

Note

Analysis of data from amino acid and other automated analysers

II. A simple Fortran program for the identification and quantitation of amino acids*

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A number of routines have been devised for the integration of amino acid and other chromatograms¹⁻⁵. These vary from relatively simple programs which calculate only peak areas to very complex routines which require a large computer.

We have recently described a system for recording data from an amino acid analyser on magnetic tape cassettes⁶. An advantage of this method is that data from up to eight 7-h analyses can be recorded on a single cassette. As it is normal practice to include an appropriate standard mixture of amino acids in each group of analyses, the computer is thus provided with sufficient information in each batch of data to enable it to identify the majority of amino acids in the samples. A simple program was therefore developed to name and quantitate amino acids on the basis of the single standard analysis.

MATERIALS AND METHODS

Amino acid analysis was carried out with a Jeol JLC-6AH amino acid analyser (Jeol, London, Great Britain) using a two-column system. The integrator output of the 570-nm channel was recorded on a magnetic tape cassette by a Digitronics AL-4 data logger (Digitronics, Milton Keynes, Great Britain). Data were recorded every 8 sec and "scan count" was included. The electronic device described previously⁶ was used to provide an indication of the start of each run in the logged data.

The data were transcribed to paper tape using a modified replay unit (Digitronics ADR-1) and fast tape punch (Facit 4070)⁶. Data in this form were input into a remote computer (ICL 1906A) for processing.

THE PROGRAM

The program is based on a general purpose integration program⁷ modified

* Copies of the program described may be obtained from the authors.

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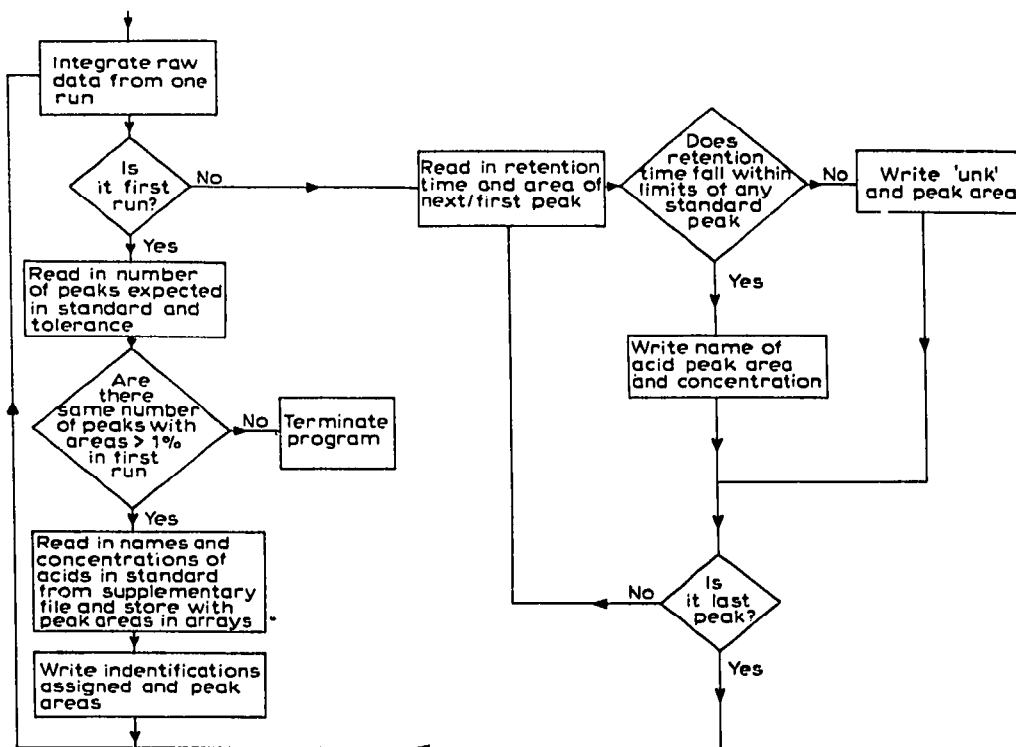


Fig. 1. Flow chart of the identification and quantitation routine.

to prevent the program being halted by erroneous characters in the raw data. The additional modifications comprising the naming routine are shown in Fig. 1. In addition to the raw data, two other files (input channels) provide supplementary data to run the program. The first of these contains the control parameters for detection of peaks by the integration segment together with the expected variation in retention times in the same batch of analyses (tolerance). The second contains the number of peaks in the standard with areas greater than 1% of the total integrated area, followed by a list of four-character abbreviated names for these peaks, in the order in which they are eluted, together with their molar concentration as $\mu\text{moles/ml}$.

GLY 0.1
 ALA 0.1
 CYS 0.05
 VAL 0.1
 MET 0.1
 ILEU 0.1

If the number of peaks with areas greater than 1% found in the standard run is not the same as that given in the supplementary data, an error message is output, and the program terminated. When the correct number of peaks are found, the inte-

grated areas are divided by the concentrations read in sequence from the second supplementary data file to give the area equivalent to 1 μ mole/ml. The name, retention time, integrated area and equivalent area are stored in arrays.

The sample runs are then integrated and the areas divided by the sample volume factor inherent to the analyser. The retention time is compared with those derived from the standard and if the difference is less than the tolerance, the integrated area is divided by the appropriate equivalent area to give the concentration in μ moles/ml in the sample. This result, together with the name of the amino acid, is then output.

If the retention time does not fall close enough to any of the identified peaks in the standard, the component is labelled "unknown" and output together with its retention time and integrated area.

DISCUSSION AND CONCLUSIONS

The program described avoids the large number of format statements normally associated with naming routines and is consequently relatively short. It could, therefore, be adapted for use with the larger-capacity desk top calculators.

The use of supplementary data files enables the same program to be used with protein hydrolysates and physiological samples. The two supplementary data files could be combined in a single file.

The existing program does not give accurate results for proline and hydroxyproline, because the maximum absorption of their ninhydrin derivatives occurs at 440 nm. Data from the 440-nm channel could be recorded using the same equipment, and this could provide a secondary check on the identification of the components, by calculating the 570/440 nm ratio and comparing this with values obtained from the standards.

REFERENCES

- 1 A. W. Boyne and W. R. H. Duncan, *J. Lipid Res.*, 11 (1970) 293.
- 2 H. L. Back, P. J. Buttery and K. Gregson, *J. Chromatogr.*, 68 (1972) 103.
- 3 H. D. Spitz, G. Henyon and J. N. Sivertson, *J. Chromatogr.*, 68 (1972) 111.
- 4 R. Taylor and M. G. Davis, *Anal. Biochem.*, 51 (1973) 180.
- 5 M. A. Fox, *J. Chromatogr.*, 89 (1974) 61.
- 6 J. M. Owen, A. D. Dale, A. Youngson and P. T. Grant, *J. Chromatogr.*, 96 (1974) 235.
- 7 K. H. Burkhardt, *General Purpose Chromatograph Peak Integration Program*, IBM Contributed Program Library, New York, 1968.