Journal of Chromatography, 101 (1974) 51–56

CHROM. 7745

DETERMINATION OF AMINO ACIDS BY GAS–LIQUID CHROMATOGRAPHY WITH THE NITROGEN-SENSITIVE THERMIONIC DETECTOR

M. BUTLER* and A. DARBRE
Biochemistry Department, King's College, Strand, London WC2R 2LS (Great Britain)
(Received June 7th, 1974)

SUMMARY

Studies with the nitrogen-sensitive thermionic detector are described. Conditions were established for the determination of trifluoroacetyl amino acid methyl esters. The thermionic detector was shown to be more sensitive than the flame ionization detector. The relative molar responses for fourteen amino acids are reported.

INTRODUCTION

The separation and determination of amino acids by gas–liquid chromatography (GLC) have been much improved in recent years and the flame ionization detector (FID) has been most widely used[1]. The presence of an alkali metal salt enhances the response of this detector to specific elements[2] and by using rubidium salt the detector was made selectively sensitive to nitrogen-containing compounds[3]. This thermionic detector (TD) has now become commercially available and has been used in our work because of its sensitivity and selectivity for nitrogen. It has enabled us to eliminate many problems due to GLC peaks caused by the presence of carbon-containing impurities derived from laboratory water samples, which interfered with amino acid determinations when working at high sensitivity with the FID[4].

MATERIALS AND METHODS

Apparatus

One gas chromatograph, Pye Series 104, Model 24, was fitted with a dual FID (Pye Unicam, Cambridge, Great Britain). A similar chromatograph was fitted with a Pye TD (rubidium chloride electrode) which was housed in a separate isothermally controlled oven. A Honeywell-Brown 10-mV, 1-sec strip chart recorder was used (Honeywell Controls, Greenford, Great Britain). Peak areas were determined with a Kent Chromalog 2 digital integrator (Kent Instruments, Luton, Great Britain). Nitrogen (99.9% "white spot" from British Oxygen, Wolverhampton, Great Britain,

* Present address: Department of Chemistry and Biology, Manchester Polytechnic, Chester Street, Manchester M1 5GD, Great Britain.

and "high-purity oxygen-free" from Air Products, New Malden, Great Britain) was used as carrier gas.

In order to maintain the necessary high nitrogen flow-rate to the TD, without changing the flow-rate through the GLC column, the outlet of the column was fitted to the detector via a T-piece. Additional nitrogen was taken directly from a nitrogen cylinder to a pressure controller then via a 40-cm length of stainless-steel capillary tubing (I.D. 0.3 mm) through the GLC oven wall and connected to the T-piece. A similar arrangement was previously described[5].

The glass GLC column (3.25 m × 2.5 mm I.D.) was packed with HP Chromosorb W, 80–100 mesh, coated with 2.5% (w/w) mixed stationary phase XE-60, QF-1 and MS-200 in the proportions 46%, 27%, and 27%, respectively (w/w)[6,7]. The initial oven temperature was 80° with programming at 2°/min to 140°. Inlet temperatures were 200°. The TD was maintained at 250°. The nitrogen carrier gas flow-rate was 15 ml/min. All sample injections were 1 μl by microsyringe.

Materials

Amino acids, acetyl chloride (AnalaR) and specially dried methanol were purchased from BDH (Poole, Great Britain). GLC materials were obtained as follows: silicone gum XE-60 from Applied Science Labs. (State College, Pa., U.S.A.), silicone oil MS-200, 100 cS, from Hopkin & Williams (Romford, Great Britain), and silicone (fluoro)-FS 1265 (QF-1) and HP Chromosorb W 80–100 mesh from F & M (Avondale, Pa., U.S.A.).

Derivatisation

The trifluoroacetyl (TFA) amino acid methyl esters were prepared as described[6,7].

EXPERIMENTAL AND RESULTS

Optimisation of conditions

The variable conditions which affected the response of the TD were previously reported[8,9]. With TFA leucine methyl ester as a test standard, an optimal response

TABLE I

RESPONSE OF THERMIONIC DETECTOR WITH INCREASING NITROGEN FLOW-RATE

GLC conditions and integration method were given under *Apparatus*. Test sample: TFA leucine methyl ester (1 μl ≡ 1 μg).

Flow-rate (ml/min)	*Response (max. = 100)*
30	1
40	8
50	53
60	92
65	100
70	88
75	76
80	68

TABLE II

RESPONSE OF THERMIONIC DETECTOR WITH INCREASING HYDROGEN FLOW-RATE

GLC conditions and integration method were given under *Apparatus*. Test sample: TFA leucine methyl ester (1 μl ≡ 1 μg).

Flow-rate (ml/min)	*Response (max. = 100)*
24	42
26	75
28	100
30	67
32	26
34	12

was obtained with a nitrogen flow-rate of 65 ml/min, as shown in Table I. Because a high flow-rate through the column prevented an adequate resolution of a mixture of amino acids, this was restricted to 15 ml/min and 50 ml/min supplied to the detector via a T-piece at the exit end of the column, as described under *Apparatus*. The optimal hydrogen flow-rate was critical and found to be 28 ml/min, as shown in Table II. Noise level at this flow-rate was very low[9]. Under these conditions the air flow-rate of 475 ml/min gave optimal response, as shown in Table III.

TABLE III

RESPONSE OF THERMIONIC DETECTOR WITH INCREASING AIR FLOW-RATE

GLC conditions and integration method were given under *Apparatus*. Test sample: TFA leucine methyl ester (1 μl ≡ 1 μg).

Flow-rate (ml/min)	*Response (max. = 100)*
400	17
450	90
475	100
500	84
550	55

The distance of the rubidium chloride annulus from the flame was adjusted daily for maximal response. The annulus required periodic cleaning[10]. A decrease in sensitivity with age, after several weeks, was also confirmed[9].

Comparison between TD and FID

A standard mixture of amino acids (Table IV, columns 1 and 2) was derivatized and chromatographed under identical conditions with analysis by TD and FID. The mean ratios of TD response to FID response obtained from five determinations made with each detector are given in Table IV. In each case the TD showed a higher response, ranging from 3.5 (phenylalanine and valine) to 21.9 (glycine). The very different ratios were probably due to selectivity of the detector varying from compound to compound[8]. Glycine had a very low FID response[7] and this accounted for the high ratio observed here.

TABLE IV

RATIO OF GLC RESPONSES OF THERMIONIC DETECTOR TO FLAME IONIZATION DETECTOR FOR TFA AMINO ACID METHYL ESTERS

GLC was carried out under identical conditions with each detector. See *Apparatus* for details. The molar ratios of the amino acids present in the test solution relative to that of norleucine = 1.00 are given in the second column. A GLC injection of 1 μl corresponded to 1.91 μg norleucine. Each detector response was the mean of five determinations.

Amino acid	*Molar ratio*	*Ratio of response TD/FID*
Alanine	0.98	16.6
Aspartic acid	0.80	9.1
Glutamic acid	0.64	5.9
Glycine	1.43	21.9
Hydroxyproline	0.94	8.3
Isoleucine	0.67	4.8
Leucine	1.01	5.7
Methionine	0.65	4.6
Norleucine	1.00	5.0
Phenylalanine	0.57	3.5
Proline	0.82	8.8
Serine	1.05	11.7
Threonine	0.87	7.9
Valine	0.73	3.5

A test for linearity of response with the TD was made with the same mixture of fourteen amino acids (Table IV). The derivatized amino acids were serially diluted over a total range ($\times$ 10) and the molar responses determined relative to that of norleucine.

The relative molar response values and standard errors given in Table V did not indicate either any changes over the concentration range tested or over the 10-day period of the experiment. The last two columns in Table V give the relative molar responses ($\pm$ S.E.) for five determinations in which the amino acid mixtures were taken through the derivatization procedure independently at a dilution ($\times$ 10). The standard errors were greater because they reflected the combined errors of methylation, trifluoroacetylation and GLC.

Theoretically, if the setting of the detector is selective for nitrogen, all the relative molar responses should be equal to unity, because all the amino acids listed possess one nitrogen atom per molecule. The low value for methionine was expected because this amino acid was reported to be partially destroyed during methylation for 90 min at 70° (ref. 1). Hydroxyproline and proline showed molar responses which possibly reflected a property of their imino groups.

DISCUSSION

There are few reports on the use of the nitrogen-sensitive TD. Swan[8] described some of the characteristics of the Pye detector with its rubidium chloride annulus and showed the need for careful positioning of this above the flame. Greenhalgh and Wilson[9] studied its performance in relation to variable factors such as conditioning, gas flow-rates and detector temperature. They showed that an increase in nitrogen flow-

TABLE V

MOLAR RESPONSES OF TFA AMINO ACID METHYL ESTERS WITH THE THERMIONIC DETECTOR

The composition of the amino acid mixture is given in Table IV. Molar responses (±S.E.) are given relative to norleucine = 1.00. A GLC injection of 1 μl corresponded to 1.91 μg norleucine at (×1) dilution. The derivatized amino acid mixture was serially diluted, except in the last set of results, where each sample was derivatized individually.

Amino acid	*Dilution*				
	×1 (n = 3)	*×2 (n = 3)*	*×4 (n = 3)*	*×10 (n = 3)*	*×10 (n = 5)*
Ala	0.98 ± 0.05	0.90 ± 0.01	0.95 ± 0.01	0.96 ± 0.01	0.99 ± 0.04
Asp	0.99 ± 0.04	1.03 ± 0.00	1.02 ± 0.01	1.02 ± 0.01	1.02 ± 0.04
Glu	0.94 ± 0.03	0.96 ± 0.00	0.99 ± 0.01	0.99 ± 0.01	0.95 ± 0.03
Gly	0.89 ± 0.02	0.87 ± 0.00	0.89 ± 0.01	0.84 ± 0.02	0.78 ± 0.03
Hyp	1.22 ± 0.03	1.27 ± 0.00	1.28 ± 0.03	1.28 ± 0.03	1.34 ± 0.05
Ile	0.82 ± 0.01	0.79 ± 0.01	0.80 ± 0.01	0.80 ± 0.01	0.87 ± 0.04
Leu	1.04 ± 0.02	1.01 ± 0.00	1.03 ± 0.01	1.03 ± 0.01	1.10 ± 0.03
Met	0.83 ± 0.02	0.81 ± 0.01	0.84 ± 0.05	0.84 ± 0.05	0.81 ± 0.03
Nle	1.00	1.00	1.00	1.00	1.00
Phe	1.13 ± 0.01	1.09 ± 0.01	1.12 ± 0.03	1.12 ± 0.03	1.03 ± 0.04
Pro	1.27 ± 0.02	1.25 ± 0.03	1.27 ± 0.02	1.25 ± 0.04	1.30 ± 0.04
Ser	0.94 ± 0.01	0.93 ± 0.00	1.00 ± 0.00	0.99 ± 0.01	1.06 ± 0.05
Thr	0.85 ± 0.02	0.82 ± 0.00	0.86 ± 0.00	0.86 ± 0.00	0.98 ± 0.03
Val	0.68 ± 0.02	0.63 ± 0.00	0.65 ± 0.00	0.62 ± 0.01	0.64 ± 0.03

rate up to 50 ml/min resulted in an increased response, but a maximal response was not shown. With the TFA amino acid methyl esters we found an optimal nitrogen flow-rate of 65 ml/min and air flow-rate of 475 ml/min. Very different carrier gas flow-rates to the detector were reported, which varied from 30 ml/min (ref. 8) to 100 ml/min (ref. 11) and air flow-rates from 85 ml/min (ref. 11) to 300 ml/min (ref. 8). Our hydrogen flow-rate of 28 ml/min gave a low noise level and was similar to that previously reported[8–11]. The positioning of the electrode was critical and it was found that with a vertical movement of 0.25 mm a ten-fold change in the detector response was obtained. Because of the variables involved, establishing the optimum conditions for this detector takes more time than with the FID. We found that reproducible values (Table V) were obtained over a 2-week period, provided that adjustments were made daily with the electrode setting. Gough and Sugden[10] also reported favourably on the stability of the detector.

The improved sensitivity obtained with the TD when compared with the FID (Table IV) was useful for the analysis of amino acids attached to transfer ribonucleic acid[4] (results to be published elsewhere).

ACKNOWLEDGEMENTS

We thank Messrs. G. D. Searle Ltd. for generous financial support and Professor H. R. V. Arnstein for his interest. M.B. gratefully acknowledges a studentship from the S.R.C.

REFERENCES

1 A. Darbre, *Biochem. Soc. Trans.*, 2 (1974) 70.
2 A. Karmen and L. Giuffrida, *Nature (London)*, 201 (1964) 1204.
3 W. A. Aue, C. W. Gehrke, R. C. Tindle, D. L. Stalling and C. D. Ruyle, *J. Gas Chromatogr.*, 5 (1967) 381.
4 M. Butler, A. Darbre and H. R. V. Arnstein, *Abstr. Commun. 9th Meet. Fed. Eur. Biochem. Soc., Budapest, 1974*, 18b56, p. 443.
5 A. Del Favero, A. Darbre and M. Waterfield, *J. Chromatogr.*, 40 (1969) 213.
6 A. Darbre and A. Islam, *Biochem. J.*, 106 (1968) 923.
7 A. Islam and A. Darbre, *J. Chromatogr.*, 71 (1972) 223.
8 D. F. K. Swan, *Column*, No. 14 (1972) 9.
9 R. Greenhalgh and M. Wilson, *Column*, No. 15 (1972) 10.
10 T. A. Gough and K. Sugden, *J. Chromatogr.*, 86 (1973) 65.
11 R. F. Coward and P. Smith, *J. Chromatogr.*, 61 (1971) 329.