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# A COMPUTER PROGRAM FOR AMINO ACID ANALYSIS

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SUMMARY

A computer program has been written which qualitatively and quantitatively determines the amino acids in a sample using the data acquired from an automatic amino acid analyzer.

The program is designed in such a manner that the operator has some flexibility in the execution of the program as several options are available. Two internal standards are employed in the analytical procedure and they have also been incorporated into the computer program.

## INTRODUCTION

The increasing demand for amino acid analysis in the field of biochemical research and other inter-related fields has prompted manufacturers to develop amino acid analyzers which are now capable of generating such large amounts of data in a 24-h period that it is no longer feasible to collect manually and calculate the acquired data.

Recent publications on the computerization of amino acid analysis[1–4] have shown how the time necessary for computation has been significantly reduced. Although their programs were acceptable for their needs, the flexibility and capabilities of the programs were somewhat limited. We have written a versatile program to accompany the various experiments which we perform daily. Several options are available in the computer program which may be employed at the discretion of the operator.

The computer program is written in Fortran IV language as implemented by the AL/COM Time-Sharing System (Applied Logic Corporation, Princeton, N.J.). The program should be adaptable not only to other ion-exchange amino acid analyzers but also with some modification to amino acid analysis by gas chromatography.

## EXPERIMENTAL

### *Equipment*

Amino acid analysis was carried out by a Beckman 121 amino acid analyzer (Beckman Instrument, Inc., Spinco Division, Palo Alto, Calif.) equipped with an integrator (Model No. CRS-110A, Infotronics Corp.) and a teletype (Teletype Corp., Houston, Texas).

*Calibration*

Calibration of the instrument was accomplished employing the Type I Standard Calibration Mixture of Beckman Instruments, Palo Alto, Calif., with L-norleucine and L-α-amino-β-guanidinopropionic acid hydrochloride (Calbiochem, Los Angeles, Calif.) as the internal standards. The basic 4-h protein hydrolyzate program (No. A-TB-033) provided by Beckman Instruments was used for all analyses.

The final amino acid calibration standard for standardization is prepared in such a manner that it will contain tryptophan (not present in the Beckman calibration mixture) and two internal standards. The reagents for the amino acid calibration standard are prepared as follows:

*Reagents*

*Citrate buffer.* Dissolve 39.2 g of sodium citrate in 1 l of distilled water. Add 33 ml of concentrated hydrochloric acid, 10 ml of thiodiglycol (Pierce Chemical Co., Rockford, Ill.) and 0.2 ml of octanoic acid (Pierce Chemical Co.); dilute to 2 l and mix well. Adjust the pH to 2.20 ± 0.10 with concentrated HCl or 50% sodium hydroxide.

*Norleucine (NLE) internal standard working solution.* Dissolve 170 mg of norleucine accurately weighed to 0.1 mg in 500 ml of pH 2.2 citrate buffer, mix well. Store in a refrigerator.

*L-α-Amino-β-guanidinopropionic acid hydrochloride (QPA) internal standard working solution.* Dissolve 118 mg of L-α-amino-β-guanidinopropionic acid hydrochloride accurately weighed to 0.1 mg in 250 ml of pH 2.2 citrate buffer, mix well. Store in a refrigerator.

*L-Tryptophan (TRP) standard solution.* Dissolve 85 mg of tryptophan accurately weighed to 0.1 mg in 100 ml of pH 2.2 citrate buffer, mix well (solution A). Pipet 5.0 ml of solution A into a 50-ml volumetric flask; dilute to the mark with pH 2.2 citrate buffer, and mix well (solution B). Store in a refrigerator. The two internal standards and the tryptophan were purchased from Calbiochem, Los Angeles, Calif.

*Calibration standard*

Into a 10-ml volumetric flask pipet 2.0 ml of the Beckman calibration mixture, Type 1, 2.0 ml of the NLE internal standard working solution, 2.0 ml of the QPA internal standard working solution, and 2.0 ml of the TRP standard solution; dilute to the 10.0 ml mark with pH 2.2 citrate buffer.

## BASIC EQUATIONS FOR AMINO ACID ANALYSIS

The use of internal standards in the analysis of amino acids necessitates the use of eqn. 1, which provides the color yield constant of each known amino acid in the calibration standard relative to the internal standards employed.

$$K = \frac{(CIS)(AKA)}{(CKA)(AIS)} \tag{1}$$

where

$K$ = color yield constant of a particular known amino acid

$CIS$ = concentration (mg/ml) of the particular internal standard in the calibration standard

$AKA$ = area (in integrator counts) of a particular known amino acid in the calibration standard

$CKA$ = concentration (mg/ml) of a particular known amino acid in the calibration standard

$AIS$ = area (in integrator counts) of the internal standard in the calibration standard.

The final calculation for the amount of each amino acid found in the sample after amino acid analysis is computed from eqn. 2.

$$\%AA = \frac{(CIS)(AKA)(DF)}{(WS)(AISS)(K)} \times 100 \qquad (2)$$

where

$\%AA$ = percent found of an identified amino acid in a sample after analysis

$CIS$ = weight (mg) of the internal standard added to the final sample solution to be analyzed

$AKA$ = area (in integrator counts) of an identified known amino acid from a sample after analysis

$DF$ = sample dilution factor

$WS$ = original weight (mg) of sample taken for analysis

$AISS$ = area (in integrator counts) of the internal standard in the sample analyzed

$K$ = same as in eqn. 1.

It should be mentioned at this point that the ion-exchange procedure employed is based on the classic MOORE–STEIN two-column concept[5]. Therefore, QPA is used as the internal standard for the basic amino acids and NLE is used as the internal standard for the acidic and neutral amino acids. The concentrations of NLE and QPA were chosen so that their respective integrated areas would be congruous with the amino acids in the calibration standard and the samples analyzed. Similarly, tryptophan was added to the calibration standard in a concentration range of our product.

For our convenience, all results are calculated on a weight–weight basis although the program can be modified to compute the results in other units.

The Beckman calibration mixture is stated to contain 2.5 $\mu$moles of each amino acid per ml of solution (except cystine, which is present at one-half this concentration). Since 2.0 ml of this solution are finally diluted to 10 ml for the preparation of the calibration standard, each milliliter contains 0.5 $\mu$mole/ml of each standard amino acid (except for cystine). To convert 0.5 $\mu$mole/ml of each amino acid to mg/ml, one simply multiplies the molecular weight of the individual amino acid by 0.0005 (except for cystine, which is multiplied by 0.00025, since it is present at one-half the concentration of the other amino acids). The resulting numbers are now stored as constants in a separate computer file for calculation of the $K$ value. Since the calibration standard is always prepared in the same manner for this given system, one needs only to type in the weights of the internal standards into the computer program. This eliminates any manual computation on the part of the operator. It should be remembered that tryptophan is not present in the Beckman calibration mixture and therefore it is placed in the calibration standard. Hence, the tryptophan number to be stored in the computer file (as a constant) along with the

other amino acid numbers is equal to the milligrams of tryptophan in 1 ml of the calibration standard. This is the only number which will vary when a new calibration standard is prepared. However, one can simply weigh out the same initial weight of tryptophan each time a calibration standard has to be prepared and thereby eliminate the need for changing the tryptophan number in this file.

## EXPLANATION OF THE COMPUTER PROGRAM

*Control tape*

Prior to solving a problem, the operator must punch a control tape. This tape is necessary to furnish the variable data that will occur with each sample analyzed. For each sample, the following information is required:

*Line 1.* Line 1 contains an alphameric sample identification for the first sample, up to four characters in length.

*Line 2.* Line 2 contains the values for the constants listed below, separated by spaces. (For simplicity, the column used to separate the basic amino acids will be referred to as the short column and the column employed for the separation of the acidic and neutral amino acids as the long column.)

(a) If the analysis is for a short- or short and long column analysis, a1 is typed. If it is only for a long column analysis, a2 is typed.

(b) The identification of the amino acids in an analyzed sample is based upon their elution times as compared to the elution times of the amino acids present in the calibration standard. Since the elution time and the integrator counts are recorded for each eluted amino acid on the teletype, one can visually compare the elution times of the amino acids in the sample to that of the standard amino acids. If the elution times of both sample and standard amino acids are the same, then the program will compute correctly.

Unfortunately, elution times may vary somewhat from sample to sample for the same amino acid. These variations may be attributed to a variety of possible small changes in the experimental parameters. To overcome this elution time problem, an "uncertainty factor" (UF), has been included in the computer program which allows the operator to choose a time factor (in minutes) to make the elution time of a known amino acid in the sample fall within an acceptable elution time range of the same kind of standard amino acid. Too high an uncertainty factor may not be acceptable as it might fall within the elution time of another peak. (This is usually the case in the threonine–serine doublet in a 4-h protein analysis.) We have found a UF of 1 to 2 to be satisfactory.

If the elution time of a sample peak does not meet the specified requirements, it will automatically be recorded as an unknown and calculated employing the $K$ value of aspartic acid, which is also recorded. This novel feature allows one to have a record of the unknown peak and provides a means for quantitation if and when it is identified since all the necessary parameters are recorded.

(c) The total number of integrated peaks in the sample run must be typed. This includes, acidic and neutral peaks in the basic column, buffer change, etc.

(d) The weight (in mg) of the short column internal standard (QPA) placed in the sample for analysis must be entered. If the short column is not being employed, enter a zero.

(e) The weight (in mg) of the long column internal standard placed in the sample for analysis must be entered. If the long column is not being employed, enter a zero.

(f) Enter the sample dilution factor.

(g) Enter the total weight of the sample (in mg).

(h) Enter the elution time of the acidic and neutral integrated peak from the short column. If none is present, enter a zero. The purpose of this step is to eliminate the calculation of this peak as an unknown.

(i) Enter the elution time of the buffer change (which integrates and occurs just before the methionine peak). If none is present, enter a zero. The purpose of this step is the same as in the previous step. If the long column is only operating, add 23 min to the elution time in this step. This number is a constant for our system. It is derived from the difference in elution times of the amino acids on the long column *vs.* the same amino acids after a short and long column analysis. Since the latter procedure is an overlapping technique (one starts the long column analysis while the short column analysis is being performed), it has a shorter overall analysis time and all the amino acids are eluted from the long column 23 min earlier than if they were analyzed only on a long column. This factor should be experimentally determined by those investigators who are going to use this program.

*Line 3.* Line 3 contains the alphameric sample identification of the next sample; if there are no more samples in this problem enter***. Enter the remaining information, if any, according to the above format.

## EXECUTION OF THE COMPUTER PROGRAM

To execute a problem the following sequence should be employed:

(1) Load the control tape into the file ACTRL·DAT. by typing COPY ACTRL·DAT = TTY: followed by CONTROL R.

(2) Put the tape reader on and, after completion, type CONTROL T, CONTROL Z.

(3) Load the paper tape punched by the integrator into the file ADATA·DAT. The data from a calibration run, if any, must precede the data from sample runs. Type COPY ADATA·DAT = TTY: followed by CONTROL R.

(4) Put the tape reader on and after completion type CONTROL T, CONTROL Z.

(5) At this point the data should be sequenced by typing SEQ ADATA·DAT. This allows one to make any changes such as deletion of undesirable data.

(6) Unsequence the file ADATA·DAT by typing UNS ADATA·DAT. Initiate the program by typing RUN AMINO.

During the execution of the program, several options will be offered, *viz.* (a) If the operator does not desire to use an option, type a carriage return. (b) Options: (1) To perform a calibration and/or sample run; (2) to change sample elution times; (3) if the opertor selects a calibration run he must enter via the teletype the weight of the short and long columns internal standards and the total number of peaks in the calibration mix data.

After satisfactory execution of the program and the data in the files ACTRL·DAT and ADATA·DAT are no longer needed, type DEL ACTRL·DAT, ADATA·DAT.

A typical report produced by the computer program is shown below:

CALIBRATION RUN

| A.A. | R.T. | K VALUE |
|---|---|---|
| TRP | 11. | 0.78076 |
| LYS | 15. | 1.62484 |
| HIS | 20. | 1.34547 |
| ACL | 26. | 3.05031 |
| ARG | 44. | 1.22331 |
| ASP | 69. | 0.88548 |
| THR | 79. | 0.93637 |
| SER | 82. | 1.25851 |
| GLU | 91. | 0.84674 |
| PRO | 97. | 0.19949 |
| GLY | 115. | 1.62089 |
| ALA | 122. | 1.43297 |
| CYS | 137. | 0.50197 |
| VAL | 152. | 1.01329 |
| MET | 168. | 0.85176 |
| ILE | 173. | 0.98310 |
| LEU | 177. | 0.95897 |
| TYR | 197. | 0.66239 |
| PHE | 203. | 0.74051 |

* * * * * * * * * *

SAMPLE BB-6

| COMPONENT | R. TIME | UF | %A.A. |
|---|---|---|---|
| 2 LYS | 15 | 0 | 0.634 |
| 3 HIS | 20 | 0 | 0.207 |
| 4 ACL | 26 | 0 | 0.854 |
| 5 ARG | 44 | 0 | 0.545 |
| 6 ASP | 68 | 1 | 1.674 |
| 7 THR | 78 | 1 | 0.590 |
| 8 SER | 81 | 1 | 0.939 |
| 9 GLU | 90 | 1 | 8.506 |
| 10 PRO | 97 | 0 | 1.005 |
| 11 GLY | 114 | 1 | 0.739 |
| 12 ALA | 121 | 1 | 1.262 |
| 13 CYS | 137 | 0 | 0.732 |
| 14 VAL | 152 | 0 | 0.519 |
| 15 MET | 167 | 1 | 0.313 |
| 16 ILE | 173 | 0 | 0.496 |
| 17 LEU | 177 | 0 | 0.841 |
| 18 TYR | 197 | 0 | 0.086 |
| 19 PHE | 203 | 0 | 0.351 |

TOTAL %AA NOT INCLUDING UNKNOWNS OR ACL = 19.438

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## DISCUSSION

The addition of tryptophan to the calibration standard to provide quantitative data for the tryptophan content in our samples is an acceptable procedure because it is added as the free acid and the sample does not require any acid or enzymatic hydrolysis. Since tryptophan is partially destroyed in such samples as proteins and

peptides during acid hydrolysis, it would have to be determined by some other procedure[6-9].

Another added feature of the program is the summation of all the calculated amino acids. In our case, this quickly gives us some idea if the amino acid content in the sample is in the desired range.

The computer program as discussed can be found in the APPENDIX. Likewise, the abbreviations employed for most of the amino acids in the program are those suggested by the International Union of Pure and Applied Chemistry and can be found in the APPENDIX listed under *Amino acid abbreviations*.

One of the main reasons for sequencing the data is to allow one to use the editing program of SEDIT. Hence, one may delete any extraneous data that are not wanted, or delete complete analyses from the punch tape. Other possible techniques are available and should be used according to the problem at hand.

The qualitative identification of each amino acid may be approached by other techniques, such as time ratios. Time ratios (which may be defined as the elution time of a particular amino acid divided by the elution time of the internal standard) may be used but the numbers obtained are difficult to visually compare to the time ratios of eluted amino acids from a sample. However, the upper and lower limits of variability of the time ratios for each particular amino acid may be determined experimentally and these ranges may be put into the computer for the identification of the amino acids[1]. Because of the possible variations in elution times and other concomitant problems, we have chosen a somewhat different approach utilizing the elution times of the amino acids which are automatically typed on the teletype. These whole numbers allow for easy visual comparison between the sample and standard amino acids. From this, we are at liberty to choose whatever uncertainty factor we desire which will produce the correct calculations and at the same time qualitatively indicate those amino acids which are present in the sample.

Using this basic program as a model, one can develop other programs of a similar nature. For example, if one does not use internal standards, then the program is further simplified. Although this program does not include the calculations of the number of residues of a particular amino acid in a sample (such as a protein or polypeptide), one can easily incorporate this into the present program by adding one or more subroutines.

The program was devised so that the operator has some flexibility in the computer operation: enough to make some choices, but restricted enough that the operator is not burdened by too much data required by the computer program. If the amino acid analyzer is operating in a continuous fashion for several days, the amount of information generated would require a full time assistant to calculate the results. In effect, this computer program eliminates the need for someone to perform the laborious routine calculations.

It should be mentioned, since the time this manuscript was prepared, some minor modifications have been introduced into the program; however, they do not affect the basic principles of this computer program.

ACKNOWLEDGEMENT

We would like to thank Miss A. HOLMAN for her technical assistance.

APPENDIX

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00100   C COMPUTER ANALYSIS OF AMINO ACID CHROMATOGRAMS
00110   C
00120           DIMENSION STIME(30),SAREA(30),XDENT(19)
00130           DATA(XDENT(K),K=1,19)/'TRP','LYS','HIS','ACL',
00140           1'ARG','ASP','THR','SER','GLU','PRO','GLY',
00150           2'ALA','CYS','VAL','MET','ILE','LEU','TYR','PHE'/
00160           DATA END/'***'/
00170           COMMON NC,CTISS,CAISS,CTISL,CAISL,CTIME(19),
00180           1CAREA(19),RFACT(19)
00190           WRITE(5,646)
00200           ACCEPT 631,IRUN
00210           IF(IRUN.NE.1) GO TO 10
00220           CALL CALIB(XDENT)
00230           GO TO 20
00240   10      CALL IFILE(2,'ADATA')
00250           CALL IFILE(3,'ACALB')
00260   C
00270   C INPUT DATA AND CONSTANTS FROM FORMER CALIBRATION RUN.
00280   C
00290           READ(3,626) NC,CTISS,CAISS,CTISL,CAISL
00300           READ(3,627)(RFACT(K),CTIME(K),CAREA(K),K=1,NC)
00310   20      CALL IFILE(1,'ACTRL')
00320   C
00330   C INPUT SAMPLE IDENTITY AND CONSTANTS.
00340   C
00350   99      READ(1,636) SAMPID
00360           IF(SAMPID.EQ.END) GO TO 999
00370           READ(1,628) LSCODE,MAXUF,NSP,SWISS,SWISL,DFACT,SWT,ANN,
00380           1BUFCNG
00390           KIL=0
00400           NS = 0
00410           NSTD = 0
00420           MM = MAXUF + 1
00430           TPCAA = 0
00440   C
00450   C IDENTIFY AND STORE SAMPLE SHORT AND LONG COL INTERNAL STDS.
00460   C
00470           DO 250 I=1,NSP
00480   222     READ(2,629) X,Y
00490           IF(EOFC(2)) 255,231,231
00500   231     IF(Y.EQ.0.) GO TO 222
00510           IF(X-ANN) 232,250,232
00520   232     IF(X-BUFCNG) 233,250,233
00530   233     IF(LSCODE.NE.2) GO TO 234
00540           IF(X.LT.47.) GO TO 250
00550           X = X + 23.
00560   234     DO 240 L=1,MM
00570           UF=L-1
00580           SHTLB=CTISS-UF
00590           SHTUB=CTISS+UF
00600           LNGLB=CTISL-UF
00610           LNGUB=CTISL+UF
00620           IF(X.LT.SHTLB) GO TO 240
00630           IF(X.GT.SHTUB) GO TO 235
00640           STISS = X
00650           SAISS = Y
00660           GO TO 250
00670   235     IF(X.LT.LNGLB) GO TO 240
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00680            IF(X.GT.LNGUB) GO TO 240
00690            STISL = X
00700            SAISL = Y
00710            GO TO 250
00720   240      CONTINUE
00730            NS = NS + 1
00740            STIME(NS) = X
00750            SAREA(NS) = Y
00760   250      CONTINUE
00770   C
00780   C DO YOU WISH TO CHANGE ANY SAMPLE RETENTION TIMES?
00790   C
00800   255      WRITE(5,630)
00810            ACCEPT 631,ICHNG
00820            IF(ICHNG.NE.1) GO TO 256
00830            WRITE(5,632)
00840            ACCEPT 633,ICMP,STIME(ICMP)
00850            GO TO 255
00860   256      IF(ICHNG.NE.2) GO TO 260
00870            WRITE(5,642)
00880            ACCEPT 631,INTSD
00890            IF((INTSD.NE.1).AND.(INTSD.NE.3)) GO TO 257
00900            WRITE(5,643)
00910            ACCEPT 644,SAISS
00920   257      IF((INTSD.NE.2).AND.(INTSD.NE.3)) GO TO 255
00930            WRITE(5,645)
00940            ACCEPT 644,SAISL
00950            GO TO 255
00960   C
00970   C WRITE TABLE HEADINGS.
00980   C
00990   260      WRITE(5,637) SAMPID
01000            WRITE(5,638)
01010   C
01020   C IDENTIFY SAMPLE PEAKS AND COMPUTE "PCAA".
01030   C
01040            DO 500 I=1,NS
01050            DO 300 J=1,NC
01060            DO 300 L=1,MM
01070            UF = L - 1
01080            IF(STIME(I)-CTIME(J)+UF) 290,270,270
01090   270      IF(STIME(I)-CTIME(J)-UF) 400,400,280
01100   280      IF(J.LT.NC) GO TO 300
01110   290      IF(UF.EQ.MAXUF) GO TO 430
01120   300      CONTINUE
01130   C
01140   400      IF(J.GT.5) GO TO 410
01150            PCAA=(100.*DFACT*SWISS*SAREA(I))/(RFACT(J)*SWT*SAISS)
01160            TPCAA=TPCAA+PCAA
01170            GO TO 420
01180   410      PCAA=(100.*DFACT*SWISL*SAREA(I))/(RFACT(J)*SWT*SAISL)
01190            TPCAA=TPCAA+PCAA
01200   420      KNOW=STIME(I)
01210            KUF=UF
01220            WRITE(5,634) J,XDENT(J),KNOW,KUF,PCAA
01230            GO TO 500
01240   430      IF(J-NC) 431,550,550
01250   431      KIL=KIL+1
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01260            KUNK=STIME(I)
01270            KUF=UF
01280            PCAA=(100.*DFACT*SWISL*SAREA(I))/(RFACT(6)*SWT*SAISL)
01290            WRITE(5,635) J,KUNK,KUF,PCAA
01300    500     CONTINUE
01310    550     WRITE(5,641)TPCAA
01320            IF(KIL.EQ.0) WRITE(5,647)
01330            IF(KIL.EQ.0) GO TO 99
01340            WRITE(5,640) RFACT(6)
01350            WRITE(5,647)
01360    C
01370    C RETURN FOR ANALSIS OF NEXT SAMPLE, IF ANY.
01380            GO TO 99
01390    C
01400    626     FORMAT(I,4F)
01410    627     FORMAT(3F)
01420    628     FORMAT(3I,6F)
01430    629     FORMAT(2F)
01440    630     FORMAT(///1X,'IF YOU WISH TO CHANGE ANY SAMPLE RET
01450            1ENTION TIMES, TYPE A 1, OR',/1X,'IF YOU WISH TO
01460            2CHANGE ANY INTERNAL STD. PEAK AREAS, TYPE A 2:  ')
01470    631     FORMAT(I)
01480    632     FORMAT(1X,'TYPE PEAK # NOT COUNTING INTERNAL STDS. OR -
01490            1OTHER EXTRANEOUS',/,1X,'PEAKS, FOLLOWED BY NEW RET.
01500            2TIME:  ')
01510    633     FORMAT(I,F)
01520    634     FORMAT(2X,I3,1X,A3,8X,I4,8X,I2,F11.3)
01530    635     FORMAT(2X,I3,' UNK',8X,I4,8X,I2,F11.3,' *')
01540    636     FORMAT(A4)
01550    637     FORMAT(/////,17X,'SAMPLE ',A4/)
01560    638     FORMAT(/1X,'COMPONENT',7X,'R. TIME',5X,'UF',
01570            16X,'%A.A.'/)
01580    640     FORMAT(//1X,'* UNK. PEAK CALC WITH ASP "K" VALUE(',F8.5,
' ).')
01590    641     FORMAT(/2X,'TOTAL %AA NOT INCLUDING UNKOWNS =',F7.3)
01600    642     FORMAT(1X,'TYPE A 1 IF SHORT COL., 2 IF LONG COL.,
01610            1 OR 3 IF BOTH:  ')
01620    643     FORMAT(1X,'NEW SHORT COL. I.S. AREA = ')
01630    644     FORMAT(F)
01640    645     FORMAT(1X,'NEW LONG COL. I.S. AREA = ')
01650    646     FORMAT(1X,'IF CALIB. RUN PRECEDES SAMPLE RUN, ',/
01660            11X,'TYPE A 1:  ')
01670    647     FORMAT(//20X,10('*  ')//)
01680    C
01690    C
01700    999     CALL EXIT
01710            END
01720    C
01730    C CALIBRATION RUN TO DETERMINE "K" VALUES.
01740    C
01750            SUBROUTINE CALIB(XDENT)
01760            DIMENSION CFACT(19),XDENT(19)
01770            DATA(CFACT(K),K=1,19)/.01708,.0731,.07758,.02675
01780            1,.08711,.06655,.05956,.05255,.07357,.05757,.03754
01790            2,.04455,.060075,.05858,.07461,.06559,.06559,.09059
01800            3,.08260/
01810            COMMON NC,CTISS,CAISS,CTISL,CAISL,CTIME(19),
01820            1CAREA(19),RFACT(19)
01830            CALL IFILE(2,'ADATA')
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01840            CALL OFILE(3,'ACALB')
01850            WRITE(5,604)
01860            ACCEPT 600,CWISS
01870            WRITE(5,606)
01880            ACCEPT 600,CWISL
01890            WRITE(5,607)
01900            ACCEPT 608,NP
01910            WRITE(5,609)
01920            WRITE(5,610)
01930            NC = 0
01940            DO 100 J=1,NP
01950    104     READ(2,601) X,Y
01960            IF(EOFC(2)) 210,105,105
01970    105     IF(X.EQ.0.) GO TO 104
01980            IF(J.EQ.1) GO TO 100
01990            IF(J.EQ.17) GO TO 100
02000            IF(J.NE.6) GO TO 110
02010            CTISS = X
02020            CAISS = Y
02030            GO TO 100
02040    110     IF(J.NE.21) GO TO 120
02050            CTISL = X
02060            CAISL = Y
02070            GO TO 100
02080    120     NC = NC + 1
02090            CTIME(NC) = X
02100            CAREA(NC) = Y
02110            IF(NC - 19) 100,210,210
02120    100     CONTINUE
02130    210     WRITE(3,602) NC,CTISS,CAISS,CTISL,CAISL
02140            DO 200 K=1,NC
02150            IF(K.GT.5) GO TO 220
02160            RFACT(K) = (CWISS*CAREA(K))/(CAISS*CFACT(K))
02170            GO TO 230
02180    220     RFACT(K) = (CWISL*CAREA(K))/(CAISL*CFACT(K))
02190    230     WRITE(3,605) RFACT(K),CTIME(K),CAREA(K)
02200            WRITE(5,603) XDENT(K),CTIME(K),RFACT(K)
02210    200     CONTINUE
02220            WRITE(5,611)
02230    C
02240    600     FORMAT(F)
02250    601     FORMAT(2F)
02260    602     FORMAT(I,F5.0,F8.0,F5.0,F8.0)
02270    605     FORMAT(F,F5.0,F8.0)
02280    603     FORMAT(1X,A5,F5.0,F10.5)
02290    604     FORMAT(1X,'WT. OF SHORT COL. I.S. = ')
02300    606     FORMAT(1X,'WT. OF LONG COL. I.S. = ')
02310    607     FORMAT(1X,'TOTAL # OF PEAKS IN CALIB. MIX = ')
02320    608     FORMAT(I)
02330    609     FORMAT(///3X,'CALIBRATION RUN'/)
02340    610     FORMAT(1X,'A.A.',3X,'R.T.  K VALUE'/)
02350    611     FORMAT(//20X,10('*  ')//)
02360    999     RETURN
02370            END
```

*Amino acid abbreviations*

TRP = Tryptophan
LYS = Lysine
HIS = Histidine
ACL = Ammonia
ARG = Arginine
ASP = Aspartic acid
THR = Threonine
SER = Serine
GLU = Glutamic acid
PRO = Proline
QPA = L-α-Amino-β-guanidinopropionic acid hydrochloride
GLY = Glycine
ALA = Alanine
CYS = Cystine
VAL = Valine
MET = Methionine
ILE = Isoleucine
LEU = Leucine
TYR = Tyrosine
PHE = Phenylalanine
NLE = Norleucine

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