Waters
Lab Highlights
An Internal Communication of Applications and Techniques
LAH 0224 3/85
AN/LS/MD/AA/OT

AMINO ACID ANALYSIS: A METHOD FOR EVERY NEED

No single method can meet the needs of the diverse groups of users interested in amino acid analysis. In order to help people choose the appropriate method for their application, three tables have been constructed.

Table 1 shows the two major types of amino acid applications: 1) protein hydrolyzates and 2) physiological. The boxes above these sections are refinements by the sample type of these two segments and where these sample types would be found.

TABLE 1

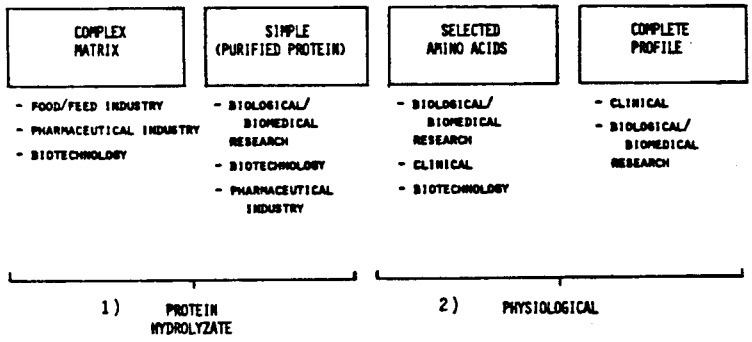

Table 2 is a grid of sample type vs methods (ion exchange with post-column ninhydrin, ion exchange with post column OPA, pre-column OPA, and pre-column PITC.)

TABLE 2

		POST - COLUMN DERIVATIZATION		PRE - COLUMN DERIVATIZATION	
SAMPLE \ METHOD		ION EXCHANGE NINHYDRIN	ION EXCHANGE OPA/HYPO	REV. PHASE AUTO-OPA	PICO-TAG
PROTEIN HYDROLYZATE	SIMPLE PURIFIED PROTEIN	YES	YES	YES (1° ONLY)	YES
	COMPLEX FEED, FOOD, ETC.	YES	YES	YES (1° ONLY)	YES
PHYSIOLOGICAL	COMPLETE PROFILE	YES	NO	NO	?
	SELECTED AMINO ACIDS	YES	YES	YES	YES

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Table 3 looks at the trade-offs of chromatographic specifications.

TABLE 3

METHODOLOGY / SPECIFICATIONS	POST - COLUMN DERIVATIZATION		PRE - COLUMN DERIVATIZATION	
	ION EXCHANGE NINHYDRIN	ION EXCHANGE OPA/HYPO	REVERSE PHASE AUTO/OPA	PICO-TAG
SPEED OF ANALYSIS	90 min	90 min	30 min	12 min
SENSITIVITY ROUTINE USAGE DETECTION LIMIT	1 nmole 100 pmoles	1 nmole 50 pmoles	250 pmoles 0.5 pmoles	250 pmoles 1 pmole1
DETECTION	1° + 2° amines, lactones, etc. 570 nm for 1° amines; 440 nm for 2° amines	1° + 2° amines, Fluorescence 338 nm ex; 425 nm em	1° amines only Fluorescence 338 nm ex; 425 nm em	1° + 2° amines, Absorb at 254 nm
REPEATABILITY (RETENTION TIME)	0.2% RSD	0.2% RSD	2% RSD	0.5%RSD
REPRODUCIBILITY (QUANTITATION)	1-2% RSD (@ 1-2 nmole)	3-5% RSD (@ 1-2 nmole)	5-10% RSD (@50-100 pmole)	< 4% (@ 250 pmole)
SAMPLE PREP (ALL REQUIRE PROTEIN HYDROLYSIS)	CLEANUP NO DERIVATIZATION	CLEANUP NO DERIVATIZATION	CLEANUP AND AUTOMATED DERIVATIZATION	CLEANUP AND BATCH DERIVATIZE 10 min/12 samples
TRADITIONAL METHOD	YES	NO	NO	NO
HARDWARE: STANDARD GRADIENT LC PLUS:	amino acid ion exchange column and post - column reaction kit for ninhydrin	amino acid ion exchange column and post - column reaction kit , Fluorescence Detector	Resolve C18 or Nova-Pak C18 column, tri-Module system , Fluorescence Detector	PICO-TAG WORK STATION and CHEMISTRY PKG