

## Amino Acids Separation Column for LC-MS

The world's first specialty column for intact amino acid analysis via LC-MS

Compatible with conventional LC-MS, providing fast and easy amino acid analysis

# Intrada Amino Acid

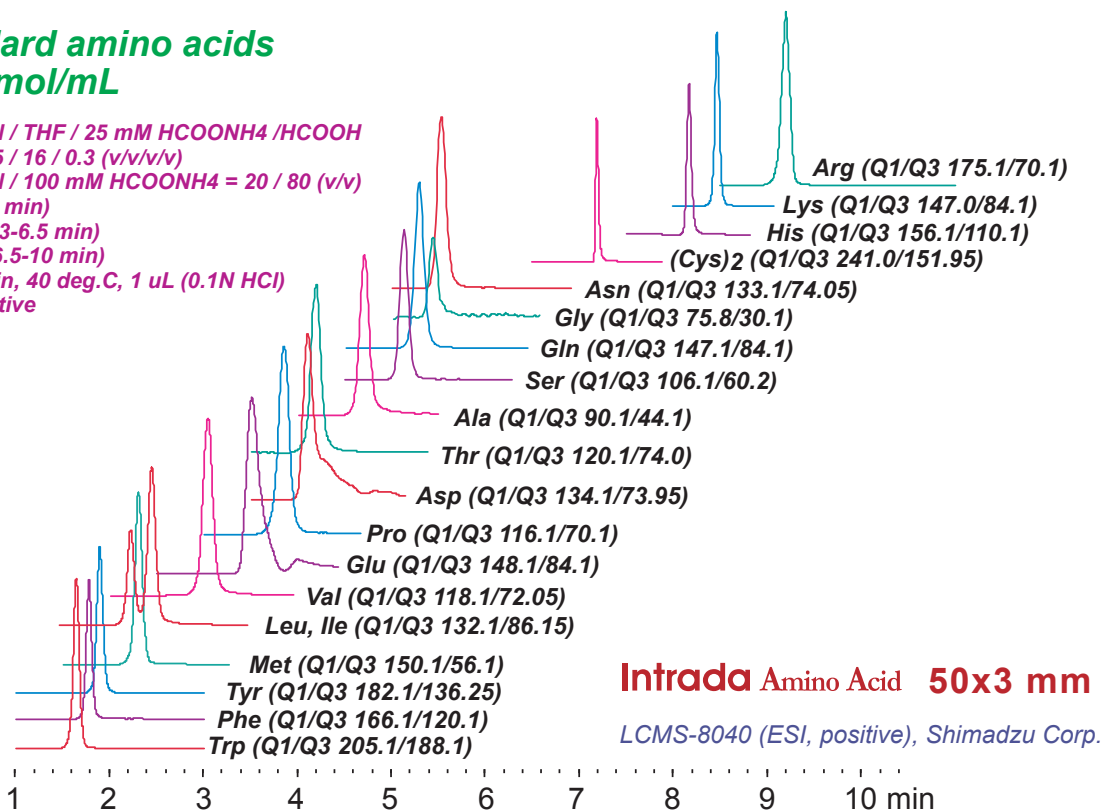
LC-MS analysis of amino acids  
 Amino Acid analysis without sample derivatization  
 Ability to separate isobaric amino acids such as Leu and Ile  
 High-throughput (<1 minute) analysis for selected amino acids  
 5-10 minutes for standard amino acids analysis

Pure spherical silica / 3µm particles / unique stationary phase designed for amino acids

## 10min analysis using LC-MS/MS

### Standard amino acids 100 nmol/mL

**A:** CH<sub>3</sub>CN / THF / 25 mM HCOONH<sub>4</sub> / HCOOH  
 = 9 / 75 / 16 / 0.3 (v/v/v/v)  
**B:** CH<sub>3</sub>CN / 100 mM HCOONH<sub>4</sub> = 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 µL (0.1N HCl)  
 ESI, positive



Imtakt has developed a novel column for the analysis of amino acids that couples well with MS detection and does not require use of an amino acid analysis system.

- When coupled to LC-MS the Intrada Amino Acid column achieves high-throughput analysis without requiring tedious pre- or post-labeling methods.
- Use of various column dimensions can aid in optimizing analytical time and resolution for different amino acid samples.
- Separation of leucine and isoleucine isomers, GABA isomers, and dipeptides analysis is now possible.
- Separate free amino acids in mixtures!
- Study protein amino acid composition!
- Isolate amino acid bio-markers!

## LC-MS analysis for 55 amino acids in 10 min

- \* Stationary phase is specially designed for amino acid and dipeptide retention and separation.
- \* No pre- or post-labeling methods required
- \* High throughput analysis via LC-MS
- \* Separation of isobaric amino acids on LC-MS systems is finally possible.

### 55 Amino Acids (standard samples)

**Intrada Amino Acid, 50 x 3 mm**

**A: acetonitrile /tetrahydrofuran /25mM ammonium formate /formic acid  
= 9 / 75 / 16 / 0.3**

**B: acetonitrile / 100mM ammonium formate = 20 / 80**

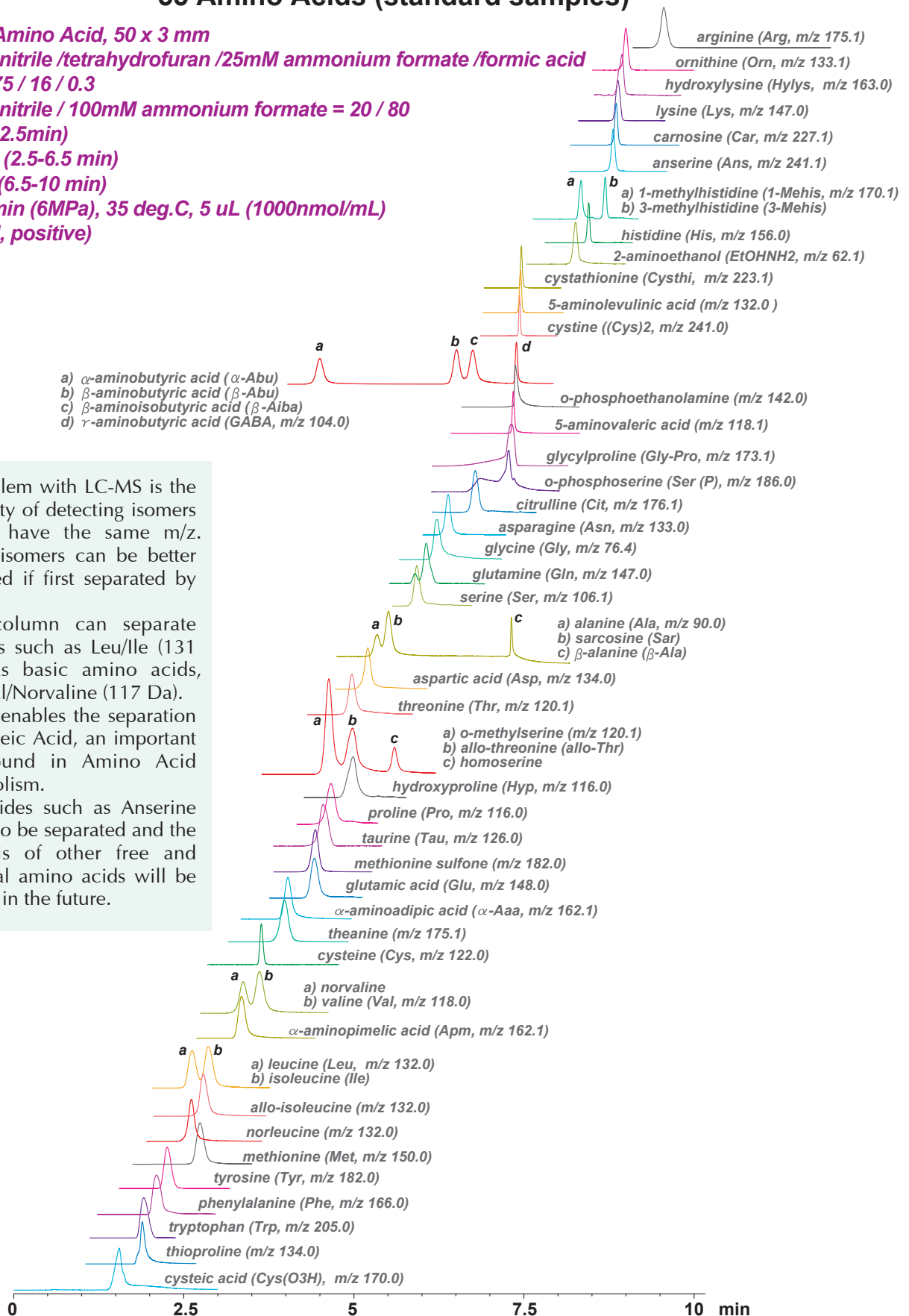
**0 %B (0-2.5min)**

**0-17 %B (2.5-6.5 min)**

**100 %B (6.5-10 min)**

**0.6 mL/min (6MPa), 35 deg.C, 5 uL (1000nmol/mL)**

**ESI (SIM, positive)**



A problem with LC-MS is the difficulty of detecting isomers which have the same m/z. These isomers can be better detected if first separated by LC.

This column can separate isomers such as Leu/Ile (131 Da), as basic amino acids, and Val/Norvaline (117 Da).

It also enables the separation of Cysteic Acid, an important compound in Amino Acid metabolism.

Dipeptides such as Anserine can also be separated and the analysis of other free and artificial amino acids will be shown in the future.

## Various column dimensions enhance scalability and flexibility of analysis methods

Intrada Amino Acid columns allow more flexibility:

- Manipulate the mobile phase composition and gradient method to accommodate a variety of amino acid samples, sensitivity requirements, and run times.
- Use of a shorter column allows for one minute analysis of non-isomer amino acids.

### Example of simple elution method

**Intrada Amino Acid, 50 x 3 mm**

**A: acetonitrile / HCOOH = 100 / 0.1**

**B: 100mM HCOONH<sub>4</sub>**

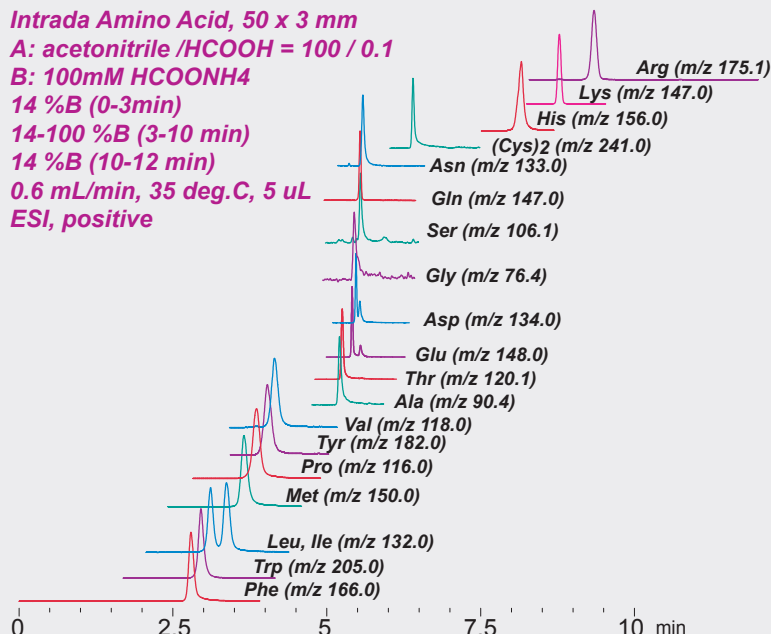
**14 %B (0-3min)**

**14-100 %B (3-10 min)**

**14 %B (10-12 min)**

**0.6 mL/min, 35 deg.C, 5 uL**

**ESI, positive**



Left: An application for protein amino acid analysis. Typically THF/acetonitrile organic solvent mobile phases are used, however in this application simple elution using acetonitrile is acceptable.

Leu/Ile isomers separate using these mild conditions. However, mid-sized amino acids such as Asp(133Da)/Asn(132Da) and Glu(147Da)/Gln(146) elute at similar times on the column, so high performance MS detection is ideal.

### Analytical conditions protocol

**A: acetonitrile / HCOOH = 100 / (0.1 - 0.5), v/v**

**B: (50-200mM) HCOONH<sub>4</sub>**

**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**

Analytical condition protocol to handle Intrada Amino Acid column is described in left table.

Analysis condition for various kinds of analysis purpose should be optimized changing gradient profile or solvent concentration etc.

### Separation of GABA (103Da) isomers

**Intrada Amino Acid, 100 x 3 mm**

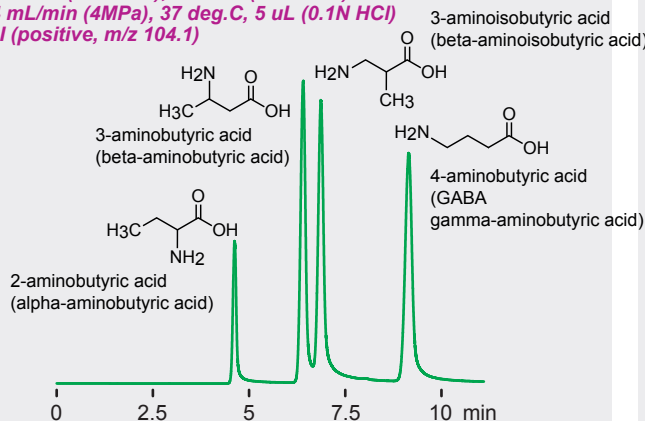
**A: acetonitrile / formic acid = 100 / 0.3**

**B: 100mM ammonium formate**

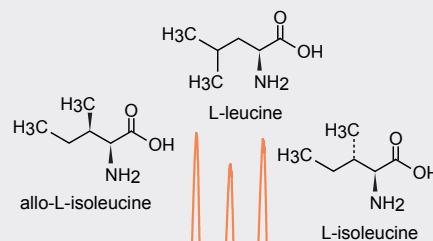
**25-30 %B (0-12 min), 100 %B (12-15min)**

**0.4 mL/min (4MPa), 37 deg.C, 5 uL (0.1N HCl)**

**ESI (positive, m/z 104.1)**



### Separation of Leucine (131Da) isomers



**Intrada Amino Acid, 150 x 3 mm**

**A: methanol / water / formic acid = 85 / 15 / 0.3**

**B: acetonitrile / 100mM ammonium formate = 20 / 80**

**12-13 %B (0-12 min)**

**100 %B (12-15 min)**

**0.4 mL/min (11 MPa)**

**35 deg.C, 5 uL (0.1N HCl)**

**ESI (positive, m/z 132.0)**

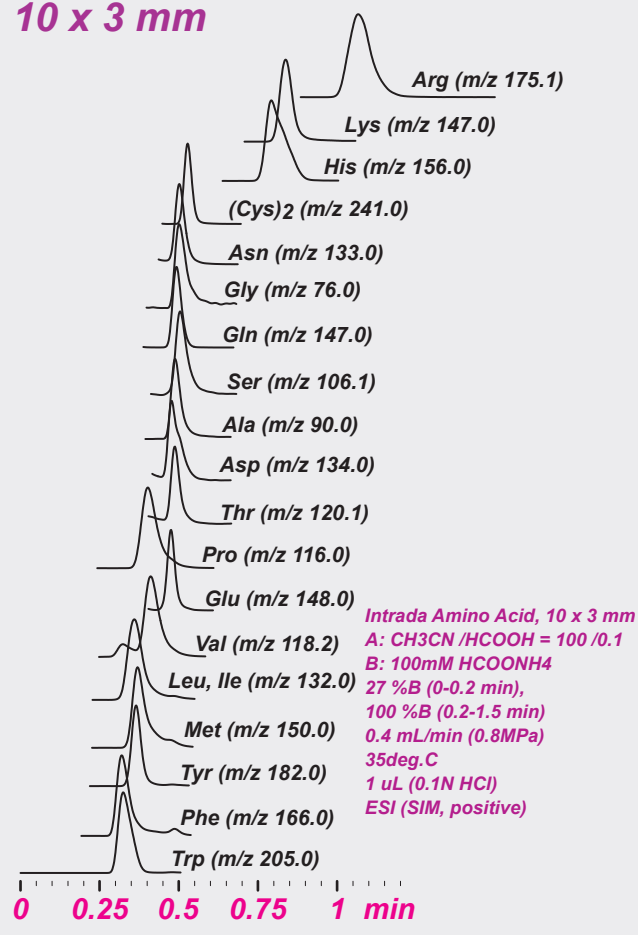


Intrada Amino Acid column separates amino acid isomers quickly by using an optimized column length.

Above: Figures show aminobutyric acid isomers(103Da) and leucine isomers(131Da) using 100-150mm length columns.

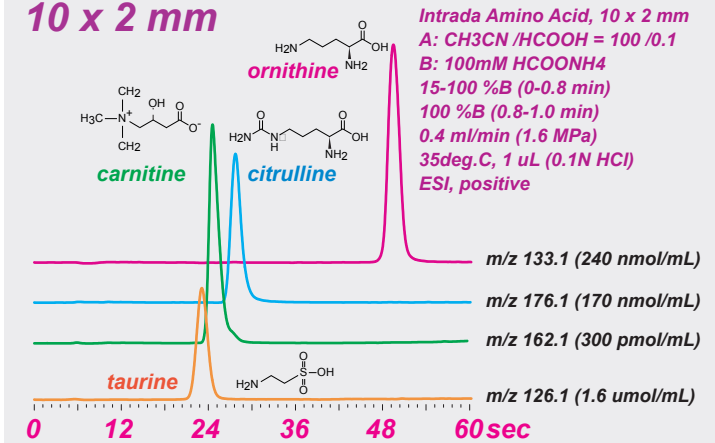
## High-throughput analysis of standard amino acids

10 x 3 mm



## One-minute analysis of related compounds

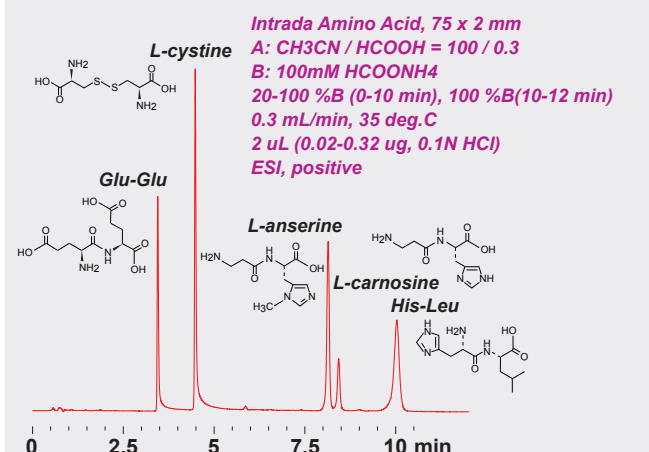
10 x 2 mm



Above: One minute ultra high-throughput analysis can successfully be performed on a 10mm length column.

Intrada Amino Acid high-throughput columns offer the next generation amino acid analysis method for clinical amino acid biomarkers, fermented materials, botanical amino acids and related compounds providing amazing speed, selectivity and convenience.

## Dipeptide analysis



Intrada Amino Acid is applicable not only for amino acid analysis, but also for polar dipeptides which are difficult to retain and separate in conventional HPLC.

Analysis of longer chain peptides that require high ionic strength mobile phases should use the Scherzo SS-C18 multi-mode ODS column.

## Column Recommendations

- The Intrada Amino Acid column should be used only for LC-MS in order to achieve adequate peak identification. This product is not recommended for applications involving UV or ELSD instruments.
- Note that detection sensitivity is highly dependent upon MS instrument performance. LC-MS instruments should be carefully chosen to yield adequately sensitive data.
- In order to achieve optimized analytical data, method development (column dimensions, gradient conditions, sample preparation, standard curve, etc.) and validation are required.
- Please refer to general methods for sample preparations for amino-acid composition analysis.

## Product Information

### Intrada Amino Acid

Column I.D.

3mm, 2mm, 1mm  
0.5mm - 0.075mm

Column Length (depends on I.D.)

10mm, 20mm, 30mm  
50mm, 75mm, 100mm  
150mm, 250mm

Guard column system is not available for this product.

