

Automated and Parameter-Free Peak Integration for LC/MS/MS Quantitation

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Overview

- > Novel algorithms to calibrate LC/MS/MS peak shape, enabling parameter-free peak integration
- > New mathematical solutions to deconvolute overlapped LC/MS/MS peaks
- > Better quantitation accuracy and precision and S/N ratios demonstrated by LC/MS/MS quantitation of chiral compounds and the analyte overlapped by an interference peak

Introduction

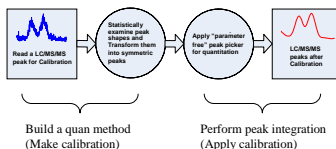
Although LC/MS/MS quantitation using multiple reaction monitoring is highly selective, peaks with the same ion transition do overlap from time to time due to the interference from endogenous peak or inadequate separation for chiral compounds. In addition, low limits of quantitation are always desirable for the quantitation support for clinic trials. As opposed to hardware approaches, we report novel post acquisition data processing methodologies with unique calibration algorithm to improve the selectivity and lower quantitation limits. The proposed algorithm not only performs noise filtering and baseline correction, but also calibrates LC/MS/MS peak shape variations and deconvolutes overlapped peaks. We will demonstrate the enhancement of quantitation performance through three examples.

Methods

Chiral compounds of C1-XBL100705, C2-XBL100705, C3-XBL100705, and C4-XBL100705 were extracted from human plasma or urine. LC/MS/MS of the compounds was performed on AB/SCIEX API-4000 tandem mass spectrometer employing turbo-ion spray ionization in the positive ion mode along with multiple reaction monitoring (MRM).

The data processing technology from MSIntegrity™, was used for all the peak integration and quantitation calculation. With no needs to set up any peak integration parameters, the integration procedure began by first establishing a peak shape calibration from one chromatogram from within the same series before applying the calibration to all other chromatograms. The peaks in the calibrated chromatograms can now be integrated accurately without the usual set of empirical parameters, achieving truly automated peak integration.

MassWorks LC/MS/MS Calibration



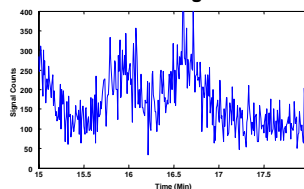
Results and Discussion

Issues w/Conventional Peak Integration

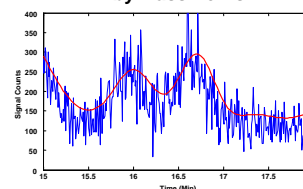
- Baseline interference
- Overlap peak interference
- Poor noise filtering
- No peak shape compensation
- Highly empirical & unreliable
- Poor integration accuracy

Results and Discussion

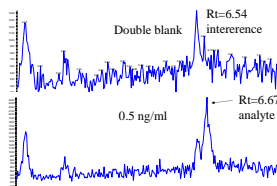
Challenging Case #1: weak signal



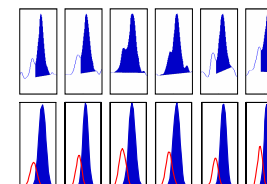
S/N Improvement by MassWorks



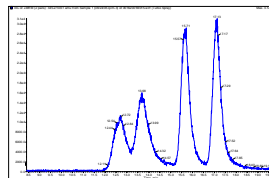
Challenging Case #2: interference peak



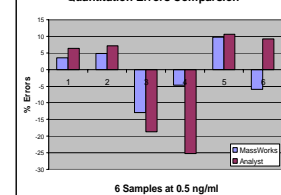
Peak Integration by Analyst and MassWorks



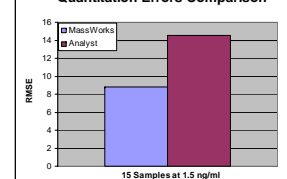
Challenging Case #3: Chiral Separation



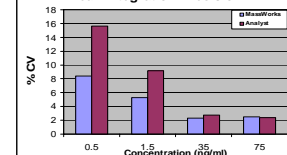
Quantitation Errors Comparison



Quantitation Errors Comparison



Peak Integration Precision



Conclusions

MassWorks calibration will:

- > Standardizes peak shapes
- > Improves S/N
- > Deconvolutes overlapped peaks
- > Improves quantitative accuracy and precision
- > Provides automated and parameter-free LC/MS/MS Quantitation through a single operation