Real-Time Collisional Energy Optimization on the Orbitrap Fusion Platform for Confident Unknown Identification

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ABSTRACT

Purpose: Real-time selection of optimal collision energy for compound fragmentation.

Methods: Perform a series of hidden, ion-trap scans to generate a breakdown curve for each precursor and select the best energy for fragmentation during the proceeding analytical scan.

Results: Optimal selection of collision energies in real-time helps improve data quality while needing fewer analyses, increasing throughput, and reducing time spent optimizing fragmentation settings for each compound.

INTRODUCTION

Small molecule fragmentation can be challenging because classes of compounds can have radically different optimal fragmentation conditions. For complex mixtures, one fixed collision energy (CE) may not generate information-rich mass spectra for all compounds, and often reinjection of samples is required to test multiple energy levels. Applying too little or too much fragmentation energy can be detrimental to quality data and accurate characterization. Here, we describe the development of real-time CE optimization to automatically select an appropriate collision energy for each compound during acquisition, without the need of reinjection. During real-time optimization, the amount of unreactive precursor is reduced while forming useful product ions and in effect resulting in more confident identifications. We call this acquisition strategy Assisted Collision Energy.

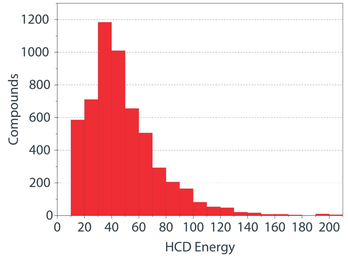


Figure 1. Distribution of optimal HCD energies for 5,575 compounds in the mzCloud database. Optimal energy is determined by the MS^2 spectrum with the most intense fragment ion current. The mean HCD energy was 43±27 NCE and shows that optimal fragmentation conditions are highly compound-specific.

MATERIALS AND METHODS

A mixture of two herbal extracts, Red Chinese Ginseng (*panax ginseng)* and Eleuthero Root (*eleutherococcus senticosus*), were combined 1:1:1 with water and 5 µL was injected onto a Thermo Scientific™ Accucore™ C18 column (2.1 x 100 mm), and analyzed using a Thermo Vanquish™ UHPLC system in-line with a Thermo Scientific™ Orbitrap ID-X™ Tribrid™ mass spectrometer (Figure 6). The mixture was analyzed using a FTMS^1 (60K), top speed (0.6 s) ddFTMS^2 (30K), top 2 ddFTMS^3 (15K, 30 Fixed HCD) method. First with stepped HCD collision energies of (25, 35, and 50) and then with HCD assisted collision energies of (25, 40, and 55) at the MS^2 stage.

RESULTS

Scan Sequence

The assisted collision energy acquisition strategy uses a series of hidden ion trap scans to determine the optimal CE to use in the subsequent analytical scan. These IT spectra only scan a small mass range around the precursor ion (±2 Th) to increase the analysis speed. The TIC of these IT scans are compared to the TIC of an IT scan of 0 collision energy to produce a pseudo-breakdown curve of the precursor. Once the amount of unreacted precursor crosses a user-defined threshold (e.g., 20%), the analytical MS/MS scan uses that 'optimal' collision energy.

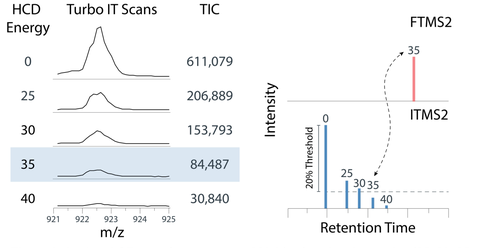


Figure 2. Assisted CE scan for precursor 923.01 m/z using 4 energies (25, 30, 35 and 40). The optimal collision energy of 35 was selected by the instrument as having reduced the precursor below the 20% threshold.

This analysis is performed on each precursor during a method acquisition, so that each precursor may have its own optimal energy Figure 3 shows a single MS-MS/MS cycle where 17 precursors were sampled and analyzed using FTMS^2 with assisted collision energy at 25, 30, 35, and 40 HCD energies. Prior to each FTMS^2 acquisition, five ion trap scans were performed and analyzed, and the resulting optimal collision energy was used in the FTMS^2 acquisition. Visually, the breakdown curves of each of these precursors can be seen in the ITMS^2 trace below, where HCD energy is increasing with time.

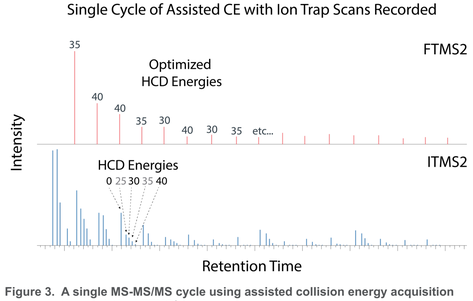


Figure 3. A single MS-MS/MS cycle using assisted collision energy acquisition results in the analytical FTMS^{2}s using a range of collision energies.

Acquisition Speed

Since the assisted collision energy strategy uses additional ion trap scans to evaluate the data in real-time, it is necessary to make this an efficient process so that overall duty cycle is not affected. The parallel nature of the Tribrid architecture allows the ion trap scans to occur concurrently with the acquisition of the FTMS transient. By choosing the appropriate settings for maximum injection times and number of assisted collision energies analyzed, one can effectively parallelize the whole acquisition with minimal reduction in duty cycle.

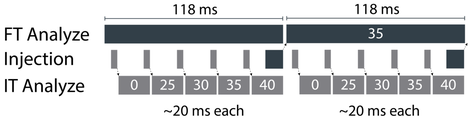
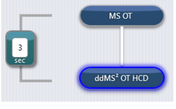


Figure 4. Pipelining of the ion trap scans for assisted collision energy determination with 60K FTMS2s. The five ion trap injections and analyses can occur during the preceding FT transient period and are used to calculate the optimal energy for the next FT injection and analysis step.

To ascertain the effect of using assisted collision energy on overall duty cycle, the following experiment was conducted. Five instrument methods were constructed using a top speed (3 s) FT MS^1/MS^2 method at 120K and 60K OT resolution, respectively. The FTMS^2 AGC target was set to 1e4 with a maximum injection time of 20 ms, with the 'inject for all parallelizable time' option disabled. Each method ran for 2 minutes on calibration mixture sample ionized using the HESI source, and the resulting data was generated.



Type	Mode	# FTMS1	# FTMS2	Energies
CID	Fixed	42	780	30
CID	*Assisted*	*42*	***657***	*25,30,35*
HCD	Fixed	42	780	30
HCD	Stepped	42	780	25,30,35
HCD	*Assisted*	*42*	***780***	*25,30,35*

Figure 5. The effect of assisted collision energy on the duty cycle of an MS/MS experiment using different collision energy modes. The slight decrease in CID assisted mode is due to the CID activation time (10 ms) adding an appreciable amount of time to the hidden ion trap scans.

Platform

The Orbitrap ID-X mass spectrometer leverages the trusted Tribrid architecture and new automated data acquisition and processing routines to be the center of the revolutionary small molecule workflow.

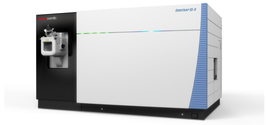
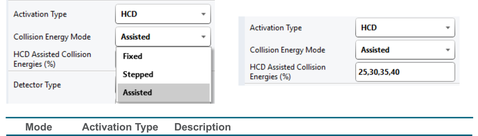


Figure 6. The Thermo Scientific Orbitrap ID-X Tribrid mass spectrometer equipped with assisted collision energy real-time optimization.

User Interface

Assisted collision energy is now an available feature on all mass spectrometer models of the Tribrid architecture, software version 3.1. Through the Method Editor GUI, users can select different 'Collision Energy Modes' for both HCD and CID activation types in the 'Scan Properties' panel.



Mode	Activation Type	Description
Fixed	CID	Single energy used in every scan
Assisted	CID	Selects single optimal energy from list of energies
Fixed	HCD	Single energy used in every scan
Stepped	HCD	Three collision energies combined into a single scan
Assisted	HCD	Selects single optimal energy from list of energies

Figure 7. Options for collision energy mode as shown in the Method Editor GUI.

Additional settings can be accessed from Tune's Diagnostic tab: *'Tools / Method / Assisted Collision Energy Settings.'* The threshold percentage for both HCD and CID can be explicitly set. This is the threshold for selecting the optimal CE when compared to a CE at 0.In addition, the AGC target for the evaluation ion trap scans can be explicitly set. By default, the ion trap scans are not displayed or recorded in the raw file.

Parameter Name	Parameter Value
CID Remaining Precursor Threshold (%)	20
HCD Remaining Precursor Threshold (%)	20
Evaluation Ion Trap Scan AGC Target	5000
Display Evaluation Ion Trap Scans	0

Figure 8. Global Assisted Collision Energy settings as shown in Tune's Diagnostic tab.

Ginseng Extract

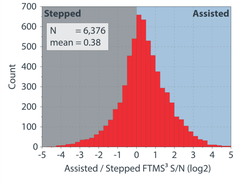


Figure 9. When 6,376 matching FTMS^3 spectra were compared between Stepped and Assisted HCD on the Ginseng extract, the average signal-to-noise of the FTMS^3 spectra increased ~30% when using Assisted HCD for the MS^2 stage.

Often in MS^N analyses for compound characterization stepped HCD fragmentation is used to get a broad distribution of fragment ions. However, with stepped HCD, only 1/3rd of the total injection is spent at each collision energy, and that could leave to non-optimal ion distributions. We thought that Assisted CE could be useful in these analyses as the total injection is fragmented under one, optimal condition, which could improve the data quality.

We analyzed the Ginseng extract sample with both stepped and assisted HCD at the MS^2 stage and compared the signal-to-noise (S/N) of the resulting FTMS^3. Figure 9 shows the FTMS^3 S/N ratio of those compounds that were sampled in both the stepped and assisted acquisitions.

Ginseng Extract

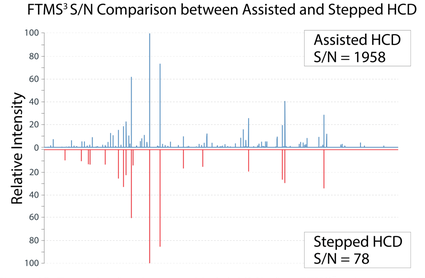


Figure 10. Example of an increased in total S/N of an FTMS^3 using Assisted HCD (25, 40, 55) at the MS^2 stage versus Stepped HCD (25, 35, 50). Assisted collision energy selection is useful for deep spectral analysis as it optimizes the fragmentation conditions for each MS-stage in real-time. The assisted spectrum contains a richer set of ions that could be used to characterize the compound better. The stepped spectrum contains many of the same ions, but at much lower S/N, and a large portion of ions doesn't appear at all.

CONCLUSIONS

- Assisted CE can help select the best collision energy per compound on the chromatographic timescale.
- The method is robust and can be optimized to have a limited impact on the overall duty cycle of the instrument.
- Available for all Tribrid instrument models in the standard method editor application (version 3.1).

REFERENCES

- mzCloud Database (https://www.mzcloud.org/).

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TRADEMARKS/LICENSING

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