Automatic Optimization of MS and MS/MS Assays with Dilute Samples or Weak Transitions

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Overview

Purpose: To optimally tune an MS/MS or single ion monitoring (SIM) assay using a dilute sample or weak signal.

Methods: Kernel intensity function estimation is used to recover the relationship between intensity and tuning from a noisy raw signal.

Results: Optimal collision energies can be recovered from full range collision energy scans even if the signal consists of as few as ten to thirty single-ion events registered at the detector.

Introduction

SIM and MS/MS assays must typically be optimized for each compound to be tested. Optimal tunings of ion optics devices like the S-lens (SRIG or ion funnel) can vary from compound to compound. CID collision energy tuning, especially, must be tuned for each MS/MS assay since it is particular to each transition.

When the concentration of the compound to be optimized is limited due to cost or scarcity or the informative CID transitions are weak, generation of smooth breakdown or tuning curves with clear optima can be costly in time and resources.

The method presented here uses kernel intensity function estimation, a statistical algorithm related to kernel density estimation, to optimize collision energies or other assay parameters using signals that are very sparse, resulting from the detection of as few as tens of ions even in the neighborhood of the optimal instrument tuning.

The problem (kernal intensity function estimation):

Given steady production of ions at the source and stable filtration of ions by quadrupole mass analyzers, arrival of ions at a triple quadrupole’s mass detector is approximately a Poisson process and the detector signal itself obeys Polya statistics. The relative error varies with intensity and when the ion current is weak the observed signal is often 0: the signal is a series of discrete single- or few-ion arrivals (Fig 1).

Complicating matters further, the relationship between collision energy or instrument tuning voltage (“tuning curve”) and signal intensity is not a simple function consistent from compound to compound. We cannot simply fit a regression curve to the data even taking into account the non-homoscedastic noise of the signal; we do not know what kind of curve to fit.

Methods

Sample Preparation

Testosterone and estradiol samples were prepared by serial dilution in 50% methanol, 50% water, 0.1% formic acid from 1 mg/mL stock solutions.

Mass Spectrometry

Samples were infused to the HESI source of a Thermo Scientific™ TSQ Endura™ mass spectrometer at a rate of 5 µL per minute. Ionization voltage was set to 3500 V. Sheath and auxiliary gas flow were set using the mass spectrometer’s source optimization routines.

Data Analysis

Kernel intensity function estimation was performed on-instrument using algorithms coded for use in compound optimization in version 2.0 of the control software of the TSQ Endura™ MS and Thermo Scientific™ TSQ Quantiva™ mass spectrometer. Further analysis of the results was performed offline using the Scipy statistics library (www.scipy.org).

Conclusion

A preliminary version of this method was used for data cleanup during compound presentation when running compound optimization or QuickQuan

Acknowledgements

More application-specific methods of kernel bandwidth selection (requiring novel presentation when running compound optimization or QuickQuan

FIGURE 1. At low signal intensities, observed tuning curves become trains of discrete single- or few-ion arrival events.

FIGURE 2. A sample kernel intensity estimate. For each ion arrival event, represented by the green X, a kernel (blue curves) is added to the reconstructed tuning curve (red curve.)

Here, the kernel bandwidth is an arbitrary 10 units wide. In practice, the kernel bandwidth is set by considering the statistical structure of the data.
Samples were infused to the HESI source of a Thermo Scientific™ mass spectrometer. The mobile phase was 50% water, 0.1% formic acid from 1 mg/mL stock solutions.

Purpose:
Overview

Automatic optimization of MS and MS/MS assays with dilute samples or weak transitions. Further analysis of the results was performed offline using the SciPy statistics library (www.scipy.org).

Data Analysis

Kernel intensity function estimation was performed on-instrument using algorithms that optimize routines.

Sheath and auxiliary gas flow were set using the mass spectrometer's source mass spectrometer at a rate of 5 µL per minute. Ionization voltage was set to 3500 V.

Methods

Few as tens of ions even in the neighborhood of the optimal instrument tuning. When the concentration of the compound to be optimized is limited due to cost or tuning curves with clear optima can be costly in time and resources.

Results

FIGURE 3. Kernel intensity estimation applied to a strong signal (here, 1 µg/ml estradiol, in positive mode) is a kind of nonparametric regression or smoothing.

At high signal strengths, kernel intensity function estimation becomes a kind of nonparametric regression, similar to LOWESS, kernel-weighted local regression, or spline regression: a way to fit a smooth curve to the noisy data without making strong assumptions about shape or the source of the noise. For consistency, we use kernel estimation across the range of signal intensities.

Optimal collision energies can be recovered from full-range collision energy scans even if the signal consists of as few as ten to thirty single-ion events registered per unit time.

Analysis of the raw detector signal results in a systematic bias away from the optimal collision energy, resulting from the asymmetric shape of the breakdown curve. (Fig. 6-7) Kernel-based optimization removes this bias.

FIGURE 5. Summary of the result of 250 collision energy optimizations for the 97 amu and 109 amu CID transitions of testosterone. (1 pg/ml solution, S-Lens detuned to produce low, spiky signal.)

Optimal collision energy was determined by analysis of a 1 µg/ml solution with a well-tuned S-lens.

Mean number of events per trial:

97 amu: 54.4
109 amu: 34.1

As seen in figure 4, kernel estimation based on several hundred detected ions across a full-range collision energy scan can recover the coarse features of a breakdown curve. Differences in the recovered optimum, at their extreme (purple curve), correspond to 10% weaker signal than the true optimum.

The relatively broad shape of the breakdown curve for the 289 to 109 amu transition (and 289 to 97 amu transition) of testosterone makes it a somewhat challenging case due to the high probability of detecting ions far away from the peak. Below (figure 5) we see that this method allows some degree of optimization with tenfold fewer detected ions.

Mean number of events per trial:

97 amu: 54.4
109 amu: 34.1

FIGURE 6. Breakdown curve for 289 to 97 amu CID transition of testosterone.

FIGURE 7. Breakdown curve for 289 to 109 amu CID transition of testosterone.
Conclusion

- Using kernel intensity function estimation, it is possible to tune MS/MS collision energies using a weak signal consisting of several hundred to as few as (on average) 35 single-ion arrival events.
- More application-specific methods of kernel bandwidth selection (requiring novel mathematical development) will allow even greater accuracy in recovery of tuning and breakdown curves. This is an area of ongoing research.
- Version 2.0 of the TSQ Endura and TSQ Quantiva MS instrument control software uses the optimization method used to generate the data in this presentation when running compound optimization or QuickQuan™.
- A preliminary version of this method was used for data cleanup during compound optimization as implemented in version 1.1 of the TSQ Endura and TSQ Quantiva MS control software.

References


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