Notes

CHROM. 4535

Calculation of amino acid analyses with a desk-top computer

This communication describes an improved method for simple and quick calculation of amino acid analyses using an Olivetti Programma 101 desk-top computer. The program of MONDINO[1] has been modified to incorporate the use of an internal standard in the calculation of the analyses and to express the results in a variety of ways *e.g.* 10^{-n} moles, or percentage amino acids, or percentage nitrogen, or as the number of residues in some particular molecular weight.

The method of calculation was

$$\frac{A_x \times M_s}{A_s \times C.F.}$$

where A_x and A_s are the areas of the amino acid x and the internal standard calculated either by the "height–width" method, or by the height method (proposed by MONDINO[1] and NAUMAN[2] and verified in this laboratory).

M_x and M_s are the amounts in 10^{-n} moles (usually μmoles) of amino acid x and internal standard applied to the column.

$C.F.$ is the colour factor defined as $(A_x/A_s) \times (M_s/M_x)$ obtained from a standard chromatogram.

The initial problem was to store the colour factors on a single program card and the results in the computer between the insertion of program cards. This was accomplished by restricting the colour factors and the results to three digits and using a special stacking technique to enable six 3-digit numbers to be stored as a single number of eighteen digits. Using this technique it was possible to store eighteen colour factors in the three available stores for numbers on the program cards. A limit of eighteen amino acids appears at first to be some restriction but as this does not include ammonia or the internal standard this restriction will be prohibitive in only a very few cases.

The basic program calculates the results in 10^{-n} moles (usually μmoles) for each amino acid, stores them and prints them in a single column as is shown in Fig. 1. After entering the first program card and entering the norleucine height, width (as number of dots) and amount in μmoles, the norleucine constant is printed out. This constant should be stable from one run to the next. Using the second card which contains the colour factors, the height and width of the eighteen amino acids (in order) are entered into the computer. The third card prints out the amount of each amino acid in a single table, and after the initial print-out duplicate sets of results may be printed out and/or the results can be printed out as residues of each amino acid with one particular amino acid set to be one residue. A further two cards suffice to calculate the ammonia and any uncommon amino acids, total the nitrogen and give the recovery from the column. The data are still held in the computer in the form of three 18-digit numbers and it has proved possible to devise programmes that can

			V
	H	0.332	S
Norleu	*W*	18.5	S
μmoles		0.125	S
Norleu factor		49.136	A ◇
			V
	H	0.016	S
Lys	*W*	18.0	S
His		0	S
		0	S
Arg		0.128	S
		24.3	S
CMCys		0.952	S
		13.3	S
Asp		0.076	S
		15.4	S
Thr		0.349	S
		16.7	S
Ser		0.401	S
		16.0	S
Glu		0.383	S
		19.1	S
Pro		0.114	S
		19.8	S
Gly		0.330	S
		19.7	S
Ala		0.132	S
		22.3	S
½Cys		0.010	S
		10.0	S
Val		0.114	S
		31.8	S
Met		0.006	S
		10.5	S
Ile		0.164	S
		15.1	S
Leu		0.125	S
		16.8	S
Tyr		0.069	S
		28.0	S
Phe		0.036	S
		33.3	S

Initial print-out (μmoles)			
			W
			V
Lys	0.006	A	◇
His	0.000	A	◇
Arg	0.072	A	◇
CMCys	0.324	A	◇
Asp	0.026	A	◇
Thr	0.120	A	◇
Ser	0.129	A	◇
Glu	0.162	A	◇
Pro	0.184	A	◇
Gly	0.148	A	◇
Ala	0.075	A	◇
½Cys	0.002	A	◇
Val	0.082	A	◇
Met	0.002	A	◇
Ile	0.052	A	◇
Leu	0.045	A	◇
Tyr	0.037	A	◇
Phe	0.023	A	◇
			V
Norleu factor	49.136		S
H	0.564		S
Ammonia *W*	27.6		S
Colour factor	0.890		S
μmoles	0.355	A	◇
			W
			V
Total N in μmoles	2.066	A	◇
Dilution factor	10		S
Measured N in mg	2.91		S
% Recovery of N	99.500	A	◇

Fig. 1. Print-out of the routine calculation of the analysis (five cards). S indicates numbers entered to the computer and A◇ indicates print-out by the computer. V and W are instructions to the computer.

% μmoles			Residues to given 10,000 mol. wt.			% N	
				V	Total μmoles		V
				V	of N	1.711	A ◇
				V	Ammonia	0	S
				W	New total	1.711	A ◇
				V			
			10000	S	% Ammonia	0.000	A ◇
		V					
Lys	0.402	A ◇	0.348	A ◇		0.701	A ◇
His	0.000	A ◇	0.000	A ◇		0.000	A ◇
Arg	4.835	A ◇	4.186	A ◇		16.832	A ◇
CMCys	21.759	A ◇	18.841	A ◇		18.936	A ◇
Asp	1.746	A ◇	1.511	A ◇		1.519	A ◇
Thr	8.059	A ◇	6.978	A ◇		7.013	A ◇
Ser	8.663	A ◇	7.501	A ◇		7.539	A ◇
Glu	10.879	A ◇	9.420	A ◇		9.468	A ◇
Pro	12.357	A ◇	10.699	A ◇		10.753	A ◇
Gly	9.939	A ◇	8.606	A ◇		8.649	A ◇
Ala	5.036	A ◇	4.361	A ◇		4.383	A ◇
½Cys	0.134	A ◇	0.116	A ◇		0.116	A ◇
Val	5.507	A ◇	4.768	A ◇		4.792	A ◇
Met	0.134	A ◇	0.116	A ◇		0.116	A ◇
Ile	3.492	A ◇	3.023	A ◇		3.039	A ◇
Leu	3.022	A ◇	2.616	A ◇		2.630	A ◇
Tyr	2.484	A ◇	2.151	A ◇		2.162	A ◇
Phe	1.544	A ◇	1.337	A ◇		1.344	A ◇

Fig. 2. Print-out of the analysis results in percentage μmoles (one card), residues to a given molecular weight (10,000 in this case) (two cards of program and two containing constants) and percentage nitrogen (two cards) with facilities to add ammonia or amide nitrogen if required.

extract these numbers in such a way as to calculate the percentage of nitrogen, the percentage of amino acids and the number of residues to any given molecular weight by simply adding program cards to the computer (Fig. 2).

It is essential that the colour factors be arranged in the correct form for storage on the program card. A program has been written which will calculate the colour factors from a standard chromatogram (even when the amount of amino acid is not the same as that of the internal standard) and then arrange them in the correct way. A simple manipulation suffices to store these colour factors on the routine analysis card. The program has to accept colour factors between 0 and 2 because colour factors greater than 1 are encountered. It does this by dividing the colour factor by 2 before storing it as a 3-digit number. This is shown in Fig. 3. The inaccuracy introduced here, if any, will always be less than 0.4% and since 3% is the usual experimental accuracy associated with amino acid analysis this will not be serious.

Since most amino acid analysers work in the range of 0–1 μmoles the program has been designed to work in this range. Consequently the primary result is stored and printed out as $0 \cdot abc$ μmoles. However for analysers working in the range 0–2 μmoles the result can be stored as half its real value and multiplied by two immediately prior to print-out.

The results shown in Fig. 3 are calculated from a standard run, with a standard made up in these laboratories, on a Beckman 120C amino acid analyser using the two column procedure and the modified ninhydrin reagent made up in dimethylsulphoxide.

		V
H	0.363	S
W	18.9	S
Norleu area	6.8607	R ◇
μmoles	0.125	S
Norleu constant	54.885	A ◇

H	0.406	S		0.408	S		0.199	S
W	17.8	S		17.3	S		30.6	S
Lys μmoles	0.126	S	Ser	0.125	S	Val	0.120	S
Colour factor	1.053	A ◇		1.028	A ◇		0.924	A ◇
	0.377	S		0.321	S		0.470	S
	17.5	S		19.4	S		13.5	S
His	0.126	S	Glu	0.128	S	Met	0.126	S
	0.955	A ◇		0.886	A ◇		0.918	A ◇
	0.243	S		0.084	S		0.420	S
	24.4	S		19.5	S		15.8	S
Arg	0.124	S	Pro	0.126	S	Ile	0.123	S
	0.872	A ◇		0.236	A ◇		0.982	A ◇
	0.448	S		0.305	S		0.369	S
	11.8	S		20.1	S		17.5	S
CMCys	0.120	S	Gly	0.126	S	Leu	0.125	S
	0.802	A ◇		0.886	A ◇		0.942	A ◇
	0.397	S		0.266	S		0.265	S
	14.9	S		22.3	S		28.5	S
Asp	0.125	S	Ala	0.123	S	Tyr	0.127	S
	0.862	A ◇		0.878	A ◇		1.084	A ◇
	0.408	S		0.194	S		0.218	S
	17.0	S		33.7	S		31.2	S
Thr	0.128	S	½Cys	0.255	S	Phe	0.122	S
	0.988	A ◇		0.468	A ◇		1.016	A ◇

		W
Colour factors as	526.4774364014314 94	A ◇
	514.4431184434392 34	C ◇
stored on program card	462.4594914715425 08	B ◇

Fig. 3. Calculation of the colour factors from height, width and amount of norleucine and the eighteen amino acids requiring only a single card. A◇ (in each case) is the print-out of the colour factor. At the bottom is the print-out of the colour factors arranged for transfer to the routine analysis program card. These are stored as half the appropriate colour factor to accommodate numbers greater than one and the numbers A◇, C◇, B◇ are generated from the 1st, 2nd and 3rd columns, respectively.

The results in Fig. 1 and 2 are from a protein hydrolysate run on the same columns with the same procedure.

The program published by MONDINO[1] calculates the amount in μmoles and μg of each amino acid, the total weight of amino acid, the total weight and percentage of nitrogen and the percentage composition of the amino acids in the mixture. To do this it requires twenty-three program cards, one each for the twenty amino acids (including ammonia) and three extra cards to carry out the final computations. However, this does not include an internal standard and uses only the height as the index of area; these two drawbacks could limit its use in many laboratories.

The present program uses three cards for the standard analysis using either the height–width or the height method—which is only valid where small sample volumes less than 0.2 ml are used (MONDINO[3])—and two for summing the nitrogen values and calculating the nitrogen recovery, and a single card for expressing the results on a percentage basis. Thus six cards suffice for the twenty-three cards of MONDINO's program while the results are always retained in the computer for presentation in the other ways shown in Fig. 2.

Copies of these programmes (to calculate either in the range 0–1 or 0–2 μmoles) are available from the author.

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1 A. MONDINO, *J. Chromatog.*, 41 (1969) 156.
2 L. W. NAUMAN, *J. Chromatog.*, 41 (1969) 456.
3 A. MONDINO, *J. Chromatog.*, 39 (1969) 262.

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Quantitative gas chromatographic determination of glutamic acid, 2-pyrrolidone-5-carboxylic acid and 2-pyrrolidone*

Work in our institute on the thermal and catalytic lactamization of glutamic acid (Glu) and on photochemical reactivity of 2-pyrrolidone-5-carboxylic acid (PCA) has made it necessary to develop analytical methods for the simultaneous quantitative determination of the two substances in the presence of their degradation products, such as 2-pyrrolidone (PYR).

Previous research has revealed the presence of PCA in many vegetable extracts and in biological fluids[1–3]. According to some authors, this acid is partially or totally formed from Glu as a result of the analytical methods used, particularly when they involve heat treatment[4–7]. A useful method for the qualitative and quantitative determination of the two substances is therefore of general interest.

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