

Unknown Identification Using KnowItAll™ Adaptive MS and IR Search “Real World Example”

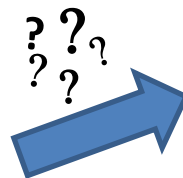
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White Powder



WILEY

KnowItAll™ Attributes

Vendor-neutral integrated solution to identify unknowns

- **Techniques:** IR, Raman, MS, NMR, and UV
- **Supported File Formats:** >170
- **Structure Drawing Program:** ChemWindows
- **Large Number of Reference Spectra:**

264K IR

25K Raman

1.25M EI Mass Spec

894K NMR

30K UV

~2.5 M total!

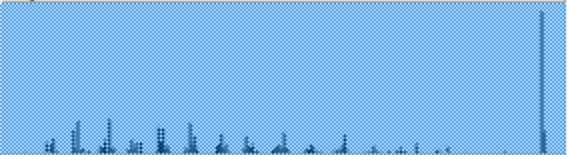
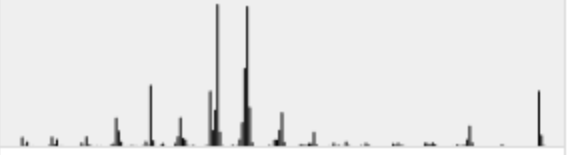
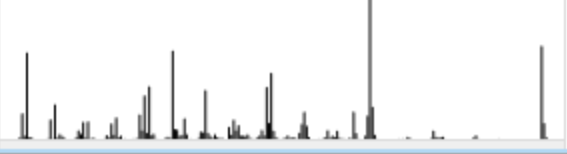
- **Ability to Create User Libraries!**

Standard Identity MS Search Fails

- Standard identify MS search
- Yields no useful results by visual comparison
- Hit Quality Index (HQI) very low
- Usually >70 to be useful

MS (GC)

Table Plot Related Compounds View

	HQI ▼	R.HQI ↗	Tag ↗	DE ↗	ID ↗	Name ↗	Spectrum <auto> (MS (GC)) ↗
1	53.89	60.06		MSX	133153	4-(Adamantan-1-yl)-3-nitro-1,2-oxazole	
2	53.44	54.19		MSX	133806	1,5-Diphenylbicyclo[3.2.0]heptane	
3	53.34	55.52		MSX	133423	Acetic acid, 1-cyclohexenyl-1-(1-cyclopenten-3-one-1-	

KnowItAll Adaptive Search (*Patented*)

- Finds similar compounds where a molecular fragment is *absent* or *present* in the reference spectrum compared to the unknown
- Presence or absence designated by its Δ_{mass} , difference of nominal molecular weight of known vs. reference
- Peaks in the unknown spectrum compared to the reference spectrum *shifted* by Δ_{mass}
- The “hybrid” hit quality index value reflects *both* the shifted and unshifted peaks

Results: Adaptive search *routinely* yields *useful results* not found with the more traditional identity search if the component is *not present* in the reference libraries

Thus: Greatly extends the *dynamic range* of *all* reference and *even* user libraries

KnowItAll Adaptive Search Yields Very Useful Results


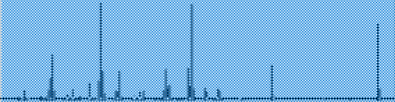





- Top 3 hits very useful
- Hit Quality Index (HQI) now >70
- Δm [μ] field shows delta mass associated with result
- Replacement field gives suggestion for fragment added or lost
- “Left clicking” on “i” icon, , shows how reference library spectrum shifted to calculate HQI versus unknown spectrum

Table		Plot		Related Compounds View							
	HQI	R.HQI	Tag	DF	ID	Name	Spectrum	< auto> (MS (GC))	Δm [u]	Δm Info	Replacement
1	81.13	83.03		MSX	114140	1-propionyl-4-anilinopiperidine			16		In unknown, Methoxy group replaces Methyl group in reference. In unknown, Phenol replaces
2	73.96	82.69		MSX	51415	N-Phenyl-4-piperidinamine			72		In unknown, Methoxycarbonylmethyl group replaces Hydrogen in reference.
3	73.50	75.14		MSX	127948	1-Ethoxycarbonyl-4-piperidinamine, N-			4		In unknown, Fluorine replaces Methyl in reference. In unknown, O group

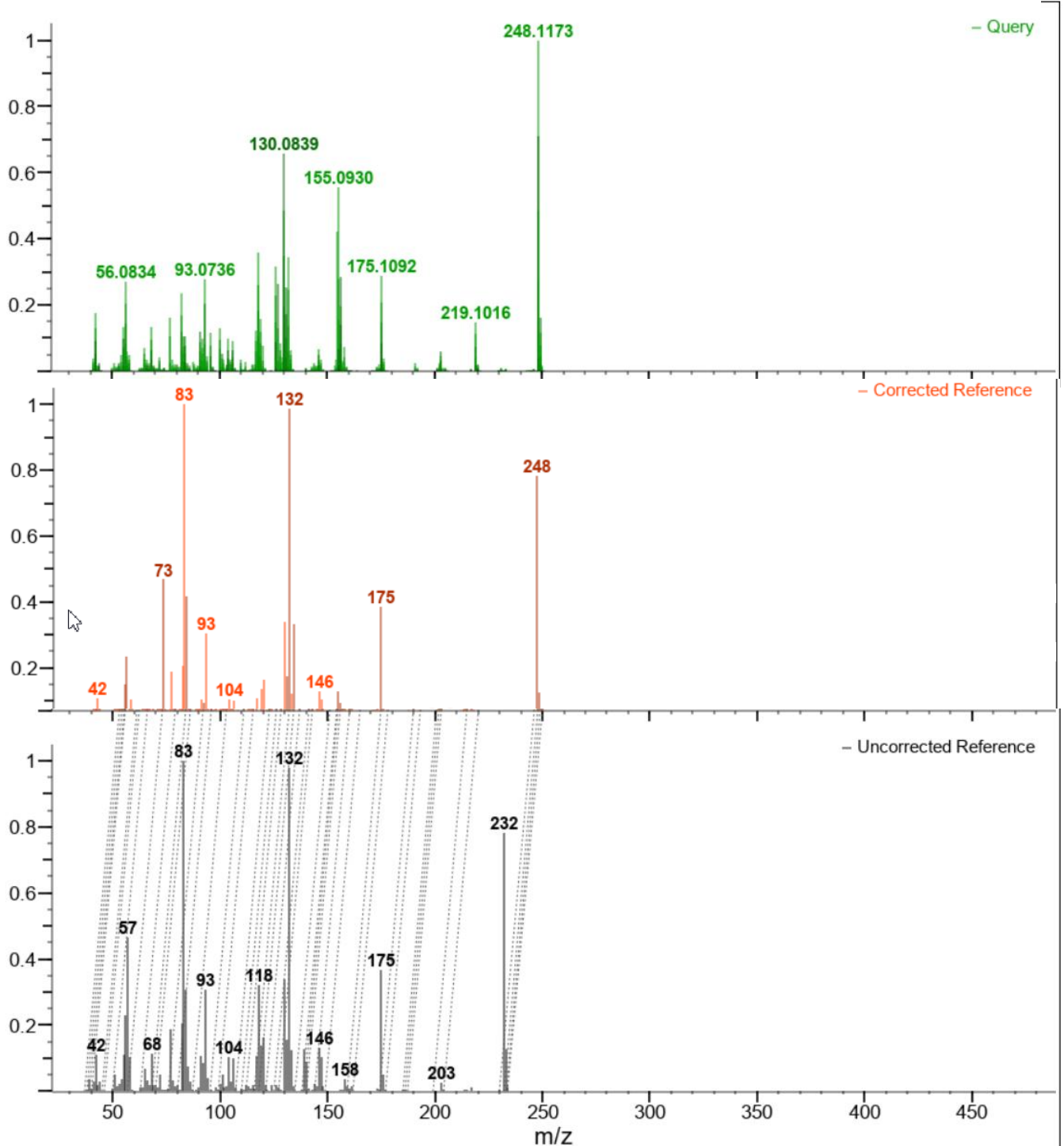
Shifted Reference Versus Unknown for Hit Quality Index Calculations

Adaptive Corrections

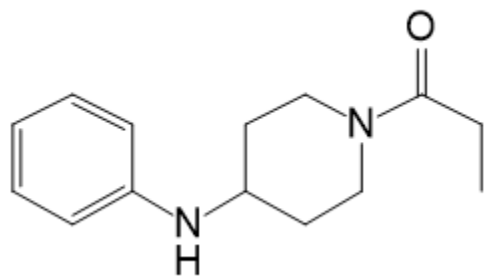
The following correction was applied to the reference spectrum to get optimal results:

Some peaks in the reference spectrum were offset horizontally by 16 u

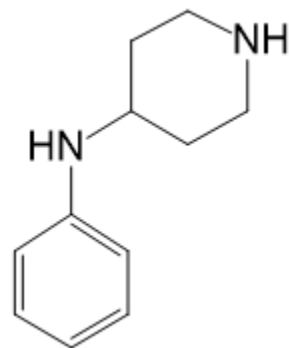
The top spectrum is the unknown spectrum, the bottom spectrum is the unshifted reference spectrum, and the middle one is the shifted spectrum. In the shifted spectrum, the darker red peaks are additions of unshifted peaks and shifted peaks. Dotted lines between shifted and reference spectrum link shifted peaks to their original positions.



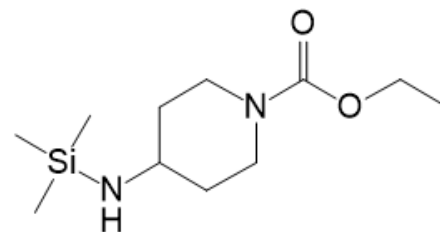
Top 3 Hits in Adaptive EI Mass Spec Search Unknown White Powder



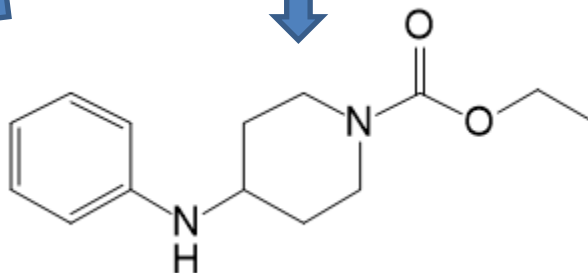
MW 232
 Δm 16
+ "oxygen"



MW 176
 Δm 72
+ "CO₂CH₂CH₃"



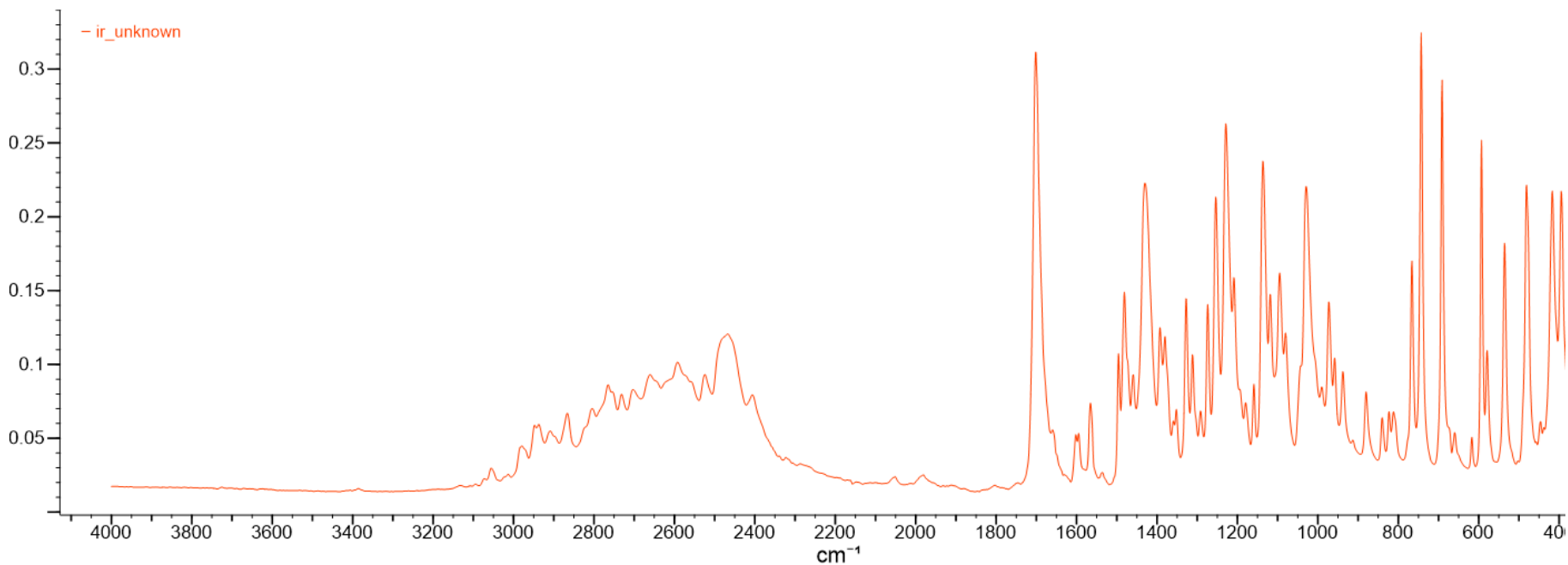
MW 244
 Δm 4
- "Si(CH₃)₃" + "phenyl"



Proposed Structure
MW 248

IR Analysis of Solder Using KnowItAll

- IR of Unknown white powder
- Broad band from 2300-2900 was somewhat unusual
- Possibly a mixture
- Used mixture capabilities of KnowItAll IR search



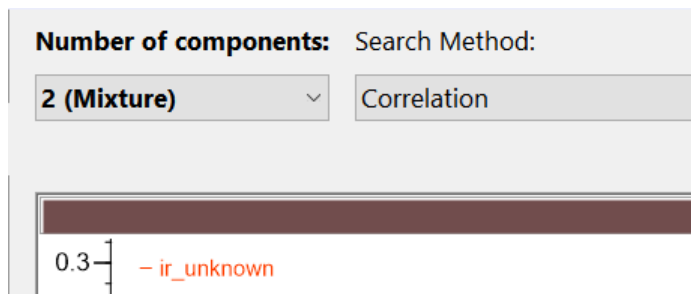
Results of IR Search Using “Mixture 2” Option

- IR search results with “Mixture 2” option

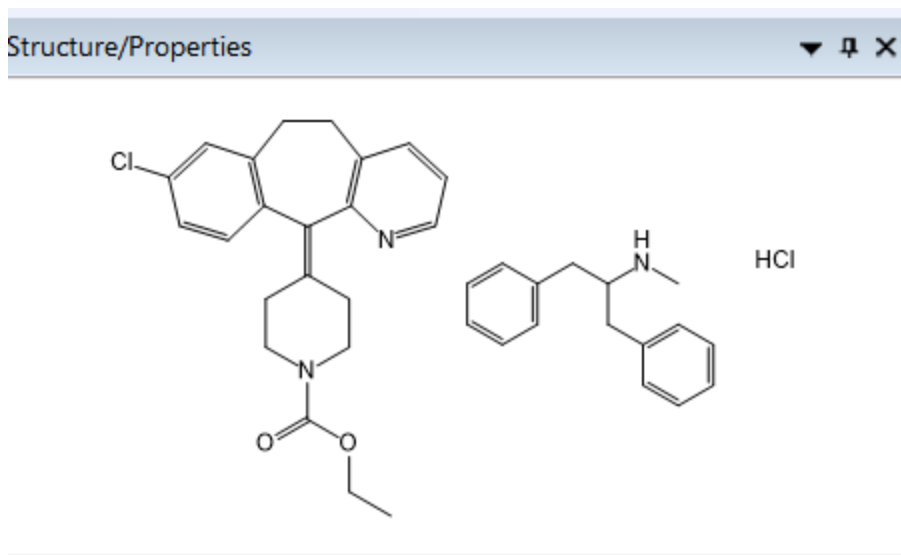
Number of components: Search Method:

2 (Mixture) Correlation

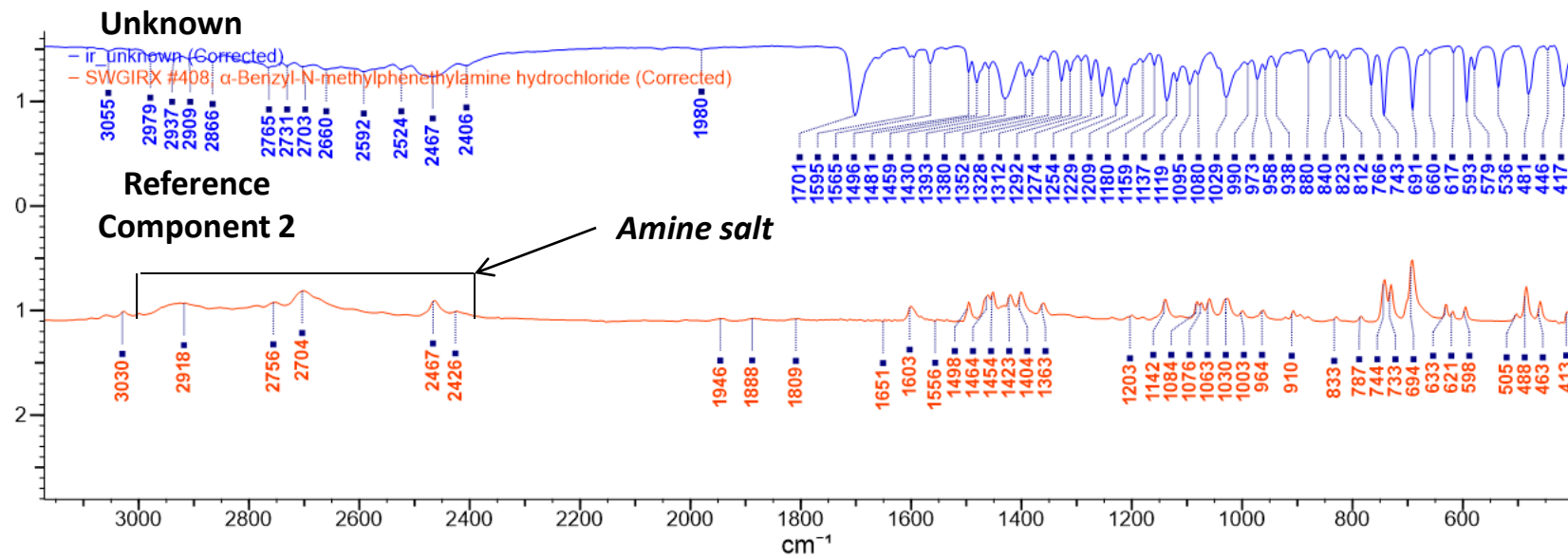
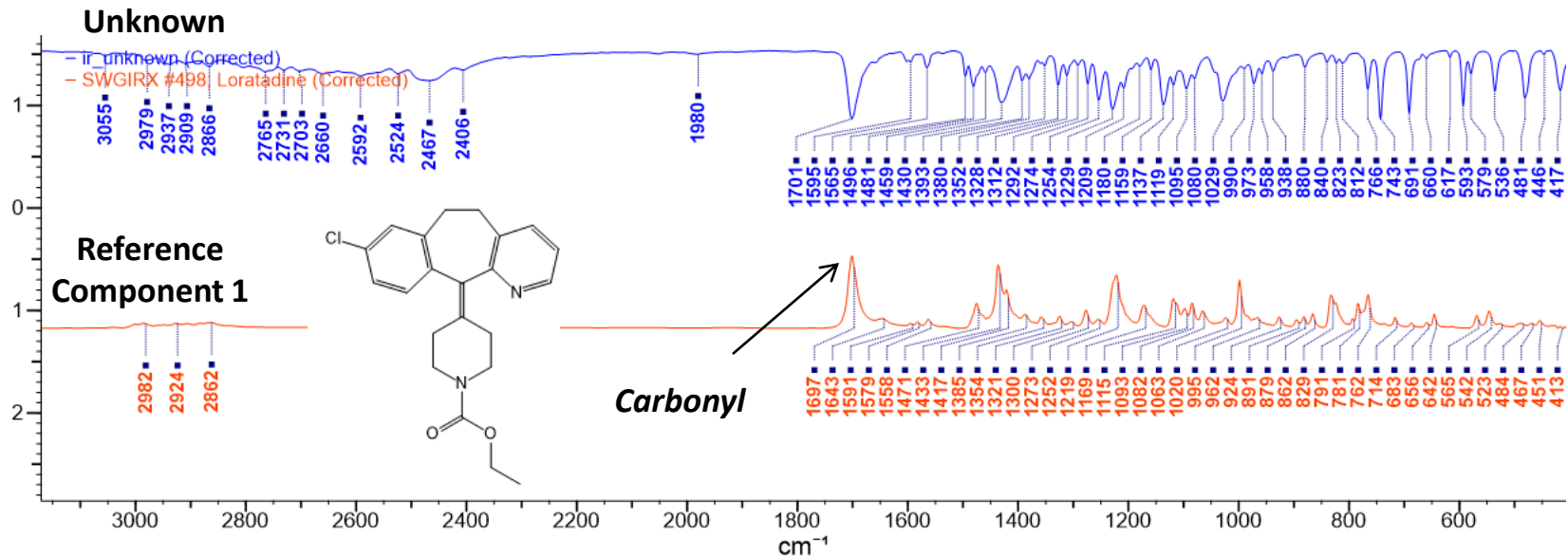
0.3 - ir_unknown



- Top hit indicates mixture of a carbamate and a hydrochloride salt

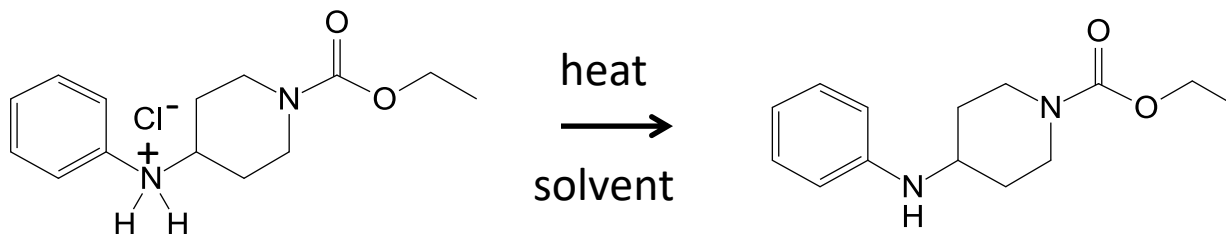


Comparing IR Spectra of Unknown to Components 1 and 2 in Mixture Result



Conclusions

- Unknown white powder tentatively identified as a fentanyl-related species
- Likely Present as hydrochloride in solid, and free base on GC column
- Confirmed at later date by reference spectra
- Adaptive search critical in the identification process for components *not found* in reference EI libraries
- Demonstrates *power* of *multi-spectral* capabilities of KnowItAll in unknown characterization



IR
HCl Salt

EI GC-MS
Free Base

Contributors

- Stewart Millen, IR Expert, Eastman Chemical Company
- Michelle D'Souza, KnowItAll Product Manager, Wiley
- Steve Stein *et al.*, NIST, EI MS Hybrid Search*

*"Combining Fragment-Ion and Neutral-Loss Matching during Mass Spectral Library Searching: A New General Purpose Algorithm Applicable to Illicit Drug Identification," A. Moorthy, W. Wallace, A. J. Kearsley, D. Tchekhovskoi, and S. Stein, *Analytical Chemistry* **2017** 89 (24), 13261-13268.