Automatic Dilution Factor Determination for Quadrupole LC/MS and Its Application to Open Access Formula ID in Drug Discovery

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Overview

➢Automatically detecting saturated MS spectra while providing a suggested sample dilution factor provides efficient and highly confident open access MS compound confirmation for medicinal chemists.

➢When signal saturation occurs, the saturated MS scans can be automatically detected and excluded from analysis so that the accurate mass and spectral accuracy confirmation of known compounds or unknown formula identification remains feasible.

➢Due to ion suppression in the presence of signal saturation, an adjustment factor **F_a** was needed for the calculation of sample dilution factor. The dilution factor provides medicinal chemists with practical sample dilution guidance so as to obtain optimal signal without saturation at 97% confidence level.

Introduction

In early drug discovery open access LC/MS is widely used for compound confirmation/identification of possible drug candidates, intermediates, and other products. Enabled by unique instrument line shape calibration technology, open access LC/MS quadrupole systems can routinely achieve high mass accuracy and high spectral accuracy for the purpose of compound confirmation/identification. However, this great performance can be compromised by highly saturated MS signal caused by overly high concentration of samples. We have worked out a solution to provide open access MS users with useful results even with any data containing saturated signal and a suggested sample dilution for best possible formula confirmation/identification results.

Methods

➢**Data Acquisition:** All data were acquired in a Waters SQD LC/MS system in positive ionization and continuum mode. Three high concentration samples were collected and subsequently diluted by factors of 4, 7, 8, 10, 15, 20, and 30.

➢**Adjustment Factor F_a :** Data analyses for 28 ions were first conducted by comparing actual dilution factors with observed signal before and after dilution. Using statistical analysis on the compared results, a suggested dilution was proposed for end users to obtain the optimal signal to achieve high mass and spectral accuracy.

➢**AutoID processing**: For the saturated data, AutoID took a scan-by-scan examination so that only non-saturated spectra would be used to provide the best possible confirmation/identification results.

Results and Discussion

Signal saturation is a very common and challenging problem when trying to achieve high mass accuracy and high spectral accuracy in any mass spectrometers. This problem can often be resolved by manually adjusting sample concentration or "de-tuning" instrument to get less abundant signal. These approaches are tedious and sometimes take trained expertise to achieve, and therefore are not feasible for medicinal chemists working in a highly automated, open-access MS environment who need quick turnaround of results.

Our automated software-based approach is to remove saturated signals and provide a sample dilution guide for open access compound confirmation as described in a flowchart (Fig. 1). To compensate for signal saturation, our solution is based on investigation of observed signal change ratio (SCR) for 28 ions in a test set using the calculation below.

$$SCR = \frac{\text{signal intensity before dilution}}{\text{signal intensity after dilution}}$$

Table 1 shows SCRs varied widely depending on specific ions. Based on statistical analysis, we introduced an adjustment factor (F_a) for calculating the recommended dilution. F_a was calculated for each ion in the test set using the calculation below. The average F_a was 2.6 and standard deviation was 1.6.

$$F_a = \frac{\text{Actual Dilution}}{\text{SCR}}$$

To calculate the recommended dilution when running open-access samples, first AutoID examines every single scan within the retention time specified by XIC, marks the saturated scans, and finds the most abundant unsaturated and saturated scans. Then the ratio of those two scans is multiplied by F_a which has been set at 2.6 based on the test sample set. The resulting suggested dilution factor is included in the sample report to provide medicinal chemists with practical sample dilution guidance. Table 2 shows AutoID data processing achieved high mass accuracy and spectral accuracy by removing saturated spectra or further diluting samples.

Since F_a = 2.6 was determined experimentally using a Waters SQD detector, while other detector models may behave somewhat differently near their saturation thresholds, an appropriate F_a for other detectors can be calculated using a test set of compounds and the equations shown above. The resulting F_a can be stored within an AutoID configuration file and then be used in automated sample processing.

Fig.1 A Flowchart of Processing Saturated Signals in Open-Access MS

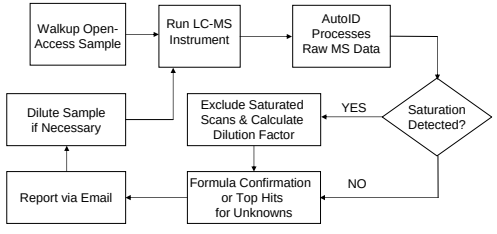


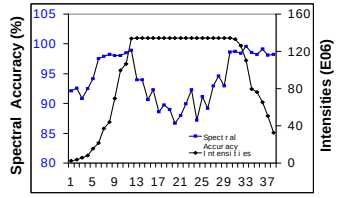
Table 1. Calculation of Dilution Adjustment Factor

Sample #	Actual Dilution	Mass	XIC Peak Height		SCR	F_a
			Before Dilution	After Dilution		
Sample 1	4	608	1.3E+09	6.5E+08	2.0	2.0
		362	6.2E+08	2.4E+08	2.6	1.5
		296	3.1E+08	7.2E+07	4.3	0.9
		414	4.6E+08	3.5E+08	1.3	3.1
	7	608	6.6E+08	1.8E+08	3.7	1.9
		362	6.8E+08	1.7E+08	4	1.8
		296	3.5E+08	5.5E+07	6.4	1.1
		414	3.9E+08	2.5E+08	1.6	4.5
	8	608	6.6E+08	1.6E+08	4.1	1.9
		362	6.8E+08	1.4E+08	4.9	1.6
		296	3.5E+08	3.5E+07	10	0.8
		414	3.9E+08	2.4E+08	1.6	4.9
	10	608	6.6E+08	1.7E+08	3.9	2.6
		362	6.8E+08	1.2E+08	5.7	1.8
		296	3.5E+08	3.4E+07	10.3	1.0
		414	3.9E+08	1.5E+08	2.6	3.8
Sample 2	20	483	1.0E+09	1.0E+08	10	2.0
		356	5.0E+08	2.5E+07	20	1.0
	30	483	1.0E+09	1.4E+08	7.1	4.2
		356	5.0E+08	1.5E+07	33.3	0.9
Sample 3	15	440	6.0E+08	2.2E+08	2.7	5.5
		441	7.5E+07	3.4E+07	2.2	6.8
		249	1.3E+08	1.3E+07	9.9	1.5
		279	1.1E+09	1.1E+08	10.4	1.4
	30	440	6.0E+08	5.0E+08	1.2	24.9
		441	7.4E+07	1.2E+07	6.2	4.9
		249	1.3E+08	8.9E+06	14.4	2.1
		279	1.1E+09	1.5E+08	7.9	3.8

Table 2. AutoID Processing on Saturated Peaks

	Initial Results		Dilution Factor		Results after Dilution	
[M+H] formula	Mass error (mDa)	Spectral Accuracy (%)	Suggested	Actual	Mass error (mDa)	Spectral Accuracy (%)
C14H19N2O5	-10.2	98.2%	3.9	4	3.1	98.9%
C21H29O5	-15.1	97.8%	3.7	4	-0.6	99.0%
C14H19N2O5	-1.2	98.5%	8.2	8	-1.3	98.4%
C21H29O5	6.5	97.8%	11.4	10	1.9	98.8%
C27H31ClN3O3	-14.1	97.5%	18.1	20	-10.0	98.1%
C22H30N3O3	-10.6	98.3%	4	3	1.6	98.8%

Fig. 2 Spectral Accuracy of a Saturated Peak at Different Scans



We tested our approach using a sample with saturated signal at m/z 436. By examining each scan of the LC/MS peak, we found that saturated signals have a flat peak-top with signal intensity about 1.34E+08 counts. We also examined spectral accuracy of m/z 463 in every single scan of the saturated spectra. It is clear that spectral accuracy is a good measurement to show signal saturation as demonstrated in Fig 2. Using 1.34E+08 counts as a cutoff to separate saturated and non-saturated signals, we calculated spectral accuracy for the spectra averaged with saturated scans included and for the spectra averaged with the saturated scans excluded. As expected, spectral accuracy significantly improved from 93.6% to 99.3%

Conclusions

➢With AutoID processing, it is possible to achieve high mass accuracy and high spectral accuracy automatically even with saturated signals by detecting saturated scans and including only un-saturated scans for analysis.

➢Signal saturation threshold can be visualized by a flat-top peak shape in Waters quadrupole instrument and can be used conveniently to determine saturated MS scans.

➢With suggested adjustment factor added, a realistic dilution factor can be calculated leading to a high likelihood of success within a single dilution.

References

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