA	$CH_3CH=\overset{+}{N}HCOCF_3$ (m/e 140)	$CD_3CD(NH_2)COOH$	$CD_3CD=\overset{+}{N}HCOCF_3$ (m/e 144)
VAL	$i-C_3H_7CH=\overset{+}{N}HCOCF_3$ (m/e 168)	$i-C_3D_7CD(NH_2)COOH$	$i-C_3D_7CD=\overset{+}{N}HCOCF_3$ (m/e 176)
GLY	$CH_2=\overset{+}{N}HCOCF_3$ (m/e 126)	NH_2CD_2COOH	$CD_2=\overset{+}{N}HCOCF_3$ (m/e 128)
ILEU	$C_2H_5CH(CH_3)CH=\overset{+}{N}HCOCF_3$ (m/e 182)	$C_2D_5CD(CD_3)CD(NH_2)COOH$	$C_2D_5CD(CD_3)CD=\overset{+}{N}HCOCF_3$ (m/e 192)
LEU	$i-C_3H_7CH_2CH=\overset{+}{N}HCOCF_3$ (m/e 182)	$i-C_3D_7CD_2CD(NH_2)COOH$	$i-C_3D_7CD_2CD=\overset{+}{N}HCOCF_3$ (m/e 192)
PRO	(structure) $\overset{+}{N}-COCF_3$ (m/e 166)	(deuterated ring structure)	(structure) $\overset{+}{N}-COCF_3$ (m/e 173)
PHE	$C_6H_5CH=CHCOOH]^{+}$ (m/e 148)	$C_6D_5CD_2CD(NH_2)COOH$	$C_6D_5CD=CDCOOH]^{+}$ (m/e 155)
ASP	$BuOOCCH_2CH=\overset{+}{N}HCOCF_3$ (m/e 240)	$HOOCCD_2CD(NH_2)COOH$	$BuOOCCD_2CD=\overset{+}{N}HCOCF_3$ (m/e 243)
GLU	$HOOCCH_2CH_2CH=\overset{+}{N}HCOCF_3$ (m/e 198)	$HOOCCD_2CD_2CD(NH_2)COOH$	$HOOCCD_2CD_2CD=\overset{+}{N}HCOCF_3$ (m/e 203)
LYS	$CH_2=CHCH_2CH_2CH=\overset{+}{N}HCOCF_3$ (m/e 180)	$NH_2(CD_2)_4CD(NH_2)COOH$	$CD_2=CDCD_2CD_2CD=\overset{+}{N}HCOCF_3$ (m/e 188)
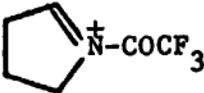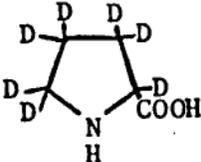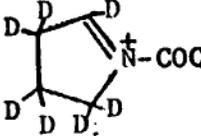
Fig. 1. Standard curve for the quantitation of Glutamic acid.

Fig. 2. Typical ion chromatogram of soil amino acids.

Fig. 3. Mass Fragmentography MS for Quantitation of Ala, Val and GLY.

Table II.   ANALYSIS OF AMINO ACIDS IN SOIL (µg/g SOIL)

Amino Acid	Amino Acid Analysis	Mass Fragmentography		
		#1	#2	#3
Ala	206.5	198.7	202.7	198.3
Val	148.3	151.0	150.5	149.9
Gly	215.4	196.8	201.6	201.3
Ileu	95.4	100.4	100.2	92.3
Leu	154.2	152.1	149.7	154.2
Pro	143.4	141.4	142.8	141.2
Phe	80.3	80.5	80.7	80.0
Asp	218.3	217.1	219.8	219.9
Glu	227.0	217.2	215.6	214.1
Lys	129.7	115.3	113.5	114.9
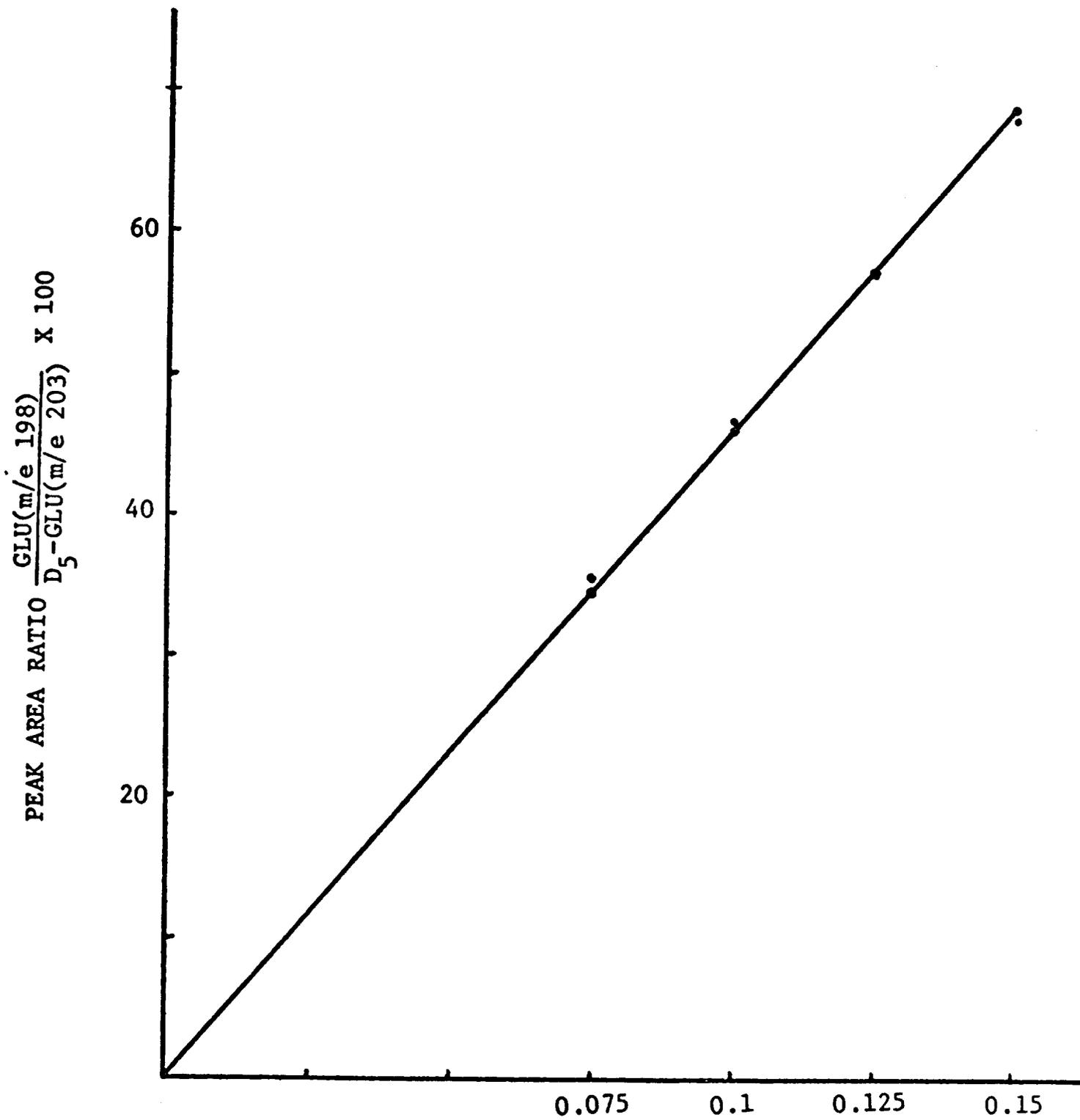
Fig. 1.

$\dfrac{GLU}{D_5-GLU}$ Added

FIG. 2.

ION CURRENT

SPECTRUM NUMBER

ALA VAL GLY THR LEU PRO PHE ASP GLU LYS

1 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500 520 540 560 580 600 620 640 660 680 700 720 740 760 780 800 820 840 860 900

FIG. 3

Ala D$_4$

Ala D$_0$

Val D$_8$

Val D$_0$

Gly D$_2$

Gly D$_0$

p-619
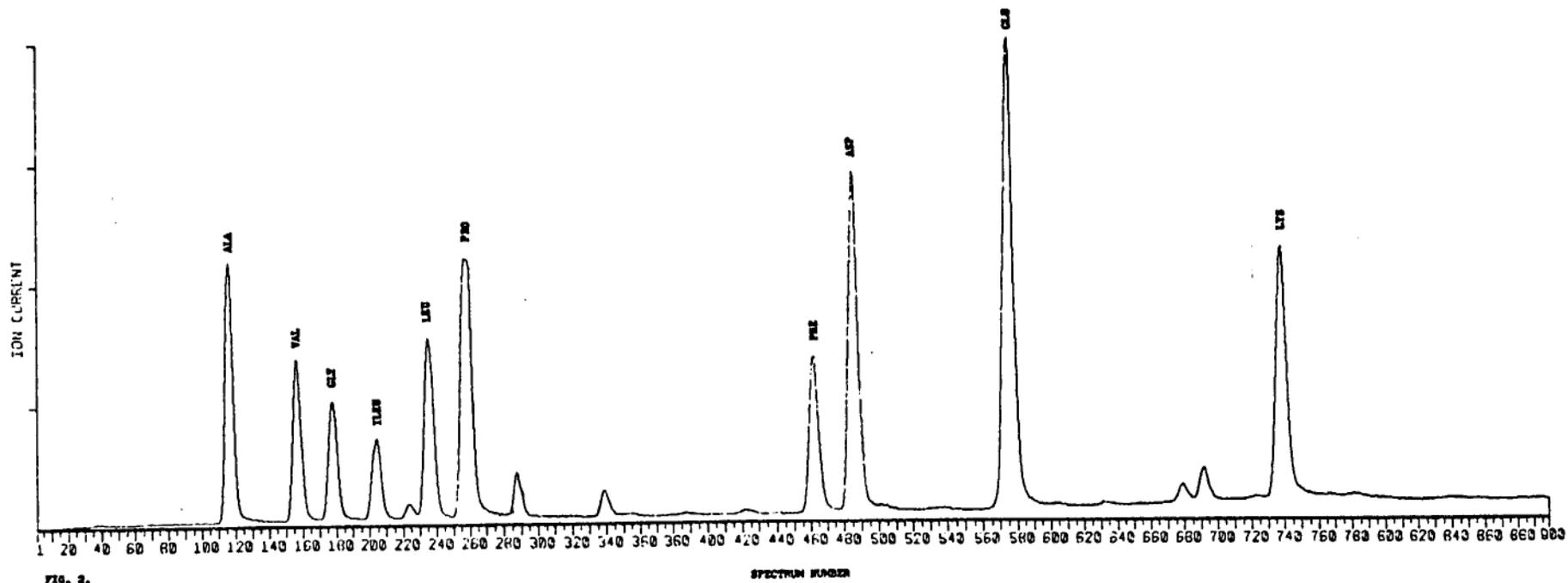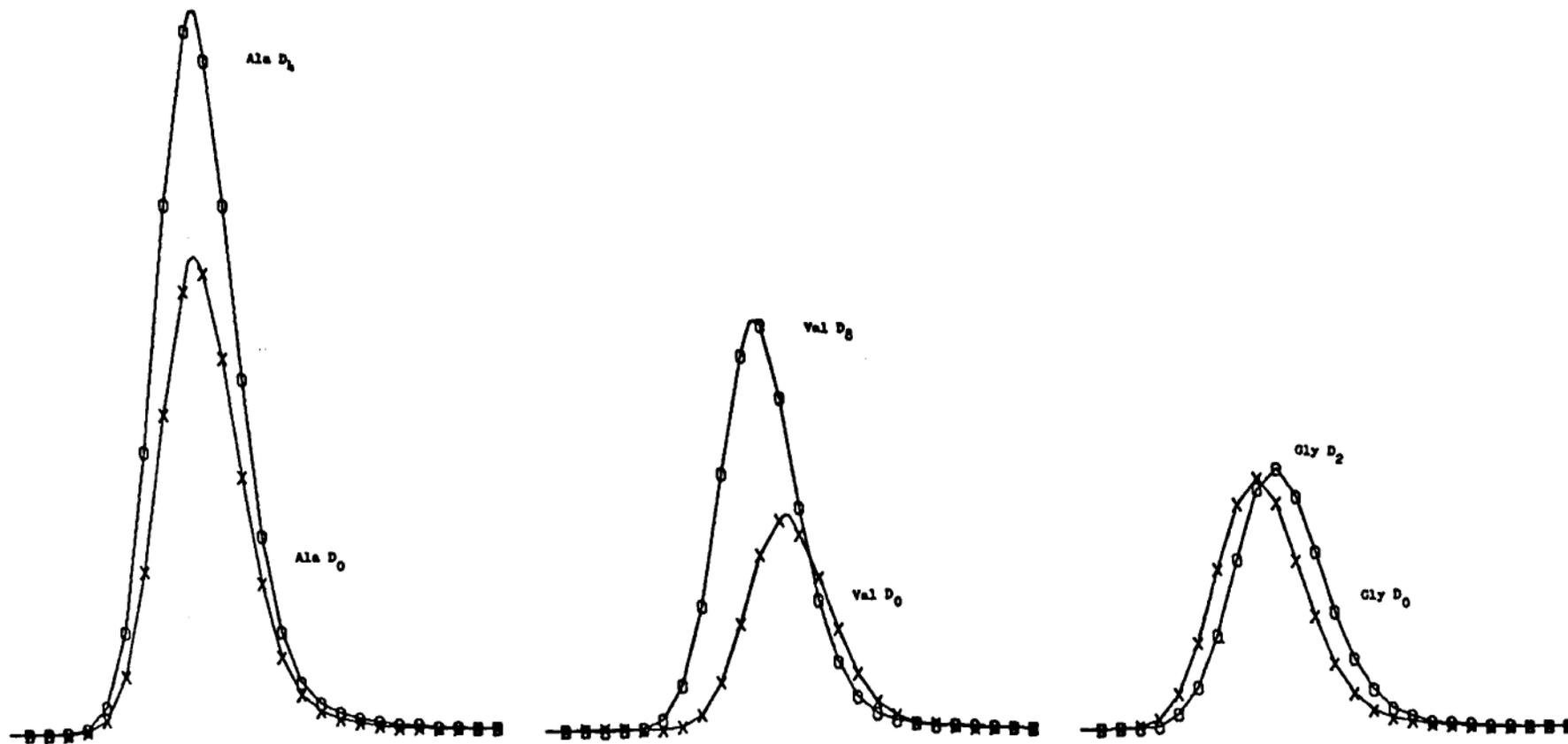
A Computer Operated Mass Spectrometer System

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An integer resolution mass spectrometer–computer system has been developed in which the computer controls the "scan" of a mass spectrometer. In this system, the computer queries the user for operating parameters which are then translated into control functions which operate the mass analyzer. The spectral information acquired from the mass spectrometer is made available to the chemist within minutes in an on-line graphic system. Examples of the processing of GLC effluent are given.

THE USE OF MASS SPECTROMETRY has been hampered by the lagging development of a fast and convenient method of reducing the spectral output of the mass spectrometer (MS) to numerical data. Usually the operator must convert a MS chart recording, which is an analog plot of intensity $vs.$ time, to a digitized plot of intensity $vs.$ mass number. Because of instrument instabilities, wide range of signal amplitudes, large amounts of data, and other operational difficulties, (1, 2), it is often difficult and time-consuming to establish all the correct mass peak identifications. One aid is to use a reference compound (3) either prior to the run or as an internal standard with the unknown sample. By counting from known mass peaks, unknown spectral peaks can be identified. However, the processing of data by this technique is still a formidable task and it may take several days to accumulate all the information from a gas chromatograph–mass spectrometer (GLC–MS) run.

Several workers have demonstrated MS-computer systems in which the computer monitors and records digital data from a MS. In most of these applications the mass spectrometer has operated independently of the computer, scanning in some time dependent mode, measuring ion intensities at all points within the range of (500 to 5000 samples per second) and afterward performs the computations required to reduce the large amounts of digital data to useful information (4–7). Much instrument time and sampling effort is expended in the intervals between integer peak positions where there is little or no information. One system that improved upon this latter inefficiency used step switches to step the scan from position to position (8).

We now describe a MS-computer system, suitable for routine laboratory use, in which the computer controls the operation of a quadrupole mass spectrometer (9, 10). In this system the "scan" is calibrated by relating known mass positions of a reference compound to a computer generated control voltage (V_c). R_c is generated as the result of a number N, sent from the computer to a Digital-to-Analog (D-to-A) converter in a MS–computer interface. The parameters of this V_c, or the N for each integer mass position, are determined by a computer program and stored in memory. The subsequent use of this information allows the computer-directed MS output to be recorded directly as mass-charge (m/e) $vs.$ intensity. On request, this data is then made available to the operator in an on-line system.

The use of this computer–MS interaction, combined with the decision-making ability of the operator, permits a significant saving in data processing costs. Furthermore, a much larger duty cycle of analyzer "on peak" time is obtainable, resulting in the detection of more ions for a given mass position than is possible in conventional time based scanning.

The new MS-computer system has at least three unique features. There is a hardware control interface to connect the MS intimately with the computer; there is an improved efficiency of information acquisition from spectral peaks that are limited in ion production rates; and there is a user-oriented control and data presentation system that conceals the foregoing details from the operator, but presents the user with prompt and concise data which include normalized mass spectral plots.

The described system has evolved through three mass spectrometers, three computers, and two basic computer programs (11, 12). The later systems have greater range, sensitivity, and convenience, but they all have a common concept. Therefore the description that follows will be conceptual rather than specific to any one configuration.

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INSTRUMENTATION

The present system is operating with a Finnigan 1015 quadrupole MS and a Varian Aerograph 600D chromatograph. The same computer programs and a similar interface were also operated successfully with a Bendix Time-of-Flight (T-o-F) MS (13) and an EAI quadrupole (14) MS. In all cases the GLC–MS, the teletypewriter, and the digital plotter were situated in a wet chemical laboratory.

A schematic diagram of the GLC–MS combination is shown in Figure 1. The effluent from the gas chromatograph, equipped with a flame ionization detector, first passes through a variable splitter that diverts between $^1/_3$ and $^1/_2$ of the flow through a Biemann separator (15) and into the MS. A solenoid-actuated valve in this line helps to keep the large initial solvent peak from entering the MS system. A reference gas reservoir containing a fluorine compound at a vapor pressure of approximately 3×10^{-7} Torr is also incorporated in the system and is connected to the MS by another solenoid valve. The computer, *via* the interfacing electronics, has direct control of gas valves, and can valve in or shut off the reference gas whenever it is needed for the calibration routine.

These valves were constructed in our shops in such a way that the back side is open to the vacuum system when the valve is closed. This avoids the common pressure burst when conventional valves are opened to a vacuum.

The right side of Figure 1 illustrates the major components and functions of the interface. This computer–MS interface was built in our Instrumentation Research Laboratory (16) and contains all the electronics not normally supplied with a standard configuration MS or computer. All of the operating parameters of the MS are, or may be, controlled by a digital word (binary number) sent from the computer. The principal control is *via* the "N" register to the D-to-A converter. The analog signal, V_c, from the D-to-A sets and holds the mass analyzer to pass ions of a predetermined *m/e*. Alternately the digital output may be coded to operate auxiliary control functions, such as actuate valves, set amplifier gains, the low speed multiplexer, or enable the digital plotter.

The characteristic method of controlling the *m/e* passband and taking measurements while the mass analyzer dwells upon a *m/e* value converts what is normally measured as a time dependent parameter, to a stationary signal. This statistically stationary property of the signal enables the employment of full integration to enhance signal to noise. Both the electrometer and the integrator are standard commercial FET operational amplifiers of the \$50.00 class. The time allowed for integration and the operation of the integrator reset are controlled by signals (numbers) from the computer to the "T" register. The output of the integrator is sampled, held, and read *via* the Analog-to-Digital (A-to-D) converter.

Auxiliary signal sensing is provided by the low speed multiplexer. This is useful to determine the automatic settings for self calibration, or may be used to record temperature, pressure, etc. These sense functions, plus some valve

(13) D. B. Harrington and R. S. Gohlke. "High Resolution Time of Flight Mass Spectrometers." 10th *Ann. Conf. Mass Spectrometry Allied Topics*, ASTM E-14. New Orleans. La., 1962.

(14) W. Paul, H. P. Reinhard, and U. von Zahn, *Z. Phys.*, **152**, 143 (1958).

(15) J. T. Watson and K. Biemann, ANAL. CHEM., **37**, 844 (1965).

(16) W. E. Reynolds, J. C. Bridges, R. B. Tucker, and T. B. Coburn, "Computer Control of Mass Analyzers." 16th *Ann. Conf. Mass Spectrometry Allied Topics*, ASTM E-14, Pittsburgh. Pa., 1968.

Figure 1. The GLC-MS instrumentation and the electronics interface to enable computer systems integration

control and checkout functions, are controlled by the "C" register.

There are no manual operator control functions in any of the above steps. The control is accomplished at the teletype writer keyboard. This keeps the system flexible and makes it independent of the idiosyncrasies of individual computer

COMPUTER DIALOGUE *(The user's response is underlined)*	COMMENT
OPTION = collect	The user requests the data collection phase.
EXPERIMENT# = test	A catch-all name, "test", is given; the spectrum will be used simply for a systems check.
T = 35	An integration time of 35 milliseconds per peak is requested.
MODE = single	Only one spectrum will be taken (single mode). The data is acquired after this answer.
PLOT, TYPE, or FILE = plot	The user can plot, type, or file the data collected; here a plot is requested.
BY MASS, AMP, or ENTIRE = entire	The user can plot selected masses, the highest intensities, or the entire spectrum within requested limits.
FROM MASS = 1 TO = 510	The user indicates the limits.
QUICK = n	A QUICK plot omits annotation, etc. Note that "y" and "n" mean "yes" and "no". The user completed the checkout and now wishes to proceed with the experiment.
PLOT, TYPE, or FILE = /	The "/" is used to backup through the conversation.
T = /	
EXPERIMENT# = MS26x15	An existing experiment name is given here.
CONTINUATION = yes	The user confirms that the spectra are to be added to the existing experiment file.
T = 6	
MODE = continuous	The user requests that spectra be collected continuously until 130 are taken.
# OF SPECTRA = 130	
FILED IN POSITIONS 940 to 1070	Data collection is complete.
T = /	
EXPERIMENT# = /	The user then wishes to sum the elements of each spectrum to produce a "total ion" plot, analogous to a GLC trace.
OPTION = sum	
EXP# = MS26x15	
FROM SPECTRUM = 940	
TO = 1020	
FROM MASS = 40	Mass position 40 to 500 are summed for each of the collected spectra.
TO MASS = 500	
PLOT = yes	The "total ion" curve is now plotted. (Figures 6 and 7 are illustrative of this sample dialogue.)
EXP# = /	Guided by the total ion plot, the user will plot interesting mass spectra.
OPTION = plot	
EXPERIMENT# = MS26x15	Only the spectrum filed in position 957 is chosen.
FROM SPECTRUM = 957	
TO = 957	
FROM MASS = 1	The plot (Figure 17) is drawn and normalized to the base peak, m/e = 31.
TO MASS = 340	

Figure 2. An example of the user–computer dialogue during operation

interrupt lines and/or individual computer characteristics. This straightforward system definition makes the software design much like conventional computer programming rather than encouraging intricate techniques highly dependent upon the specific hardware.

Thus the system is not oriented specifically to any given computer. It has operated on an early model LINC (*17*) computer with 2K words of 12 bits, memory, and on a time-shared, locally programmed, IBM 360/50, buffered with an IBM 1800 (*18*). In all cases the computer was somewhat remote, separated by some 500 ft of cable from the rest of the instrumentation. The system is very economical of computer resources. Most of today's small general purpose computers would be able to operate the described functions if it were desired to avoid time-shared computer dependency. Some

sort of magnetic storage for object code programs and data storage is most desirable. DEC-type tapes have been used on the LINC system and disc packs on the IBM system.

THE SOFTWARE STRUCTURE

The objectives of the software are to operate and control the MS, acquire data from the MS, process and present this data in a manner useful to the chemist, and provide certain control and information to aid in maintaining and servicing the instrument.

With the program loaded into the computer, the user requests any one of several functions (see Table I) by typing the name of that function. The computer responds with a series of prompts (see Figure 2) to elicit user macrocommands. The computer then generates the detailed control functions to perform the assigned task. At the completion of the task, requests are made for new parameters. By striking the slash ("/"), the user can "backup" through any conversation to correct errors or to go to a different function. This conversation technique makes the system both flexible and reasonably self-instructing.

(17) R. W. Stacy and B. Waxman, "Computers in Biomedical Research," Vol. II, Academic Press, New York, N. Y., 1965, pp 35–66.

(18) W. J. Sanders, G. Breitbard, G. Wiederhold, *et al.*, "An Advanced Computer for Medical Research," *Fall Joint Computer Conf. Proc.*, ACM, Anaheim, Calif., 1967, p 497.

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Table I. A List of Program Options

(1) **CALIBRATE:** Creates an accurate N Table. The N numbers which correspond to the peaks in the reference gas are used as the end points of a piecewise linear interpolation procedure for calculating a complete N Table.

(2) **COLLECT:** Is the primary data collection step. It is here that the 750 N Table values are sent to the MS and the 750 m/e intensities recorded. This operation can be repeated at five-second intervals as the data are filed on disk under an experiment name.

(3) **TYPE:** Allows the user to print out spectral data by indicating what spectra in a given file are to be reviewed. The user can request that the amplitudes of particular m/e positions be typed; that a given number of the highest amplitudes be typed, or that a consecutive number of them over a given range be typed.

(4) **PLOT:** Enables the user to have bar graphs produced by the computer controlled digital plotter. The amplitudes to be plotted can be selected with the same flexibility as described in TYPE.

(5) **SUM:** Produces a plot of the total ion current over a series of gathered spectra. All responses of a spectrum are summed to produce one datum point on the plot. This plot corresponds closely with the GLC output when running with the GLC.

(6) **TRACE:** Produces a record of a spectrum similar to the normal chart recording output. The analyzer is sampled at all N values (about 10 per amu) over a given range and the result is plotted as a "broken line." (Used for system check out)

(7) **MONITOR:** Provides for inspecting the peak profiles by sampling the spectrum around a given m/e position. The gathered data are then typed out. (Normally used for system service or service log)

(8) **DISPLAY:** Enables the user to display a given mass position (or N number) in the center of the console oscilloscope. (Used in the adjustment of the mass spectrometer)

(9) **GAS:** Allows the user to remotely turn the reference gas on or off. This is helpful when operating the system from a remote position.

The example of a user–computer conversation given in Figure 2 represents the day-to-day computer-researcher dialogue given to direct the system's operation. Deeper level programming may be done at the terminal to redefine these functions or to add new modes. Additional system development may be done by the chemist-user, or his programmer in a manner typical of general purpose computer software. In normal daily practice, the user first requests the calibrate function and then proceeds to data acquisition, analysis, and presentation. Usually the calibration is done once every four hours.

It is this calibration subsection of the program that assigns to each integer mass position a value N which when sent to the D-to-A converter in the interface, will set the mass analyzer to pass that particular species of m/e. During this calibration phase a reference compound (perfluorotributylamine, FC-43), is introduced into the MS. The calibration procedure in addition to determining the N values, makes data available that will aid the operator in making qualitative judgments about the stability, sensitivity, and resolution of the MS. Also a service or maintenance record plot is available (see Figure 3), that, at least indirectly, shows these and other important instrument conditions. Figure 3 is actually 12 traced segments of a complete spectra, each segment covering a span of about 4 amu and each taken at a different integration time (gain). The m/e value, its position, and a parameter indicating the gain is automatically printed below each peak. The date and time is printed by the computer, but at present the operator must insert the sample pressure and ionization parameters. The file of these plots represents an excellent record of the instrument's serviceability. The calibration is automatic and its use less complicated than the description. It takes about 5 minutes, after which the reference gas is pumped out of the system. In the IBM 360-1800 system, the time is used to compile the main program.

M	31	69	100	131	169	219	264	414	464	502	576	614
N	415	1272	2090	2587	3327	4348	5381	10362	11402	12223	13637	14457
T	4	.0690	.7900	.2800	1.300	.1200	.5800	2.800	2.700	2	0	8

PRES ___3×10^{-7} torr___   T FACTOR   40

MICA · ___40 μa___   123351   time

IONV ___20 volts___   691218   date
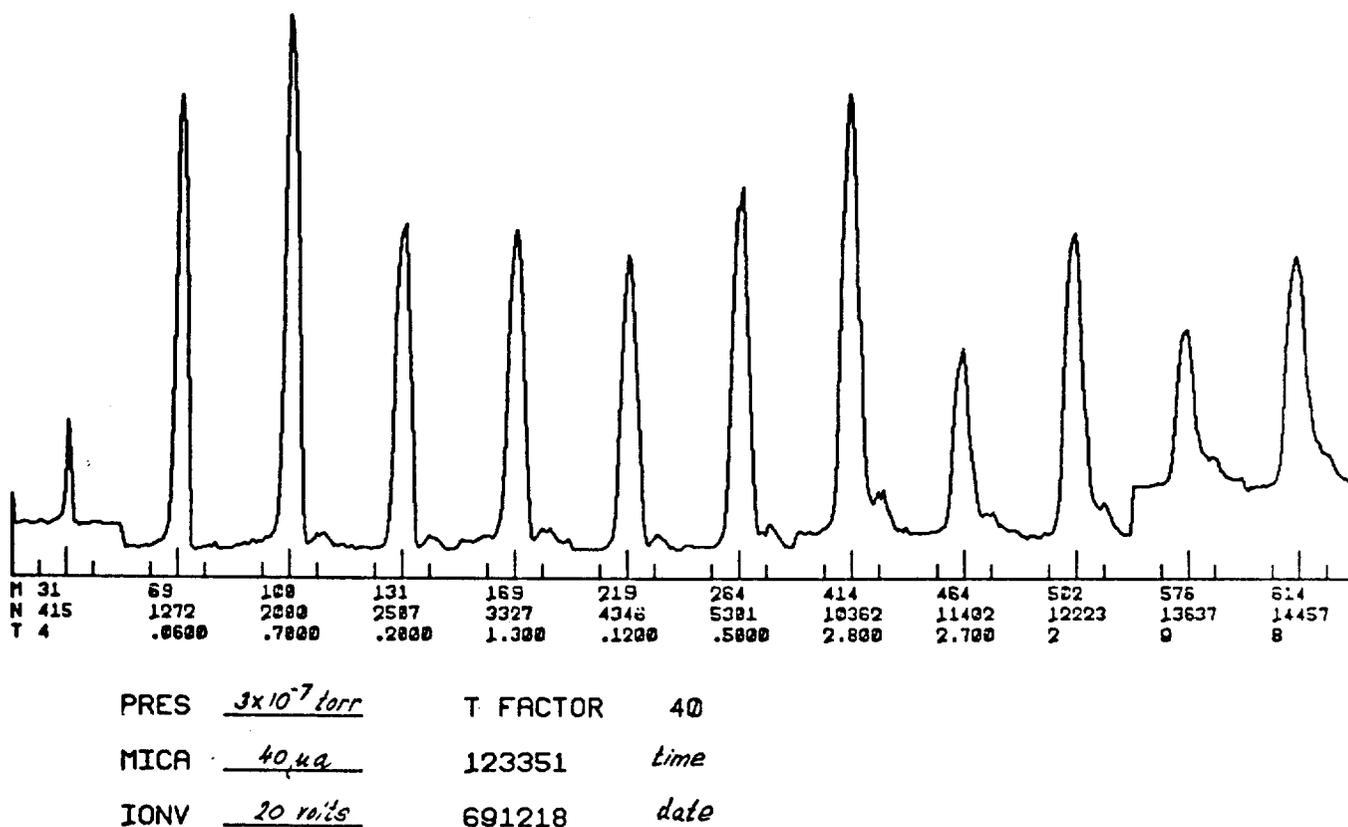
Figure 3. A monitor plot indication of instrument serviceability

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Total spectral data acquisition time depends upon the number of m/e positions measured and the integration time allowed per position. It may be calculated:

$$\text{Spectrum acquisition time} = P \times (T_t + T) \qquad (1)$$

where P is the number of m/e positions to be measured (they do not have to be contiguous or sequential), T_t is a transition time (2 msec in our system), and T is the integration time per peak (nominally 6 to 17 msec, but we have usefully used 1 to 1000 msec).

Normally data are collected at each integer m/e position 1 through 750. The 750 N values are sent through the D-to-A converter to the MS and the 750 responses (a full spectrum) are recorded by the computer *via* the A-to-D converter. This process can be repeated approximately every 5 to 10 seconds for an arbitrary number of times. The spectra thus gathered are stored by the computer on magnetic disks or tapes. Program changes may be made to measure any subset of the 750 m/e positions and thus achieve faster repetitive spectra. Conversely more measurements may be made at any specific peak position, a technique which may be used for accurate isotopic ratio measurements.

Since many spectra are taken and stored during a GLC run or a solid probe experiment, the user requires fast methods to evaluate the data. The more useful data abstracting programs we use are:

THE MATRIX SEARCH. The user specifies which group of spectra, what range of mass values in each spectra, and how many large peaks he wants abstracted from each spectrum. An abstract of these highest peaks is then typed out and in many cases this abstract contains useful chemical information or at least indicates the spectrum of interest.

THE TIME PRESENTATION (PLOT). This is a computer drawn plot of certain peak intensities or a sum of all peak intensities of each spectrum (total ion current) plotted against time. The latter gives a good reproduction of the GLC curve and also indexes the spectra of interest (*19*).

NORMALIZED SPECTRUM PLOTS. Conventional bar graphs of mass *vs.* time, normalized and annotated, are routinely available.

All data outputs are in the laboratory and are available immediately after data acquisition. All spectra are filed and may be recalled at a later time or date and reprocessed in any way desired.

Involved programs of these magnitudes are specifically dependent upon the language of a given computer. The logic may be easily transferred, but in general the specific program may not. We have about 4 man-years of programming invested in this system.

THEORY OF OPERATION

During spectrum data acquisition, the computer directs the mass analyzer to a program-selected mass position and reads the output intensity of the MS. The mass analyzer is not swept in a conventional sense. As indicated in Figure 1, it is controlled by a voltage (V_c) such that

$$m/e = f(V_c) \qquad (2)$$

where m/e is the mass/charge ratio and $f(V_c)$ is a monotonic function characterized by the MS. For every M_i, ($M = m/e$), to be passed by the mass analyzer, the computer has (according to the prior run calibration program) a digital number N_i

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(19) R. A. Hites and K. Biemann, "Advances in Mass Spectrometry IV," Elsevier Publishers, Amsterdam, 1968, p 37.

which is transformed by the D-to-A converter to the voltage V_{c_i}.

The determination of these values, N_i, is accomplished by the calibration program. The value of N for 12 key peaks of the reference compound are known to approximately 1 amu from prior calibrations. The actual N value for the centroid of each of these peaks is then determined by detailed examination of the m/e continuum in each of these areas. Sufficient detail is obtained by designing the D-to-A resolution to be 10 or more values per peak width.

After determining these exact 12 N values, linear interpolation, superimposed upon the analytical function, $m/e = f(V_c)$, is used to expand the list of 12 experimentally determined values to a full table of 750 entries. (The analytical function of m/e to control voltage is linear for the quadrupole and parabolic, $m/e = k(V_c^2)$, in the case of the T-o-F MS.)

Thus the procedure to measure the intensity at any M_i is as follows: *a.* The number N_i which corresponds to the selected m/e ratio (M_i) is loaded from the computer into the D-to-A converter. This sets the control voltage, V_c, to the mass analyzer. The output of the mass spectrometer is proportional to the quantity of ions, M_i, passed from the sample.

b. An analog circuit, reset and released by the computer, integrates the output of the MS.

c. Several milliseconds after the integrator is released, (the choice of integration time was initially supplied by the user upon program request), the computer samples the output of the integrator by means of an A-to-D converter. This digital value is stored as the intensity of M_i.

Steps (*a*) through (*c*) are repeated to acquire a complete spectrum.

The fundamental restraint upon this system is the drift of the function $m = f(V_c)$ following calibration. Our experience with the Finnigan 1015 and a Bendix T-o-F instrument and our interface, is that this drift causes an error in N of less than $1/8$ the value from one N entry to the next in a 1-hour period. This is sufficiently small to allow an unambiguous mass identification.

Table II contains comparisons of signal-to-noise ratios and the following defined figures of merit. The comparisons are made between the described control system, a linear scan in time, a parabolic scan in time such as the T-o-F, and the exponential time scan characteristic of magnetic instruments.

Uniform conditions are used to give realistic values for comparison; it is assumed that in each case the peak shapes are uniform if scanned in time, and that they are gaussian, and that the resolution is commensurate with the 10% valley (5% points on a single peak side) definition (*3*). In order to give typical comparison figures, it is further assumed that a spectrum will be taken from mass 50 to 500 in 4500 milliseconds.

The first column in Table II is the time the mass analyzer is on or about the mass position. In the case of the computer control system, the 4500 milliseconds is divided equally into 450 periods of 10 milliseconds each. Two milliseconds are allowed for each transition, and the mass analyzer will dwell on the peak position for 8 milliseconds. In the case of a conventional linear scan, the analyzer will enter a peak area and leave it 10 milliseconds later. By the 10% valley convention, this means the time from the beginning 5% level to the end 5% level of a single peak. However for the parabolic case (the T-o-F) it will be found that the resolution of the instrument will have to be set for the work case, peaks 499 and 500. It will be found that there is 6.6 milliseconds between these peak

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Table II. A Comparison of Attributes Affecting Signal-to-Noise Efficiencies

Typical Operation Condition: Scan from m/e 50 to 500 in 4500 Milliseconds

Type of scan	Time on, or between 5% points, of a peak, msec	Time constant of amplifiers, msec	Ions detected: (Peak intensities of n ions/msec)	Effective noise bandwidth, Hz	Figure of merit: $1000/n$ × detected ions/bandwidth
Control and integrate	8	N/A	$8.0\,n$	40	200
Linear scan $m = kt$	10	2	$5.1\,n$	80	64
Parabolic scan $m = kt^2$	6.6	1.3	$3.4\,n$	120	28
Exponential scan $m = m_o e^{kt}$	3.9	0.8	$2.0\,n$	200	10

centers, and the theoretical 5% heights must be at the midpoint. Since all peaks are similar, all peaks of the scan will be just 6.6 milliseconds from 5% point to 5% point. During 34% of the scan time, the analyzer is not in the area of any peak position at all; but is mostly between peaks in the low mass range. The case of the exponential scans is similar. In this case it will be found that the peaks are 3.9 milliseconds wide and 61% of the time no information can get through the instrument.

The next column indicates the time constant (τ) of the amplifier channel appropriate to the scan parameters. The control system uses a full integrator, so the entry is not applicable. In the conventional scanning system, the time constant is usually chosen as large as skewing permits to integrate signal and discriminate against noise. The relationship between τ and the 3-db bandwidth (f_{bw}) of an amplifier is simply $\tau = 1/(2\pi f_{bw})$. If τ is chosen to be large, peak skewing and broadening as illustrated in Figure 4 will occur. If τ is chosen small, the bandwidth with its attendant noise is excessive and there is little integration of the signal.

This is the dilemma always faced by the user of linear amplifier circuits: the desire to limit amplifier bandpass to smooth the signal, as opposed to the need for a wide bandpass to pass

the signal without distortion. Since the purpose here is to compare our described amplifier and integrator system with conventional linear amplifiers, a τ of 0.2 is assumed for the conventional case. This τ is still large enough to cause degradation of resolution in the conventional output signal (25 to 35% depending upon the definition used). It is felt that this choice represents a fairly typical operational parameter. The assumption of a rigorous lower value would result in an unnecessary, and perhaps unrealistic, comparison advantage for the described control and integrate signal system.

The column "Effective Noise Bandwidth" is the f_{eq} for the time dependent scans. However an equivalent 3-db bandwidth is not as well defined for the integrator. It can be shown that for an integration interval, T, (8 milliseconds in this example) an f_{eq} may be determined such that a linear amplifier of bandwidth f_{eq} would pass the same amount of "white" noise as the integrator. The actual bandpass of an integrator is a $\sin(x)/x$ type function.

The white noise power passed by either system may be expressed as an integration of the white noise model, e^{-x^2}, (20)

(20) W. B. Davenport, Jr., and W. L. Root, "Random Signals and Noise," McGraw-Hill, New York, 1958, p 88.
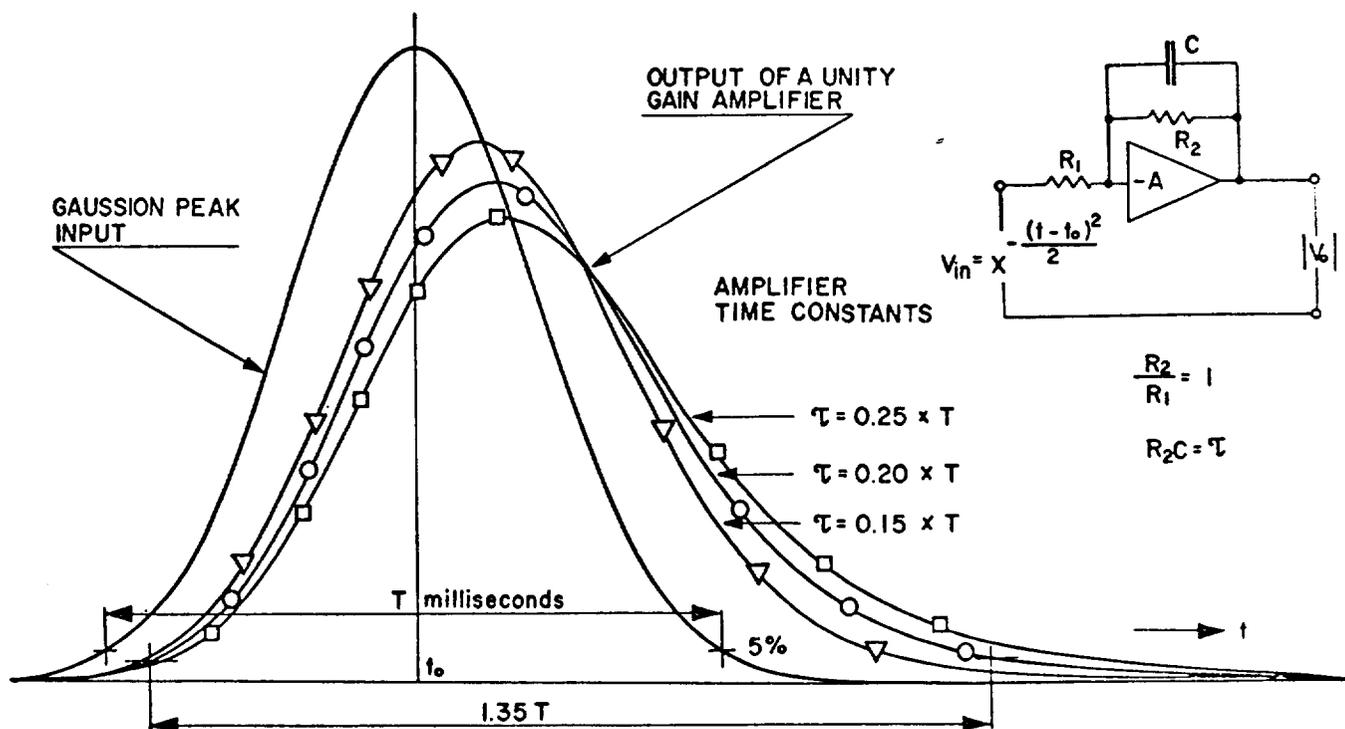
Figure 4. Peak broadening and skewing effect of narrow bandwidth amplifiers