Improving the Sensitivity for Linear Ion Trap Tandem Mass Spectrometry with Novel Automatic Gain Control (AGC)

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ABSTRACT

Purpose: Improve the sensitivity for linear ion trap tandem mass spectrometry with a novel automatic gain control (AGC) method.

Methods: Determine the ion injection time according to the fluxes of both precursor ions and product ions.

Results: Up to 100x sensitivity improvement for MSn experiments is achieved.

INTRODUCTION

In order to reduce and control the space charge effects that can degrade the performance of mass analyzers, particularly ion trapping devices, Automatic Gain Control (AGC) was developed. AGC determines an appropriate ionization/accumulation time utilizing a prescan to assess the incoming ion current. Although the established AGC techniques have been proven to work well, a significant improvement potential exists due to the fact that these techniques regulate on the precursor ion flux only. However, for maximum sensitivity for MS/MS and MSn experiments, it is ideal to regulate on the actual product ions. This would assure that the filling of the trap to the spectral space charge limit is with the product ions of interest.

MATERIALS AND METHODS

Sample Preparation

Bovine whole blood was purchased from Innovative Research (Novi, MI). Methanol and water were purchased from Fisher Scientific (Pittsburgh, PA) and used to prepare the spray solvent mixture (50/50, v/v). Levetiracetam, vancomycin, and Ciclosporin were purchased from Fisher Scientific (Pittsburgh, PA). Their respective deuterated internal standards were purchased from Fisher Scientific (Pittsburgh, PA) and Sigma-Aldrich (St. Louis, MO).

Test Method(s)

The novel AGC method performs the traditional AGC prescan that assesses the precursor ion current, but adds an additional prescan that performs activation on the isolated precursor ions and assesses the product ion current. The ion flux of the precursor and product ions are both used to calculate the optimum ionization/accumulation time. Two target values are used, $AGCTARGET_{Precursor}$ and $AGCTARGET_{Product}$, which assures maximum precursor ion accumulation and optimum product ion formation while the respective space charge limits for trapping, isolation, and activation are not exceeded. The space charge limits for the processes of ion storage, isolation, activation, and mass analysis are explored in this work to assure no deleterious effects of these limits come into play in the analytical scans.

$$\text{Injection Time} = \text{Min}(Injection\ Time_{Precursor}, Injection\ Time_{Product}, Injection\ Time_{Max})$$

where

$$Injection\ Time_{Precursor} = \frac{\text{AGCTARGET}_{\text{Precursor}}}{\text{Prescan TIC}_{\text{Precursor}}} * \text{Prescan Injection Time}$$

$$Injection\ Time_{Product} = \frac{\text{AGCTARGET}_{\text{Product}}}{\text{Prescan TIC}_{\text{Product}}} * \text{Prescan Injection Time}$$

$$Injection\ Time_{Max} = \ a\ number\ assigned\ by\ the\ user$$

Data Analysis

A full characterization of the space charge limits for storage, isolation, activation, and analysis has been done. Having understood the linear ranges of each we know the relative order of each of the space charge limits. The measured values for the storage and spectral limits for the Thermo Scientific™ LTQ Velos Pro™ linear ion trap mass spectrometer is found to be ~3E7 and ~3E4 ions, with the isolation/activation limits being in between these values.

Figure 1. The order of each of the space charge limits. In general, spectral space charge limit<isolation space charge limit<activation space charge limit<storage space charge limit.

Storage: how many ions can be stored in the trap
Fragmentation: how many ions can be in the trap and still get quality spectrum
Isolation: how many ions can be in the trap and still get efficient isolation of ions of interest
Spectra: how many ions can be in the trap and still get representative product ion spectra

RESULTS

Characterization of the space charge limits for storage, isolation, and activation

The measured values for the storage and spectral limits for the linear and 3D traps are shown in Table 1 and indicate their difference by three orders of magnitude, with the isolation and activation values being in between (not shown since there is dependence on the exact method for performing these steps). Knowing this, it is clear that the ion trap can certainly be filled with much higher numbers of ions than the spectral space charge limit, which is what the current AGC process targets. As long as the isolation and activation steps reduce the ion abundance to be equal to or less than the spectral limit, then the data will be valid, and can therefore contain many more product ions than the current AGC process would yield.

Table 1. Measured values of the Storage and Spectral Space Charge Limits for the Linear and 3D Traps.

	2D-LTQ	3D-LCQ
Storage Space Charge Limit:	~3x10^7	~1.5x10^6 (7x10^{10} Scaled TIC)
Spectral Space Charge Limit:	~3x10^4	~1.5x10^3 (6x10^7 Scaled TIC)
AGC Target:	~1x10^4	~5x10^2 (2x10^7 Scaled TIC)

* Units of ions

Figure 2. The configuration of the modified Thermo Scientific™ Velos Pro linear ion trap mass spectrometer used in this study.

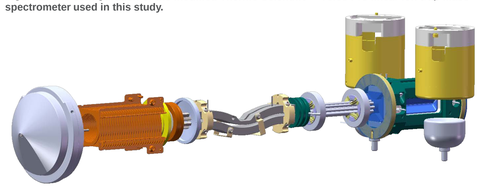
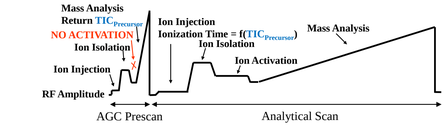
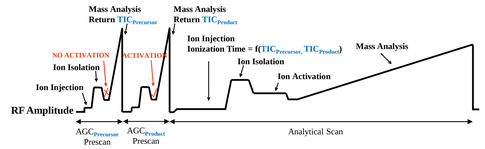


Figure 3. Automatic gain control (AGC) to regulate the ionization time according to the a) precursor ion current, and b) precursor/product ion current.

a) Conventional Full Scan MS/MS Scan Function with AGC On



b) New Full Scan MS/MS Scan Function with AGC On

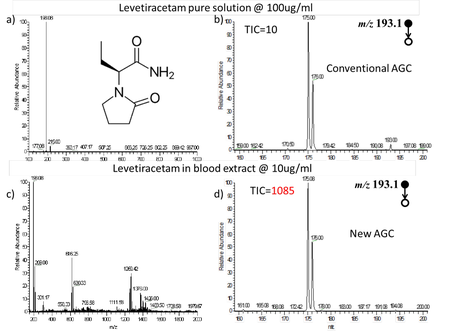


Higher sensitivity of MSn

The specificity and signal to noise ratio, of multiple stage tandem mass spectrometry can greatly be improved from the currently used form of AGC which regulates on precursor ion signal only, since the ion losses in the fragmentation and isolation steps are compensated for.

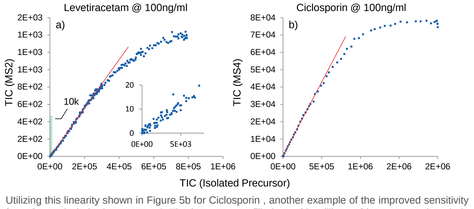
In the MS/MS analysis of compounds of low fragmentation efficiencies, such as Levetiracetam that has a fragmentation efficiency of ~1%, the signal of the product ions is usually very weak or sometimes not observed at all even with highly concentrated pure samples. With the novel AGC methods, the abundance of precursor ion can be accumulated as high as 100x the conventional AGCTARGET for the precursor ions; so that the product ion signals are improved by ~100x with a sample of only 10% of the normal concentration.

Figure 4. MS2 analysis of Levetiracetam [M+Na]+. a) Full spectrum of Levetiracetam at 100ug/ml in pure solution. b) MS2 spectrum with conventional AGC scan function with AGCTARGET of 1E4. c) Full spectrum of Levetiracetam of 10ug/ml in blood extract. d) MS2 spectrum with conventional AGC scan function with $AGCTARGET_{Precursor}$ of 5E5 and $AGCTARGET_{Product}$ of 1E4. The isolation window width is set to be 10 amu.



The linearity of the precursor ions and the product ions shown in Figure 5 demonstrates that the linear ion trap is capable of accumulating, isolating, and fragmenting precursor ions up to ~3E5 and still be linear.

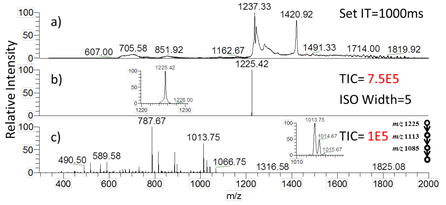
Figure 5. Linearity of TIC(Product Ions) and TIC(Isolated Precursor) of a) Levetiracetam and b) Ciclosporin. The isolation window width is set to be 5amu. Sample is infused to the Thermo EASY-SPRAY Nano ESI source at a rate of 0.35 ul/min. Spray voltage = +2kV.



Utilizing this linearity shown in Figure 5b for Ciclosporin , another example of the improved sensitivity for MSn analysis is demonstrated. The ion trap was filled up with millions of ions across the whole mass range in Figure 6a (severely space charged spectrum). However, by performing waveform isolation during the ion injection and isolation steps, the precursor ions of 1225, 1226, and 1227 (sodium adducts) can be isolated from the background as shown in Figure 6b. The total ion count is 7.5E5, which exceeds the spectral space charge limit and so isotopes are not resolved in the spectra.

However, after the multiple stages of isolation and fragmentation of the selected precursor ions, the MS4 productspectra is obtained with a TIC of 1E5, which is the $AGCTARGET_{Product}$ used, and therefore not space charged. Figure 6c shows ~100x more ions compared to the conventional method of AGC with MSn AGCTARGET of 1E4.

Figure 6. MS4 mass analysis of 1000 ng/ml Ciclosporin [M+Na]+ with $AGCTARGET_{Precursor}$ of 1E6 and $AGCTARGET_{Product}$ of 1E5. a) Full scan spectra with injection time of 1000ms calculated by the corresponding AGC Prescans. b) Isolated precursor ions with an isolation width of 5amu. c) MS4 mass spectra of the selected precursor ions.



More resistant to background interference

In scenarios having strong background interferences typically observed when directly analyzing complex samples such as biological fluids, the presence of background ions in the precursor window will suppress the precursor of interest leading to a weak MSn signal using the standard AGC method. With the new AGC method, an example of a MS3 analysis of Vancomycin in blood extract produces ~30x higher product ion signals versus the standard AGC method.

Figure 7. Mass analysis of Vancomycin in blood extract. a) Full spectrum of Vancomycin of 50ug/ml in blood extract with zoomed insert of precursor m/z window showing doubly charged Vancomycin precursor ion clusters. b) MS2 spectrum of 725.8 doubly charged precursor ion showing the presence of interfering product ions from background.

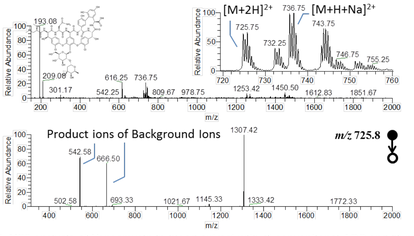
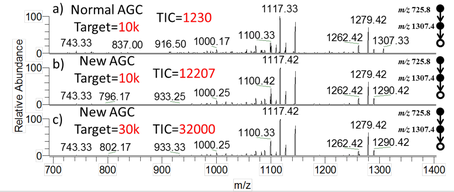


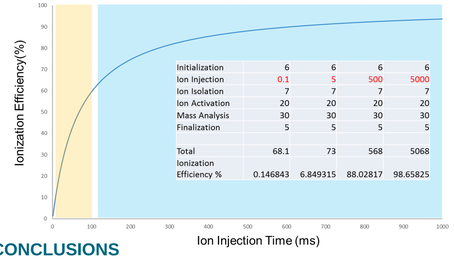
Figure 8. MS3 analysis of Vancomycin in blood extract with a) conventional AGC and b) new MS^n AGC with $AGCTARGET_{Product}$ = 1E4 and $AGCTARGET_{Precursor}$ = 5E5 and c) with $AGCTARGET_{Product}$ = 3E4 and $AGCTARGET_{Precursor}$ of 5E5. Vancomycin is spiked into the solution at a concentration of 50ug/ml.



Higher duty cycle

The new MSn AGC is higher in duty cycle, the ratio of $\frac{Injection\ Time}{Overall\ Time}$ for the same period of time, thus, more ions can be analyzed in the same time window. This is offset somewhat due to the extra time of the prescans.

Figure 9. The Efficiency of ion utilization (% Duty Cycle) with various ion injection time.



Initialization	6	6	6	6
Ion Injection	0.1	5	500	5000
Ion Isolation	7	7	7	7
Ion Activation	20	20	20	20
Mass Analysis	30	30	30	30
Finalization	5	5	5	5
Total	68.1	73	568	5068
Ionization Efficiency %	0.146843	6.849315	88.02817	98.65825

CONCLUSIONS

- A new MSn AGC scheme produces significantly higher sensitivity MSn analysis.
- The abundance of precursor ions can be accumulated as high as 100x the conventional AGCTARGET for the precursor ions. Consequently, An improvement of up to 100x sensitivity can be expected.
- This AGC method is more resistant to background interference. Regulating the injection time according to just the precursor ions can suppress the sensitivity for MSn analysis when the background interference in the precursor window of interest is high. However, regulating the injection time according to the product ions can greatly eliminate the sensitivity suppressions caused by the background interference that is in the same isolation window.
- Although a slightly longer prescan is utilized, a higher duty cycle is typically achieved. The ratio of $\frac{Injection\ Time}{Overall\ Time}$ is higher, thus, more ions are analyzed for the same time window.
- This method is especially applicable for applications where time is not a significant factor. For example, paperspray ionization biased analysis typically gives 10s of minutes of ionization, and therefore more intelligent prescanning can be performed without compromise due to the extra time of the prescan process.

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