Applications of High-Resolution Mass Spectrometry in the Analysis and Characterisation of Peptide APIs

Anna Klimek, MEng.
Ipsen
AGENDA

• A brief word on API Development in Ipsen

• Introduction to Mass Spec and HRMS in Ipsen

• Overview of chemical synthesis of peptide APIs and analytical challenges

• 4 case studies on the use of HRMS in Ipsen

• Conclusions

• Acknowledgements
A Brief Word on API Development within Ipsen

• A part of the CMC Peptides function
  • CMCP responsible for all drug product and API development activities for small molecules and peptides

• Based in Dublin, Ireland
  • APID responsible for SM / peptide APIs in Development and for technical support to commercial peptide APIs
    • Process development, scale-up, supply, analytical development
Basic theory of Mass Spectrometry

• Traditional UV detection replaced/ supported by Mass Spectrometry
  • Allows quantitation of atoms or molecules and provides structural information by the identification of distinctive fragmentation patterns

• Mass spectrometers use the difference in mass-to-charge ratio \((m/z)\) of ionized atoms or molecules to separate them
  • Create gas-phase ions
  • Separate the ions in space or time based on their mass-to-charge ratio
  • Measure the quantity of ions of each mass-to-charge ratio

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Ionization sources
- Chemical Ionisation (CI)
- Atmospheric Pressure CI (APCI)
- Electron Impact (EI)
- Electrospray Ionization (ESI)
- Fast Atom Bombardment (FAB)
- Field Desorption/Field Ionisation (FD/FI)
- Matrix Assisted Laser Desorption Ionisation (MALDI)
- Thermospray Ionisation (TI)

Analyzers
- quadrupoles
  - Time-of-Flight (TOF)
  - magnetic sectors
  - Fourier transform
  - and quadrupole ion traps

Detectors
- electron multiplier
- Faraday cup
Quadrupole Time-of-Flight (QToF) Mass Spectrometry in Ipsen

- UPLC® compatible mass resolution
- Matrix-tolerant dynamic range
- Accurate mass, quantitative performance
- Rapid analysis with a variety of options:
  - Infusion – Used when pure sample is available
  - UPLC® – Used when impurity detection is required
  - MALDI – Used for fragile, easily fragmented species
Overview of Chemical Synthesis of Peptide APIs and associated Analytical Challenges

Assembly via Solid Phase, Liquid Phase, or convergent synthesis

• Sample matrix effects – multiple components
• Low concentration of impurities
• Multiple enantiomeric impurities may be present
• Increasingly complex APIs – ligations with small molecules, PEG etc.

Preparative scale purification

• Impurity identification and fate determination
• Tracking the fate of multiple synthetic reagents to ensure their removal

Isolation by lyophilisation, spray drying, or precipitation

• Confirmation of structure of potentially fragile / easily fragmented molecules
• Identification of unknown low level impurities in isolated peptide APIs

Case Study 1

• Impurity identification and fate determination
• Tracking the fate of multiple synthetic reagents to ensure their removal

Case Study 2

• Confirmation of structure of potentially fragile / easily fragmented molecules
• Identification of unknown low level impurities in isolated peptide APIs

Case Studies 3 and 4

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Case Study 1: Ligation of a peptide to a small molecule

Case study description

- Early stage development project
- A crude, impure sample of a peptide is coupled to an unstable small molecule intermediate
- Use of UPLC®-MS for in-process control to monitor reaction completion

Challenges

- Complex sample matrix
- Unstable intermediate and no available reference material
- Reaction site specificity an issue
Case Study 1: Ligation of a peptide to a small molecule

UV IPC UPLC-MS method

HRMS – extracted MS

Dimer RT 18.10 min

Isomer RT 16.11 min
Case Study 2: Tracking the fate of Synthesis reagents and by-products during purification

- **Case study description**
  - Multiple peptide projects utilising a wide range of amino acid coupling reagents and their by-products (Generally assumed to be removed during solid phase resin washes)
  - Development of an IPC to ensure removal

- **Challenges**
  - 14 different coupling reagents in use in various projects
  - Wanted a single UPLC method to “see” everything – 7 different HPLC methods previously available
  - Mass spec (Single ion monitoring) used as an orthogonal tool to speed development of this single method and facilitate low level detection

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IPC UPLC-MS method

[Image of chromatogram showing various peaks and compounds identified: 6-ChloroHOBT, COMU, Oxyma Pure, HCTU, etc.]

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Case Study 3: Impurity Identification in Peptide APIs facilitating Yield Improvement

- Case study description
  - Improvements in analytical methods resulted in improved resolution of low levels of unidentified impurities
  - Although present at low levels can cause issues during purification due to co-elution with API during preparative chromatography

- Challenges
  - Identification of multiple peaks at relatively low levels in the API (< 0.1 %)
  - Orthogonal impurity identification techniques also required (Isolation, NMR, impurity synthesis)

Impurity Identification by UPLC-MS, Isolation, and NMR

1. Deletion Impurity
2. Multiple point enantiomeric impurity

Deletion Impurity

1. Insertion impurity
2. Methylated amino acid impurity

Degradation impurity

1. Double deletion impurity
2. Multiple point enantiomeric impurity
3. Formylated deletion impurity

C-terminal methylamide impurity

Methylated amino acid impurity

Synthetic yield improvement also seen
Case Study 4: Identification of “fragile” species

- **Case study description**
  - Pegylated synthetic peptide API requiring conclusive identification by mass

- **Challenges**
  - Prone to fragmentation by routine MS analysis due to presence of PEG moieties
  - MALDI ion source used in combination with QToF

- **Matrix-assisted laser desorption/ionization (MALDI)**
  - Soft ionization technique used in mass spectrometry, allowing the analysis of biomolecules and large organic molecules (e.g., PEG) which tend to be fragile and fragment when ionized by more conventional ionization methods

- **The complex and polydispersed PEG moiety and extensive charge states generated from the PEG molecule and PEGylated API make ESI-MS analysis nearly impossible, even with high resolution instruments**

- **Use of MALDI-MS generated a simpler form of the distribution data**

- **Additionally, MALDI-MS provided confirmation of the PEG position on the peptide backbone**
Conclusions

High resolution Mass Spec is an essential tool in the development and manufacture of synthetic peptide APIs facilitating the following:

• **Impurity identification improving regulatory compliance and supporting process development / yield improvement initiatives**

• **Rapid development of reliable in-Process Control (IPC) methodology where development of HPLC / UPLC® only methodology can be tedious**

• **Identification of fragile, easily fragmented species such as Pegylated peptides**
Acknowledgements

• Tom Loughman, Ph.D.
  • Director of API Development

• Marion King, Ph.D.
  • Analytical Development Manager

• Katarzyna Wegner, Ph.D.
  • Senior Chemical Process Development Chemist

• Deborah Barry, M.Sc.
  • Regulatory Affairs Specialist
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Thank you & Questions?