Optimization and Selection of Assigned Charge State Conditions for LC/MS/MS High Throughput Bioanalysis

Wayne Lootsma[2]; Mary Piotrowski[1]; Julie Keefer[1]; Hui Zhang[1]; Joe Janiszewski[2]; Steve Ainley[2]

[1]Pfizer Global Research & Development, Groton/New London Laboratories, Pfizer Inc, Groton CT 06340, [2]Sound Analytics, Niantic, CT

ABSTRACT

Electrospray Ionization (ESI) typically induces a range of charge states for multiply charged based analytes. Charge states of 2+, 3+, 4+, 5+ and greater are commonly found depending on a species molecular size and properties. Selection of optimal MRM methods can be time-consuming as each species may have multiple precursor ions and each produces numerous fragments. The issue is further complicated in a screening context when a plate (N=96) of unique substrates needs MRM methods assigned quickly, prior to High-throughput bioanalysis. We report on development of an automated approach wherein each analyte is tuned at multiple charge states and resultant MRM sensitivity and chromatography are evaluated. The top MRM transitions are indicated in the central database and easily retrieved for high throughput bioanalysis.

Objective

Improved bioanalytical throughput of multiple charge state species by optimizing and assigning best conditions to use for high throughput analysis.

METHODS

- A Sciex 5500 triple quadrupole mass spectrometer was coupled to an LC system consisting of an Agilent UHPLC pumps and a LeadSampler (LS-I) autosampler.
- LC mobile phases consisted of water containing 0.1% formic acid and acetonitrile containing 0.1% formic acid. HALO C18 columns were used for separation.
- Data were acquired and processed with Analyst via LeadScape software.
- Several peptides were used for testing. Including LFSGDVVLTAR; MW 1177.66 and EYGGLDVLVNNAGIAFK; MW 1780.39.
- Import lists were created in Excel, containing information necessary to run and process data, including molecular formula and/or peptide sequence.

Compound ID	MW	Formula	Peptide	Vial Position	Plate Location	Charge State
P1+1	1177.66	C53H88N14O16	LFSGDVVLTAR	1	Plate 2	1
P1+2	1177.66	C53H88N14O16	LFSGDVVLTAR	1	Plate 2	2
P1+3	1177.66	C53H88N14O16	LFSGDVVLTAR	1	Plate 2	3
P2+2	1780.39	C81H126N20O25	EYGGLDVLVNNAGIAFK	2	Plate 2	2
P2+3	1780.39	C81H126N20O25	EYGGLDVLVNNAGIAFK	2	Plate 2	3
P2+4	1780.39	C81H126N20O25	EYGGLDVLVNNAGIAFK	2	Plate 2	4

Figure 1. Example of Import File used for Optimization. Either Formula, Peptide or Monoisotopic MW field is needed to run optimization at multiple charge states.

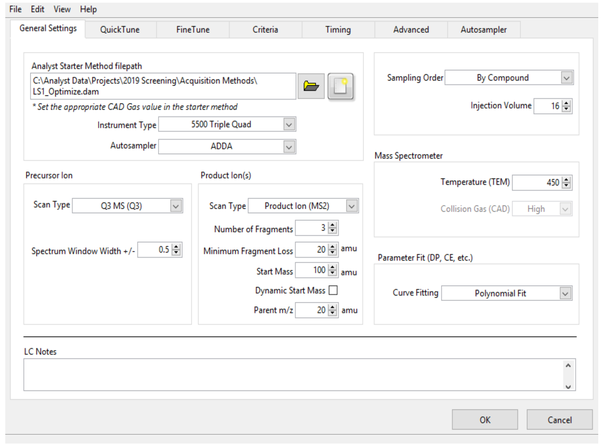

Figure 2. Tuning template configured for optimal results.

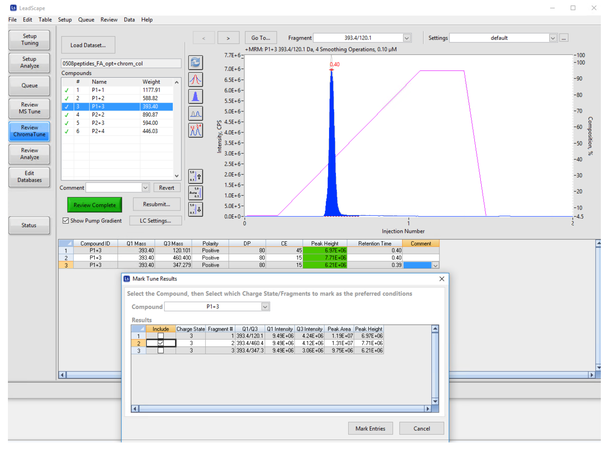

Figure 3. ChromaTune Review. Charge states can be viewed and best MRM marked. For peptide P1 charge state +3 and 2nd MRM was chosen.

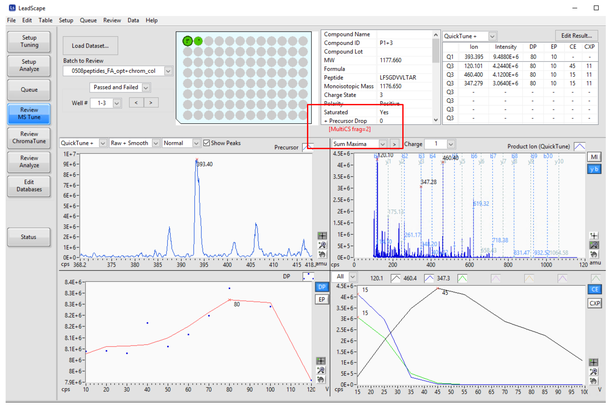

Figure 4. Example of Optimize screen viewing a multiple charged species. MRM marked in ChromaTune is indicated in red. If peptide sequence is included in the import file, y and b ions are displayed. For the peptide P1, the y4 ion is the most abundant. Choosing a specific y or b ion, which are more specific, can yield a better signal in matrix.

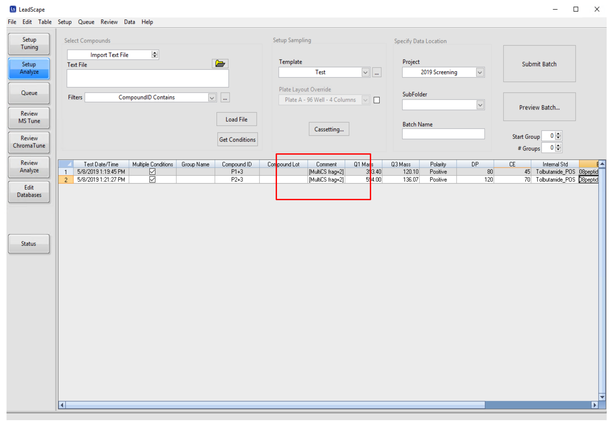

Figure 5. Marked conditions are retrieved for high throughput bioanalysis in Setup Analyze screen. Get Conditions button is pressed and the preferred conditions from the database are automatically selected based on the tune comment field. The comment field then shows that the best MRM was chosen.

RESULTS

Our group routinely uses DiscoveryQuant to optimize hundreds of new molecules weekly. The optimized MRM conditions are loaded into a central database and used to assay high throughput ADME studies. High quality conditions are needed to successfully complete the analysis of samples in a timely manner. Multiply charged species, such as peptides, often require additional consideration during the optimization process in order to be successful.

Here we report an improved workflow to optimize multiply charged species. FIA-MRM Optimization using Discovery Quant is performed at multiple charge states. This is followed by LC/MS/MS chromatography using the previously determined precursor and fragment ions in the LeadScape ChromaTune module. Multiple LC conditions can be run, a matrix sample can be included to further optimize the selection of best conditions. The LC performance and response is compared at each charge state/MRM condition and the optimal conditions are marked and uploaded to the database. The marked MRM are downloaded from DiscoveryQuant database and used for subsequent high throughput bioanalysis.

CONCLUSIONS

- An automated workflow to support LC/MS/MS high throughput bioanalysis of multiply charged analytes using Leadscape has been developed.
- MRM's for multiple charges states are generated. For peptides, y or b ions can be evaluated in the MS Tune review screen.
- Multiple LC conditions and if needed multiple extraction conditions can be evaluated using this workflow.
- Optimal conditions stored in a database, are easily retrieved by the bioanalyst running high throughput samples.

AKNOWLEDGEMENTS

Paul Harradine, Merck & Co., Inc
Wilson Shou, Bristol-Myers Squibb Company