# Characterization of Clindamycin using TurboDDS on the Agilent 500 Ion Trap LC/MS

Application Note

Pharmaceuticals


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## Abstract

The structures of antibiotics such as clindamycin can be explored and confirmed with the use of the 500 Ion Trap LC/MS.


## Introduction

Clindamycin is a lincosamide antibiotic that is used against gram-positive and gram-negative anaerobic pathogens and gram-positive aerobes. Its mode of operation is inhibition of bacterial protein synthesis and it is mainly used for antimicrobial purposes. Since the synthesis of clindamycin from lincomycin can result in impurities such as clindamycin B, 7-epiclindamycin and lincomycin, it is useful to be able to characterize the pure drug in the bulk drug form to certify that it has a high level of purity.

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## Instrumentation

The following instruments were used in this study:

- Agilent 500 Ion Trap LC/MS
- Agilent 212-LC Binary Solvent Delivery Modules (2)
- Built-in 6-port valve and syringe infusion pump

## Materials and Reagents

Methanol (p/n A456-4) and water (p/n W6-4) (Optima LC/MS grade) were purchased from Fisher Scientific (Pittsburgh, PA). Clindamycin was provided by Agilent Technologies.

## Sample Preparation

A clindamycin standard was prepared at 10 ppm by dissolving 1 mg in 100 mL of 50:50 methanol:water.

### LC conditions

| | |
|---|---|
| LC program: | Isocratic |
| Flow rate: | 200 µL/min |
| Injection volume: | 20 µL |

### MS parameters

| | |
|---|---|
| Ionization mode: | ESI positive |
| Nebulizing gas: | 20 psi |
| Drying gas: | 50 psi at 250 °C |
| Needle: | 6000 V |
| Shield: | 600 V |
| Capillary: | 56.4 V |
| RF loading: | 65 |
| Scan parameters: | Full scan: *m/z* 100 to 450 |
| µscans averaged: | 3 µscans; 2.20 s/scan |

### TurboDDS parameters

| | |
|---|---|
| Survey scan range: | *m/z* 100–450 |
| Capillary: | 56.4 V |
| RF loading: | 65% |
| $MS^n$ depth: | n=5 |
| Auto mass range: | On |
| High resolution: | On |
| High resolution mscans: | 3 µscans |
| Isolation window: | 3 *m/z* |

| | Breadth | mscans | Start mass | End mass | Threshold |
|---|---|---|---|---|---|
| $MS^2$ | 1 | 3 | 50 | 2000 | 20000 |
| $MS^3$ | 1 | 3 | 50 | 2000 | 860 |
| $MS^4$ | 1 | 3 | 50 | 2000 | 860 |
| $MS^5$ | 1 | 3 | 50 | 2000 | 860 |

### TurboDDS trigger conditions

| | |
|---|---|
| Threshold: | 200,000 counts |
| Minimum intensity ratio: | 1% |
| Isotopic equivalence: | 3 *m/z* |

## Results and Discussion

Full scan MS spectra, MS/MS spectra of the protonated molecule and $MS^3$ spectra of prominent product ions were first generated by infusing the standard at 20 µL/minute for two minutes using the built-in syringe pump. The TurboDDS run was made by flow injection of 20 µL on the built-in six-port valve through a flow of 200 µL/min to produce peaks for compound recognition.

The standard was infused in MS mode to yield the molecular weight and some structural information to trace the fragmentation of clindamycin.

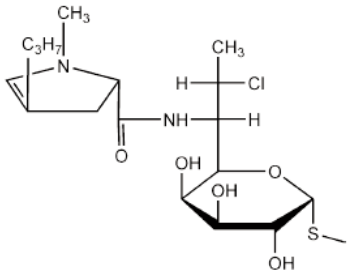



*Figure 1. Clindamycin MW 424 u.*

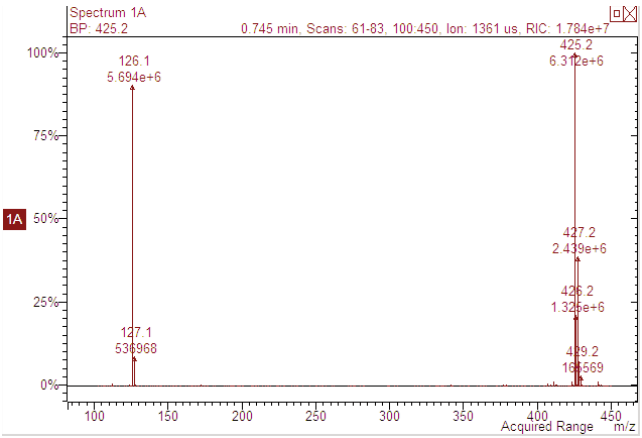
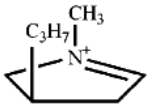



*Figure 2. Protonated molecule m/z 425.2 is $[M+H]^+$.*




***m/z* 126**

*Figure 3. Clindamycin fragment m/z 126.*

An easily generated fragment at *m/z* 126.1 was observed in source at optimum capillary voltage for the protonated molecule. There are many possible pathways to this fragment.

To explore the structure further, MS/MS spectra were collected for clindamycin (precursor *m/z* 425) to investigate the most abundant fragments (Figure 4).

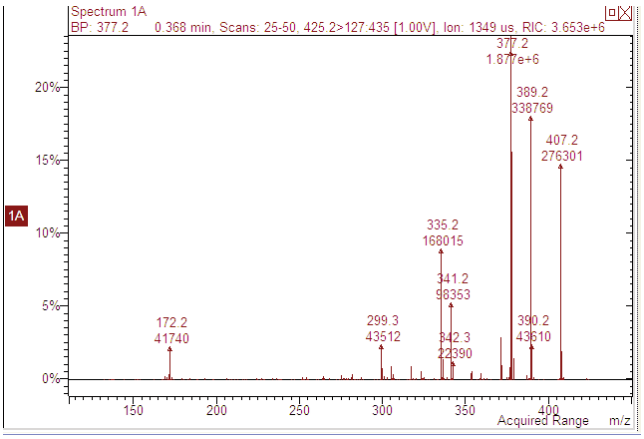



*Figure 4. MS/MS spectrum of clindamycin.*

The most likely chemical compositions of the product ions include:

- *m/z* 407 $[M+H-H_2O]^+$
- *m/z* 389 $[M+H-HCl]^+$
- *m/z* 377 $[M+H-HSCH_3]^+$
- *m/z* 341 $[M+H-HSCH_3-HCl]^+$
- *m/z* 335 $[M+H-CHOHCHSCH_3]^+$
- *m/z* 299 $[M+H-HCl-CHOHCHSCH_3]^+$

$MS^3$ spectra were collected for each major MS/MS product ion, and the structural cleavage was correlated with those fragments to further explore the fragmentation pathways. Figures 5 through 15 show the $MS^3$ spectra collected for clindamycin.

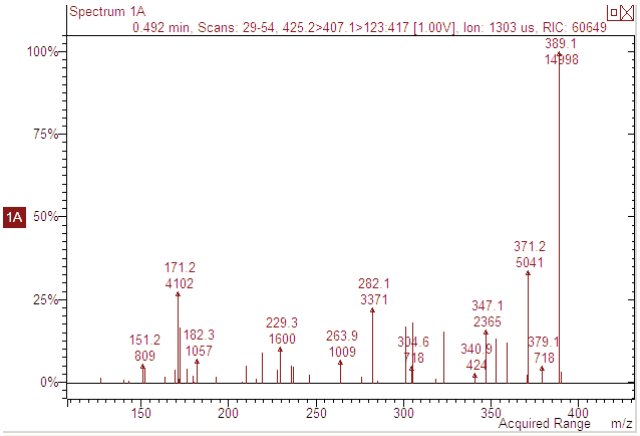



*Figure 5. $MS^3$ spectrum of m/z 407.*

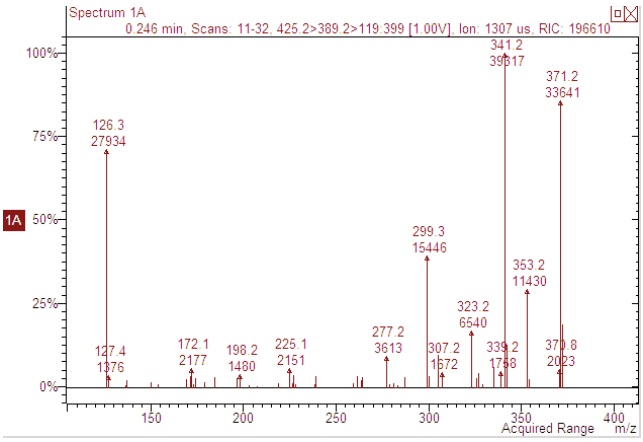



*Figure 6 MS³ spectrum of m/z 389.2.*

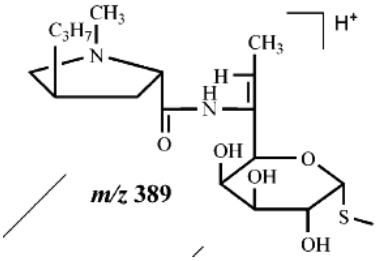



*Figure 7. Structure for clindamycin fragment with m/z 389.*

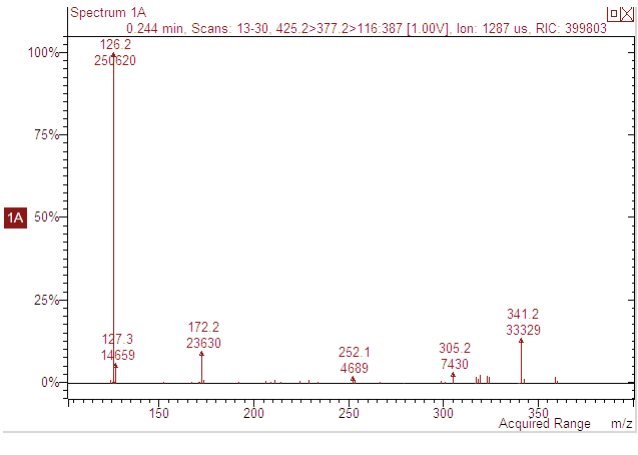



*Figure 8. MS³ spectrum of m/z 377.2.*

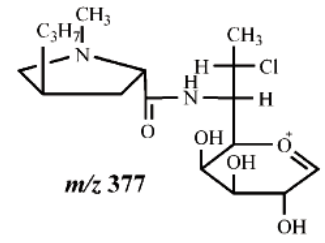



*Figure 9. Structure for clindamycin fragment with m/z 377.*

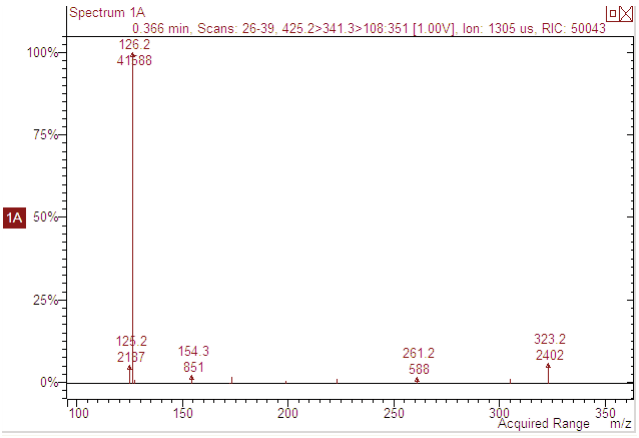



*Figure 10. MS³ spectrum of m/z 341.3.*

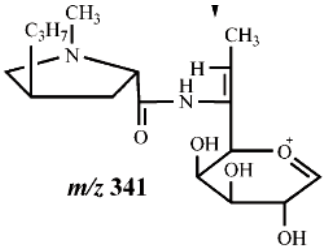



*Figure 11. Structure for clindamycin fragment with m/z 341.*

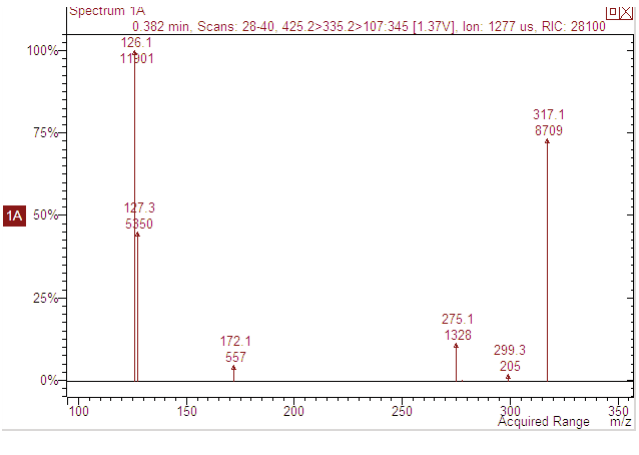



*Figure 12. MS³ spectrum of m/z 335.2.*

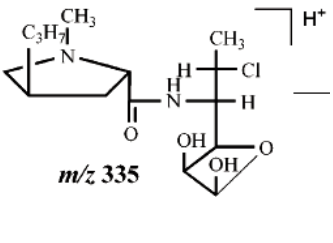



*Figure 13. Structure for clindamycin fragment with m/z 335.*

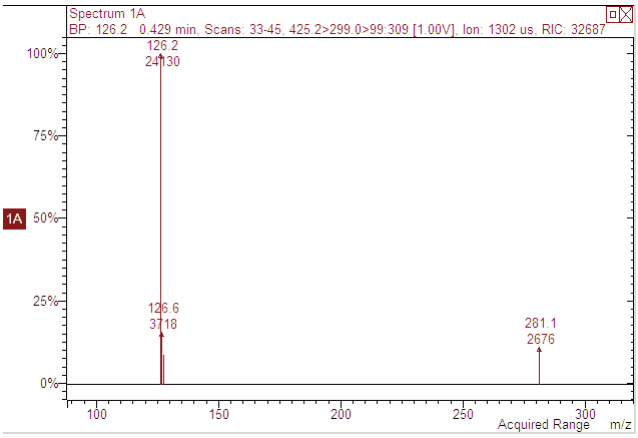



*Figure 14. MS³ of m/z 299.*

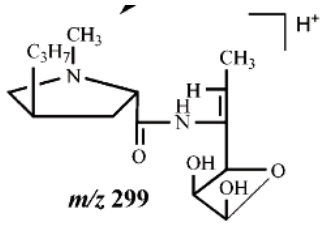



*Figure 15. Structure for clindamycin fragment with m/z 299.*

## High Resolution and Data Dependent Scanning

TurboDDS is a data dependent scanning program with useful features for assisting in structural elucidation:

- High-resolution display of the molecular ion isotope pattern.
- Automatic triggering of the $MS^n$ spectra acquisition when a chromatographic peak of sufficient signal is encountered.
- Organization of the series of spectra acquired in convenient cascading displays.
- Ion Tree report to highlight major product ions and neutral losses.

A high-resolution display of the molecular ion allows confirmation of naturally occurring isotopes in the formula. The

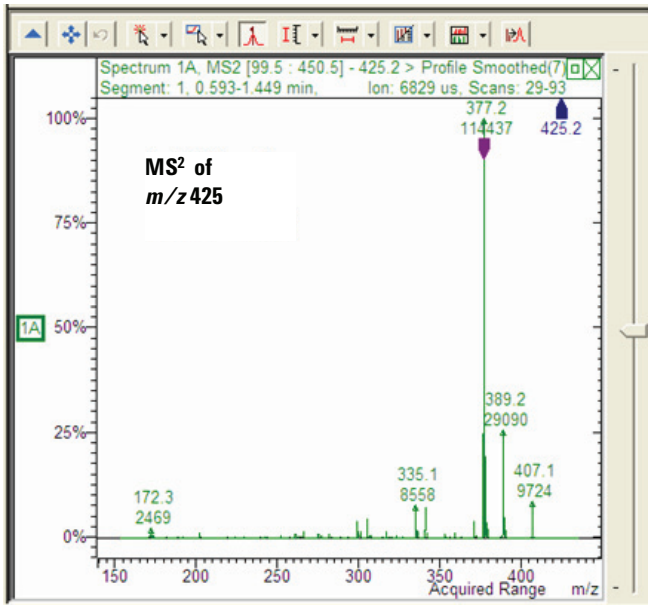



*Figure 16. MS/MS spectrum of m/z 425. The carrot shown at m/z 377.2 peak indicates that further $MS^n$ scans were performed for that ion.*

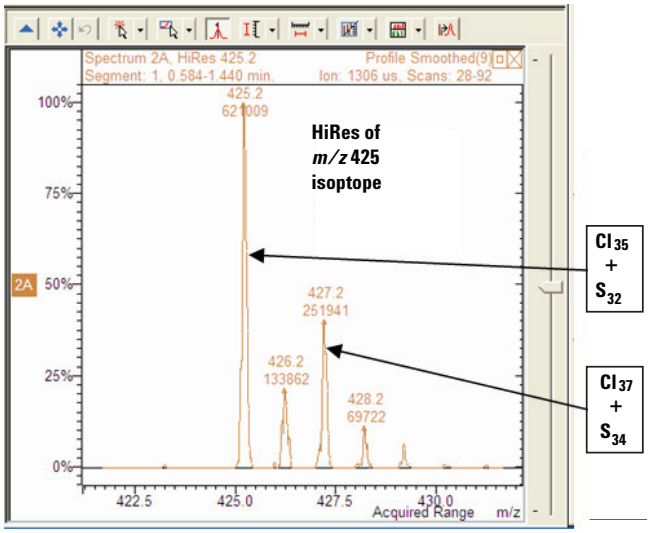



*Figure 17. High-resolution spectrum for m/z 425.2.*

chlorine and the sulfur in the formula of clindamycin constitute a combined 32.4 and 4.4 percent relative abundance of the $[M + 2]^+$ ion. Oxygen also contributes 0.2 percent per atom.

The product ion tree report combines all of the data acquired in a TurboDDS analysis. It allows the organization of all of ions elucidated and gives ion and neutral loss patterns to help confirm the structure.

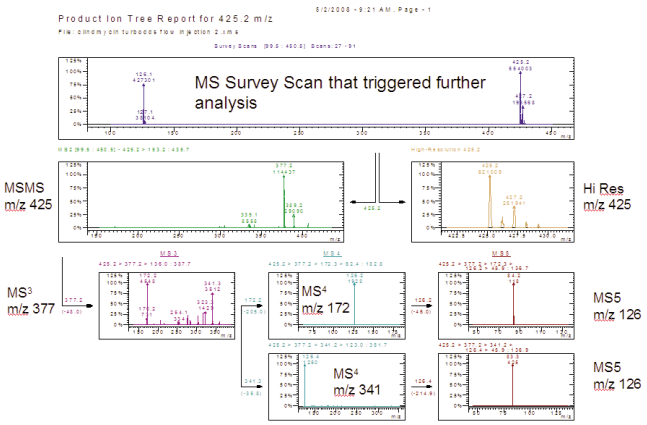



*Figure 18. Product ion tree report for m/z 425.*

## Conclusion

The structures of antibiotics such as clindamycin can be explored and confirmed with the use of the Agilent 500 Ion Trap LC/MS. With the addition of TurboDDS software, all the necessary components can be acquired on an unknown sample where the chromatographic peak triggers further analysis and provides clear report organization of $MS^n$ and high-resolution data.

## Reference

1 "Separation and characterization of clindamycin and related impurities in bulk drug by high-performance liquid chromatography-electrospray tandem mass spectrometry." *Journal of Pharmaceutical and Biomedical Analysis* 41, **2006,** 1116–1123.

## For More Information

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