Journal of Chromatography, 194 (1980) 69–75
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 12,730

Note

Program for processing amino acid data with a programmable pocket calculator

MARCELLO DURANTI
Department of General Biochemistry, University of Milan, Via G. Celoria, 2, I-20133 Milan (Italy)
(First received August 10th, 1979; revised manuscript received January 29th, 1980)

The quantitative evaluation of a chromatogram obtained from an automatic amino acid analyzer is a time-consuming repetitive operation comprising integration of the area of each peak, after comparison with an appropriate standard. These operations can be accomplished by desk-top calculators[1] and electronic integrators[2,3]. Several authors have described the use of these devices in this field and how to reduce analysis costs[4], how to simplify the software even in the case of analyses of complex mixtures such as those of physiological fluids[5], how to minimize the effects of noisy outputs and variable retention times[6], how to detect the critical points of the chromatogram and how to prevent fluctuations in the base line[7]. However, the price of these devices (which represents 15–25% of the total cost of the apparatus, even for the simplest models) and the need for some knowledge of computer language put them out of reach of most laboratories.

Such repetitive routine calculations can be performed with the new programmable pocket calculators, whose prices are much lower than those of the instruments mentioned. Of course, in this case the calculator cannot be interfaced with the amino acid analyzer. Thus some parameters of the chromatogram peaks, such as height and width, must be measured manually, but once this is done the time required to process data is drastically shortened and the possibility of error is greatly diminished compared to full manual evaluation.

Buchanan[8] reported a program for processing amino acid data with a Hewlett-Packard HP25 calculator; however, this machine has a limited number of program steps and memories, and it can process the information for only one amino acid at a time. For each peak, 1.5–2.0 min are required to evaluate the amount in nmoles, *i.e.*, the procedure must be repeated eighteen times for protein hydrolysates, making the full elaboration of data tedious and time-consuming.

The program described in this paper is written for a Texas Instruments TI59 calculator which offers a larger number of steps and memories. It enables calculation of the whole amino acid composition just by entering in separate steps the data calculated manually (total heights, baselines and widths), which are then processed automatically. The program is divided in two sections: one for calculating correction factors from a calibration run with a standard mixture (this section requires 83 steps and 58 memory registers); and another to evaluate the amino acid composition of the sample (this section requires 150 steps and 59 memory registers).

0021-9673/80/0000–0000/$02.25

In the procedure proposed, the amino acid content is expressed as a percentage of the total recovered amino acids and as mg per g N (N = nitrogen). However, the program can easily be modified according to specific needs and the results can be expressed as desired (*e.g.*, residues per mole of protein, g per 100 g of protein, mg per g N, residues percent, g per 16 g N). Partial results can be displayed in any step of the program in order to record them on data sheets. The program described is designed for the evaluation of the amino acid composition of fully hydrolyzed samples (*i.e.*, eighteen amino acids), but can be modified to process more amino acids, as is required in the case of physiological fluid analysis.

The calculator needs only a few seconds to process all the data; of course, more time is required to manually enter the parameters of each peak. However, the whole procedure takes less than 6 min.

In our program the amount of each amino acid is expressed either as a percentage of the total recovered amino acids or as mg amino acid per g N, in which case the nitrogen content is determined by direct analysis of the sample. This method of expressing data is particularly useful in the analysis of food proteins. Moreover, by relating the determined data to the nitrogen content separately assayed, instead of to the total recovered amino acids, it is possible to correct the results for losses during the preparation and hydrolysis of the sample.

Table I shows the sequence in which the data for eighteen amino acids are processed. It also lists the memory addresses for total heights, net heights, baselines, widths, mg $\times 10^2$ of each amino acid, expansion scale and optical pathway, nitrogen

TABLE I

MEMORY ADDRESSES OF AMINO ACID DATA

Expansion scale factor and optical pathway*: 19. g N/ml* (only if 1 ml is the injected volume): 59. Total mg $\times 10^2$: 39. Counting memories: 00, 20 and 40.

$C = (\text{Height}_{\text{standard a.a.}} \times W_{\text{standard a.a.}})/(\text{Nanomoles}_{\text{standard a.a.}} \times \text{MW}) \times 10{,}000.$

Amino acid	*Total height*, Net height*	*Baseline*, Width**	*Correction factor, C*	*Amount (mg × 10^2)*
His	01	21	41	01
Lys	02	22	42	02
Arg	03	23	43	03
Asp	04	24	44	04
Thr	05	25	45	05
Ser	06	26	46	06
Glu	07	27	47	07
Pro	08	28	48	08
Gly	09	29	49	09
Ala	10	30	50	10
Cys	11	31	51	11
Val	12	32	52	12
Met	13	33	53	13
Ile	14	34	54	14
Leu	15	35	55	15
Tyr	16	36	56	16
Phe	17	37	57	17
Trp	18	38	58	18

* These data must be entered by the operator.

content of the sample and percentages of total recovered amino acids. Notice that when one amino acid is absent, the values 0 for total height, baseline and width and 1 for correction factor must be entered.

A schematic diagram of the program is shown in Fig. 1. Further details are given in Table II.

Program steps	Data Entry	Total heights and base lines
000–039	Calculation of net heights and net half heights	Net half heights displayed, net heights stored
	Data Entry	Widths, correction factors, selected scale and optical pathway, g N/ml
040–079	Calculation of the amount of each a.a.	Result stored
080–099	Calculation of the amount of recovered amino acids	Result stored
100–140	Calculation of amino acid % and mg amino acid/gN	Final results displayed

Fig. 1. Block diagram of the program.

With the suggested procedure the calculator first computes and displays net half-heights, which indicate where to evaluate the widths of the peaks and, after these data have been measured and entered, it calculates and displays the percent of each amino acid to two decimal places and the values of mg per g N approximated to an integer. However, other partial results can be displayed if the instruction "2nd Pause", which interrupts the program for 0.5 sec, is inserted after the sequence of instructions which define them.

Correction factors for each amino acid are calculated by modifying the main program after step 039, as shown in Table III. In this case the molecular weights of the amino acids must be entered in memories 41–58. Once calculated, correction factors are automatically stored in the same memories.

The performance of the main program is checked by two different types of tests. A preliminary run can be done: if, for all total heights, the digit 2 is entered and the digit 1 for baselines, widths, correction factors, expansion scale and g N, the program should display, in turn, 5.56% and 1. Alternatively, in routine operation, the number 18 displayed at the end of each cycle indicates the correct completion of a program section. After each section, the key-stroke run/stop must be pressed in order to move the program forward.

Once correction factors are calculated and stored, eighteen peaks in a chromatogram are processed in less than 6 min, including entering the data, but excluding the manual evaluation of baselines, total heights and widths which depend on the skill of the operator.

The program steps can be recorded on magnetic cards for quick reuse. In

TABLE II

PROGRAM FOR THE CALCULATION OF AMINO ACID COMPOSITION

Program steps	*Key*	*Comments*
000	0	Values for counting memories are set.
001	STO	
002	00	
003	2	
004	0	
005	STO	
006	20	
007	2nd Lbl	The first loop is labeled. 18 is put in t register.
008	A	
009	1	
010	8	Counting program.
011	x≥t	
012	1	
013	SUM	
014	00	
015	SUM	
016	20	
017	RCL 2nd IND	Total heights recalled; base lines subtracted.
018	00	
019	−	
020	RCL 2nd IND	
021	00	
022	=	
023	STO 2nd IND	Net heights are stored and divided by 2; base lines summed.
024	00	
025	:	
026	2	
027	÷	
028	RCL 2nd IND	
028	20	
030	=	
031	2nd Pause	Net half heights diplayed
032	2nd Pause	The digit in memory 00 is
033	RCL	compared with t.
034	00	If the value is ∠18 the
035	INV	cycle is repeated, if =
036	2nd x=t	18, is stopped.
037	A	
038	R/S	
039	0	
040	STO	Values for counting memories are reset.
041	00	
042	2	
043	0	
044	STO	
045	20	
046	4	
047	0	
048	STO	
049	40	
050	2nd LBl	The second loop is labeled.
051	B	
052	1	Counting program.
053	SUM	
054	00	
055	SUM	
056	20	
057	SUM	
058	40	

TABLE II (*continued*)

Program steps	*Key*	*Comments*
059	RCL 2nd IND	Net heights recal-
060	00	led, multiplied by
061	*	widths, divided by
062	RCL 2nd IND	correction factors,
063	20	multiplied by expan-
064	:	sion scale factor.
065	RCL 2nd IND	
066	40	
067	*	
068	RCL	
069	19	
070	=	
071	STO 2nd IND	mg * 10^2 stored. The
072	00	digit in memory 00 is
073	RCL	compared with t.
074	00	If the value is < 18
075	INV	the cycle is repeated,
076	2nd x=t	if = 18 is stopped.
077	B	0 is set in the coun-
078	R/S	ting memory.
079	0	Memory 39 is cleared.
080	STO	
081	00	
082	STO	
083	39	
084	2nd LBl	The third loop is la-
085	C	beled.
086	1	Counting program.
087	SUM	
088	00	
089	RCL 2nd IND	mg * 10^{+2} recalled,
090	00	summed and stored.
091	SUM	
092	39	The digit in memory
093	RCL	00 is compared with
094	00	t. If the value is
095	INV	< 18 the cycle is re-
096	2nd x=t	peated, if = 18, is
097	C	stopped.
098	R/S	
099	0	0 is set in the coun-
100	STO	ting memory.
101	00	
102	2nd Lbl	The fourth loop is la-
103	D	beled.
104	1	Counting program.
105	SUM	
106	00	
107	RCL 2nd IND	mg * 10^{+2} recalled, di-
018	00	vided by total mg *
109	:	10^2, multiplied by 100.
110	RCL	
111	39	
112	*	
113	I	
114	0	
115	0	
116	=	
117	2nd Fix	Amino acid percentage
118	2	to 2 decimal places
119	2nd Pause	displayed.
120	2nd Pause	
121	2nd Pause	
122	2nd Pause	

(Continued on p. 74)

TABLE II *(continued)*

Program steps	*Key*	*Comments*
123	RCL 2nd IND	mg ≡ 10^{+2} recalled
124	00	and divided by gN/ml.
125	:	
126	RCL	
127	59	
128	=	
129	2nd Fix	Results to integer
130	0	displayed.
131	2nd Pause	
132	2nd Pause	
133	2nd Pause	
134	2nd Pause	
135	RCL	The digit in memory
136	00	00 is compared with
137	INV	t. If the value is
138	2nd x=t	<18 the cycle is re-
139	D	peated, if = 18 is
140	R/S	stopped.

TABLE III

PROGRAM FOR THE CALCULATION OF CORRECTION FACTORS

Program steps	*Key*	*Comments*
000-038	The same as in the main program	
039	0	Value for counting
040	STO	program are set.
041	00	
042	2	
043	0	
044	STO	
045	20	
046	4	
047	0	
048	STO	
049	40	
050	2nd Lbl	The second loop is
051	B	labeled.
052	I	Counting program.
053	SUM	
054	00	
055	SUM	
056	20	
057	SUM	
058	40	
059	RCL 2nd IND	Net heights recalled,
060	00	multiplied by widths,
061	*	multiplied by 10000 and
062	RCL 2nd IND	divided by nanomoles
063	20	of each aa/ml of cali-
064	*	bration mixture (=40),
065	4	divided by molecular
066	0	weights, multiplied by
067	:	expansion scale factor.
068	RCL 2nd IND	
069	40	
070	*	
071	RCL	
072	19	
073	=	
074	STO 2nd IND	Correction factors to
075	40	2 decimal places sto-
076	2nd Fix	red.
077	2	The digit in memory 00
078	RCL	is compared with t. If
079	00	the value is <18, the
080	INV	cycle is repeated, if
081	2nd x=t	= 18, is stopped.
082	B	
083	R/S	

addition, if the printing device PC100 is available, the program itself and partial or final results can be printed. Thus, by avoiding any break in the program to write down the data, the time needed to process data is further reduced.

REFERENCES

1 A. P. Damoglou, *J. Chromatogr.*, 47 (1970) 257–261.
2 R. E. Exss, H. D. Hill and G. K. Summer, *J. Chromatogr.*, 42 (1969) 442–451.
3 J. M. Wilkinson and J. E. Fox, *Anal. Biochem.*, 76 (1976) 387–391.
4 B. M. Nair, *J. Chromatogr.*, 155 (1978) 249–259.
5 J. H. Brown, S. Walker, L. Casto and R. R. Howell, *J. Chromatogr.*, 116 (1976) 293–304.
6 G. D. Bacon, *J. Chromatogr.*, 172 (1979) 57–66.
7 H. L. Back, P. J. Buttery and K. Gregson, *J. Chromatogr.*, 68 (1972) 103–109.
8 J. H. Buchanan, *J. Chromatogr.*, 137 (1977) 475–480.