

# Thermo Scientific LC IsoLink™



## *The Link from HPLC to Isotope Ratio Mass Spectrometry*



Carbohydrates



Amino Acids, Nucleotides



Organic Acids



Pharmaceuticals

# The Link from HPLC to Isotope Ratio MS...

The Thermo Scientific LC IsoLink is the first high sensitivity interface connecting HPLC with Isotope Ratio MS (IRMS) for reproducible and accurate on-line determination of  $^{13}\text{C}/^{12}\text{C}$  isotope ratios. All organic compounds eluting from an HPLC column are analyzed while maintaining the chromatographic resolution, opening up a new frontier in the application of isotope ratio mass spectrometry.

## Unique Features of the LC IsoLink:

- On-line coupling of HPLC and Isotope Ratio MS
- Isotope ratio data acquired on an HPLC time scale
- Preserves the integrity of chromatographic resolution
- Sample analysis in the low nanomole range
- Accurate and reproducible results
- Direct injection of reference materials
- Maintenance-free operation
- High throughput
- Capability for full automation

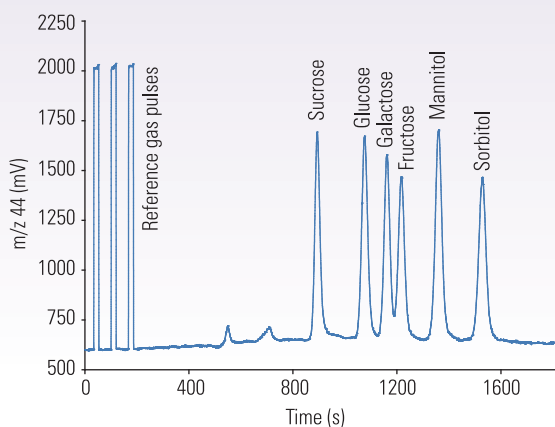




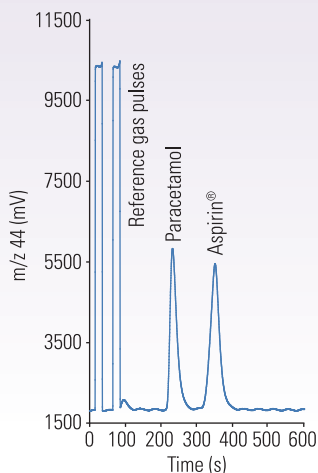
**Carbohydrates** are one of the largest and most common families of molecules in life. They serve as energy resource, backbone of DNA and RNA, carrier of biologically active compounds, and as structure building material in plants. Sugars can carry information of their origin and processing. If isotopically labeled, they tell us about their pathways and use.



Because **pharmaceuticals** are produced at various sites with different prices and different formulations, it is essential for both the producers and consumers to be able to distinguish between originals and generics. Aspirin® and Paracetamol are representatives of mass produced drugs with a wide range of origin and price.



Sugars on a cation exchange column.



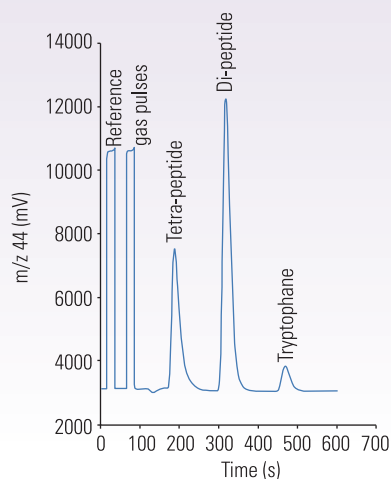
Drugs on a HyPURITY AQUASTAR™ HPLC column.

# ...for a wide Range of Applications...

The Thermo Scientific LC IsoLink is built to directly address the analysis of polar and thermolabile compounds. Most of these compounds (such as amino acids, peptides, carbohydrates, nucleotides, alcohols, organic acids, sulfa drugs, and many more) can be chromatographically separated in aqueous phase using inorganic buffers on ion exchange, size exclusion, or specific reversed phase HPLC columns. The LC IsoLink enables the on-line high precision determination of the  $^{13}\text{C}/^{12}\text{C}$  isotope ratio of each separated component.

- **Metabolism Studies**
  - *Tracer experiments with amino acids, lipids, carbohydrates*
  - *Nucleotide analysis for DNA synthesis rates*
- **Food and Food Ingredients**
  - *Authentication of honey and fruit juices*
  - *Determining origin of flavors and nutrients*
  - *Analysis of phospholipids, carbohydrates, amino acids and vitamins*
- **Pharmaceuticals**
  - *Authenticity control of drugs*
  - *Investigation of agents and additives*
- **Doping control**
  - *Analysis of steroids and hormones*
- **Biogeochemistry**
  - *History of biomolecules in sediments and soils*

**Amino acids and peptides** are the basic elements for building proteins. Their structure and metabolism are of major interest in medicine and biology. Either naturally occurring isotope differences or labelling experiments can be used to give insight into their pathways.



Short-chained peptides and amino acid on a C18 reversed phase endcapped column.

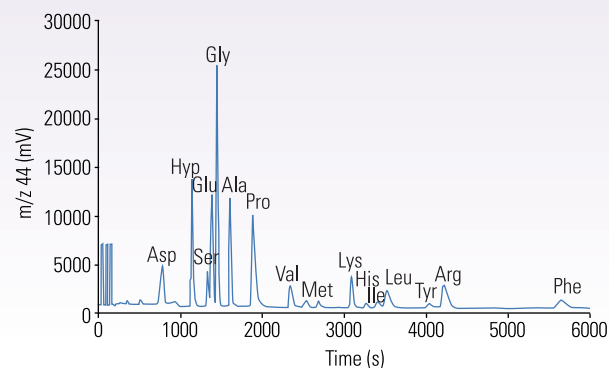
## Archaeological Bone Collagen

The reconstruction of ancient diets using body tissue stable isotope values is an important aspect of paleodietary studies which provide archaeological science with a quantitative understanding of what people consumed in the past.

Isotopic studies of diet are based on the principle “You are what you eat”, and collagen, being a well-preserved protein found in bone, has been most often used for these purposes.

Isotopic signatures of individual amino acids can differ consistently and widely from each other. More dietary information is potentially available at the individual amino acid level.

Separation of up to 18 amino acids can be performed on a single HPLC column.



*irm*-LC/MS chromatogram of archaeological bone collagen hydrolysate.

## The Concept of HPLC Coupling to IRMS

Until now, the analysis of organic samples for  $^{13}\text{C}/^{12}\text{C}$  isotope ratios was restricted to either on-line GC separation followed by combustion (isotope ratio monitoring GC/MS, *irm*-GC/MS) or bulk combustion of samples in an organic elemental analyzer. Biologically or pharmaceutically important compounds are often not GC-volatile or too thermolabile to be separated by gas chromatography.  $^{13}\text{C}/^{12}\text{C}$  isotope ratio determination of these compounds in real analytical applications, typically within complex matrices, was so far either impossible or extremely labor intensive.

The patented LC IsoLink\* leapfrogs the hurdle that, until now, has prevented successful isotope ratio monitoring LC/MS, namely the quantitative, fractionation-free elimination of the liquid phase prior to the oxidative conversion of the sample into  $\text{CO}_2$ .

In the LC IsoLink, the liquid phase is not removed from the sample prior to oxidation but the sample is oxidized while still within the aqueous solution, and only afterwards the  $\text{CO}_2$  is separated from the liquid phase for isotopic analysis. This process is quantitative and fractionation-free in the LC IsoLink.

The oxidation reagent consists of two solutions, the oxidizing agent and phosphoric acid. Both are pumped separately and added to the mobile LC phase.

All individual organic compounds eluting from the HPLC column are oxidized quantitatively into  $\text{CO}_2$  within this mixture when passing through a heated reactor. In a downstream degassing unit, the  $\text{CO}_2$  is removed from the liquid phase and entrained into a stream of He.

The individual  $\text{CO}_2$  peaks in He are subsequently dried in an on-line gas drying unit (Nafion®) and then admitted to the Isotope Ratio MS via an open split interface.

Since the liquid phase is not vaporized at all, inorganic buffers, e.g. phosphate buffers, have no influence on the operation of the interface, and can not accumulate anywhere in the system. This is in marked contrast to any other LC/MS interface and makes the LC IsoLink essentially maintenance free.

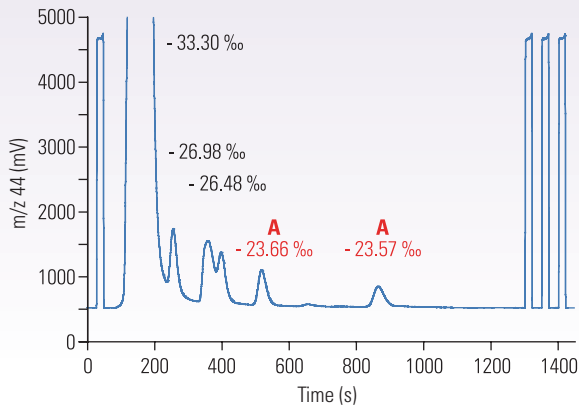
\*GB2388800 B, US7213443 B2



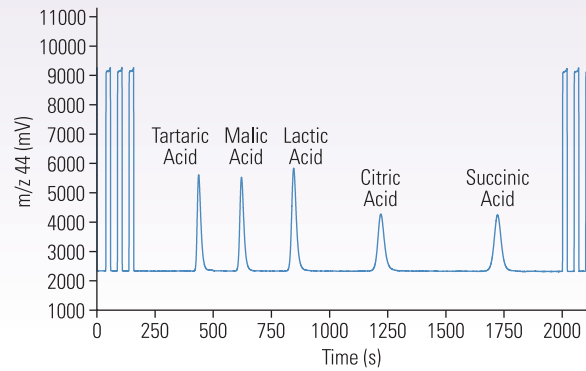
**RNA Nucleotides:** Molecular biological methods based on detection, sequencing, and phylogenetic identification of ribosomal RNA (rRNA) and the genes encoding it (rDNA) allow description of natural microbial communities without isolation and culturing of individual species. By carbon isotopic characterization of rRNA species identity can be linked with carbon source utilization.



**Organic acids** play an important role in the metabolism of plants. They are involved in various anabolic and catabolic processes of metabolic pathways, such as citric acid cycle.

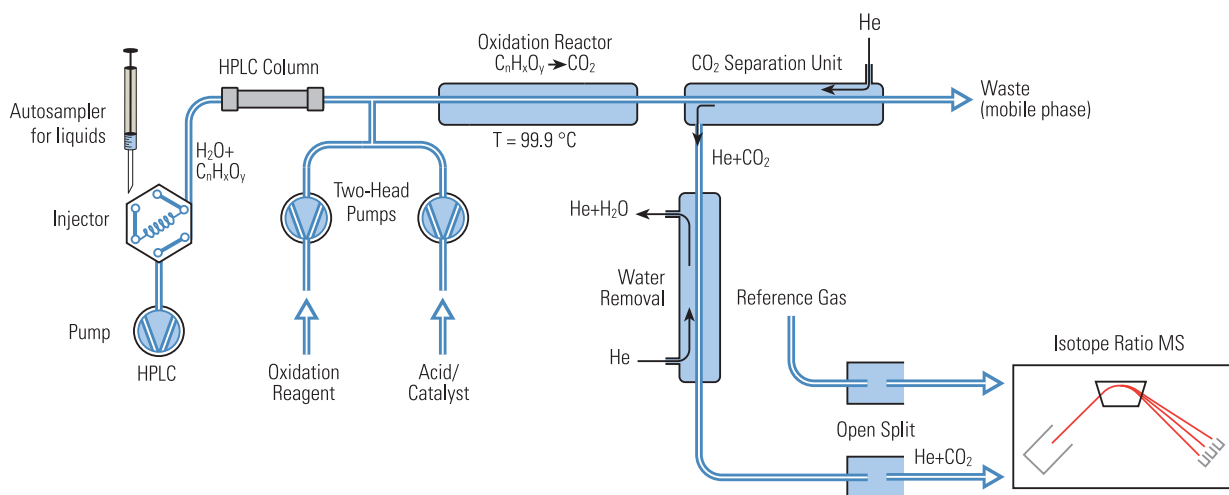


*irm*-LC/MS analysis of NaOH-hydrolyzed *E. coli* RNA, LB-grown.



*irm*-LC/MS chromatogram (*m/z* 44 trace) of an organic acid mixture.

## Principle of HPLC Coupling to IRMS

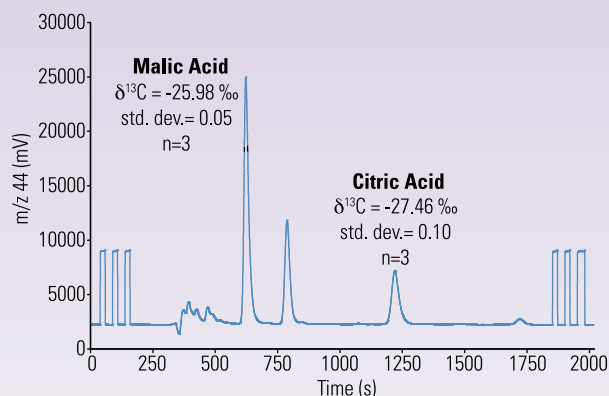




### Analysis of an Extract of Geranium Pratense

Changes in the isotopic signature of organic acid are studied in the process of ontogenesis of plants. Investigations can be important to understand development of plant metabolism and to estimate the impact of environmental factors.

The most dominant organic acids in an extract of Geranium Pratense are malic acid and citric acid. Malic acid is isotopically heavier than citric acid.



Plant metabolism study of organic acids.

## Isodat Software Suite

The *irm*-LC/MS system is fully automated and controlled by the Isodat software suite. The Thermo Scientific Surveyor™ LC is completely integrated, other HPLC systems are synchronized by contact closure.

The LC IsoLink and the mass spectrometer are entirely under full computer control, resulting in analyses with no user interaction. When coupled to any autosampler, the system can be operated fully unattended for high sample throughput and high productivity.

The *irm*-LC/MS does not require any manual intervention during operation except for refilling of the reagents. Data evaluation and report generation are fully automated by Isodat software suite. Since the interface has only few variables, e.g. flow of oxidizing agent and He-flow, method development and operation are also exceedingly simple.

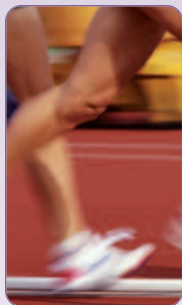
- Easy and fast method and sequence setup for Isotope Ratio MS and LC IsoLink
- Complete control and automation of all interface functions before and during data acquisition
- Automated peak and background detection
- Fully automated ion correction for isobaric ion contribution such as  $^{12}\text{C}^{17}\text{O}^{16}\text{O}$  on  $^{13}\text{C}^{16}\text{O}^{16}\text{O}$
- Full access to all raw and processed data
- Complete raw data integrity and secure sample identity
- Software suite flexible export of evaluated data to Excel, other spreadsheet programs and databases (LIMS)
- Customized printouts and data export
- Full network compatibility with direct and fast access to Windows® tools
- Complete Isodat software suite system backup using the Isodat Version Handler
- Based on the unique “Plug and Measure” concept of Thermo Scientific Isotope Ratio MS (Thermo Scientific DELTA V Advantage, DELTA V Plus and MAT 253), the LC IsoLink is immediately recognized and operational

The screenshot displays the Isodat software interface with three overlapping windows:

- HPLC Sequence (seq):** A table with columns: Line, AS Sample, AS Method, HPLC Method, Identifier 1, and I6. It lists 10 lines of data for Leucine analysis.
- Surveyor AS Method:** A configuration window for the autosampler with various parameters:
  - Injection volume (µl): 20.00
  - Needle height from bottom (mm): 2.0
  - Syringe speed (µl/s): 30
  - Flush volume (µl): 400
  - Flush/Wash source: bottle
  - Wash volume (µl): 0
  - Flush speed (µl/s): 250.00
  - Injection valve switch time (min): 0.0
  - Loop loading speed (µl/s): 100
  - Injection Mode:  Partial loop,  Full loop,  No waste
  - Tray Temperature Control:  Enable tray temperature control, Temperature (°C): 30.0
  - Column Oven Control:  Enable column oven control, Temperature (°C): 30.0
- Gradient Program:** A window showing a Gradient Table and a Gradient Profile.
 

min	A%	B%	C%	D%	µl/min
0	0.00	40	0	60	1000
1	30.00	0	0	100	1000
2	100	0	0	0	1000





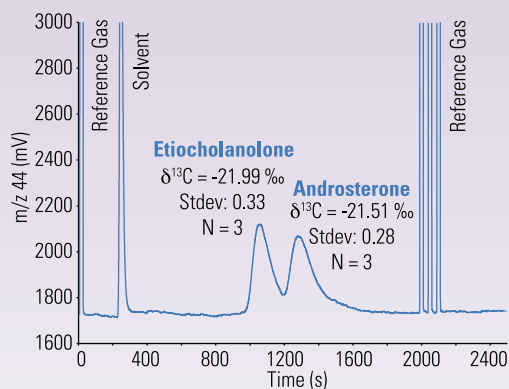
### Confirmation of Synthetic Testosterone

The administration of synthetic testosterone is detected by the testosterone/epitestosterone (T/E) ratio in urine using GC/MS. Confirmation is performed by *irm*-GC/MS.

The <sup>13</sup>C/<sup>12</sup>C ratio of synthetic testosterone differs from the <sup>13</sup>C/<sup>12</sup>C ratio of endogenous testosterone.

The range of applications for the LC IsoLink can be extended by high

temperature HPLC. At elevated temperatures, water acts like a weak polar organic solvent. Therefore, it's also capable of separating mid polar and even non-polar analytes. Steroids can be separated under 100% aqueous conditions, selectivity can be tuned by temperature.



Analysis of steroids in urine (LC fraction) by high temperature HPLC coupled to IRMS.

## Analytical Performance

### Accuracy, Precision and Sensitivity

Accuracy and precision has been tested using standards with certified isotope ratios. The  $\delta^{13}\text{C}$  value of 400 ng of benzoic acid analyzed by repetitive loop injection has been shown to be correct within 0.08‰ with a reproducibility of 0.11‰. These values compare favorably with the accuracy and precision achievable with bulk analysis by Elemental Analyzer Isotope Ratio MS – but require 100 times less sample.

The sensitivity of the integrated *irm*-LC/MS system is sufficient to allow the isotope ratio determination of <sup>13</sup>C/<sup>12</sup>C on sample amounts as low as 50 ng in direct injection mode. The minimum amount in HPLC mode depends on the background created by the HPLC column bleed.

### Acceptance Specifications

After installation, the system performance of the LC IsoLink will be demonstrated by direct loop injection, to show an external precision of < 0.3‰ for  $\delta^{13}\text{C}$  (n=5). This requires an organic-free HPLC pump.



### Mass Spectrometer DELTA Series and MAT 253

#### LC IsoLink Basic Performance

##### 10 pulses of reference gas (amplitude 3V), $\delta$ notation

		Precision (1 $\sigma$ )	Linearity
CO <sub>2</sub>	<sup>13</sup> C	0.06‰	0.02‰/nA

#### External precision using the LC IsoLink (n=5, 1 $\sigma$ ), $\delta$ notation

##### Benzoic Acid (direct loop injection)

400 ng in 10  $\mu\text{L}$  of water

	3 nmol Benzoic Acid		
	21 nmol (250 ng) C	as CO <sub>2</sub>	< 0.3‰

# Installation Requirements

## Power

230 V single phase, 8 A

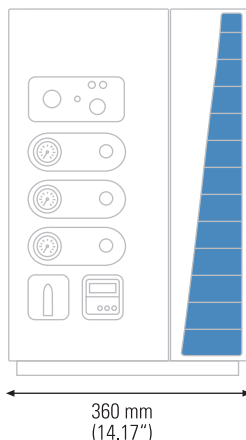
## Gases

- He, better 99.999%
- CO<sub>2</sub>, better 99.995%

## Supplies

- Water (HPLC grade)
- Reagent
- Catalyst

## Thermo Scientific LC IsoLink Dimensions



## Recommended Supplies

- Thermo Scientific Surveyor HPLC system with MS pump
- Thermo Scientific columns

## Literature

Krummen, M., A. W. Hilkert, D. Juchelka, A. Duhr, H.-J. Schlüter and R. Pesch: A new concept for isotope ratio monitoring liquid chromatography/mass spectrometry. *Rapid Commun. Mass Spectrom.* **2004**; 18: 2260-2266. USA, 1991, 21.-26.05.1994, 875

Analytical data is available in the LC IsoLink Application Notes.

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