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Lab Highlights
An Internal Communication of Applications and Techniques
LAH 0265 10/85
DP/LS/MD/AA/DV

PICO•TAG™ METHODOLOGY QUANTITATES TRYPTOPHAN

Waters™ now announces a simple method for tryptophan analysis using the PICO•TAG™ method with methanesulfonic acid hydrolysis. Quantitative analysis of tryptophan content in peptides and proteins is complicated by its instability to hydrolysis in HCl. The most common means of hydrolysis have used base (e.g. sodium hydroxide), sulfonic acids such as p-toluenesulfonic acid or most commonly methanesulfonic acid (MSA), or additives in HCl that protect against destruction. Most common of the latter is thioglycolic acid. Because each of these methods uses a nonvolatile constituent that cannot be readily removed from protein /peptide hydrolyzates, there can be possible interference in the coupling reaction of the PICO•TAG™ method. In the case of MSA this is easily dealt with by neutralization after hydrolysis (see below).

A major modification of the procedure, shown in the step-by-step description below, is that the MSA reagent must be added directly to the 6 X 50 mm sample tubes due to the acid's nonvolatility. Batch processing for the hydrolysis and derivatization steps is still streamlined through the use of Waters™ exclusive PICO•TAG™ Work Station.

Procedure for MSA Hydrolysis/Analysis

1. Add 20 µl of 4M MSA containing 0.2% (w/v) tryptamine HCl (Sigma P/N M4141) to each 6 X 50 mm sample tube containing dried sample.
2. Add 100 µl of water to the reaction vial.
3. Seal for hydrolysis using the usual PICO•TAG™ procedure.
4. Hydrolyze at 110°C for 20-24 hours.
5. Cool, open vial and add 22 µl of 4M KOH (sufficient to neutralize) to each sample tube.
6. Dry under vacuum
7. Redry using 20 µl of redrying reagent, methanol-water-triethylamine (2:2:1)
8. Derivatize using the modified PICO•TAG™ derivatizing reagent, methanol-water-triethylamine-PITC (7:1:1:1).
9. Dry under vacuum.
10. Reconstitute in sample diluent, filter through a Millipore HV4 filter and inject.

Tryptophan is easy to quantitate by the PICO•TAG™ Method as it gives far better peak shape than by ion-exchange analysis, and is baseline resolved from the other amino acids and reagent peaks (Figure 1). Values for tryptophan (Table I) are excellent, although for best quantitation of all the amino acids it is recommended that both HCl and MSA hydrolyses be performed.

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TABLE I

PICO·TAG™ ANALYSIS FOR TRYPTOPHAN QUANTITATION
MSA HYDROLYSIS[a]

AMINO ACID	HEXAPEPTIDE	PORCINE ACTH	EGG WHITE LYSOZYME
ASP		2.19 (2)	21.15 (21)
GLU		4.86 (5)	5.47 (5)
SER		1.52 (2)	10.26 (10)
GLY		3.32 (3)	12.85 (12)
HIS		0.97 (1)	1.08 (1)
ARG	0.82 (1)	3.51 (3)	12.55 (12)
THR		0. (0)	8.10 (7)
ALA	0.98 (1)	2.97 (3)	12. b
PRO		4.02 (4)	2.82 (2)
TYR		1.77 (2)	3.24 (3)
VAL		2.86 (3)	5.19 (6)
MET	0.81 (1)	0.54 (1)	1.44 (2)
ILE		0. (0)	5.09 (6)
LEU	1.00 (1)	2.18 (2)	7.66 (8)
PHE	1.02 (1)	2.76 (3)	3.08 (3)
TRP	0.90 (1)	0.76 (1)	5.46 (6)
LYS		3.96 (4)	4.56 (6)

a) Values in parentheses are based on the actual sequence
b) Lysozyme values are normalized to the theoretical Ala content

FIGURE 1

CHROMATOGRAM OF LYSOZYME MSA HYDROLYZATE