The development and validation of QC analytical techniques for the study of peptides and complex peptide mixtures

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The development and validation of analytical methods for peptides is governed by Annex 5 of ICH Q2 (R1).

Many laboratories have traditionally worked with small molecules and biologics but peptides, whether they are small molecule peptides ~5-20 aa, large peptides >100 aa or complex peptide mixtures often offer unique challenges that make analytical method development difficult.

Common tools and techniques (e.g. chromatography, MS) are used but it is more important for the scientists developing methods and analysing samples to understand the attributes of the peptides they are working with and the requirements and parameters for each assay that will affect successful development.

>50 assays developed and validated

- **Assay/purity coupled with UV and MS detection**
- **Aggregate methods coupled with UV**
- **Preservative and excipient methods**
- **Residual solvent analysis**

Project lifecycle management – method development

Define method development objectives

- Understand the chemistry
- Chemical properties
- Potential degradation products
- Sample matrix
- Analytes
- Resolution
- LOQ
- Precision
- Accuracy

Define initial method conditions

- Sample preparation
- Selection of solvent
- Accuracy
- Precision
- LOQ

Standardisation

- Approach based on results
- Overall accuracy
- Method optimisation/robustness

Characteristics that may be included:

Specificity
- Identification
- Assay and impurity test(s)

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Linearity

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Range

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Accuracy
- Assay
- Drug substance
- Drug product
- Impurities (quantitation)

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Precision

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Repeatability
- Intermediate precision
- Reproducibility

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Detection limit
- Based on visual evaluation
- Based on signal:noise
- Based on standard deviation of the blank
- Based on calibration curve

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Quantitation limit
- Based on visual evaluation
- Based on signal: noise approach
- Based on standard deviation of the response and slope
- Based on standard deviation of the blank
- Based on calibration curve

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Robustness

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System suitability testing

Project lifecycle management – method validation

- Understand objectives of analytical procedure
- Ensure procedure is fit for its intended purpose
- Structure and prepare validation protocol

Methods should not be validated as a one-time situation but validated and designed to ensure ruggedness and robustness throughout the life of the method.

Materials and methods

All development and validation of methods are controlled through SOPs and are integral parts of our Pharmaceutical Quality System that governs all aspects of studies.

Methods developed:

- Assay/purity UPLC UV coupled with MS detection for peptide API's and complex mixtures
- Aggregate methods UPLC UV coupled with MS detection
- Preservative and excipient methods
- Residual solvent analysis
- Moisture deterimination for peptide API's and drug products

Technologies used:

- Waters Acquity UPLC-TUV with MS
- Waters Acquity H-Class UPLC-PDA with MS
- Waters Acquity Analytical Columns
- Waters Empower^3 software

Case Study 1 – Purity method development for peptide drug substances

Our client was developing a novel peptide drug product and required quality control analytics of the API's to be used for manufacture. We were trusted to develop a novel method for determination of peptide purity to be used for batch release and stability.

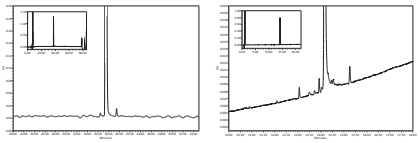
The development was completed within a design protocol to build quality and reliability into the development activities and included the following goals:

- Utilise UHPLC technology
- Primary detection source to be UV but the method must be MS compatible
- Separation of critical peaks must be obtained
- Reduce run time and improve sensitivity
- Automated scouting/screening using Acquity H Class combined with UV and MS detection

The method identified from scouting was to be optimised and validated.

Benefits of developing the new method:

- Increased efficiency from a reduced run time
- Increased separation, resolving power of method increased x5
- Increased sensitivity, method x10 more sensitive to detecting impurity peaks
- Typically >90% purity claimed; Actually 60-70% found
- Redevelopment brought advantages of cost and time saving coupled with confidence and accuracy in the data produced
- Method robustness displayed with adoption for long term stability projects
- Method used for regulatory submission



Pre-Optimisation Post-Optimisation

Tepnel Pharma Services is an independent CRO with >30 years experience of pharmaceutical testing

cGMP analytical services – regulatory compliant analytics in support of small molecules and biologic APIs, IMPs and finished products.

Biomarker and CDx development – uniquely placed to support early pre-clinical research and biomarker discovery through to the development, manufacture and regulatory approval of a CDx test.

Peptide speciality

- >50 assays developed and validated
- >10 years experience in peptide analytical method development
- Large biotech to small biotech clients
- Experience with 1 product start-ups that now have marketable products

Case Study 2 - Development of peptide aggregation method for combined peptide drug product

We were trusted to re-develop a method for determination of peptide aggregation to be used for peptide product batch release and stability. Existing HPLC-UV methodology was available but this had the disadvantages of:

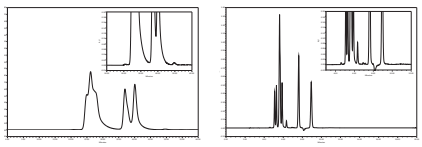
- Not MS compatible
- Time consuming (40 mins run time)
- Poor separation resulting in poor specificity
- Could not identify dimers or aggregates accurately

The development was completed within a design protocol to build quality and reliability into the development activities and included the following goals:

- Utilise UHPLC technology
- MS compatible
- Decrease run time to improve efficiency, sensitivity and separation to increase specificity

Benefits of new methodology

- Reduced run time (x3 increase in efficiency)
- SEC method MS compatible allowing identification of eluting peaks coupled with UV detection or quantitation thus increasing specificity
- Increased separation, resolving power of method increased x5
- Increased sensitivity, method x10 more sensitive to detecting impurity peaks
- Redevelopment brought advantages of cost and time saving coupled with confidence and accuracy in the data produced



Pre-Optimisation Post-Optimisation

Conclusion

Tepnel Pharma Services has developed superior, complex, difficult methods for a variety of peptide products.

Clients who have had issues with these methods, now have reliable, accurate assays saving time and money whilst generating their desired results.

Over 10 years experience in peptide analytical method development and validation.

- Developed and validated >50 stability indicating chromatography applications
- An extension of your laboratory and critical member of your team
- Involved in project decision milestones for large pharma or small biotech
- We take ownership of projects, by placing emphasis on quality, communication and scientific understanding

Our motto is **'Together we are better'**.

Case Study 3 – Development of stability indicating method for combined peptide drug product

Our client was developing a novel drug product and required quality control analytics of the drug product. We were trusted to re-develop a novel method for determination of peptide content and related impurities to be used for batch release and stability.

Existing HPLC-UV methodology was available but this had the disadvantages of:

- Poor separation resulting in poor specificity as identification and resolution of all key degradants could not be achieved
- Poor sensitivity
- Quantitation of all degradants was dependent on an orthogonal separation
- Time consuming (90 mins run time)

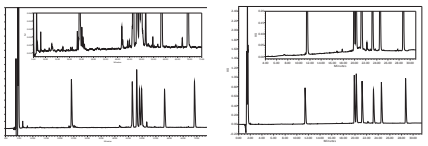
The development was completed within a design protocol to build quality and reliability into the development activities which included the following goals:

- Utilise UHPLC technology
- MS compatible
- Decrease run time to improve efficiency and improve sensitivity and separation to increase specificity
- Increase resolving power to include all known key impurities

The method identified from scouting would then be optimised and validated

Benefits of re-development:

- Reduced run time (x3 efficiency saving)
- Increased separation and resolution of important degradants (all critical separations have been achieved)
- Increased specificity (x5 increase in resolving capability)
- Increased sensitivity (x10 increase in detection limit)
- Redevelopment brought advantages of cost and time saving coupled with confidence, reliability, and accuracy in the data produced
- Method robustness displayed with adoption for long term stability projects used for regulatory submission



HPLC UPLC

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