Waters
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Pico-Tag® Method Provides Accurate and Reproducible Amino Acid Compositions - Even in the Hands of Inexperienced Users.

Much of the routine amino acid analysis work in the Applications Development Laboratory is performed by university co-op students. Their training includes doing compositional analysis, and the success of that training is assessed by their ability to get accurate compositions on standard proteins and peptides.

Since co-op students generally possess good basic laboratory skills, but little or no HPLC or Pico-Tag experience, their performance is a good indicator of what can be expected in a new customer's lab.

Standard proteins and peptides were obtained from Sigma or an equivalent source. Hydrolysis and derivatization were performed using the Pico-Tag Workstation. The LC system consisted of two M6000 pumps, M440 detector @254 nm, M710B WISP™Autosampler, Temperature Control Module, Eluent Stabilization System, and an M840 Data and Chromatography Control Station with Waters Expert™ software, Rev. 4.0. The column was a Pico-Tag Free Amino Acid Column, 3.9 X 300 mm; eluents were standard Pico-Tag® Eluents A and B. The column was maintained at 46°C; the gradient was as previously published (1), with the times doubled.

The table below presents results obtained for five replicate hydrolyses of 5 µg hen egg white lysozyme. Values are expressed in residues, and have been normalized to PHE = 3. Deviations as a percent of actual are shown in the last column. No corrections for loss of serine, threonine, or methionine have been applied. The average deviation (not including CYS) from expected is 7.8%. The average of the relative standard deviations (not shown) is 3.09%.

AA	LYSO-1	LYSO-2	LYSO-3	LYSO-4	LYSO-5	AVG.	THEOR.	% DEV.
ASP	22.16	21.06	21.10	21.49	21.42	21.45	21	2.13
GLU	5.39	5.74	5.10	5.16	5.46	5.37	5	7.42
SER	8.08	8.17	8.10	8.23	8.34	8.18	10	18.15
GLY	13.47	13.47	12.80	12.84	13.74	13.26	12	10.52
HIS	1.04	1.02	1.20	0.91	1.08	1.05	1	4.98
ARG	11.20	10.85	11.20	11.30	11.70	11.25	11	2.29
THR	6.12	6.00	6.20	6.07	6.36	6.15	7	12.14
ALA	12.55	11.94	12.20	12.14	12.54	12.27	12	2.28
PRO	2.45	2.49	2.50	2.44	2.58	2.49	2	24.60
TYR	2.63	2.74	2.60	2.79	2.94	2.74	3	8.61
VAL	5.76	5.62	5.70	5.44	5.58	5.62	6	6.35
MET	1.78	1.79	1.80	1.74	1.80	1.78	2	10.93
CYS	2.33	2.04	0.80	2.23	2.94	2.07		
ILE	5.69	5.55	5.10	5.51	5.64	5.50	6	8.34
LEU	8.63	8.36	7.90	8.37	8.70	8.39	8	4.92
PHE	3.00	3.00	3.00	3.00	3.00	3.00	3	0.00
LYS	6.12	5.94	5.90	6.07	6.24	6.05	6	0.89
						AVG. % DEV.		7.78

T. Tarvin/S. Cohen

Conclusions:

The results of this study confirm that the Pico-Tag method does give reproducible, accurate compositions at the 5 μg level for proteins; for peptides equivalent accuracy can be obtained at the 1 μg level.

Examination of values obtained for other standard proteins (BSA, aldolase, ribonuclease, insulin, myoglobin, etc.) indicates that the results of this study are typical of what can be expected of the Pico-Tag method with normal good laboratory practices. It has previously been shown that when laboratory procedures and environment are optimized for high sensitivity work, good compositions can be obtained on as little as 100 ng of sample (2,3).

References:

1. B. A. Bidlingmeyer, S.A. Cohen, and T.L. Tarvin, *J. Chromatogr.* (Biomed. Appln.) 336 (1984), 93.

2. B. A. Bidlingmeyer, S. A. Cohen, and T.L. Tarvin, in METHODS IN PROTEIN SEQUENCE ANALYSIS 1986, Ed. by Kenneth A. Walsh, Humana Press, Clifton NJ, (1987), 229-245.

3. D. J. Strydom, J.W. Harper, and R.R. Lobb, *Biochem.*, 25 (1986), 945.

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