The Intrada Amino Acid is a separation column for non-labeled amino acids and related compounds using LC-MS systems.
The column provides LC-MS analysis for intact amino acids and related compounds. Various column dimensions may be selected in order to optimize analyses across different combinations of amino acids.

Product Specification

Product Name	Intrada Amino Acid
Base Materials	Pure spherical silica
Particle Size	3µm
Ligand	Special phase for amino acid

HPLC column usage and directions

The Intrada Amino Acid column should be used only for LC-MS. Analytical sensitivity depends on the specific LC-MS instrumentation.
Please see the reverse side of this instruction manual for details on analytical conditions and warnings.

Column usage

Dropping, shaking, striking, or any other extreme actions with this column may lead to decreased column performance and efficiency. Please do not tighten or loosen the column end-fitting as this can lead to fluid leakage and abnormal column pressure.

Column connection and release

There is a "FLOW" mark on the column label. Please connect the column according to the directional flow of the elution. When using a wrench to connect or release the column, please place the wrench near the end fitting closest to the line. Please do not place the wrench on the column body itself as this can lead to problems including, but not limited to, eluent leakage.

Mobile phase and sample filtration

Please filter the mobile phase and sample solution through either a 0.45um or 0.2um membrane filter prior to injection. If there is any floating or deposited material, it can cause higher pressure via column clogging. In particular, please be careful of microbial pollutants in the aqueous mobile phase.

Column enclosed solvent

The column's enclosed solvent is **92%acetonitrile, 8%water**. It can be directly substituted with the initial mobile phase of the amino acid analysis.

Mobile phase preparation

This column requires gradient elution with two kinds of mobile phases. To retain ionic amino acids, pH modifiers like formic acid, ammonium formate, or ammonium acetate are required. Organic solvents can be used such as acetonitrile, methanol, tetrahydrofuran (THF), etc., with concentration of 0-100%.

Flow rate

In general, please set the flow to levels similar to conventional 3um columns. In order to achieve best results, please optimize flow rate around the desired separation characteristics, solvent consumption, column pressure and temperature.

RECOMMENDED FLOW RATE (depends on column length and conditions)

Column I.D.	1mm	2mm	3mm
Recommended Flowrate (mL/min)	0.03-0.2	0.1-0.6	0.2-1.0

Pressure

Peak pressure is approximately 50MPa.

Temperature

Column temperature range is suitable for 15-65 degrees Celsius. Please adjust the temperature according to the desired peak shape, retention and separation characteristics. However, please note that extended exposure to high temperature and high pH can decrease column lifetime.

pH

The typical pH usage range is 1.5-8. However, if the column is used extensively on the alkali side, column lifetime can differ depending upon analytical conditions such as organic solvent density, pH adjusting agent composition, temperature, and eluent structure. Please adjust the pH according to your own personal objectives.

If storing the column after use under alkali conditions, please store the column after replacing the preservation solution. Please note that extensive exposure to alkali conditions can shorten column lifetime.

Cleaning the column

100mM ammonium formate is a general column washing solvent. Wash column with 100mM ammonium formate for one hour, then wash with water for 30 minutes, followed by initial gradient mobile phase for conditioning. Polymers like protein may cause coagulation in the column and be difficult to wash out, so this column cleaning procedure is strongly recommended pre-treatment.

Column preservation solution

After the column has been used for analysis, please replace with the initial gradient mobile phase and store at room temperature.

AUTHORIZED DISTRIBUTOR
MZ-Analysentechnik GmbH, Barcelona-Allee 17• D-55129 Mainz
Tel.+49 6131 880 96-0, Fax.+49 6131 880 96-20
e-mail: info@mz-at.de, www.mz-at.de
MZ ANALYSENTECHNIK

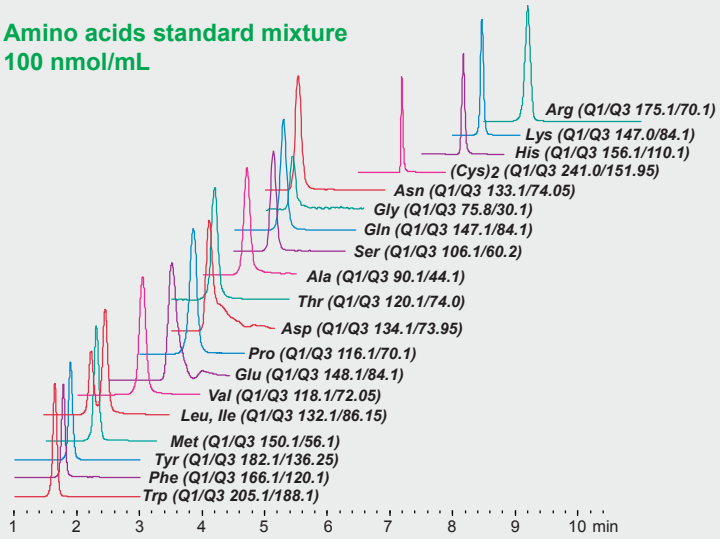
Imtakt Intrada Amino Acid Analytical Conditions and Warnings

WARNINGS

- *The Intrada Amino Acid column should be used only for LC-MS. This product is not recommended for applications with UV or ELSD detection systems, in order to avoid peak identification failure.*
- *Because detection sensitivity depends highly on MS instrument performance, proper LC-MS instruments should be carefully chosen for individual analysis purposes or sensitivity.*
- *Method development (column dimensions, gradient conditions, sample preparation, standard curve, etc.) and validation are required to achieve an optimized analytical method.*
- *Amino acid surrogate compounds using an isotope internal standard may be useful for high sensitive probe assays*
- *Injection solution requires acidic conditions using 0.1N HCl or 0.1-2% HCOOH etc. for peak shape and reproducibility.*
- *Final 0.2N HClO4 for sample preparation is recommended to remove proteins from serum.*
- *Please refer to general methods for sample preparations for amino-acid composition analysis.*

Mobile Phase Preparation (General conditions for protein amino acids)

Amino acids standard mixture
100 nmol/mL

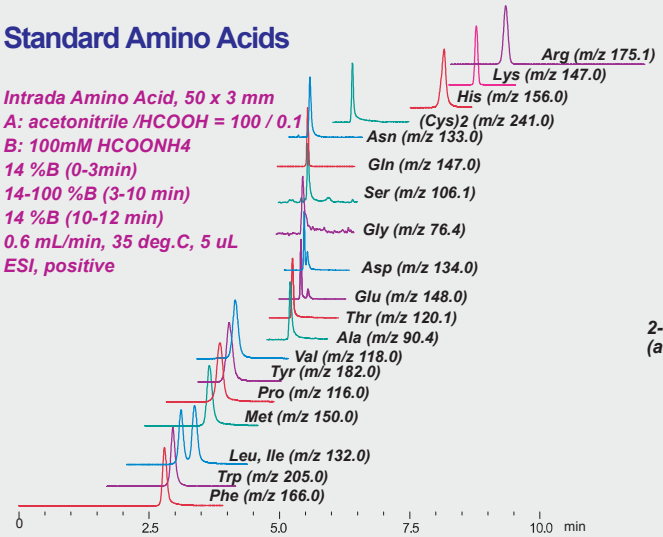


Intrada Amino Acid, 50 x 3 mm
A: CH3CN / THF / 25 mM HCOONH4 /HCOOH = 9 / 75 / 16 / 0.3 (v/v/v/v)
B: ACN / 100 mM HCOONH4 = 20 / 80 (v/v)
0 %B (0-3 min)
0-17 %B (3-6.5 min)
100 %B (6.5-10 min)
0.6 mL/min, 40 deg.C, 1 uL (0.1N HCl)
ESI , positive

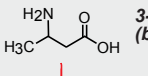
Examples for simple gradient elution

Standard Amino Acids

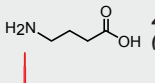
Intrada Amino Acid, 50 x 3 mm
A: acetonitrile /HCOOH = 100 / 0.1
B: 100mM HCOONH4
14 %B (0-3min)
14-100 %B (3-10 min)
14 %B (10-12 min)
0.6 mL/min, 35 deg.C, 5 uL
ESI, positive



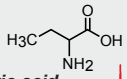
Amino Acid Isomers



3-aminobutyric acid (beta-aminobutyric acid)
m/z 104.1



4-aminobutyric acid (GABA, gamma-aminobutyric acid)
m/z 104.1

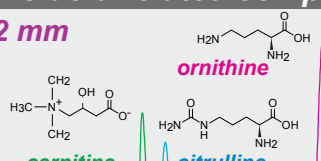


2-aminobutyric acid (alpha-aminobutyric acid)
m/z 104.1

Intrada Amino Acid, 75 x 2 mm
A: acetonitrile / HCOOH = 100 / 0.3
B: 100 mM HCOONH4
15-100 %B (0-8 min)
15 %B (8-10 min)
0.3 mL/min, 35 deg.C
5 uL (0.1N-HCl aq.)
ESI (positive, m/z 104.1)

Amino acid related compounds

10 x 2 mm

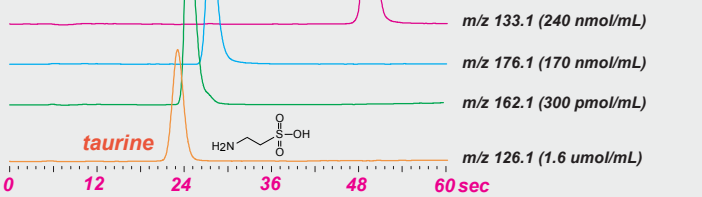


ornithine

carnitine

citrulline

Intrada Amino Acid, 10 x 2 mm
A: acetonitrile /HCOOH = 100 /0.1
B: 100mM HCOONH4
15-100 %B (0-0.8 min)
100 %B (0.8-1.0 min)
0.4 ml/min (1.6 MPa)
35deg.C, 1 uL (0.1N HCl)
ESI, positive

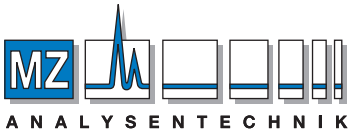


taurine

ANALYTICAL CONDITIONS PROTOCOL

Several parameters below should be tried for analytical optimization.

A: acetonitrile /HCOOH = 100 / (0.1 - 0.5), v/v
B: (50-200mM) HCOONH4
Initial - Final %B (Gradient Time)
Flow Rate: depends on column I.D.
Temperature: up to 65deg.C
Injection Solution: 0.1N HCl or 0.1 - 2% HCOOH
MS detection: ESI, positive



AUTHORIZED DISTRIBUTOR
MZ-Analysentechnik GmbH, Barcelona-Allee 17• D-55129 Mainz
Tel +49 6131 880 96-0, Fax +49 6131 880 96-20
e-mail: info@mz-at.de, www.mz-at.de