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Fast and Effective Optimization of MRM Methods for LC-MS/MS Analysis of Peptides

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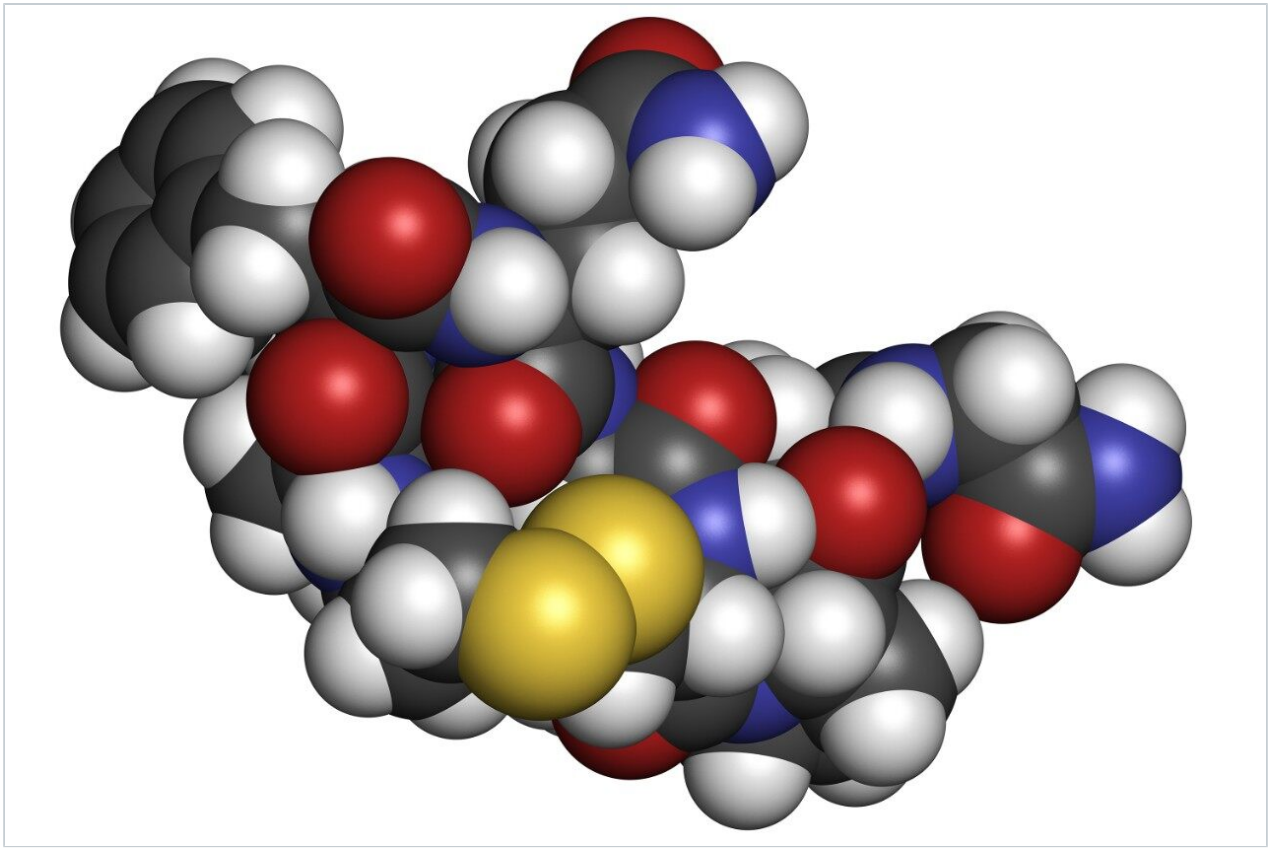

Abstract

In this application note, we describe the use of Waters' innovative IntelliStart Software and its use with the ACQUITY UPLC and Xevo TQ MS systems to rapidly develop an MRM method for peptides and small proteins.

Introduction

Peptide-based biotherapeutic compounds are used in a wide range of fields including endocrinology, hematology, and neurology to treat a variety of conditions from acromegaly to Parkinson's disease.

Biotherapeutic drugs are often synthetic or modified versions of an endogenous peptide, designed to mimic or enhance activity of these naturally-occurring compounds, and they are therefore welltolerated by the body. They exhibit high specificity, efficacy, and low toxicity profiles.[1] Bioanalysis must be performed to measure pharmacokinetics/pharmacodynamics (PK/PD), metabolic fate, and bioequivalence for regulatory submissions. There are currently more than 40 peptide drugs on the market as well as more than 400 in late stage clinical trials.[2]

Peptides have traditionally been analyzed by ligand-binding assays such as ELISA or RIA, which can be time-consuming and expensive to develop the antibodies and assays.

LC-MS/MS is a well-understood technique for the analysis of small molecules; use of the same platform for bioanalysis of peptides offers:

- Broad dynamic range – 3 to 4 orders of magnitude
- Accuracy
- Universal Method – One analytical technique for many diverse peptides
- Fast method development

Analytical challenges

Determining optimum LC-MS/MS operating conditions for peptide bioanalysis generally requires some trial and error, which for nonexperts can be time-consuming and daunting. In particular, choosing the optimum

ionization mode has traditionally involved physically changing the MS source. To further complicate matters, it is common to see 2+, 3+, 4+, and 5+ charge states, depending on the size of the peptide. This can make decisions in multiple reaction mode (MRM) MS more challenging as product ions may appear at higher *m/z* values than precursors. Thus a wider *m/z* range needs to be scanned for fragment identification. A mass range of up to 2000 amu is required to have the ability to select higher m/z precursors for fragmentation, for example, 3+ precursor ions of larger peptides.

In this application note we describe the use of Waters' innovative IntelliStart Software and its use with the ACQUITY UPLC and tandem quadrupole Xevo TQ MS systems to rapidly develop an MRM method for peptides/small proteins, with subsequent incorporation into an LC-MS/MS method for separation, detection, and quantitation.

Figure 1. ACQUITY UPLC and Xevo TQ MS.

Results and Discussion

A generalized workflow for the development of a peptide MRM method in bioanalysis is shown in Figure 2. IntelliStart incorporates software and hardware that greatly simplify the task of selecting the best parameters for monitoring peptides.

Figure 2. Workflow for the development of a peptide MRM method.

IntelliStart will automatically:

- Monitor system parameters
- Perform mass calibration and set resolution
- Tune source conditions to each compound
- Generate an MS Method for SIR or MRM
- Check LC-MS system performance
- Enable automated generation of optimized SIR and/or MRM methods for new compounds

Setting up IntelliStart to select the optimum conditions for MRM monitoring of a peptide is easily carried out. Since there can be many charged states, the first step is to use the Molecular Mass Calculator embedded within MassLynx Software (Figure 3) to calculate the possible charge state *m/z* values for a compound.

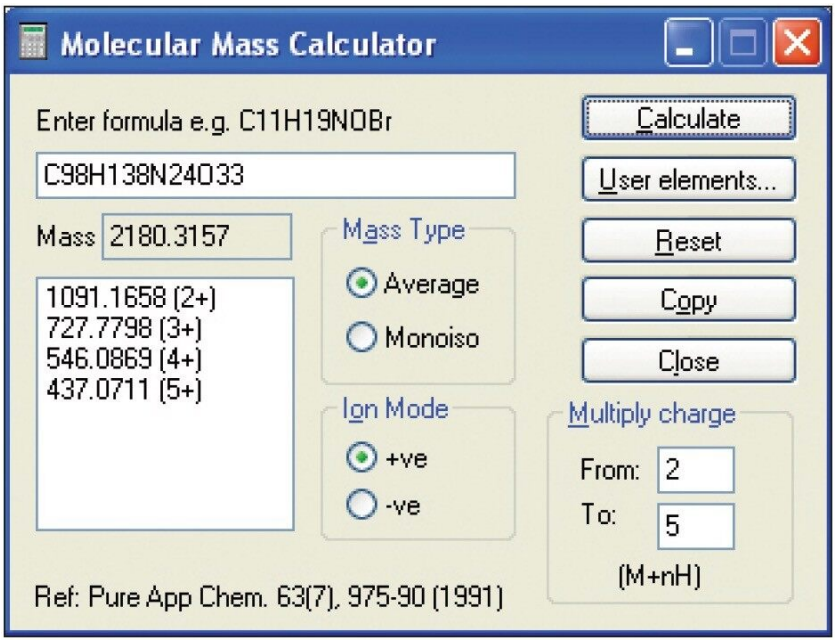

Figure 3. Molecular Mass Calculator in MassLynx Software.

After entering a compound's formula, the number of required charge states is generated, dependent on the peptide's size. The calculator will then display the possible *m/z* values.

The next step is simply to input the name of the compound, the *m/z* values, and check the Multiply Charged Parents box in the IntelliStart setup page (Figure 4).

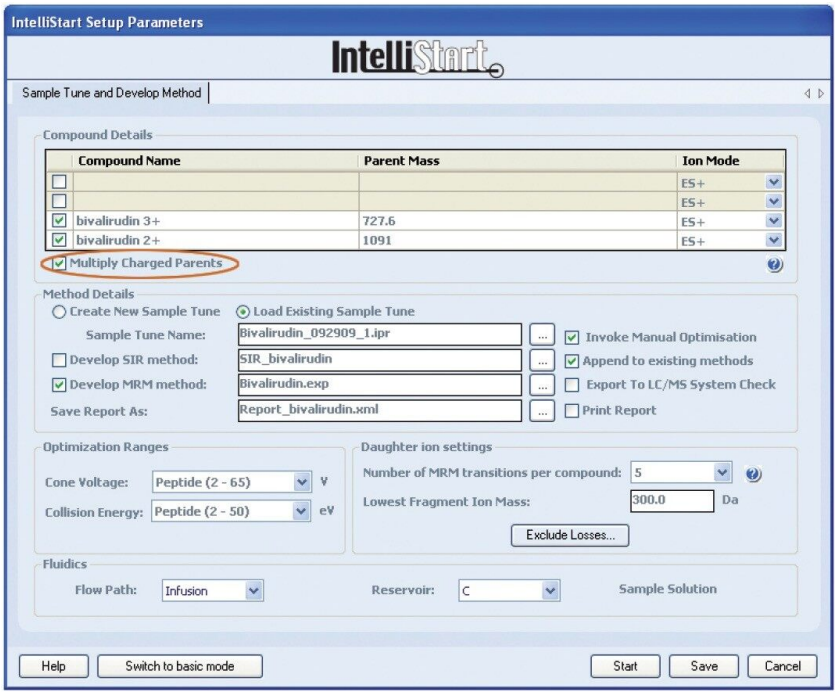

Figure 4. IntelliStart's Sample Tune browser.

IntelliStart will now optimize conditions as detailed in Figure 2 and generate a report (Figure 5) detailing the conditions for the best MRM for the peptide. The report is automatically saved for future reference and incorporation into routine analysis.

In this example, compounds are sampled from an on-board vial via fluidics, so no external syringe driver is required.

Tuning can be carried out in "combined infusion" mode to optimize the method at an appropriate LC flow rate and solvent composition. As peptides form different charge states depending on pH, a thorough investigation of mobile phase pH will allow the optimum conditions to be generated.

Compound	Formula/Mass		Parent m/z	Cone Voltage	Daughters	Collision Energy	Ion Mode
bivalirudin	727.6	1	727.63	16	943.80	18	ES+
		2	727.63	16	1025.35	16	ES+
		3	727.63	16	766.14	16	ES+
		4	727.63	16	356.23	26	ES+
		5	727.63	16	721.69	12	ES+
bivalirudin	1091	1	1090.84	44	356.23	48	ES+
		2	1090.84	44	650.40	24	ES+
		3	1090.84	44	1531.07	36	ES+
		4	1090.84	44	519.30	34	ES+
		5	1090.84	44	1025.22	24	ES+

Figure 5. Section of an IntelliStart report.

The IntelliStart report details parameters determined by the system. This report is automatically saved for future reference. IntelliStart also automatically generates an MS method that can then be used in routine analysis of mobile phase pH will allow for the optimum conditions to be generated.

Conclusion

- IntelliStart Software provides an efficient, rapid, reproducible, and effective approach to developing MRM methods for peptide assays
- The software intelligently scans for multiple charge states of the parent peptide and scans for product ions at higher *m/z* values than the parents
- The Xevo TQ MS allows a wide mass range, up to 2000 Da, to be scanned for fragment identification of larger peptides

References

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