Pharmaceutical Structure Confirmation Using Mass Spectrometry and Nuclear Magnetic Resonance Spectroscopy

Proof of Structure – Clindamycin

Application Note

Pharmaceuticals

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Abstract

This note investigates the structure of clindamycin in an integrated fashion using an Agilent 500 Ion Trap LC/MS and an Agilent 400-MR NMR spectrometer. The data allows for the rapid confirmation of the chemical structure.

Introduction

One of the basic spectroscopic tasks widely performed in the pharmaceutical industry is confirmation of structure for a known compound. This is a critically important function with relevance in many areas, including medicinal chemistry, process research, pharmaceutical development, manufacturing, and numerous regulatory operations.

Two of the key spectroscopic techniques used to accomplish this task are mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR). These methods provide a wealth of complementary structural information. Mass spectrometry is used to determine the molecular formula for a given compound and can provide structural information through MS^n analysis. Nuclear magnetic resonance spectroscopy is used to determine the type and connectivity of individual atoms within a structural framework. When used in an integrated fashion, these techniques can unambiguously establish the chemical identity of a pharmaceutical compound.

Clindamycin is a widely prescribed lincosamide antibiotic that acts through inhibition of bacterial protein synthesis. This active pharmaceutical ingredient is a semi-synthetic material derived from lincomycin, a natural product isolated from the actinobacterium *S. lincolnensis*. Careful characterization of the bulk drug is important as the synthetic process is known to result in impurities such as clindamycin B, 7-epiclindamycin, and unreacted lincomycin.

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As an example of a typical confirmation of structure analysis, we have investigated the structure of clindamycin in an integrated fashion using an Agilent 500 Ion Trap LC/MS and an Agilent 400-MR NMR spectrometer.

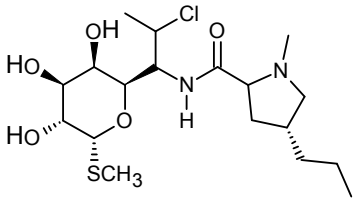

Figure 1. Clindamycin.

Results and Discussion

Primary mass spectral investigations were performed using LC/MS analysis. The MS data were collected with an Agilent 500 Ion Trap LC/MS using TurboDDS data-dependant scanning software. This software allows for collection of a full scan "survey" as well as MS^n data for structural analysis. As shown in Figure 2, the analyte displayed a signal representing the protonated molecule at *m/z* 425.2, consistent with the expected molecular formula of $C_{18}H_{35}ClN_2O_5S$.

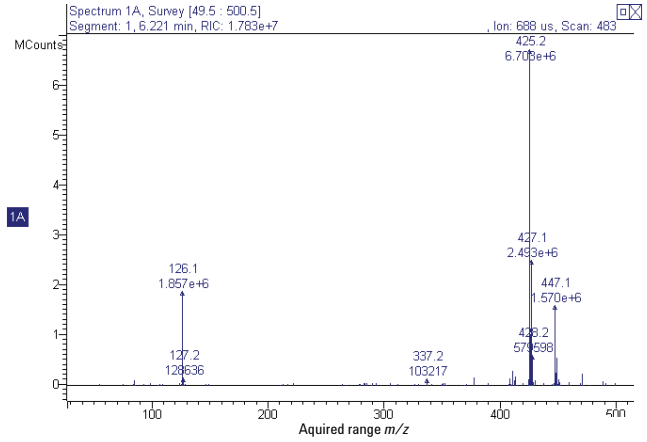

Figure 2. Full scan spectrum of clindamycin.

The molecule is also well behaved magnetically and displays NMR spectra that are nearly first-order, even at the modest 9.4 Tesla field of a 400-MR spectrometer (Figure 3).

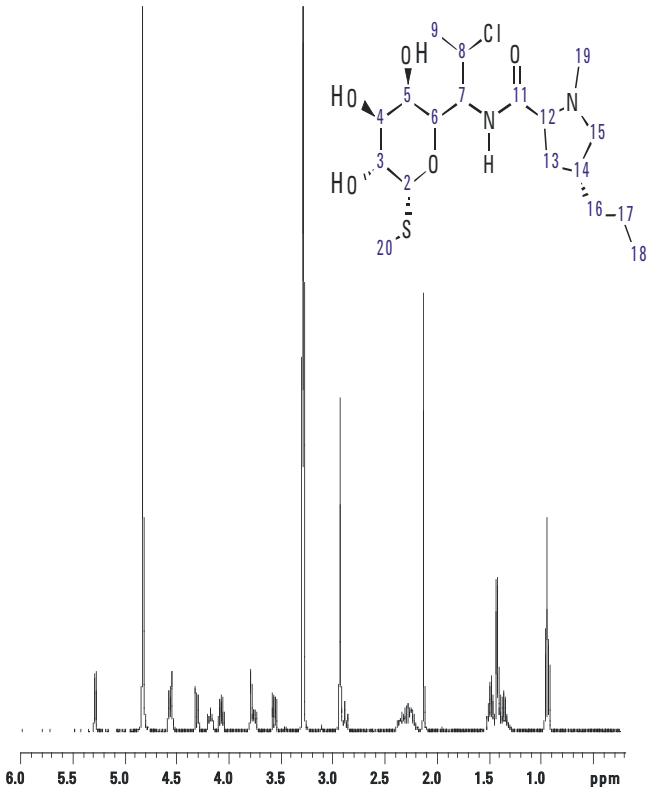

Figure 3. Proton NMR spectrum of clindamycin. These data can be interpreted to provide information about the number of ^1H atoms in a given chemical environment and structural relationship between those atoms.

Clindamycin displays several interesting chemical motifs. The presence of the substituted pyrrolidine ring is revealed by the observation of an *m/z* 126 ion in the full scan mass spectrum of clindamycin. The NMR data also confirm this assignment and show the presence of a contiguous seven-spin system (for example, H12 through H18). The Heteronuclear Single Quantum Coherence (HSQC) data set allows identification of the two methine, four methylene, and one methyl resonances expected for this carbon chain, while the responses observed in the Correlated Spectroscopy (COSY) spectrum establish molecular connectivity between the heteronuclear spin pairs. The chemical shifts observed for H/C12 and the strongly anisochronous H/C15 pair are consistent with their attachment to the cyclic pyrrolidine nitrogen atom. Finally, the H/C19 methyl group yields a three-bond HMBC response to both C12 and C15, thereby completing this structural fragment.

The second major structural fragment in this molecule is the thiosaccharide ring with its pendant chloroethyl tail. The observation of fragment ions at *m/z* 389 and *m/z* 377 are indicative of the loss of HCl and $HSCH_3$, respectively. A fragment ion was observed at *m/z* 407, corresponding to the loss of water from the protonated molecule. This *m/z* 407 ion loses a second molecule of water to yield an *m/z* 389 ion that is distinct from the previously observed facile loss of neutral HCl from the $[M+H]^+$ ion. The process by which these ions are generated can be differentiated by independent observation of the fragmentation pathway for the ^{35}Cl and ^{37}Cl isotopes of the parent compound. In a similar fashion, a fragment ion is observed at *m/z* 335, consistent with the neutral loss of 2-(methylsulfanyl) ethanol from the thiosaccharide ring (Figure 4).

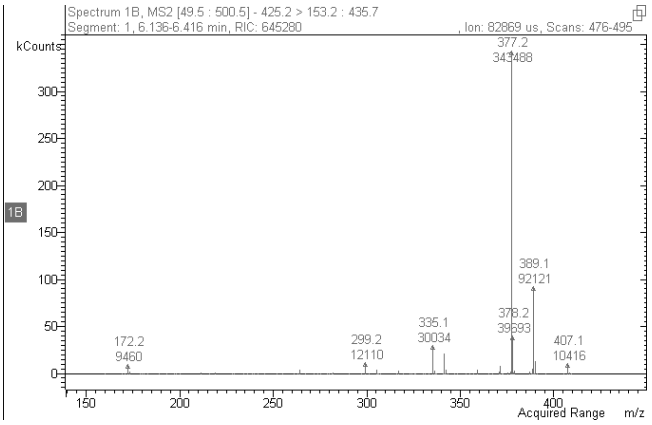

Figure 4. MS/MS spectrum of clindamycin. Observation of fragment ions such as m/z 335 provide structurally significant information about the atomic structure of the precursor ions.

The ^1H NMR spectrum of clindamycin displays the cluster of methine resonances between 3.5 and 5.3 ppm expected for a sugar moiety. The COSY and HSQC data sets, when used in conjunction, allow unambiguous assignment of the contiguous eight-carbon spin system beginning with H/C2 and extending through to the terminal H/C9 methyl resonance. The large 11.1 Hz coupling constant observed between H3 and H4 indicates the expected 1,2-*trans*-diaxial relationship of these two proton atoms. All the other coupling constants in the carbohydrate ring are more modest (for example, ~ 4.2-5.9 Hz), as predicted based on the stereochemistry of the molecule. Further confirmation of the relative stereochemistry in the carbohydrate ring is provided by the 500 ms 2-D Nuclear Overhauser Enhancement Spectroscopy (NOESY) experiment. As shown in Figure 5, H4 and H6 display reciprocal dipolar relaxation as predicted based on their 1,3-*cis*-diaxial relationship.

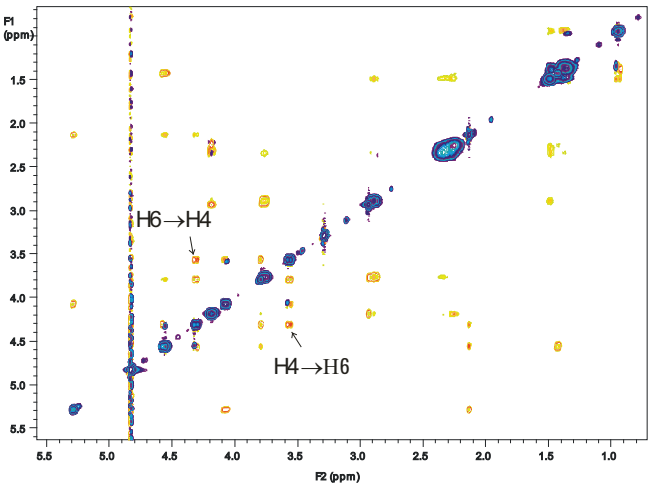

Figure 5. Two-dimensional NOESY spectrum of clindamycin. Dipolar relaxation data such as these provide information about the spacial relationships between proton atoms. The H4 to H6 1,3-cis-diaxial interaction is labeled.

Finally, connectivity between the two halves of the molecule is provided by responses in the Heteronuclear Multiple Bond Correlation (HMBC) data set. The H7 and H13 resonances are both observed to respond with a carbon resonance at 167.9 ppm. This is the chemical shift expected for the only quaternary carbon found in clindamycin, the C11 carbonyl atom.

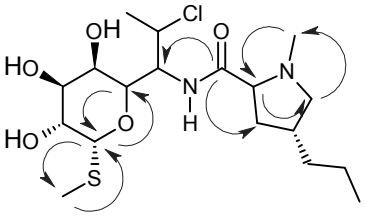

Figure 6. Structurally significant three-bond HMBC responses (H → C) observed for clindamycin.

Conclusion

When used in an interdisciplinary fashion, modern spectroscopic techniques provide extremely powerful tools for the investigation of chemical structures. The integration of NMR and MS data interpretation allows for the rapid confirmation of chemical structure with very high confidence in the result. As shown here using clindamycin as a example, the structural data required to document a characterization report were acquired in an automated fashion using an Agilent 400-MR NMR spectrometer and Agilent 500 Ion Trap LC/MS.

For More Information

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