Application Note: 540

# Multiple Fragmentation Methods for Small Molecule Characterization on a Dual Pressure Linear Ion Trap Orbitrap Hybrid Mass Spectrometer

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**Key Words**

- Orbitrap Velos Pro
- Higher-Energy Collisional Dissociation
- Collision Induced Dissociation
- Fragmentation
- Structural Elucidation

## Introduction

A key advantage of the linear ion trap (LIT) mass spectrometer is its ability to perform multiple stage MS/MS (or $MS^n$) fragmentation on a single precursor and its product ions to yield extensive amounts of structural information and the linkage between them. Coupling a Thermo Scientific Orbitrap mass analyzer to the back of an LIT not only enables parallel data acquisition with high mass accuracy and resolution, but also provides opportunities for post-LIT ion manipulations. Higher-Energy Collisional Dissociation (HCD) was introduced on the Thermo Scientific Orbitrap Velos Pro mass spectrometer as an alternative dissociation method (Figure 1). HCD MS/MS has been demonstrated to display fragment ions resulting from multiple steps of activation.[1] It can also determine low *m/z* product ions. Having access to both dissociation methods can be a significant advantage for small molecule structural elucidation. The two dissociation methods can provide different energy pathways to access fragmentation fingerprints due to the intrinsic mechanistic difference of ion dissociation. Combined with ultra-high resolution and accurate mass, such a platform can offer comprehensive information for confident small molecule structure characterization.

## Goal

To determine if two ion dissociation techniques are complementary by comparing and contrasting the MS/MS and $MS^n$ capabilities of Collision Induced Dissociation (CID) and Higher-Energy Collisional Dissociation (HCD) with a mass analyzer with high-resolution, accurate mass capabilities, including fragmentation pattern, efficiency, sensitivity, and spectral quality.

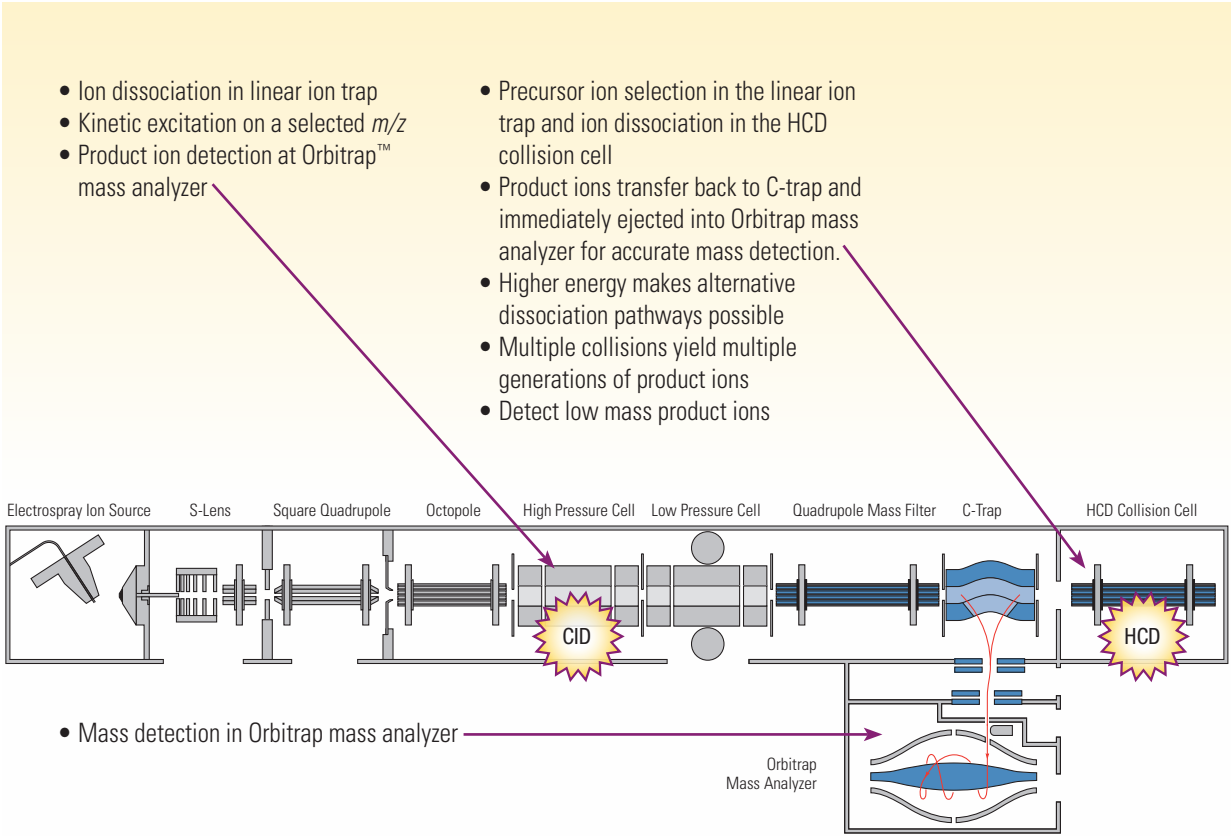


Figure 1. Schematic of the Orbitrap Velos Pro mass spectrometer. The multiple collision regions are highlighted.

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## Experimental

### Sample Preparation

The eleven compounds, as shown in Table 1, were purchased from Sigma-Aldrich® and Sequoia Research Products. Stock solutions of 1 µg/µL of each compound were prepared in DMSO. A mixture containing 10 ng/µL of each of the eleven compounds was prepared by diluting the stock solutions with 1:1 acetonitrile: water. A working solution of 1 ng/µL was prepared by 10-fold dilution from 10 ng/µL solution using water with 5% acetonitrile (v/v).

The 1 ng/µL matrix-spiked samples were prepared from 10 ng/µL solution with supernatant from protein precipitation of acetonitrile and human plasma. Human plasma was purchased from Bioreclamation.

### HPLC Separation

HPLC separation was performed on a Thermo Scientific Accela 1250 UHPLC pump and an Accela™ autosampler.

| | |
|---|---|
| Column: | Thermo Scientific Hypersil GOLD 100 x 2.1mm, 1.9 µm particle size |
| Column temperature: | 50 °C |
| Injection volume: | 1 µL (1 ng/µL) |
| Mobile phase A: | Water/0.1% formic acid |
| Mobile phase B: | Acetonitrile/0.1% formic acid |

***Gradient***

| Time (min) | A% | B% | Flow rate (µL/min) |
|---|---|---|---|
| 0.0 | 95 | 5 | 700 |
| 2.0 | 70 | 30 | 700 |
| 5.0 | 65 | 35 | 700 |
| 7.0 | 10 | 90 | 700 |
| 7.5 | 10 | 90 | 700 |
| 7.6 | 95 | 5 | 700 |
| 8.5 | 95 | 5 | 700 |

### Mass Spectrometry Conditions

The mass spectrometric analysis was performed on an Orbitrap Velos Pro™ mass spectrometer with an HCD cell in positive ion mode. The system was equipped with a Thermo Scientific Ion MAX API source housing and a heated electrospray ionization (HESI-II) probe. The following normalized collision energy settings (CE%) were used for both CID and HCD: 25, 30, 35, 40, 45, 50, 60, and 70. Spectral annotation was performed using Thermo Scientific Mass Frontier software.

## Results and Discussion

The elution profile for the 11 compounds is provided in Figure 2. The fragmentation data show that the sensitivities of the CID and HCD spectra are comparable on the Orbitrap Velos Pro mass spectrometer. In general, 35% normalized collision energy for CID is efficient for fragmenting the majority of small molecule compounds. The optimal collision energy for HCD varies depending on the structural features and molecular weight of the compounds. HCD tends to produce EI-like fragmentation and records ions from multiple steps of collision as seen in Figure 3. HCD can be used to determine the low mass product ions, while the CID $MS^n$ preserves the structural linkage between fragments as shown in Figure 4.

For about 50% of the compounds tested under different collision energy settings, a significant difference in fragmentation pattern was observed between CID vs. HCD MS/MS spectra, as shown in Figure 5 for prednisone.

The CID and HCD spectra of reserpine (Figure 3) show significant differences in their fragmentation pattern at collision energy 35%, while the CID $MS^n$ spectra of reserpine preserve the spectral linkage between the fragments (Figure 4). Compared to CID spectra, the fragmentation pattern varies more significantly with collision energy settings in HCD spectra (Figure 6). At their optimized energies, CID MS/MS and HCD MS/MS display comparable sensitivity in the spiked human plasma sample (Figure 7).

| Peak # | Name | Formula | [M+H]+ | RT min. | CAS# | Note |
|---|---|---|---|---|---|---|
| 1 | Zolmitriptan | $C_{16}H_{21}N_3O_2$ | 288.17065 | 1.30 | 139264-17-8 | Sequoia Research p/n SRP01300Z |
| 2 | Sulfamethazine | $C_{12}H_{14}N_4O_2S_1$ | 279.09102 | 1.63 | 57-68-1 | Sigma-Aldrich p/n S6256-25G |
| 3 | Sulfamethoxazole | $C_{10}H_{11}N_3O_3S_1$ | 254.05939 | 2.03 | 723-46-6 | Sigma-Aldrich p/n S7507 |
| 4 | Bupropion | $C_{13}H_{18}Cl_1N_1O_1$ | 240.11497 | 2.45 | 31677-93-7 | Sigma-Aldrich p/n B102-50MG HCl Salt |
| 5 | Propranolol | $C_{16}H_{21}N_1O_2$ | 260.16451 | 2.75 | 318-98-9 | Sigma-Aldrich p/n P0884-1G HCl salt |
| 6 | Prednisone | $C_{21}H_{26}O_5$ | 359.1853 | 2.84 | 53-03-2 | Sigma-Aldrich p/n P-6254 |
| 7 | Erythromycin | $C_{37}H_{67}N_1O_{13}$ | 734.46852 | 3.45 | 114-07-8 | Sigma-Aldrich p/n E-6376 |
| 8 | Alprazolam | $C_{17}H_{13}N_4Cl_1$ | 309.09015 | 4.20 | 28981-97-7 | Sigma-Aldrich p/n A8800-10MG |
| 9 | Reserpine | $C_{33}H_{40}N_2O_9$ | 609.28066 | 4.85 | 50-55-5 | Sigma-Aldrich p/n R-0875 |
| 10 | Loperamide | $C_{29}H_{33}N_2O_2Cl_1$ | 477.23033 | 5.92 | 34552-83-5 | Sigma-Aldrich p/n L4762-5G HCl salt |
| 11 | Terfenadine | $C_{32}H_{41}N_1O_2$ | 472.32101 | 6.44 | 50679-08-8 | Sigma-Aldrich p/n T9652-5G |

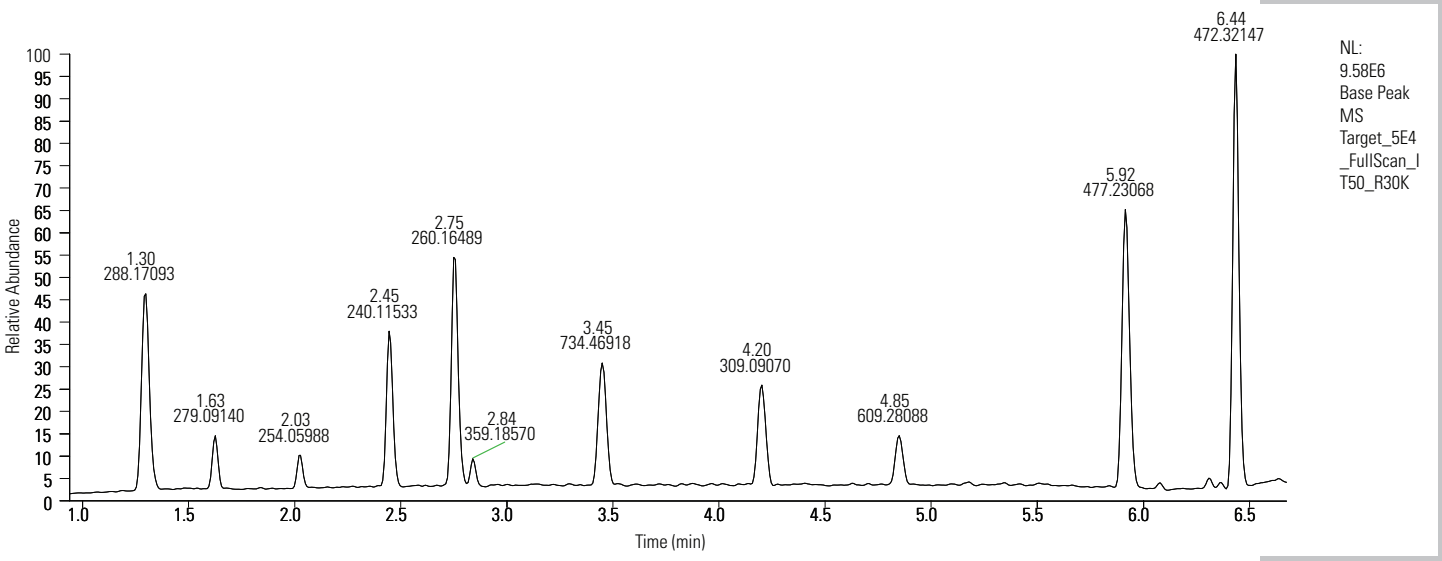


Figure 2. Base peak ion chromatograph of the 11-compound mixture. The table lists the identity of the 11 compounds.

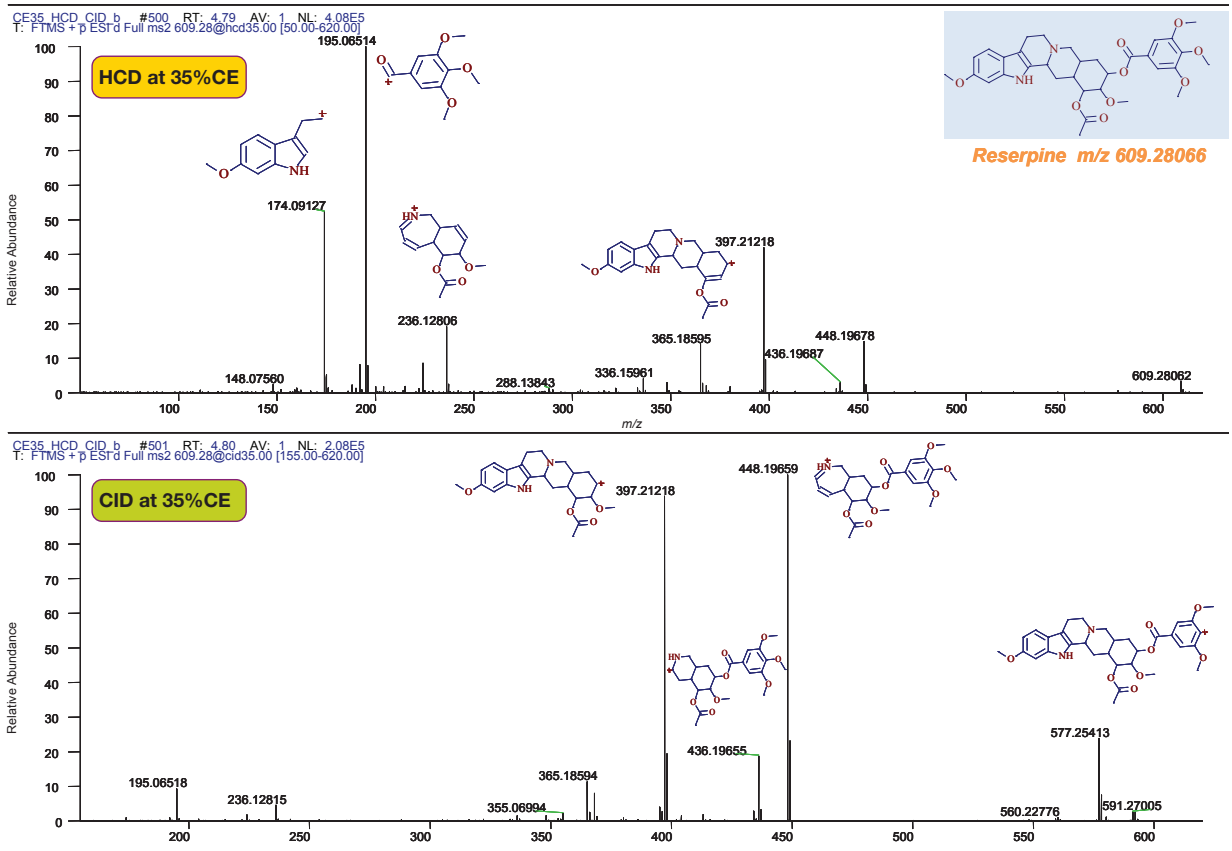


Figure 3. HCD and CID spectra of reserpine at 35% collision energy

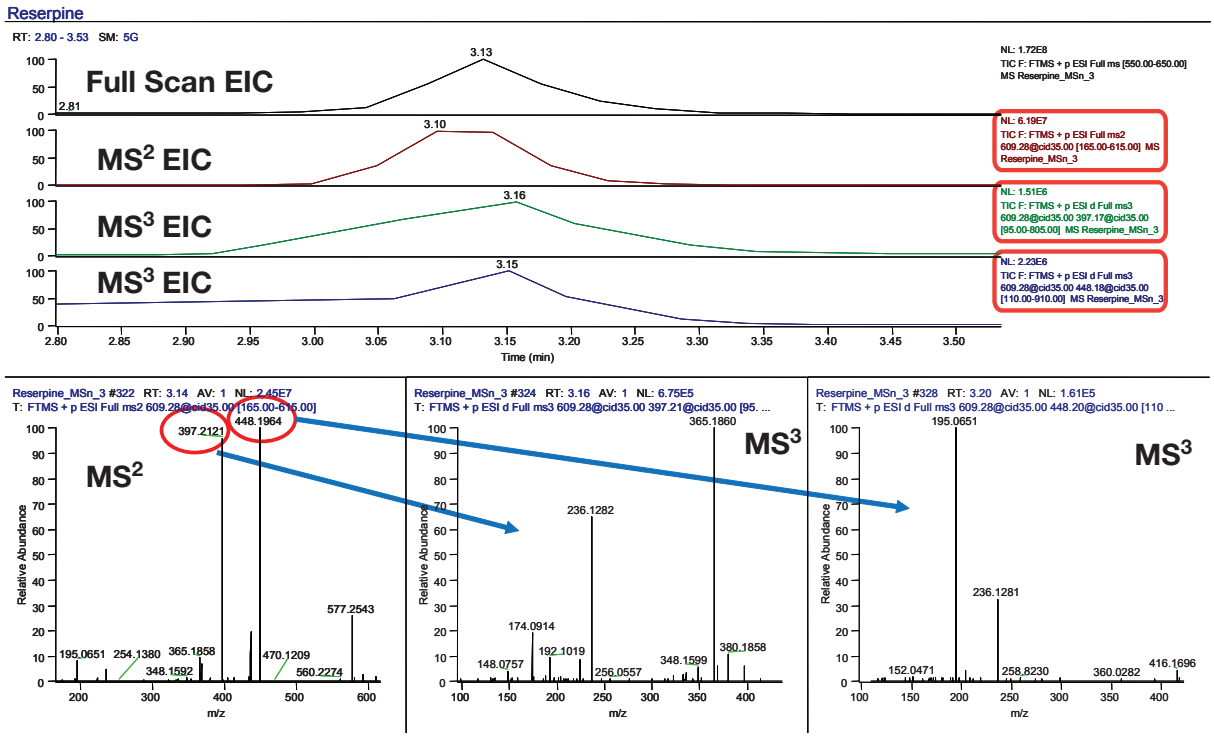


Figure 4. Reserpine CID $MS^n$ spectra preserve the structural linkage between fragments.

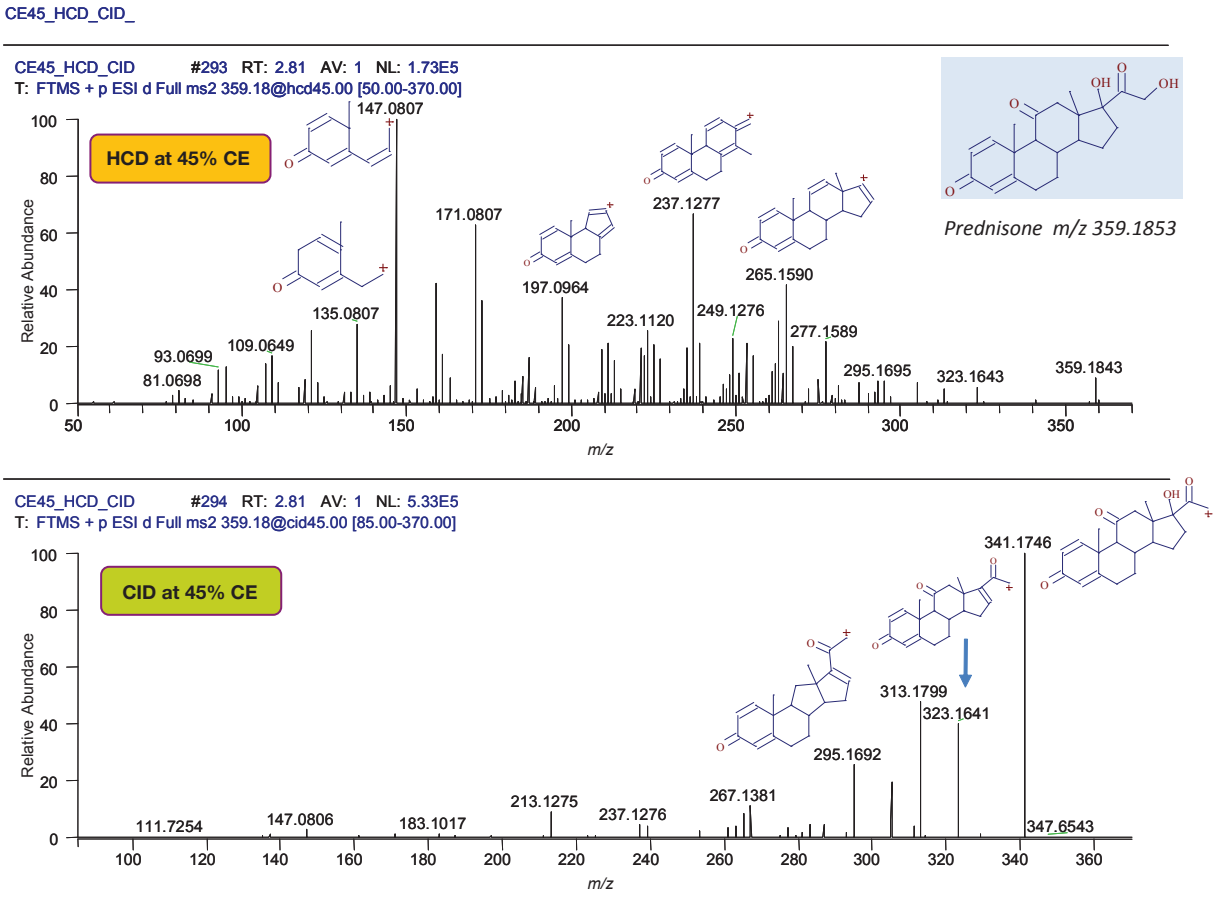


Figure 5. HCD and CID spectra of prednisone at 45% collision energy

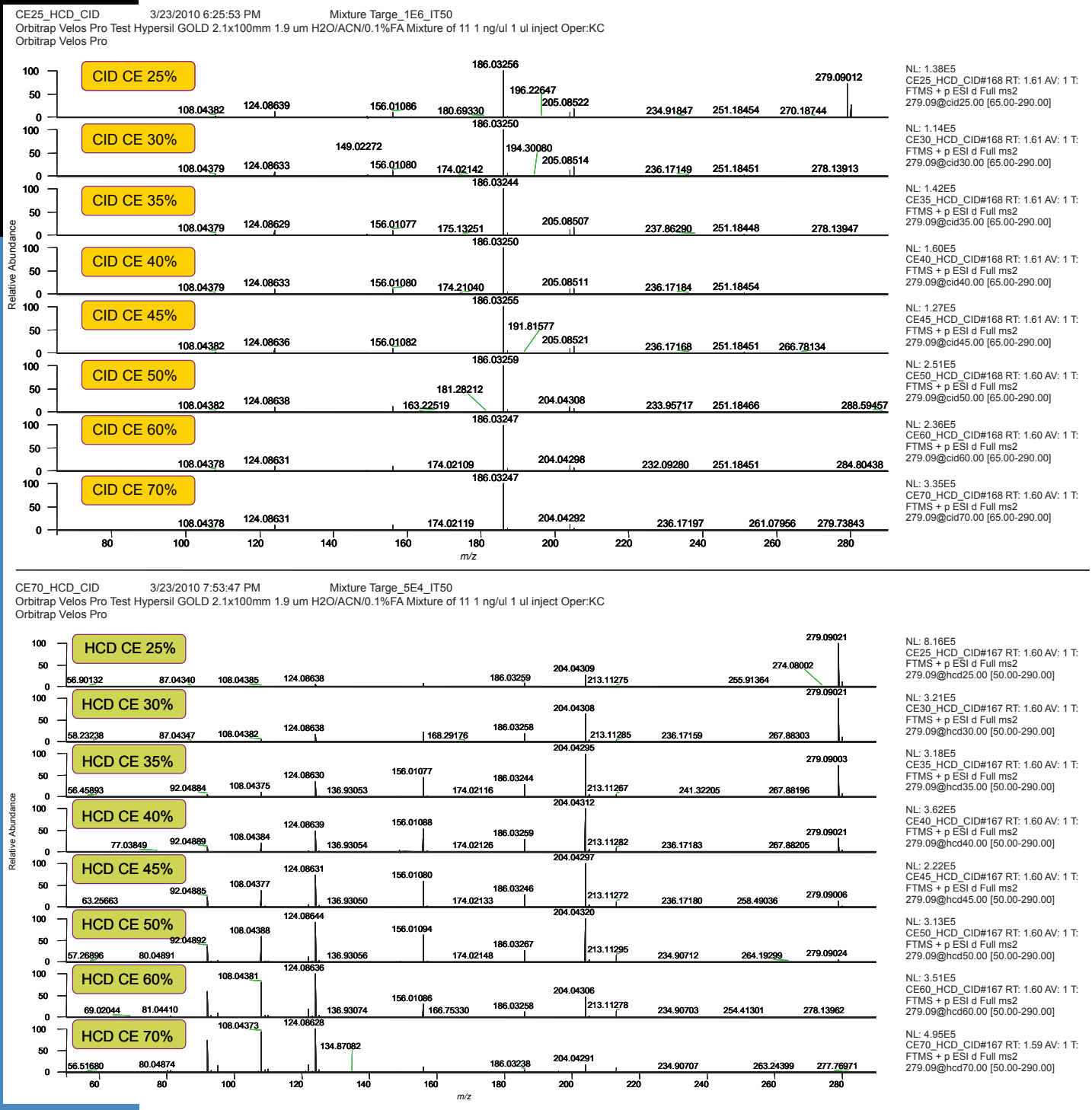


Figure 6. CID and HCD spectra of sulfamethazine at different collision energy percentages (%): 25, 30, 35, 40, 45, 50, 60, and 70

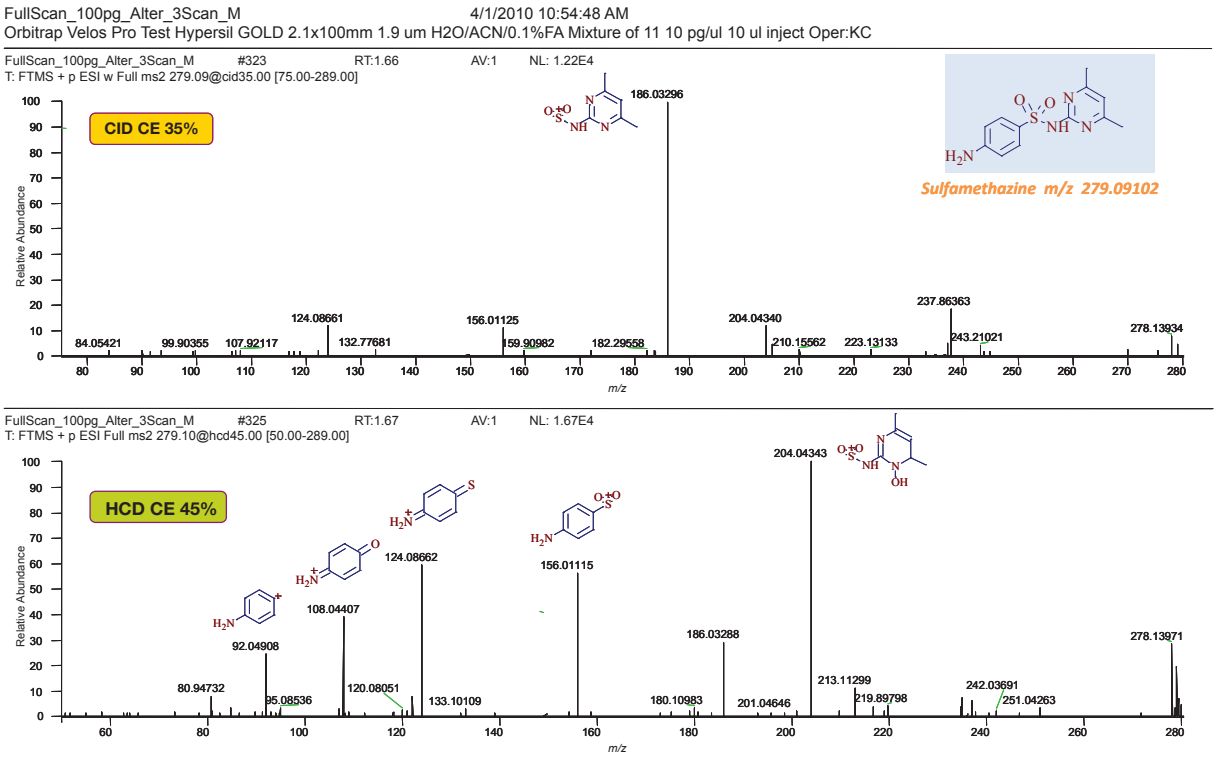


Figure 7. HCD and CID spectra of sulfamethazine from plasma spiked with 11-compound mixture using optimized CID and HCD collision energy (CID 35% and HCD 45%)

## Conclusion

- CID $MS^n$ and HCD MS/MS complement each other by providing different fragmentation pathways to generate informative, structurally significant product ions. Used in combination, the CID and HCD fragmentations enable confident small molecule structure characterization.
- Comprehensive fragmentation information from CID and HCD facilitates accurate and confident small molecule structural characterization.
- With robust ultra-high resolution, accurate mass capabilities, and multiple dissociation methods, the Orbitrap Velos Pro mass spectrometer combined with the Accela UHPLC system and Mass Frontier™ software offers a total solution for any small molecule structural elucidation applications (e.g. metabolite identification as well as impurity and degradation analyses).

## References

1. Y. Huang et al. Proceedings 18th International Mass Spectrometry Conference, Bremen, Germany, Aug 30 – Sept 4, 2009.

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