

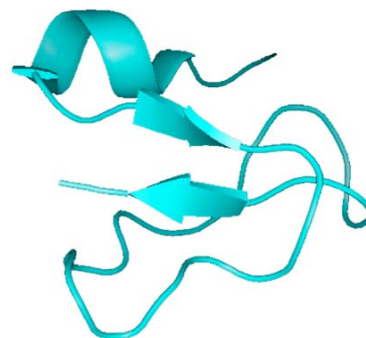
Atheris Analytical

Peptides, Proteins and Small Molecules

At Atheris labs we use state-of-the-art separation and analytical methods to offer highly specialised services to the life sciences industry, spanning from quality control to complex structural elucidation. Our team of specialists accumulates several decades of experience of investigations in the following areas:

Mass spectrometry, peptidomics and proteomics

- Electrospray MS in positive or negative ionisation mode
- On-line LC-ESI-MS, from nano-bore to preparative scale
- MALDI-TOF-MS and MS/MS in linear or reflectron mode
- NanoLC-MS/MS tandem mass spectrometry



Protein biochemistry

- Analytical quality control, purity assessment, characterisation of impurities, co-elution
- Pre-analytical sample preparation (solid phase extraction, ultrafiltration, precipitation, etc.)
- Chromatographic and electrophoretic fractionation and protein purification
- Enzymatic digestions & mass fingerprinting (in-gel and off-gel, MS & MS/MS identification)
- Protein characterisation, structural elucidation, disulfide bridges & other modifications
- Automated Edman and MS/MS *de novo* protein sequencing

Protein engineering

- Peptide synthesis (automated and manual), refolding and purification
- Chemical modifications, modified amino acids, labelling with dyes or stable isotopes
- Selective and reversible immobilisation on solid support (beads or other surfaces)
- Lead evaluation, profiling, drug design & lead optimisation

Pharmacokinetic and metabolic bio-analytics

- Proprietary peptidomics platform for biomarker discovery in any biological matrix
- Stability studies, *in vitro* & *in vivo* identification and characterisation of metabolites
- Ultra-sensitive & accurate absolute quantification of peptides & mini-proteins (pg/mL or pM)
- Original isotope dilution assay (IDA) using stable isotope labelled analogues as internal standard
- Method development, refinement & validation to FDA standards

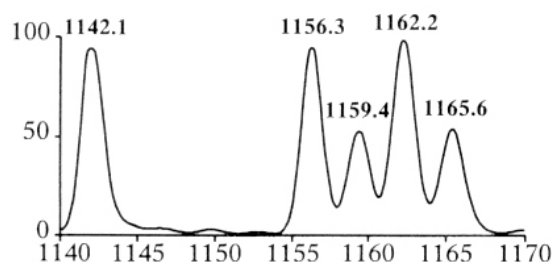
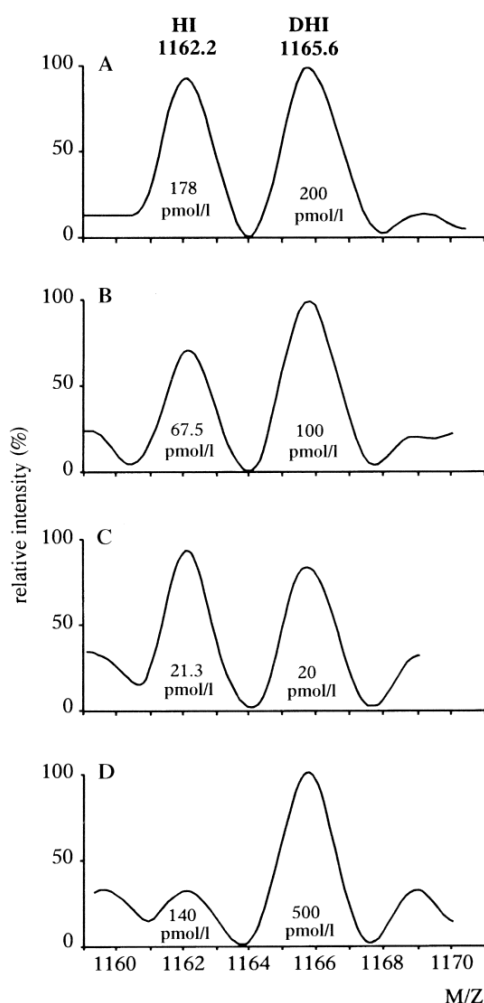


IDA - Isotope Dilution Assay

Atheris addresses the crucial issues of Pharmacokinetics and Pharmacodynamics of polypeptides using an attractive method known as IDA or Isotope Dilution Assay. It was developed successfully for quantification of insulin and other peptides in blood samples at pM levels. IDA's inherent characteristics are making it the "gold standard" method for clinical work.

We have extensive experience using HPLC mass spectrometry (LC-MS) to quantify minuscule concentrations of peptides and proteins in biological fluids and tissues down to basal pM concentrations for PK/PD or ADME studies (absorption, distribution, metabolism and excretion). Highly sophisticated LC-MS and LC-MS/MS methods for investigations related to insulin, proinsulin, C-peptide, NPY or LHRH analogues for example have thus been developed successfully. They allow fast, sensitive and precise measurements of the target compound and its metabolites.

Studies of protein metabolism have so far relied heavily on the use of radioactive tracers or on immunoassays. However, both methods suffer from inherent drawbacks: on the one side a certain aversion to use radioactive labels or difficulties to produce specific antibodies and, on the other side, an indirect detection and cross-reactions often leading to ambiguous or incomplete results. In many cases, Atheris likes to address the crucial issues of *in vivo* metabolic studies of polypeptides using analogues of the target compound labelled with stable isotopes. These non-radioactive analogues have different molecular masses and can thus specifically be followed by mass spectrometry independently of the native unlabelled form of the drug of interest. This drastically facilitates tissue distribution studies and the identification of metabolites of the injected peptide drug.



Above: A mixture of different analogues of the target compound can be used to generate a specific MS pattern that can be followed through the body. The mass spectrum shown corresponds to a 1:1:0.5:1:0.5 mixture of desAlaB30 insulin (DAI, m/z 1142.1); porcine insulin (PI, m/z 1156.3); octadeutero-PheB1-octadeutero-ValB2 porcine insulin (DPI, m/z 1159.4); human insulin (HI, m/z 1162.2), octadeutero-PheB1-octadeutero-ValB2 human insulin (DHI, m/z 1165.6).

Left: Principle of Isotope Dilution Assay (IDA): examples of mass spectra of clinical samples in physiological pM concentrations. The mass spectra for the quantification of human insulin (HI, on the left hand side) were obtained after extraction from clinical serum samples using a known amount of the hexadecadeuterated analogue as internal standard (DHI, on the right hand side).

The ionised species carry five protons (H) and so appear at m/z (mass-to-charge) values given by $(M_r + 5H)/5$, where M_r is the relative molecular mass. HI ($M_r = 5807.6$ Da) for example is responsible for the signal at a mass-to-charge ratio of 1162.2.

Absolute quantification down to basal levels

Dr Reto Stöcklin pioneered the use of stable isotope for *in vivo* PK-PD studies by IDA for the quantification of insulin C-peptide and proinsulin in blood samples down to low pM levels (a few pg/mL). IDA allows the most accurate and sensitive detection of proteins or peptides by mass spectrometry. In addition, it enables a precise quantification on the basis of the relative intensities of the observed signals (principle of isotope dilution, see figure above). IDA completely avoids the use of radioactive material and is not susceptible to errors arising from immunological dosages. Furthermore, this method can clearly discriminate endogenous and injected forms, thus allowing for simultaneous *in vivo* quantitative investigations of both endogenous and injected compounds. Similar strategies can be used to detect, identify and characterise degradation fragments.

IDA method development and validation

IDA can thus be developed specifically for a given peptide or protein for its detection and quantitative studies in animal or human blood plasma, possibly also in other body fluids or solid tissues. The methodology will need to be adapted to achieve an acceptable limit of detection (LOD) and lower limit of quantification (LLOQ) in biological samples. Analytical and data treatment processing time will also need to be optimised. Complete or partial validation of the method (selectivity, accuracy, precision, recovery, calibration/standard curve, stability under various analytical conditions, quality controls, etc.) are in principle performed according to the guidance for industry - bioanalytical method validation. Determination of the LOD and of the LLOQ for the compound of interest, as well as determination of the linearity of the response in standard plasma samples spiked with known amounts of the target peptide are usually achieved prior to be eventually developed and validated again for each of the other tissues investigated.

In vivo PK-ADME studies

IDA allows to detect the peptide of interest present in the blood stream down to its LOD values, and to quantify it precisely down to its LLOQ values in samples generated by *in vivo* animal or human studies. IDA also allows determining the *in vivo* half-life of a peptide in blood, whatever administration method is used. *In fine*, this approach also allows investigating whether an orally administered peptide penetrates the blood stream across the gut, and if so at what levels at with which kinetic parameters. The use of stable isotope labelled analogues further opens the doors to additional investigation such as metabolite identification and characterisation or biodistribution studies.



research & development

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Atheris Analytical 2012 - page 3/3