Targeted and untargeted analysis of an LC-MS drug mixture

Introduction

The detection and identification of drugs is an analytical problem with diverse applications. For example, in forensic science, one may use it identify illicit drugs aiding in law enforcement and public safety. In sports, it ensures fair and safe competitions by detecting doping substances. Clinically, a crucial role for improving patient care is accurately identifying therapeutic drugs and monitoring their metabolites.

Drug mixtures present a particular challenge, especially in complicated matrices. Liquid chromatography-mass spectrometry (LC-MS) is ideally suited to analyzing mixtures due to its ability to efficiently separate compounds and the specificity of the detection technique. Furthermore, the analyses can be either targeted or untargeted.

Wiley's KnowItAll[1] provides an integrated solution to identify, analyze, and manage analytical data. It supports multiple techniques and vendor formats, including LC-MS, with LC Expert for automated untargeted analysis. Users can also create or import a structure database for targeted analysis in the MineIt application.

In this application note, an LC-MS drug mixture sample was analyzed using first a targeted workflow with a user-created structure database. Next, it was searched against a tandem MS library of drugs and poisons to demonstrate the ease of KnowItAll's LC-MS workflow and the integration of both targeted and untargeted search results. The complementary nature of targeted and untargeted searches provides greater confidence of having identified the correct drug.

Results and Discussion

The data were analyzed using Wiley's KnowItAll Analytical Edition (Version 2025). Using knowledge of the drugs in the mixture, a target structure database was created with the **MineIt** application. For each drug, the name and structure were entered sequentially. This was done using integration with ChemWindow to enter the structure as a SMILES string. The resulting eight compounds formed the target structure database. (Figure 1).

Taking advantage of the multi-vendor nature of KnowItAll, a raw LC-MS data file was opened in the LC Expert* application. This was a Thermo Q Exactive Orbitrap .RAW file acquired in data-dependent acquisition (DDA) mode.

LC Expert automatically deconvoluted the LC-MS data and displayed it as Extracted Ion Chromatogram (EIC) peaks in the LC panel. LC Expert performed a targeted search against the data file and found all eight compounds. The results of the accurate mass search were displayed in the Peak table (Figure 2).

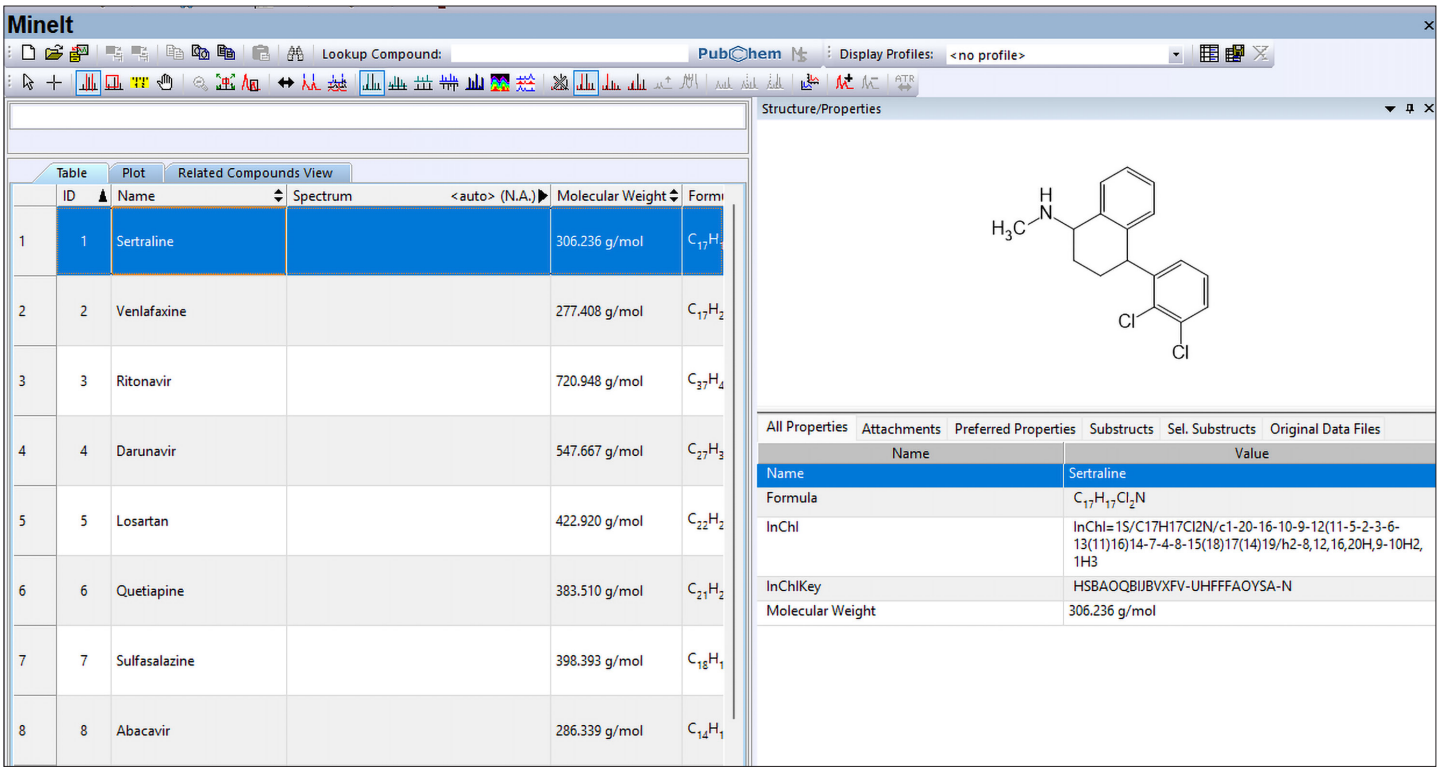

Figure 1. *The complete drug mixture target structure database in MineIt.*

Peaks

	RT [min]	Peak Area	Peak Area [%]	Peak Height	FWHM [	Base Ion [m/z	Annotated Name	Molecular Formula	Match Score	Mass Accuracy [ppm
12	3.4477	19752513	2.70	1426710945.5	-3.5198	287.1611	Abacavir	$C_{14}H_{18}N_6O$	81.18	-1.34
13	5.5588	2604851	0.36	818136839.5	0.0069	278.2103	Venlafaxine	$C_{17}H_{27}NO_2$	97.94	-4.04
14	5.9388	147686	0.02	15464412.7	0.0878					
15	6.0598	38518463	5.27	2138906469.3	0.0323	384.1739	Quetiapine	$C_{21}H_{25}N_3O_2S$	70.64	-0.34
16	6.9092	3386770	0.46	130448939.6	0.0447					
17	7.7026	20747572	2.84	267944370.6	0.0890	399.0737	Sulfasalazine	$C_{18}H_{14}N_4O_5S$	74.37	-5.29
18	7.9662	285812429	39.14	4470222186.1	0.0420	306.0798	Sertraline	$C_{17}H_{17}Cl_2N$	74.76	-4.34

Figure 2. *The* ***Peaks*** *table displays the results of an accurate mass search against the target structure database. It lists each component with its characteristic information such as retention time (RT), Peak Area, and Peak Height, as well as Annotated Name from the search.*

It is also possible to perform an untargeted search against a commercially available drug database. Then the targeted and untargeted results can be compared for additional confidence in the results. In this example, the sample file was then searched against the *Maurer/Meyer/Helfer/Weber LC-HR-MS/MS Library of Drugs, Poisons, and Their Metabolites, 2nd Edition*[2] database containing over 5,500 spectra from over 2,700 compounds (Figure 3).*

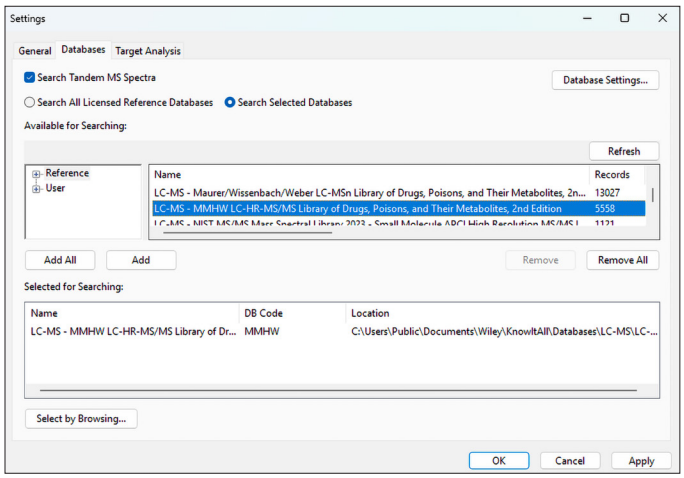

Figure 3. *In this untargeted analysis, the Maurer/Meyer/Helfer/Weber LC-HR-MS/MS Library of Drugs, Poisons, and Their Metabolites, 2nd Edition database was selected as the reference database for searching.*

The **Database Search Bar** displays the search progress and spectral matches with their scores (Figure 4). The **Database Match** panel displays the raw spectrum against the best match from the database including the Database Abbreviation and its Record ID as well as a structure, if available (Figure 5). Because a targeted search was performed earlier, corresponding accurate mass hits for Abacavir and Venlafaxine are visible at the same retention times in the **Peaks** table (Figure 6).

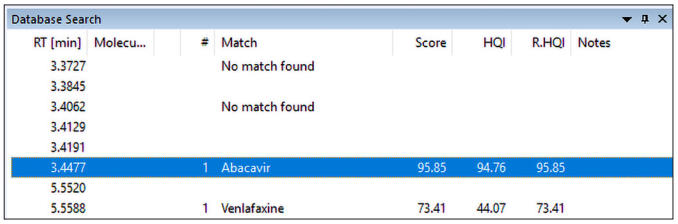

Figure 4. *The **Database Search Bar** displays untargeted matches with their scores.*

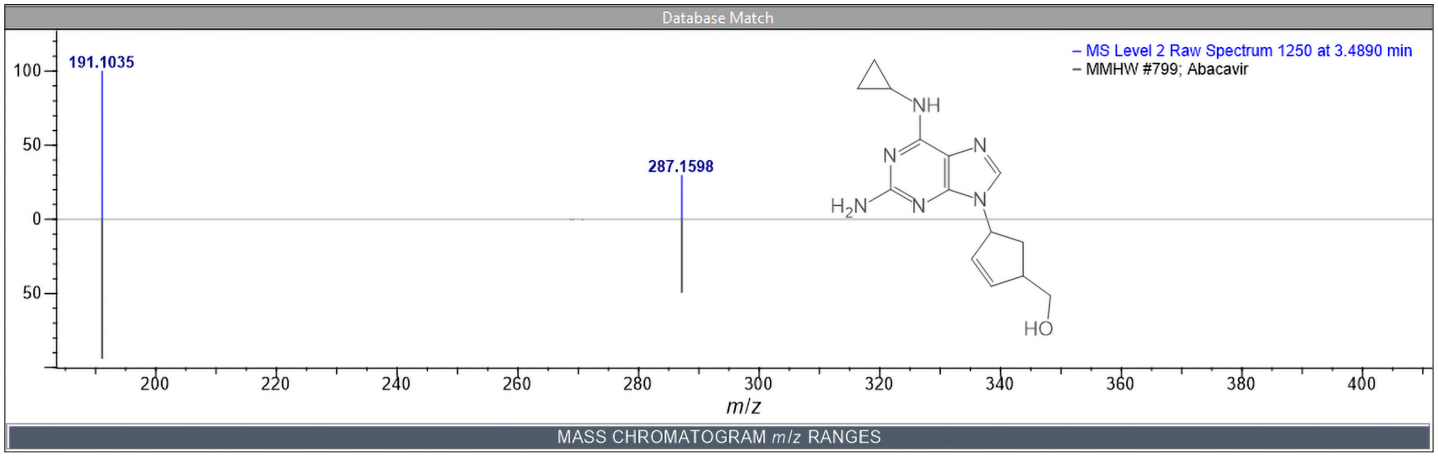

Figure 5. *The **Database Match** panel displays the matched spectra and a structure for the hit.*

Peaks

	RT [min]	Peak Area	Peak Area [%]	Peak Height	FWHM [	Base Ion [m/z	Annotated Na	Molecular Formula	Match Score	Mass Accuracy [ppm
12	3.4477	19752513	2.45	1426710945.5	-3.5198	287.1611	Abacavir	$C_{14}H_{18}N_6O$	96.55	-1.34
13	5.5588	2604851	0.32	818136839.5	0.0069	278.2103	Venlafaxine	$C_{17}H_{27}NO_2$	99.34	-4.04

Figure 6. *The **Peaks** table has corresponding targeted hits at the same retention times as the untargeted matches.* The results for the data file were saved as an LC Expert file, allowing quick access to the integrated targeted and untargeted search results.

Conclusion

The results demonstrate both the automated and streamlined workflow of Wiley's KnowItAll Analytical Edition (Version 2025), and that it can easily be applied to quickly perform both targeted and untargeted analyses. The drug mixture was deconvoluted into component peaks upon loading into the LC Expert application. It was then searched against a user structure database created in the MineIt application in minutes, and the *Maurer/Meyer/Helfer/Weber LC-HR-MS/MS Library of Drugs, Poisons, and Their Metabolites, 2nd Edition*. The combined targeted and untargeted search results give greater confidence in the identifications within the mixture.

References:

1. *KnowItAll Analytical Edition*, version 2025.1: John Wiley and Sons, Inc.: Hoboken, NJ, 2025; https://sciencesolutions.wiley.com/knowitall-analytical-edition-software/ (accessed 2025-03-19).
2. Maurer, H.; Meyer M.; Helfer, A. (2025); Weber, A. *LC-HR-MS/MS Library of Drugs*, Poisons and Their Metabolites, 2nd Edition: John Wiley and Sons, Inc.: Hoboken, NJ, 2025.

∗Subscription required to *LC Expert and to search the Maurer/Meyer/Helfer/Weber LC-HR-MS/MS Library of Drugs, Poisons, and Their Metabolites database.*