

Using Multiple Mass Defect Filters and Higher Energy Collisional Dissociation on an LTQ Orbitrap XL for Fast, Sensitive and Accurate Metabolite ID

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Overview

Purpose: To evaluate the use of Multiple Mass Defect Filters (MMDFs)[™] with LTQ Orbitrap XL data for metabolite identification; to investigate the use of Higher energy Collisional Dissociation (HCD) for structural elucidation in metabolite identification experiments.

Methods: Rat hepatocyte incubation samples of Irinotecan were analyzed using an LTQ Orbitrap XL with HCD collision cell. Both Collision Induced Dissociation (CID) MS/MS and HCD MS/MS were acquired for the potential metabolites. MMDFs were then used to process the acquired raw file.

Results: MMDFs were able to filter out the vast majority of the background ions in the full scan spectra while preserving those related to the parent drug. Compared with the results gathered from using only a single Mass Defect Filter (MDF), results from MMDFs are more distinct and specific, allowing users to do faster, more sensitive and more accurate analyses. HCD provides complementary fragmentation pathways, in addition to the CID available in the ion trap, and produces low mass diagnostic ions in MS/MS spectra that are useful for metabolite structural elucidation.

Introduction

An integral part of drug discovery and development is the identification of drug metabolites formed through phase I and phase II metabolic reactions. These metabolites may have either intrinsic pharmacological activity or display specific toxicity. LC-MS has become the cornerstone in drug metabolite identification because of its sensitivity and ability to analyze complex mixtures. In particular,

LC-MSⁿ employing linear ion trap (LIT) technology has become widely used because of its speed, sensitivity and robustness in generating rich structure information. However, challenges still remain in detecting and identifying metabolites in the presence of highly complex biological matrices.

Coupling an orbitrap mass analyzer to the LIT greatly facilitates the task of metabolite identification because it not only enables parallel data acquisition with high mass accuracy and resolution, but it also provides post-LIT ion manipulations. High resolution and accurate mass help resolve and identify metabolite peaks from background matrix ions, and also allow the use of post-acquisition data processing tools like MDF to reduce the number of false positives by removing the vast majority of matrix-related background ions.^{1,2} HCD was recently introduced on the LTQ Orbitrap as an alternative dissociation method by adding a new collision cell behind the C-trap region (Figure 1). HCD can be used to generate low mass diagnostic ions in MS/MS mode, and can also be used in combination with CID in the LIT for MSⁿ experiments.

Irinotecan (CPT-11; 7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxy-camptothecin) is a water-soluble carbamate prodrug of camptothecin and is activated *in vivo* to SN-38, a potent topoisomerase I inhibitor.^{3,4} Currently, Irinotecan, combined with 5-fluorouracil and leucovorin, is approved by the U.S. Food and Drug Administration as a first-line therapy in the treatment of metastatic carcinoma of the colon or rectum.⁴ In this study, we will utilize MMDF and HCD on an LTQ Orbitrap XL to study the biotransformations of Irinotecan in hepatocyte incubation and identify its metabolites.

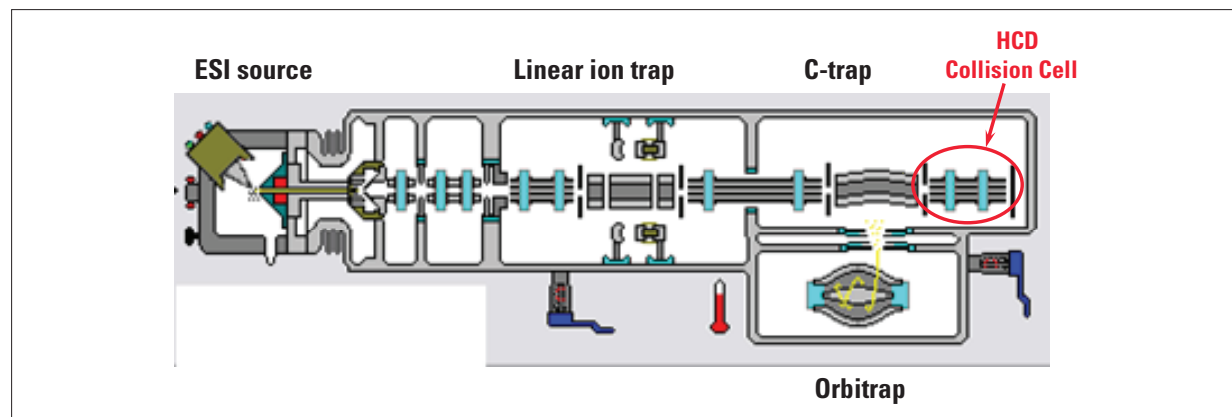


Figure 1: Scheme diagram of the LTQ Orbitrap, highlighting the HCD collision cell behind the C-trap.

Key Words

- LTQ Orbitrap XL[™]
- MetWorks[™] Software
- Accela[™] High Speed LC
- Hypersil GOLD[™] Column
- Metabolite Identification
- Multiple Mass Defect Filters[™]

Materials and Methods

Samples: Incubation was carried out using rat hepatocytes pooled from 1 male and 1 female with a cell density of 0.5 million/mL and 10 μ M of Irinotecan in the final 1 mL incubation solution. The solution was shaken overnight and quenched by cooling down on dry ice, followed by the addition of 200 μ L chilled Acetonitrile. The solution was then vortexed and centrifuged. The supernatant (~1 mL) was taken out, and 10 μ L from such solution was directly injected for each LC-MS/MS run.

HPLC: Accela High Speed LC, Thermo Scientific

Column: Hypersil GOLD C18, 1 \times 100 mm, 1.9 μ m particle size, Thermo Scientific

Gradient:	Time	A%	B%	μ L/min
	0	98	2	150
	2	98	2	150
	2.5	85	15	150
	14	75	25	150
	14.9	20	80	150
	15	98	2	150
	20	98	2	150

A = 0.1% Formic Acid in water

B = 0.1% Formic Acid in Acetonitrile

Mass Spectrometer: LTQ Orbitrap XL with HCD collision cell.

Results and Discussions

MMDF is a new feature in the latest version of Thermo Scientific MetWorks Software that combines the results from up to six different MDFs. Through the use of MMDF and the combination of HCD and CID MS/MS, thirteen Irinotecan metabolites were identified from the incubation sample using the LTQ Orbitrap XL with less than 3 ppm mass accuracy. Table 1 summarizes the 13 metabolites identified, while Scheme 1 displays their corresponding structures.

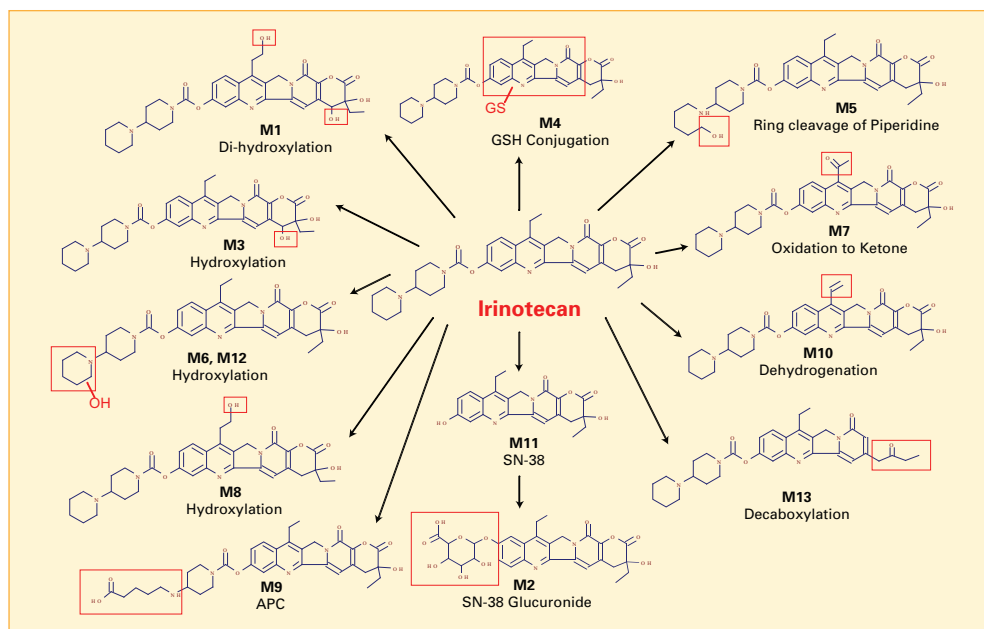
As shown in Table 1, all 13 metabolites were found with peak areas less than 1% of that of the parent.

Figure 2 shows how the base peak chromatogram from the same LC-MS/MS run changed after a single MDF and after MMDF processing. Due to the low abundance of the metabolites, in all these chromatograms the intensity (y axis) for retention time 5.7-11.2 minutes and 12.5-16.1 minutes was expanded by 50 times to better illustrate the effectiveness of MMDF. While all the peaks from the Irinotecan metabolites were well buried in the original chromatogram (Figure 2a), the most abundant metabolite peaks (e.g. M2 @ 7.44 minute, M5 @ 8.84 minute, M6 @ 9.92 minute) start to show up after applying a single MDF (Figure 2b). However, even with a single MDF, peaks from background matrix ions that are unrelated to the metabolites were still prominent (e.g. peaks at 6.55 minute, 7.85 minute, and 11.1 minute). This is due to the fact that in order to use only one single MDF to capture all the phase I and II metabolites, including those from the hydrolysis product SN-38, a relatively wide mass defect range needs to be used (-150 mmu, +70 mmu). Therefore, a portion of the background ions remains after the mass defect filtering.

When MMDFs were applied to the original data (Figure 2c), four different mass defect filters were used, whose corresponding metabolites identified were highlighted using different colors in Table 1: phase I metabolites of Irinotecan are shown with white background; phase II metabolites of Irinotecan are shown in light blue; phase I metabolites of SN-38 are shown in pink; phase II metabolites of SN-38 are shown in yellow. Compared to the results using a single MDF (Figure 2b), results from MMDF (Figure 2c) are cleaner and more specific to the metabolites related to Irinotecan, and are, therefore, easier to interpret.

MDF Template	#	R.T.	Metabolite Identity	Formula Change	Integrated Peak Area	% of Parent	Theoretical MH ⁺ m/z	Measured MH ⁺ m/z	ppm	Δ MW (Da) from Irinotecan	Δ MD from Irinotecan	Δ MD from Template
Irinotecan MH ⁺ m/z = 587.2864	M1	7.19	Di-hydroxylation	+O2	4.0E+4	0.027	619.2762	619.2755	-1.1	31.9898	- 0.0102	- 0.0102
	M3	8.45	Hydroxylation	+O	1.6E+5	0.107	603.2813	603.2805	-1.3	15.9949	- 0.0051	- 0.0051
	M4	8.48	GSH conjugation	+C10H15 N3O6S	8.7E+3	0.006	892.3546	892.3551	0.6	305.0682	+ 0.0682	+ 0.0682
	M5	8.84	Ring cleavage of Piperidine	+H2O	9.8E+5	0.653	605.2970	605.2965	-0.8	18.0106	+ 0.0106	+ 0.0106
	M6	9.92	Hydroxylation	+O	1.3E+6	0.867	603.2813	603.2800	-2.2	15.9949	- 0.0051	- 0.0051
	M7	10.34	Oxidation to Ketone	-H2+O	9.7E+4	0.065	601.2657	601.2650	-1.2	13.9792	- 0.0208	- 0.0208
	M8	10.64	Hydroxylation	+O	8.9E+4	0.059	603.2813	603.2805	-1.3	15.9949	- 0.0051	- 0.0051
	M9	11.11	APC	+O2	1.7E+5	0.113	619.2762	619.2758	-0.6	31.9898	- 0.0102	- 0.0102
	M10	11.55	Dehydrogenation	-H2	3.7E+5	0.247	585.2707	585.2699	-1.4	-2.0157	- 0.0157	- 0.0157
	P	11.68	Irinotecan	N.A.	1.5E+8	100	587.2864	587.2860	-0.7	0	0	0
	M12	12.69	Hydroxylation	+O	1.2E+5	0.080	603.2813	603.2803	-1.7	15.9949	- 0.0051	- 0.0051
	M13	15.77	Decarboxylation	-CO2	3.2E+5	0.213	543.2966	543.2960	1.1	-43.9898	+ 0.0102	+ 0.0102
	SN-38 MH ⁺ m/z = 393.1445	M2	7.44	SN-38 Glucuronide	+C6H8O6	4.0E+5	0.267	569.1766	569.1754	-2.1	176.0321	-0.1106
M11		11.95	SN-38	N.A.	6.1E+5	0.407	393.1445	393.1440	-1.3	0	-0.1415	0

Table 1. Summary of the putative metabolites identified from 10 μ M Irinotecan rat hepatocyte incubation. Those highlighted in different colors were filtered by different Mass Defect Filters.



Scheme 1. Proposed structures and biotransformation pathways of Irinotecan metabolites.

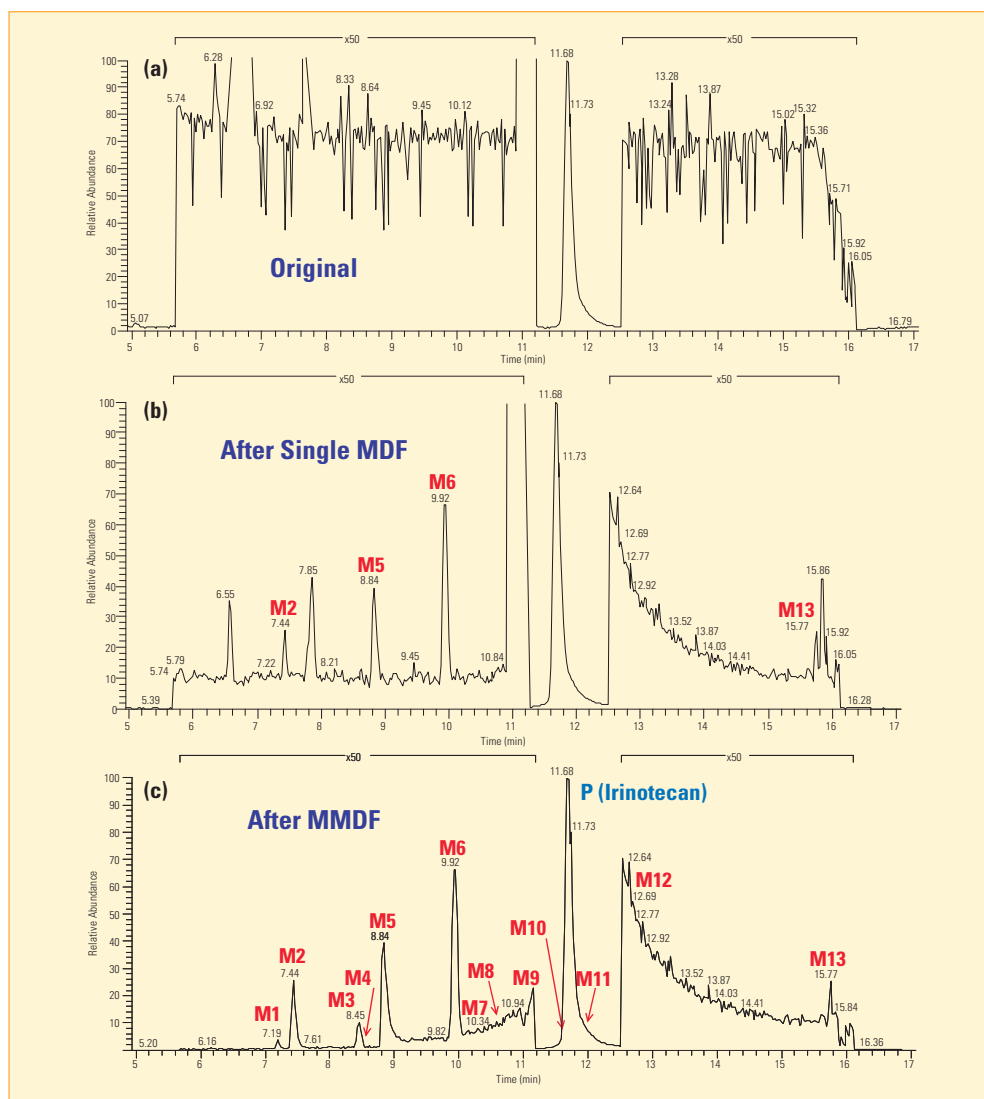


Figure 2: Base peak chromatograms of 10mM Irinotecan rat hepatocyte incubation: (a) Original; (b) After single Mass Defect Filter; (c) After Multiple Mass Defect Filters (MMDF).

Figure 3 further illustrates the power of MMDF by showing an example of how the full MS spectrum changed after the single MDF, and MMDFs were applied. The peak at 603.2805 m/z is a hydroxylation metabolite (M3) that elutes at 8.45 minute. The original full scan MS is dominated by background ions and the M3 peak only has less than 15% relative abundance (Figure 3a). After a single MDF was applied, the M3 peak becomes the base peak, however, there are still a lot of background peaks remaining in the spectrum (Figure 3b). After MMDFs were applied, only the M3 peak and trace of the parent remain in the spectrum while almost all the background ions are gone (Figure 3c).

After MMDF processing, HCD and CID MS/MS spectra of Irinotecan and its potential metabolites were analyzed, and their corresponding structures were elucidated. Figure 4a shows the CID MS/MS of Irinotecan

from the LTQ, and Figure 4b shows the HCD MS/MS acquired in the orbitrap. While all the major fragment ions in the CID spectrum were also observed in the HCD spectrum, the HCD spectrum contains additional fragment ions including low m/z ions. A portion of the parent ions still remains in the HCD spectrum. These are characteristics similar to those from a quadrupole collision cell. Better than 2 ppm mass accuracy and high resolution were obtained on the fragment ions in the HCD MS/MS spectrum because it was acquired in the orbitrap. Mass Frontier™ software, with its accurate mass capability, was used to assist spectrum interpretation. Structures of the fragment ions were assigned accordingly. The fact that HCD spectra display rich fragment ions, especially in the low mass region, as well as high mass accuracy on these product ions, greatly facilitates the MS/MS interpretation.

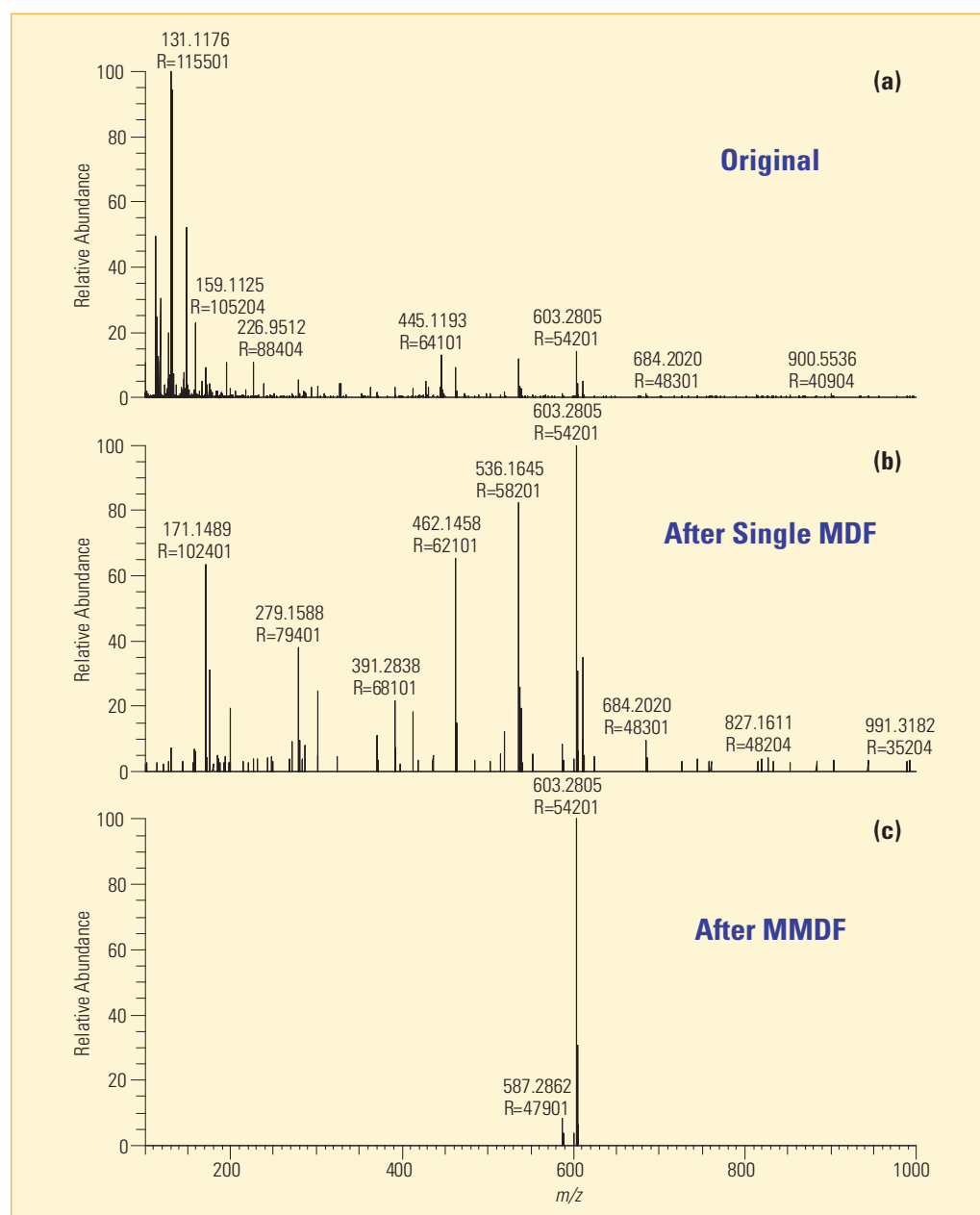


Figure 3: Full MS spectrum at 8.45 minute: (a) original, (b) after single Mass Defect Filter, (c) after Multiple Mass Defect Filters (MMDF).

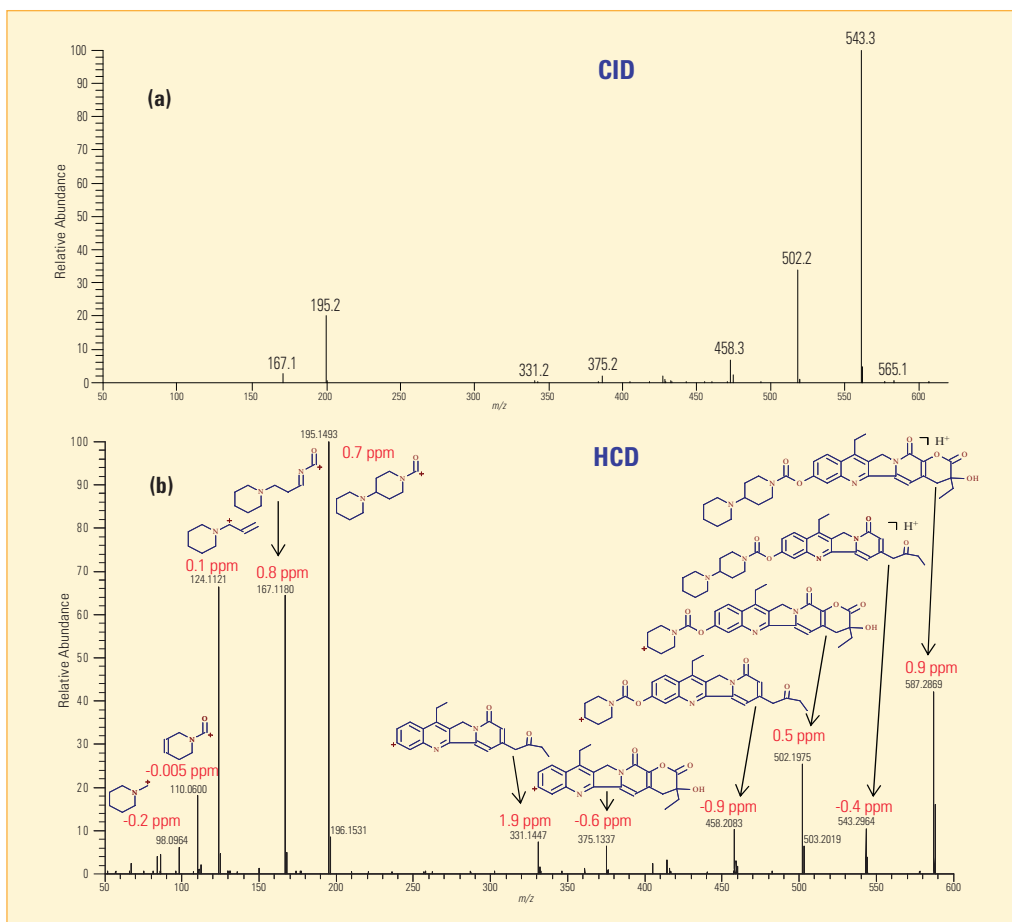


Figure 4: MS/MS spectra of Irinotecan: (a) CID MS/MS acquired in the LTQ. (b) HCD MS/MS acquired in the orbitrap.

Conclusions

With the help of MMDF and the combination of HCD and CID MS/MS, 13 Irinotecan metabolites whose peak areas were less than 1% of that of the parent were identified on an LTQ Orbitrap XL coupled to an Accela High Speed LC.

This report demonstrates that MMDF is more effective than a single MDF to uncover phase I and II metabolites specifically and concurrently. It also allows the detection of metabolites from hydrolysis or N-dealkylation, even when the products from such processes have mass defects that are significantly different from the parent. MMDF allows users to use low threshold values during data processing so that metabolites at very low levels can be easily identified. The resulting chromatogram from MMDF is accurate and specific because it is based on exact mass and mass deficiencies, which are highly specific to the parent drug compound. It provides speed, sensitivity and accuracy to facilitate the identification of drug metabolites in drug discovery and development.

HCD provides an alternative fragmentation method on the LTQ Orbitrap XL in addition to the CID in the linear ion trap. HCD spectra display characteristics similar to those from a quadrupole collision cell: rich in product ions, has no low mass cut off, and typically a portion of the parent ions still remains. The fragment ions in HCD spectra have high mass accuracy and resolution. These characteristics of HCD spectra complement the power of ion trap MSⁿ and allow easy spectrum interpretation and high confidence in structural elucidation.

References

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