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# Wiley's KnowItAll software as a spectral interpretation tool

A designer drug case study

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*Interpretation of unknown spectra using Wiley's KnowItAll software and spectral libraries has many real-world applications. An example from forensics is demonstrated by this case study featuring a hunt for an unknown and potentially dangerous compound.*

The **KnowItAll Software** is Wiley Science Solution's software package for identifying, analyzing and interpreting spectra, whether they be infrared, mass spectrometry, near infrared, NMR, Raman or UV-Vis. Combined with a subscription to the KnowItAll Spectral Libraries, it provides a powerful and easy-to-use interface that can help to solve real-world problems of chemical identification. One such problem was presented to Dr James de Haseth, Emeritus Professor of Chemistry at the University of Georgia in the U.S., in the form of an infrared spectrum of an unknown compound, of which identification was urgent.

### **A suspicious compound from Las Vegas**

Dr de Haseth was teaching a course on the interpretation of infrared and Raman spectra, when he was approached by a group of scientists from the U.S. Drug Enforcement Administration (DEA)'s Chicago Laboratory. They were puzzled by the spectrum of an unknown compound which had been sent to them by the police force in Las Vegas, Nevada.

"They said, 'we've got a spectrum,'" de Haseth recounts. "We don't have any idea what it is. It seems to be a completely unknown compound, but it's very important for us to know what it is.' They didn't give me the full story on it right away, the information was on a need-to-know basis."

Whatever it was had been found in very large quantities and was deemed suspicious by the police. The spectrum was of a pure sample and it was the only lead they had to go on. They had already searched for a matching spectrum and come up short. Mass spectrometry had also been conducted but had not provided any additional information.

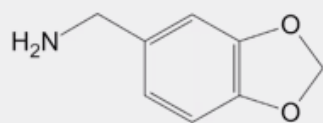
"The fact that the DEA could not determine it by searching is not surprising," de Haseth says. "Looking at KnowItAll today, I believe there are about 300,000 infrared spectra I can search, but the Chemical Abstracts Registry of what I estimate to be compounds with unique vibrational spectra is well in excess of 100 million." In other words, many more chemical compounds than confirmed infrared spectra exist.



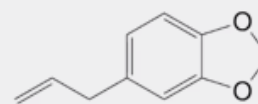
As a first step, de Haseth ran several searches using the Minelt function across the KnowItAll spectral library, including a First Derivative search that mitigates some potential baseline effects and weights peak positions over peak intensities. However, none of the searches produced particularly high Hit Quality Indices (HQI), a value out of 100 that describes the correlation between the sample spectrum and the spectra of the known compounds in the library. All HQIs were between the low sixties and the high seventies.

But one commonality among the hits stood out. Multiple of the highest hit compounds in the searches had a methylenedioxy functional group, two oxygens connected by a methylene bridge, often in a ring substructure, such as the 5-member dioxolanes in the common structures.

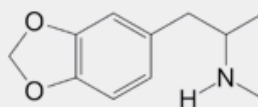
### Common Structures



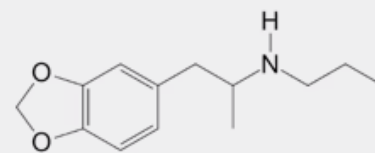
3,4-(Methylenedioxy)benzylamine



Safrole



DL-3,4-Methylenedioxymethamphetamine



3,4-Methylenedioxypropylamphetamine

Figure 1: Suggested image to show functional groups

“Now, we could have immediately turned around and said, ‘it has to be a methylenedioxyphenyl compound,’” de Haseth explains. “But it was also possible that it was something not in the database, and that these methylenedioxyphenyl compounds just happened to be the closest structures. We have to be really careful about jumping to conclusions. It was a good start, but I didn’t want to hang my hat on it.”

## A heat map reveals differences

When de Haseth overlaid the sample spectrum with the library spectra, he found that many peaks did not match, despite the same patterns cropping up again and again: methylenedioxy and amphetamine structures.

He knew that the sample was not one of the reference compounds, which meant he needed to explore the differences as much as the similarities. To do so, he selected the spectra of the compounds containing the 1,3,4-trisubstituted methylenedioxyphenyl substructure and overlaid them with the sample spectra. Then he used the heat map feature in the KnowItAll software to map out the peaks common to all the spectra so that both the differences and the commonalities became apparent.



Figure 2: Suggested image to show heat map function

“Many of the peaks correlated,” he says, “so the question I had to ask myself right away was, ‘are those peaks unique to the methylenedioxy group, or are they common peaks that would occur in many other compounds?’ The answer is the latter.”

Additionally, there were significant differences between the sample and the library spectra, particularly in the 1700-1500  $\text{cm}^{-1}$  region. Having reached the limit of what the library spectra could tell him, de Haseth turned to classic spectral interpretation. The KnowItAll software has several features to make this task as easy as possible.





## Spectral visualisation using KnowItAll

“When I interpret, I don’t look at the absorbance spectrum,” de Haseth says. “I’ve learned that the best way to look at a spectrum is to change it to percent transmission.”

The peak intensities of an absorbance spectrum are linear with concentration, whereas in a percent transmission spectrum, they are exponential. This makes smaller bands much easier to see. de Haseth also split the wavenumber scale at  $2000\text{ cm}^{-1}$  and compressed the relatively sparse  $4000\text{--}2000\text{ cm}^{-1}$  region by a factor of two, so that the important spectral features below  $2000\text{ cm}^{-1}$  could be expanded and viewed in greater detail.


“The first thing I noticed,” he continues, “was that the bands all tended to be quite sharp and narrow.” The bandwidth of spectral peaks depend on molecular structure, and needle-like peaks are indicative of constrained or rigid molecules, such as aromatics, cage molecules or other type of ring structures. “We didn’t have a lot of internal rotations in the molecule. It was constrained.”

### Peak-by-peak spectral interpretation

When interpreting spectra, it’s usual to start at the higher wavenumbers and work downwards, verifying and ticking off functional groups along the way. In this case, de Haseth suspected that the unknown sample contained an aromatic ring with a methylenedioxy functional group attached, in its simplest form a 1,2-methylenedioxybenzene. This hypothesis would need to be confirmed through peak-by-peak interpretation.

The first bands were at  $3360$  and  $3180\text{ cm}^{-1}$ , indicative of a primary amine or amide. In particular, the  $3180\text{ cm}^{-1}$  peak fit with the N-H symmetric stretch of a primary, hydrogen-bonded amide. To confirm this, de Haseth looked for bands in the carbonyl region which would be consistent with such a feature, finding two relevant bands at  $1652$  and  $1630\text{ cm}^{-1}$ .

“So, we found the carbonyl stretch and the N-H scissoring mode where the two hydrogens are just bending in towards each other,” he explains. “That’s very characteristic.” Using the Analyzelt IR function in the KnowItAll software, which lists many possible functional groups at a peak and compares peak regions, he was able to directly compare the position of the relevant sample peaks to those expected of a primary amide. He saw an acceptable overlap. The primary amide was confirmed.



The next peaks were less obvious and had to be approached more carefully. A series of bands suggested a carbon chain with a  $\text{CH}_2$  and a  $\text{CH}_3$ , and there were aromatic peaks at 1608, 1500 and  $1485\text{ cm}^{-1}$ .

"It's always a confused region," de Haseth says, "but when I looked through everything, I knew I had a primary amide and an aromatic group, and I strongly suspected a methyl group." The methylenedioxy group, however, remained unconfirmed.

### **Pinning down the methylenedioxy group**

In order to know for sure whether he was dealing with a methylenedioxy group, de Haseth had to look closer at the aromatic ring. If a methylenedioxy group featured in the unknown molecule, it would probably be attached to the ring, as it had been in the highest hit library compounds. So he started to look for peaks that would suggest a substituted benzene ring, which could accommodate both the methylenedioxy group and the carbon chain he had found in the remaining part of the spectrum.

The unknown sample spectrum matched only with the expected peaks of one substituted ring: the 1,3,4-trisubstituted benzene ring. This was strong evidence for the methylenedioxyphenyl group because all the library compounds which contained it had this exact structure.

To confirm the presence of the functional group, de Haseth checked for a specific pattern. "It's a mixed ether," he says, "aromatic on one side and aliphatic on the other." Such a group would produce peaks at  $1250 \pm 20$  and  $1100 \pm 50\text{ cm}^{-1}$ . "I went back to the heat map spectrum, and indeed, both of those bands were there."

But the  $\text{CH}_2$  in the methylenedioxyphenyl could not account for the previously spotted aliphatic band, due to its orientation with respect to the ether oxygens. "This methylene was very unusual," de Haseth says. "At  $2783\text{ cm}^{-1}$ , there was a tiny band and there's a rather special structure associated with this."

The oxygens in the methylenedioxy group each had two lone pairs in non-bonding orbitals, pointing above and below the plane of the molecule. This meant that, for each methylene C-H stretch in the functional group which were also pointing out of plane, there were two lone pairs in the trans diaxial position, i.e. pointing in the opposite direction. That changed the electron distribution around



the C-H stretch, causing its vibrational frequency to drop to a lower wavenumber – the  $2783\text{ cm}^{-1}$  peak. In other words, despite confirmation of the methylenedioxyphenyl group, there was an extra  $\text{CH}_2$  band still to assign.

### 'We're in trouble'

The intensities of the methyl and additional methylene bands were weak, indicating a low number of those groups. This allowed de Haseth to settle on two, subtly different structures that would fit the data. The structures differed only in the position of the methyl and methylene groups in relation to the rest of the structure. The  $\text{CH}_2$  was either attached next to the phenyl ring or next to the amide.

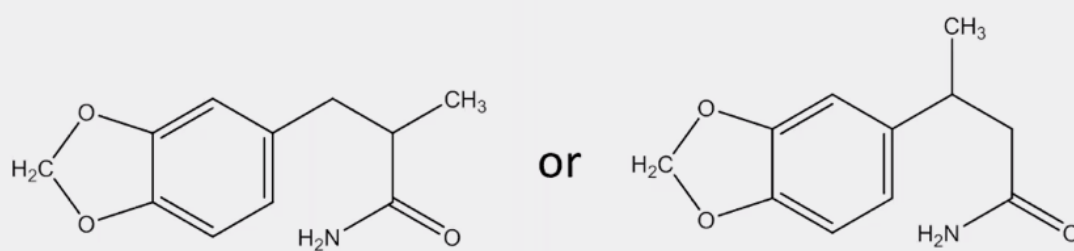


Figure 3: Suggested image to show two potential structures

Seeing as there was no information in the spectrum to distinguish between the two structures, de Haseth presented them both at the end of the course to the clients from the DEA. "It took me about eight hours to get to this point," de Haseth says. "It wasn't trivial."

One of the scientists, an organic chemist, immediately realized the second structure could quickly be converted into methamphetamine with household chemicals at a very high yield, with no toxic solvents. "At which point he said, 'we're in a lot of trouble,'" de Haseth recalls. This had to be the structure of the chemical confiscated in Las Vegas, the chemist argued. The molecule is alpha-methyl-3,4-dioxymethylphenylpropionamide [MMDPPA].

Methamphetamine, also known as crystal meth, is a potentially dangerous and highly addictive drug. Once MMDPPA was suggested as the potential identity of the compound, the DEA took swift action. Complete synthesis of the molecule was carried out and the compound was verified. They also tested its conversion to methamphetamine to make sure they knew exactly what they were dealing with. "It was immediately classified as a restricted compound, as a List 1 precursor," de Haseth says.



## From mystery sample to known precursor

Identification of compounds such as designer drugs and precursors that might otherwise fly under the radar, takes skill and robust chemical knowledge. Spectral interpretation requires complex pattern recognition and high-level problem solving on top of simply knowing which vibrational transitions produce what bands. But with technology that plays to the strength of the user, the task can be made easier.

“KnowItAll was very helpful,” de Haseth says. “I could use different searches, Analyzelt IR, heat maps and so on. It gave me a partial structure. But I needed a good understanding of how to interpret infrared and Raman spectra – well, I wish I’d had a Raman spectrum, too.”

This is a point that should be highlighted: the more evidence there is from different sources, the greater the certainty in the final assignment. KnowItAll is specifically developed to enable simultaneous analysis of all different kinds of spectra, be they infrared, Raman, near infrared, NMR or UV-Vis. This way, users avoid having to use multiple software packages — different spectral evidence can be interrogated and compared straight away.

Prof de Haseth’s identification of MMPDA as the mystery compound shows that spectral interpretation is of concrete use across a range of fields, not least in forensics, in which compound identification can have high stakes. For chemists in these and other areas, Wiley Science Solution’s KnowItAll software package and spectral libraries can be a critical tool.