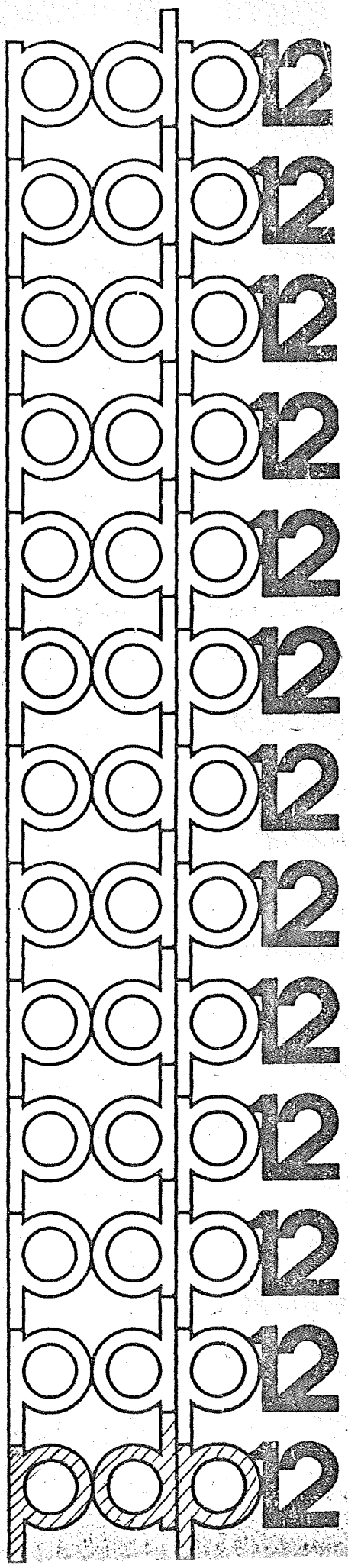
digital

TCKRAM

HIRES-MS



DEC-12-[redacted]-D

March, 1973

DEC-HIRES-MS

HIGH RESOLUTION MASS SPECTROMETER DATA SYSTEM

I. INTRODUCTION

HIRES-MS is a data system designed to interface to and acquire data from a high resolution mass spectrometer. The first phase is the acquisition of data and thresholding it such that only the peak envelopes or profiles are retained and written onto a mass storage device. The second phase of processing consists of reducing the peak profile to an ordered file of peak information consisting of the centroid, the intensity, the width, etc. The third phase is conversion of this into a mass-intensity file. The final phase is creation of an elemental composition table. The latter consists of choosing elemental compositions, including CHO & N and up to six other hetero atoms, that fit within a specified mass window and printing out all possibilities for a given mass as found in the data file.

The data acquisition portion of the system is based under the OS8/12 operating system using a monitor called OS/RT.

The data acquisition program is designed to acquire data from the AIP-12 analog subsystem at the rate of 10KC. After thresholding, the data is transferred to the mass

storage device (disk or LINCtape), where it is kept in a permanent storage file. The remaining reduction operations and printing of data, etc. are all performed under the RTPS FORTRAN system which runs with OS12. The calculation of centroid, areas, fused peak determination, mass calculations and elemental composition are all performed using FORTRAN IV.

II. THEORY OF OPERATION

The system is composed of effectively four operating programs, the first of which is acquisition creating the raw peak profile information in a stored file. The remaining three programs in FORTRAN IV reduce the peak envelope data to peak areas versus centroid, calculate masses and perform elemental composition calculations. These three are loaded by the RTPS Linking Loader as single program files. The files so used are loader image files built as described in Chapter VII. Three such files constitute the remainder of DEC HIRES-MS. The first, HRMSI, is used to perform the peak analysis of the raw data created by HRACQ and create the peak data output file containing the intensity (area), centroid, Hall voltage reading, peak type (fused or not), width (number of points from beginning to end of peak) and the maximum height observed over the peak.

HRMS2 uses the results of HRMS1 and a standard or mass calibration file to match up internal standard peaks in the data to chosen calibration peaks in the standard file thus defining the means of converting centroids to masses. Here, also, is wherein all the standard peaks are removed from the peak datafile.

HRMS3 supplies the final processing functions of intensity normalization, elemental composition calculations and building of standard or mass calibration files. These are special insofar as they are runs of pure standard components chosen such there is minimal interference with expected masses in the unknown - samples which will be calibrated by that standard. Although not required, HIRES-MS is geared to use perfluorokerosene (PFK) as the primary standard ingredient. Other materials can be added to enhance the mass coverage as desired. HRMS3 allows easy insertion of any and all desired CF fragments as calibration peaks and also allows entry of exact mass for other desired calibration peaks.

III. ACQUISITION

The first section of code, the acquisition module, is called from the OS12 monitor by typing:

.R HRACQ↵

RHACQ then types:

HRMS-12 V.1

and then brings up the command decoder of OS12 which prints an asterisk on the left margin. The user should then enter the output file name followed by a left arrow and return key.

*(output file) ←↵

When activated, first question asked is

SCAN TIME?

This is a value of between Ø and 999 in seconds. The second question is

DELAY TIME?

also with values of Ø to 999 in seconds. At this point in time, the program takes control of the mass spectrometer and will begin dummy cycles in order to equilibrate the magnetic field. Meanwhile,

THRESHOLD?

is requested from the user to which he can respond to the value of Ø to 999 in ADC units. The value should be greater than 1Ø. A minimum of 1Ø dummy cycles must be completed prior to each scan. A scan is then requested by typing G. Only if typed during delay time will the next scan be used. After completion of the acquisition scan, HRACQ will close the data file and exit back to OS12. If another scan is desired, HRACQ is again called and the output file specified, and a scan time, delay time and threshold specified.

relay light remains on Stopped

If, instead of normal exit to OS12, this error message is printed:

DATA BUFFER ERROR

the threshold value was too low and/or the ion intensity was so noisy that HRACQ could not write out data fast enough. The run is aborted and no output file is created. Try again using a larger threshold value.

The resultant peak envelope file contains for each peak the time at which threshold was penetrated and the Hall voltage reading at that instant, followed by a number of data points describing the peak envelope. Each peak has the same logical format.

| User responses to system are underlined.

IV. USE OF FORTRAN PROCESSING PROGRAMS

When a desired number of scans have been completed and assembled into the appropriately named output files, the FORTRAN IV loader is called by typing

.R LOAD↵

It responds with an asterisk on the left margin to which the user supplies the loader image file previously created followed by /D. This informs the loader to bring this file

*FILE/D↵

into core and then allow the user to specify the physical devices and file names to use for processing.

A. Use of HRMSI

For the first phase of processing, i.e., that of peak detection, integration, centroid determination, etc., specify

. R LOAD↵
*HRMSI/D↵

This is then followed by the input data file,

*(input raw data file)/5/C↵

and the output peak data file

*(peak output file)/6↵

After loading, CMD: is printed to request a command. Typing

PP↵

requests the system to search the input data file for peaks and create the output peak data file. Typing

LS↵

requests that that information be printed on the output line printer or teletype.

If PP was typed, the peak processing phase is entered. This requests

ENTER TITLE, SENS., & BASELINE:

At this point, the user is allowed to specify a 6Ø character alphanumeric title to be appended to the data file. Enter TITLE information followed by RETURN.

SENS is the minimum number of points required on each side of the peak. Values of 3 to 1Ø are nominal. Type the desired value, a decimal point and RETURN.

Then enter BASELINE, a decimal point, and RETURN. Baseline is a value of Ø-2ØØ or so to be subtracted from the data. Typically, baseline is chosen slightly less than 8 times the threshold used during acquisition.

The peak envelope input file will be fully processed creating a complete output file with masses of Ø for all peaks. After completion of the output peak data file, the program again types

CMD:

Typing LS will create the intermediate print out of the intensity, the peak width, the peak type, the starting Hall voltage, the centroid value and maximum amplitude of the peak. Peak types are Ø for fused peaks ending in a valley; 1 for peaks which ended below the threshold; and 3 for peaks designated as mass calibration peaks. Type 3 will not exist at this time. If the amplitude is Ø, this indicates a badly formed peak without consistent points increasing then decreasing.

After completion of either peak processing or listing, return is made to the beginning, requesting a new command. Typing

EX↵

will cause an orderly exit back to the OS12 monitor. If desired, the peak processing can be again performed, for example, using a different SENS and baseline values.

All numeric input to HRMS1, 2, and 3 assume one value per line with a typed decimal point and terminated with RETURN key. Zero, however, can be entered as Ø without decimal point or, more simply, just the RETURN key.

B. Use of HRMS2

When HRMS1 has successfully completed and exit is made to OS12, the mass calibration program is called by typing

.R LOAD↵

*HRMS2/D↵

Then enter the peak data file and standard file

*(peak datafile)/6/C↵

*(standard file)/7↵

If the files are successfully opened, the program enters the command state

after typing

CMD:

The mass calculation portion of the program is entered by typing MC. HRMS2 types

ENTER DELTA HV, I FACTOR, & DELTA CENT.:

requesting the user to enter DELTA HV (the Hall voltage window wherein to find the first three standard peaks), the I FACTOR (an intensity fraction of the calibration peak wherein to look for the data peak), and the DELTA CENT (the centroid window wherein to identify the standard beyond the first two).

Then a match of the standard file against the data file is performed for the first three calibration peaks based upon the Hall voltage of those peaks using the Hall voltage window and the I factor specified. In order to qualify as one of these standards, a data file peak must match up within the Hall voltage window and within plus or minus the I factor times the standard intensity. If three matching peaks were not found, a message of error 1 or 2 will appear on the teletype. Error 1 implies there are insufficient data peaks in order to match

the standard; error 2 indicates there were insufficient standard peaks matched to the data. If three peaks are correctly matched, all subsequent mass calibration peaks are matched by centroid using the delta centroid window and the same I factor employed previously. After completion of the mass calculation CMD:

is typed allowing another command.

Typing DS requests HRMS2 to match all peaks in the standard to the datafile and remove them from the datafile.

ENTER DELTA MASS & I FACTOR:

is requested wherein DELTA MASS is the mass window and I FACTOR is as defined above. If masses have not yet been inserted into the file, this error message is printed:

PLEASE CALL MASSES BEFORE CALLING STD DELETION

and HRMS2 returns to command entry mode.

If LS is the typed command, this information is printed: mass, intensity, width, type and centroid. After completion, return is made to the beginning of the program to allow new commands. This allows the user to try matching with different Hall voltage, centroid windows, and intensity factor in the event the initial attempt was unsuccessful.

The program remains in this mode, allowing attempts to match standard to data file, until

EX

is input as the command. Exit is made back to the monitor, closing the data file for subsequent use.

C. Use of HRMS3

The third phase of processing is entered from the OS12 monitor in much the same manner as the first and second phases. Type and respond to the asterisk with

.R LOAD↵

*HRMS3/D↵

The third section requires only one data file, this being the peak data file with masses calculated from Section II. , Therefore, respond with that file

*(peak datafile)/6↵

then, CMD: is typed out. Typing in

NI↵

requests normalization of the peak data file to be done. The program requests the

NORM PEAK # AND NORM VALUE

The normalization peak number is the index number printed as the result of a list command Section I or II in order to reference the desired peak for normalization The norm value is the intensity value to which to normalize all other peaks. The routine then commences to find the required normalization peak and correct all intensities relative to the norm value. After completion

CMD:

is printed and another command can be typed. Typing

LS↵

requests print out of the mass, normalized intensity and the peak type on the output line printer or teletype and exit back to command state.

If

EC↓

is typed, the elemental composition routine is entered. This prints

ENTER WINDOW, MAX H-ATOMS, MIN INT, LOW & HI MASS:

which are the mass window in amu, the maximum number of hetero atoms allowable in any fragment, the minimum intensity, and beginning and ending nominal mass limits. Enter one number per line each terminated by RETURN.

Then is printed

ENTER #C13, #0, #N, #CL, #S, #F:

to request the maximum number of carbon 13 atoms, the number of oxygens, the number of nitrogens, the number of chlorine, (equal to both Cl^{35} and Cl^{37}) the number of sulfur and the number of fluorine atoms to be allowed maximum for the fit. Type one number

per line, each terminated with RETURN. Then the user is requested to enter other new isotope names and masses by the message

ENTER UP TO n ISOTOPES: MAX #, NAME, MASS:

"n" is the allowed number after choosing Cl3, O, N, etc. where ten total isotopes, including C and H are possible. Name is a four character colmn coding. Hitting carriage return terminates this input. The program will then commence to fit the sum of elemental masses until a resultant calculated mass is within the window of the real data mass. Once successful, a line of information is printed on the output line printer or teletype indicating the number of carbon, hydrogen, oxygen, nitrogen, chlorine, sulfur, etc. that was found to fit within the mass window. If no combination of the chosen atoms

successfully fits the mass, the message is printed, NOTHING FITS! All possible elemental combinations are tried for all masses until either the number of hydrogens exceeds two times the number of carbon plus nitrogen plus oxygen plus three or the weight does not equal the window. This can result in excessive lines of output if, (1) the window is too large; (2) too many hetero atoms were allowed, i.e., maximum hetero atoms was a large number; (3) an inappropriate choice of kinds of hetero atoms were used. After completion of printout from the elemental composition program, return is made to command mode. Entering new commands allows the user to renormalize the data to a different peak if desired, get an intermediate print out again, and re-enter the elemental composition program.

E. Data Editing

If the user types

ED
DEL

a data editing mode is entered wherein any value within the data file can be

reset. The user is requested to enter the peak number, parameter index, and a value. Peak number is the number of the peaks obtained on print outs from either Section I or Section II. The parameter index is a value of 1 to 7 where 1 is intensity, 2 is mass, 3 is peak type, 4 is peak width, 5 is centroid, 6 is peak amplitude and 7 is the Hall voltage. In practice, one only manipulates intensity, mass, peak type and centroid. The value is the new value to be assigned to the chosen parameter for the chosen peak. If peak number of Ø is entered, return to command state is made. A peak number greater than the number of peaks in the file will create an end of file message and immediate return to the OS12 monitor.

F. Build Standard File

If the user types in

BS ↵

to build a new mass calibration file, C-F compositions are computed and assigned to data peak numbers. The user is requested to enter the chosen peak number,

the number of carbons, and the number of fluorines. From the number of carbons and the number of fluorines, a mass is computed which is then assigned to the peak number input by the user. The data file is corrected by inserting the computed mass for the fluorocarbon fragments and by setting the peak type to 3 thereby designating it a mass calibration peak. If the peak number is entered as Ø or carriage return, return is made to the beginning of the program again requesting the user to enter commands.

six line
carriage rtn

Pk
Ø

V.. PROCEDURAL USE AND HINTS

The first thing to be done is to build a mass standard reference file. This is done by acquiring, via HRACQ, a peak envelope file from PFK (perfluoro kerosene). This data file is then entered to the peak analyzer, HRMS1. The result of peak analysis is then placed into the output file specified at load time with all masses set at Ø. This file should be listed. Then HRMS3 is loaded and the BS command entered in order to allow computation of the fluorocarbon peaks. Proceed as illustrated in Table XXX of the manual. This table lists the masses versus carbon-fluorine elemental composition. Bear in mind that no peaks outside the range of the third and last calibration peaks should be considered reliable although extrapolation will be done to attempt to get masses. Therefore, build as large a standard file as you can totally encompassing all possible masses expected to be seen in samples to be calibrated by this file. If certain other elemental compositions other than carbon-fluorine are to be inserted, this is done with the data editing command. Here the user can refer to the chosen peak number, index 2, and the desired mass value. Also set that peak's type, index 3, to 3. The resultant peak data file will now contain all calibration

masses with each standard peak referred to with a peak type of 3. However, peaks between standards will not have assigned masses at this time. These masses are assigned by making a copy of this file via OS12 PIP and loading HRMS2.

call up PIP current out < file name/I from

To calculate all masses for the mass standard reference file, specify the partial mass calculation file as the standard file and a copy of that file as the data file. Then the file will be matched against itself and masses computed for all peaks between the standards.

PFK STD TRIAL

If all masses are not correctly calculated, this probably indicates masses were incorrectly assigned previously. This situation is rectified by again loading HRMS3 and using the editor to correct the data file, then again calling HRMS2 and repeating the mass calculation until satisfied. The resultant mass calibration file can then be used for all unknown analyses with that scan rate.

Subsequent data runs using this standard file must be run at the same scan rate and preferably for the same time period to avoid hysteresis effects. It is assumed that the

peak intensities of the mass standard reference file be similar to those of the standard peaks appearing in subsequent data files. This assumes that for all runs approximately the same amount of standard compound, i.e., PFK, is within the sample. This also indicates that the standard files should never be normalized. If, because of large differences in standard concentration, the peak matching is unsuccessful, simply normalize the data file to a standard peak with a value such that it is a more near match in intensity to the standard file then re-enter section 2 for mass calculation. The resultant data file can then be renormalized to suit.

Probably the most disastrous occurence during mass calculation is the use of a too small intensity calibration peak or (horror of horrors!) HRMS2 picks up a data peak instead. The former situation can occur if too little PFK was present. This is indicated by a peak amplitude of less than 5Ø units or so and/or a width of less than 1Ø. In that case, mass inaccuracies of .ØlØ amu can occur. The latter case can be corrected by manipulating the centroid and height windows to get separation, resetting the standard peak assignment, or setting the data peak intensity to zero (if unimportant).

VI. EXAMPLES

This is an example using Sections 1-3 of HIRES-MS. First, HRMS1 is called up to do peak processing of a file acquired by HRACQ. Commentary along right margin is for explanation of each line.

```
.R LOAD↵                                  (load HRMS1)
*HRMS1/D↵
*D18/5/C↵                                 (use D18 as peak envelope file, creating
*P18 ←/6↵                                  P18 as peak data file)

CMD:                                      (do peak processing)
PP↵

ENTER TITLE, SENS., & BASELINE:
RUN ON D18 WITH CAL18, 2/23/73            (use this as the title, SENS of 4, and a baseline
4.↵                                        of 280 units)
280.↵
CMD:                                      (then list results)
LS↵

CMD:                                      (and exit)
EX↵
 .R LOAD↵
 *HRMS2/D↵                                (load HRMS2 to do mass calculation)
 *P18/6/C↵                                (use P18 as data file and CAL18 as mass
 *CAL18/7↵                                 std file)

CMD:                                      (do mass calculation)
MC↵

ENTER DELTA HV, I FACTOR, DELTA CENT.:
10.↵                                      (locate first three calibration peaks with HV
.5↵                                        window of 10 units and require data peak be
10.↵                                       within 50% of calibration peak intensity.
                                           Then use centroid window of 10 units)

CMD:                                      (list these results)
LS↵

CMD:                                      (then exit)
EX↵
```

Finally, Section 3 is loaded to do elemental composition calculations.

```
.R LOAD↵                                  (load Section 3)
*HRMS3/D↵
*P18/6↵                                   (use datafile P18)

CMD:                                      (normalize intensities)
NI↵

ENTER NORM DATA PK # & NORM VALUE:
18.↵                                      (to peak number 18 with value of 100)
100.↵

CMD:                                      (LI is illegal command so program returns
LI↵                                        to command state)

CMD:                                      (type correct command to list)
LS↵

CMD:                                      (do elemental composition)
EC↵

ENTER WINDOW, MAX H-ATOMS, MIN INT, LOW & HI MASS:
.008↵                                     (8 millimass window, 30 heterotoms maximum,
30.↵                                       1 as minimum intensity, starting at mass 70
1.↵                                        and ending at mass 300)
70.↵
300.↵

ENTER #C13, #O, #N, #CL, #S, #F:
1.↵                                       (allow 1 carbon 13, no O, N, CL or S but up
0↵                                         to 30 fluorine)
0↵
0↵
0↵
0↵
30.↵

ENTER UP TO 6 ISOTOPES: MAX #, NAME, & MASS:
↵                                         (hit RETURN to signify no other atoms)

CMD:
EX↵                                       (then exit to monitor)
```

VII. INSTRUCTIONS FOR BUILDING HIRES-MS ON RK8 DISK

There are four functions to be performed:

1. Build OS8 on disk
2. Transfer files from LINCtape to disk
3. Build loader images of HRMS1, 2, 3 on disk
4. Build HRACQ core image file (if not present)

Starting from the LINCtape OS8 system, the program BUILD is used to specify the new disk configuration:

1. Load a standard format cartridge into drive
2. Mount the HIRES-MS LINCtape on drive Ø, write enabled
3. Set left switches to Ø7ØØ, right to ØØØØ
4. Press I/O preset and DO switches, then when tape motion stops, press START 2Ø to load OS8 monitor. It will respond with a dot on left margin.
5. Type

   .R BUILD↵

   to run BUILD program. It will respond with a $ on left margin.
6. Type

   $ SYS RK8↵

   to specify RK8 as system device.
7. Type

   $ BOOT↵

   to initialize disk. BUILD will then print DSK=

Respond with RETURN key. Then NEW DIRECTORY? is printed. Respond with YES ↵

Then is printed SYSTEM BUILT

The monitor will then be started from disk, responding with a period on the left margin. This completes building of OS8.

The OS8 program PIP is used to move files from tape to disk.

Type

.RUN␣LTA Ø␣PIP ↵

to fetch it from LTAØ and start it.

PIP responds with an asterisk on left margin. Now to move files over, type

*SYS:<LTAØ:/S ↵

PIP will ask ARE YOU SURE?

Respond with Y ↵. The tape will shuffle for a bit while disk is loaded from it. That completes file transfer. PIP will respond with asterisk. Hit CTRL/C to exit to monitor.

The RTPS program LOAD is now used to build loader images. Call it by

. R LOAD ↵

and in response to the asterisk, type

*HRMS1<HRNI,G2,NP2,NEXTPT/D ↵

to build HRMS1. When asterisk is printed again, hit CTRL/C to return to monitor.

Again, type

.R LOAD ↵

Then, type

*HRMS2 < HRN2,G2,PM3/D ↵

to build HRMS2. When asterisk is printed, again hit CTRL/C and, for the last time, type

.R LOAD ↵

then

*HRMS3 < HRN3,G2/D ↵

and CTRL/C when asterisk is printed.

This completes building of all loader images for the HIRES-MS system. Now proceed via the manual to operate the system.

In order to build HRACQ, five OS8 binaries are loaded and the core image saved as an OS8 .SV file as follows: Type

.R ABSLDR ↵

*OSRTØ2,HRINØ7,CONVT,TIMEC,PKPRØ7$

(where $ = ALTMODE)

OS8 then loads the files and returns to the keyboard monitor, typing a dot on the left margin. Now type

.SAVE SYS HRACQ Ø-7577, 1ØØØØ-11577;Ø32ØØ ↵

and OS8 will save the core image.

If it is desired to use fewer than ten pre-acquisition dummy scans, the HRAC core image can be modified using ODT as follows. Type

.GET SYS HRACQ ↵

then type

.ODT ↵

Ø4256/ 7766 (ODT types out contents of Ø4256 as 7766 and waits for a new value.)

Then type a number according to this table

777Ø for 8 prescans

7774 for 4 prescans

7777 for 2 prescans

ØØØØ for 1 prescan

Enter the number followed by CTRL/C then save the core image:

↑C

.SAVE SYS HRACQ ↵