Peptides
Quality Assistance, European leader in analytical sciences

The company
- Private independent limited company
- Located in Belgium
- Founded in 1982
- 150 highly-qualified employees
- 5200 m² of premises
- GMP, GLP, GCP/GCLP compliant

Quality Assistance provides the pharmaceutical industry with all the analytical services required by EMA and FDA regulations for the development and marketing of innovative human medicinal products.

Speed up people's access to new medicines!

All laboratories on one site
- Bioanalysis (PK/TK/Immuno)
- Bioassays
- Biochemistry
- Chromatography
- Elemental Impurities
- Mass Spectrometry
- Microbiology
- Molecular Biology
- Protein Characterisation
Peptides

Therapeutic peptides are at the crossroads of small molecules and proteins, requiring specific analytical packages depending on whether they are of recombinant or synthetic origin.

In both cases, Quality Assistance provides customised solutions in terms of analytical protocols and innovative technologies to help you move your peptide through non-clinical and clinical development towards registration.

Key figures

- **Clients**: 11
- **Projects**: 12
- **Turnover**: 1.21M€
- **Studies**:
  - Method development & validation: 39%
  - Routine testing: 35%
  - Stability studies: 25%

(2014 - June 2016)

Added value

- All laboratories on one site
- Dedicated scientific and technical support
- Customised project management
- Compliance with EMA and FDA regulations

Communication during the project

- Prospecting
- Receipt of requests
- Budget evaluation and sending of proposal
- Invoicing
- Satisfaction survey
- Client follow-up
- Scientific discussion and writing of Technical Agreement
- Protocol and description of analytical work
- Planning and respect of deadlines
- Technical supervision and follow-up
- In-project Client communication
- Reporting

POC: Point Of Contact

(1) Team Manager, Technical Leader or Project Leader depending on Project scope and context
From discovery to the market place

What's your concern?

Non-clinical
- Method development
- Method qualification
- Method transfer
- Method validation
- Preliminary stability studies
- Forced degradation studies
- GLP formulation/buffer analysis
- Non-clinical immunogenicity studies

Clinical
- DS/DP characterisation
- Photostability
- DS and DP batch analysis
- GLP formulation/buffer analysis
- Clinical PK sample analysis
- Clinical biomarker analysis

Post-registration
- Stability sample storage
- ICH stability studies
- Clinical PK sample analysis
- Clinical immunogenicity studies

Protocol writing
Quality Assurance audit
Certificate of Analysis
Statistical analysis
Data transfer
Report writing

Our solutions
CMC services

Analytical development

- Method development from scratch or fine-tuning of existing methods
- Method qualification in accordance with protocols adapted to the development stage
- Method validation according to protocols compliant with ICH, FDA and EMA requirements
- Method transfer from/to your laboratory, in accordance with customised protocols including analyst training if needed
- SOPs and development, transfer, qualification and validation reports adapted to your needs
- Statistical analysis of validation results (including total error concept)

Stability studies

- Protocol design and optimisation
- Identification of degradation products and unknown impurities
- Stability studies
  - Preliminary assessment and short-term studies
  - Forced degradation studies
  - ICH guidelines followed
  - Photostability (under controlled conditions)
- Storage
  - Cabinets, refrigerators, freezers including ultra-low temperature and liquid nitrogen vapour phase
  - 150 m³ capacity, 7 walk-in chambers
  - All ICH conditions available
  - Customised conditions for specific requests

<table>
<thead>
<tr>
<th>ICH CONDITIONS</th>
<th>OTHER CONDITIONS</th>
<th>ULTRA-LOW CONDITIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>-20°C ± 5°C</td>
<td>20°C ± 2°C</td>
<td>Vapour phase nitrogen (-196°C)</td>
</tr>
<tr>
<td>5°C ± 3°C</td>
<td>15°C ± 2°C</td>
<td>-60°C ± 10°C</td>
</tr>
<tr>
<td>25°C ± 2°C/40±5%RH</td>
<td>30°C ± 2°C/80±5%RH</td>
<td>-70°C ± 10°C</td>
</tr>
<tr>
<td>25°C ± 2°C/60±5%RH</td>
<td>30°C ± 2°C/65±5%RH</td>
<td></td>
</tr>
<tr>
<td>30°C ± 2°C/65±5%RH</td>
<td>50°C ± 2°C/40±5%RH</td>
<td></td>
</tr>
<tr>
<td>40°C ± 2°C/≤ 25%RH</td>
<td>60°C ± 2°C/40±5%RH</td>
<td></td>
</tr>
<tr>
<td>40°C ± 2°C/75±5%RH</td>
<td>22.5±2.5°C/1000 lux ±400 lux</td>
<td></td>
</tr>
<tr>
<td>30°C ± 2°C/35±5%RH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30°C ± 2°C/75±5%RH</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Batch testing (release testing)

- Customised testing, following methods transferred to Quality Assistance or developed and validated in our laboratories
- Compendial testing in accordance with Ph. Eur., USP-NF and JP
- Retained samples
  - Back-up storage facilities for your retained samples, an integral part of your disaster recovery programme
- OOS-OOT procedure compliant with FDA requirements (Full scale investigation)
- Certificates of analysis approved by a Qualified Person
# Batch release and stability testing

<table>
<thead>
<tr>
<th>GENERAL QUALITY</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Properties after reconstitution</td>
</tr>
<tr>
<td>pH / Osmolarity (if liquid)</td>
<td>Solubility</td>
</tr>
<tr>
<td>Isoelectric point</td>
<td>IcIEF</td>
</tr>
<tr>
<td>Particulate matter</td>
<td>Optical microscopy / Light obscuration / Imaging Particle Analysis</td>
</tr>
<tr>
<td>Particle size (formulation)</td>
<td>DLS</td>
</tr>
<tr>
<td>Water content / residual moisture (if lyophilised)</td>
<td>(Coulometer) KF titration</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IDENTITY</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact peptide</td>
<td>(U)HPLC (UV, RI, fluorescence, MS)</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>ESI-MS / MALDI-TOF</td>
</tr>
<tr>
<td>Peptide mapping and / or sequencing</td>
<td>UPLC (UV, MS&lt;sup&gt;2&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Immunological Identification</td>
<td>ELISA / ECL (MSD)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>QUANTITY</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay</td>
<td>UV / (U)HPLC (UV, RI, fluorescence, MS)</td>
</tr>
<tr>
<td>Determination of extinction coefficient</td>
<td>UV + UPLC (UV, Fluor) for A.A.A. (ACCQ-TAG)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PURITY &amp; INTEGRITY</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Purity profile</td>
<td>IEX / RP-(U)HPLC / (ic)IEF</td>
</tr>
<tr>
<td>High order structure</td>
<td>Circular Dichroïsm</td>
</tr>
<tr>
<td>Mass distribution profile</td>
<td>ESI-MS / MALDI-TOF</td>
</tr>
<tr>
<td>Chemical modifications</td>
<td>(U)HPLC (UV, MS) / CE(UV, LIF)</td>
</tr>
<tr>
<td>Degradation patterns (Oxidation, deamidation, truncation)</td>
<td>IEX / RP-(U)HPLC (UV, MS) / CE (icIEF, CE-SDS)</td>
</tr>
<tr>
<td>Aggregation and particle size distribution</td>
<td>A-H &amp; SEC-(U)HPLC (UV/RI/MALS) / DLS / Imaging Particle Analysis</td>
</tr>
<tr>
<td>Disulfide bridges</td>
<td>Peptide mapping (UPLC-UV/MS) / SDS-PAGE</td>
</tr>
<tr>
<td>Free thiols</td>
<td>Fluorescence, UV (Ellman)</td>
</tr>
<tr>
<td>Enantiomeric purity</td>
<td>(U)HPLC-UV / GC-MS</td>
</tr>
<tr>
<td>Counter-ion content (Mass balance)</td>
<td>(U)HPLC (UV, MS) / ICP (OES, MS)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>POTENCY</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunoassays</td>
<td>ELISA / ECL (MSD) / Biacore / FACS</td>
</tr>
<tr>
<td>Cell-based assays (Cytotoxicity / Proliferative / Cell death / Cell migration / Cell receptor binding and activation / Reporter gene assays / etc.)</td>
<td>Different read outs</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PROCESS-RELATED IMPURITIES</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbiology</td>
<td>Bioburden / Sterility (filtration, direct inoculation)</td>
</tr>
<tr>
<td>Endotoxins</td>
<td>LAL (kinetic, end point)</td>
</tr>
<tr>
<td>Elemental impurities</td>
<td>ICP-MS</td>
</tr>
<tr>
<td>Residual solvents (synthetic peptides)</td>
<td>HS-GC (FID, MS)</td>
</tr>
<tr>
<td>Residual proteins (recombinant peptides)</td>
<td>ELISA / ECL (MSD) / Luminex / UPLC-MS / Western blot / 2D-DIGE</td>
</tr>
<tr>
<td>Residual DNA (recombinant peptides)</td>
<td>QPCR / Picogreen / Threshold (to be discussed)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PACKAGING</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Leachables</td>
<td>GC (FID, ECD, MS) / ICP (OES, MS) / (U)HPLC (UV, ELSD, CAD, MS)</td>
</tr>
<tr>
<td>Container Closure System Integrity</td>
<td>Bubbling / Dye ingress (methylene blue, fluorescence) / Microbial ingress</td>
</tr>
<tr>
<td>Cytotoxicity / Biological reactivity</td>
<td>Cell-based assays / USP &lt;87&gt;</td>
</tr>
</tbody>
</table>
Peptides are situated somewhere between organic drug substances and high molecular weight biopharmaceuticals.

Amongst all studies needed to characterise peptides, Quality Assistance can develop and validate a potency assay, according to the mechanism of action of your peptide.

Two examples are given below.

**A. Potency - Gene reporter assay**

This assay is based on the affinity of the peptide for its receptor. A CHO cell line is transfected to express the peptide receptor and a CRE-NanoLuc luciferase reporter gene. Intracellular signaling, triggered by the activation of the receptor, leads to an increased production of cAMP which eventually results in the production of a nanoluciferase whose activity is measured by the addition of a luminescent substrate.

The relative potency is expressed as the EC\(_{50}\) of a reference standard compared to the EC\(_{50}\) value of the sample tested.

**Method qualification parameters:**

- Linearity
- Precision and intermediate precision
- Repeatability
- Range
- Stability indicating potential

**Application:** batch release of drug substance and drug product

The results of the relative potency decrease as the relative oxidation increases, showing that the method is stability indicating.

**B. Potency - Enzymatic assay**

This assay is based on the inhibiting activity of the peptide on an enzyme involved in inflammatory disorders. After hydrolysis, the substrate of the enzyme releases a fluorophore. This fluorescent substance is measured to assess the activity of the enzyme.

The test is made of four steps:

1. Creation of a standard curve to correlate the obtained fluorescence with the substrate concentration
2. Determination of the specific activity of the enzyme (kinetic measurement)
3. Verification of the enzyme activity under inhibition assay conditions
4. Determination of the inhibiting activity of the peptide

**Application:** batch release and ICH stability studies of drug product

The inhibiting activity was maintained during the entire stability study, demonstrating the stability of the drug product under the storage conditions tested.
Quality Assistance provides you with a full analytical package to characterise your peptide. Peptide mapping and aggregation study are routinely performed in our laboratory.

**Characterisation by mass spectroscopy**
- Primary sequence by ESI/MS\(^E\) and/or peptide mapping
- Identification of impurities by LC-MS\(^E\)
- Wide range of mass spectrometers: XEVO G2-XS QTOF and QTOF Premier (Waters), Amazon ion trap with ETD fragmentation mode and Microflex LRF-60 MALDI-TOF (Bruker)

Peaks of the chromatogram are analysed by mass spectrometry (MS\(^E\)) for molecular mass and sequence determination.

**Study of peptide aggregation**
- Quantification of aggregates by Size-Exclusion chromatography (SEC) hyphenated to UV, RI and MALLS detectors
- Confirmation of results using Field-Flow Fractionation (A4F or HF5, Eclipse DUALTEC, Wyatt Technology) as an orthogonal technique
- Analysis of sub-visible and visible particles by light obscuration, microscopy and flow imaging
Thanks to a complete analytical platform and a sound experience, Quality Assistance can provide you with all the requested methods to characterise the PK and immunogenicity of the product.

Our services

Bioanalytical methods

- Drug
- Drug metabolites
- Anti-drug antibody
- Biomarkers

Non-clinical study support

- GLP compliant
- PK/TX sample analysis
- GLP supportive analysis in vehicles/buffers
- Immunogenicity (ADA & neutralizing assay)
- Biomarkers

Clinical study support

- GCLP/GCP compliant
- PK sample analysis
- Immunogenicity (ADA & neutralizing assay)
- Biomarkers

Analysis and reporting

- Reporting in accordance with GLP/GCLP/GCP
- Deviation SOPs compliant with EMA/FDA requirements
- PK/TX reporting, expertise in WinNonlin
- Data transfer to your data base

Wide expertise, including

- handling radiolabelled samples

---

**PHARMACOKINETICS / TOXICOKINETICS / IMMUNOGENICITY / BIOMARKERS**

<table>
<thead>
<tr>
<th>Drug/Metabolites</th>
<th>UPLC (MS/MS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICP (MS)</td>
</tr>
<tr>
<td></td>
<td>ELISA</td>
</tr>
<tr>
<td></td>
<td>ElectroChemiluminescence (MSD)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Immunogenicity</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-drug antibodies (ADA)</td>
<td>Biacore</td>
</tr>
<tr>
<td>Screening and confirmatory assays</td>
<td>ElectroChemiluminescence (MSD)</td>
</tr>
<tr>
<td>Neutralizing assay</td>
<td>Cell-based with different read outs (Fluorescence, Luminescence, Flow cytometry)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vehicle/Buffers</th>
<th>UV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(U)HPLC (MS/MS)</td>
</tr>
<tr>
<td></td>
<td>ICP (MS)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>UPLC (MS/MS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ELISA</td>
</tr>
<tr>
<td></td>
<td>ElectroChemiluminescence (MSD)</td>
</tr>
<tr>
<td></td>
<td>Luminex</td>
</tr>
</tbody>
</table>
Bioanalysis - practical cases

For non-clinical and clinical studies, quantitative bioanalytical methods have to be developed for therapeutic drug monitoring or to obtain pharmacokinetic information about the peptide.

Quantitative analysis of peptides in biological matrices, however, remains a challenging task due to the complexity of both the matrix and the physicochemical characteristics of these molecules. Liquid chromatography coupled to mass spectrometry ((U)HPLC–MS/MS) is the gold standard analytical technique for peptide analysis as it allows very selective and sensitive determination.

Practical case 1: LC-MS/MS method to quantify a peptide in human plasma (Phase I clinical study)
Sample preparation method: solid phase extraction (96-well plate)
Equipment: UPLC-MS/MS (Waters I-class and Xevo TQS)
Range: 10 to 1500 ng/ml
Validation parameters:
- Precision and inaccuracy (intra and inter runs)
- Linearity
- Recovery
- LOQ/LOD
- Specificity
- Matrix effect
- Carry-over
- Dilutions
- Reliability of the method
- Stability in solution
- Stability in plasma
  - in working conditions
  - in storage conditions

Besides the quantitative determination, the immunogenicity potential of the peptide has to be investigated since peptides share with large molecules their ability to generate an immune response and trigger the formation of anti-drug antibodies (ADAs).

Practical case 2: Immunogenicity - ELISA method - Human serum (Phase I clinical study)
Assay by ELISA
Read out: SpectraMax I3, 450 nm
ADA detection with biotinylated anti-IgG
Positive control antibody for validation: polyclonal rabbit antibody
Validation parameters:
- Screening cut point
- Sensitivity
- Precision and intermediate precision
- Selectivity
- Specificity - Confirmatory assay
- Drug tolerance
- Short term and long term stability
- Freeze/thaw cycles

Drug tolerance results

The graph above shows the decrease of the detection of the ADAs as the peptide concentration increase in the sample.
Quality Assistance S.A. is a leading European Contract Research Organisation (CRO) providing the pharmaceutical industry with all the analytical services required by EMA and FDA regulations for the development and marketing of innovative human medicinal products.

Quality Assistance S.A. is an expert for the development of New Chemical Entities (e.g. PKIs, synthetic peptides, cytotoxics), Biologics (monoclonal antibodies, ADCs, recombinant proteins and peptides), Nanomedicine products, Vaccines (recombinant proteins, synthetic peptides) and Advanced-Therapy Medicinal Products (Cell-Based MPs and Gene Therapy MPs).

The company holds a unique place on the market with
- all its laboratories on one site
  - Bioanalysis (PK/TK/Immuno)
  - Bioassays
  - Biochemistry
  - Chromatography
  - Elemental Impurities
  - Mass Spectrometry
  - Microbiology
  - Molecular Biology
  - Protein Characterisation
- 150 highly-qualified professionals
- over 30 years’ expertise at the forefront of analytical sciences

Our core competencies are

QUALITY (CMC)
- Development and validation of analytical methods
- Characterisation
- Stability studies
- Batch testing

SAFETY/EFFICACY (Bioanalysis)
- Development and validation of bioanalytical methods
- PK/TK
- Immunogenicity
- Biomarkers

The Quality Assistance S.A. environment is GMP, GLP, GCLP/GCP compliant.