Comparison of untargeted database search results between different LC-MS vendor systems

Introduction

Analytical laboratories can face challenges when their lab is equipped with instrumentation from multiple vendors. Setting up streamlined workflows under these circumstances can be complex, complicating training procedures and standard operating procedures (SOPs), while necessitating the maintenance of different software packages and formats.

Wiley's KnowItAll software[1] addresses this issue with an integrated solution to identify, analyze, and manage analytical data across multiple techniques (GC-MS, LC-MS, IR, Raman, NMR, UV-Vis) and vendor formats. As instruments are added or changed in the laboratory, workflows can remain intact, allowing users to import, analyze, and store data consistently. The recent addition of the LC Expert application to KnowItAll's comprehensive suite of spectral analysis tools now enables the rapid processing of LC-MS samples in a vendor-neutral environment.

In this application note, we will demonstrate the flexibility of the KnowItAll environment using a sample analyzed by LC-MS systems from two different vendors. Despite distinct data file types, the overall workflow remained consistent, and non-targeted tandem MS database searches identified the same compound in each case. This study illustrates how LC Expert's automated workflow saves time and helps overcome the challenges posed by instrumentation from disparate vendors.

Experimental

Sample Analysis: The same sample was analyzed on two different LC-MS systems to demonstrate cross-vendor compatibility:

- Agilent 6550 LC/Q-TOF operated in data-dependent acquisition (DDA) mode
- Thermo Orbitrap Exploris 120 operated in Automated MS/MS mode

Software: Data analysis was performed using Wiley's KnowItAll Analytical Edition (Version 2025) with the LC Expert application.
Database: NIST MS/MS high-resolution libraries[2] were used for compound identification.

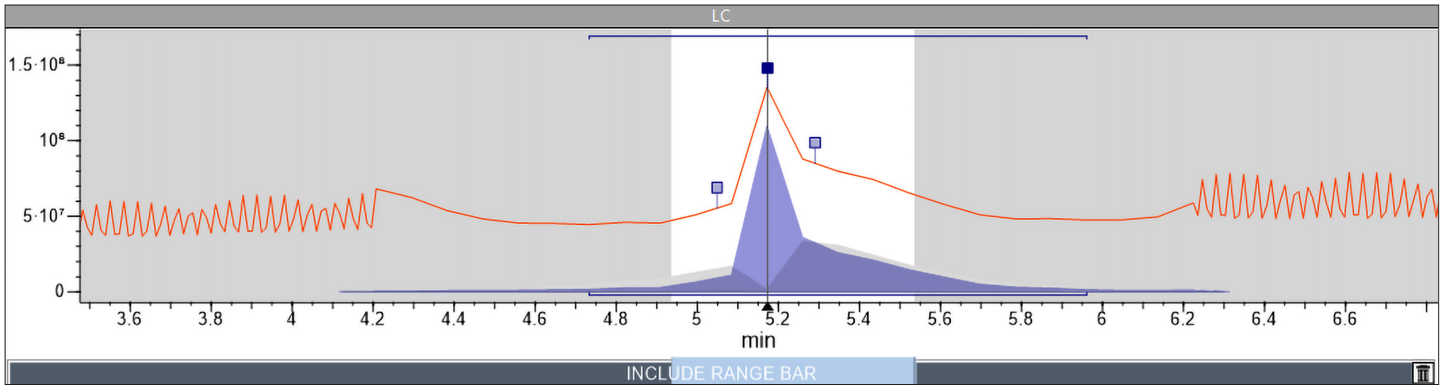
Results and Discussion

Agilent LC/Q-TOF Analysis

The raw LC-MS/MS data file from the Agilent 6550 LC/Q-TOF was opened in the KnowItAll LC Expert application. The LC-MS data was deconvoluted and displayed as Extracted Ion Chromatogram (EIC) peaks in the LC panel. The **Peaks** table listed each component with characteristic information including retention time (RT), peak area, and peak height.

The peak for the main component of interest was selected using the **Include Range Bar** underneath the LC panel (Figure 1). This peak was then searched against the NIST MS/MS high-resolution libraries.

Figure 1. *The selected peak region for analysis in the Agilent LC/Q-TOF chromatogram file.*



The **Database Search** display shows the spectral matches, along with their respective scores and MS1 centroid retention time (RT) (Figure 2). The **Database Match** panel displays the raw spectrum against the best match from the database: **2,4-Dihydroxybenzophenone**, including the MS2 scan's RT, the Database Abbreviation and its Record ID as well as a structure (Figure 3). In this case, the **Score** was calculated using 10% Hit Quality Index (HQI) and 90% Reverse HQI.

Database Search

RT [min]	#	Match	Score	HQI	R.HQI	Notes
5.0487	1	2,4-Dihydroxybenzophenone	97.54	88.82	98.51	
5.1733	1	2,4-Dihydroxybenzophenone	94.99	86.28	95.96	
5.2906	1	2,4-Dihydroxybenzophenone	94.60	78.10	96.43	

Figure 2. *The* **Database Search table** *lists matches in the Agilent LC/Q-TOF spectrum, along with their corresponding scores.*

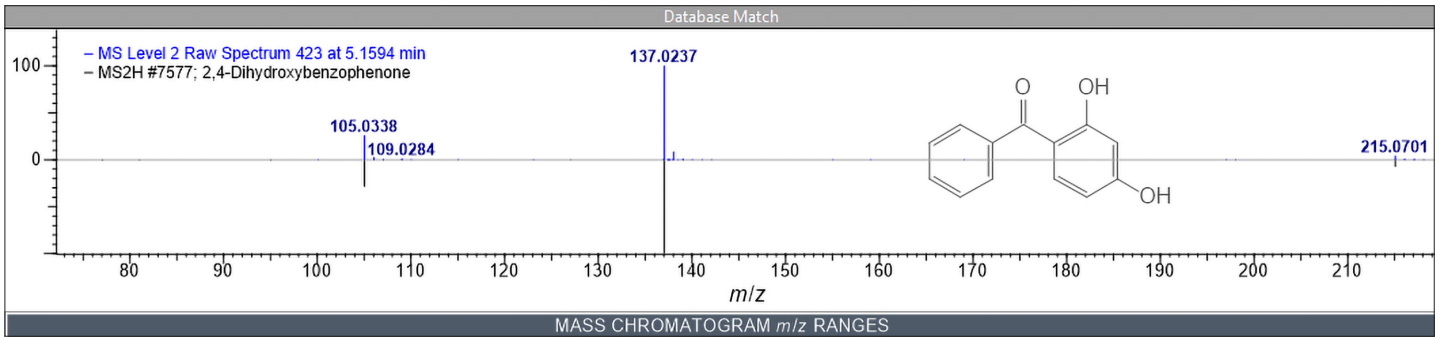


Figure 3. *The* ***Database Match*** *panel visually displays the matched spectrum and molecular structure.*

Thermo Orbitrap Analysis

To demonstrate KnowItAll's cross-vendor compatibility and workflow robustness, the same sample was analyzed using a Thermo Orbitrap Exploris 120 operated in Automated MS/MS mode. This analysis utilized different chromatographic conditions and acquisition parameters compared to the Agilent system.

The main component was selected and searched against the same NIST HRAM MS/MS libraries. The analysis yielded the same top hit (**2,4-Dihydroxybenzophenone**) with high confidence, though with a different retention time due to different chromatographic conditions employed (Figures 4 and 5).

Database Search

RT [min]	#	Match	Score	HQI	R.HQI	Notes
10.0190	1	2,4-Dihydroxybenzophenone	96.41	82.29	97.98	
10.0407	1	2,4-Dihydroxybenzophenone	95.95	81.32	97.57	
10.0586	1	2,4-Dihydroxybenzophenone	95.96	81.48	97.57	

Figure 4. *The* ***Database Search table*** *displays the Thermo Orbitrap matched spectra, showing the same identification result, but with different retention times compared to the Agilent analysis.*

utilizing instrumentation from multiple vendors and can be extended to include additional analytical techniques as workflow requirements expand. By implementing a unified analytical platform, laboratories can future proof their operations, ensuring that workflows remain consistent and training requirements minimized as new instruments are added or existing equipment is replaced with different vendor systems.

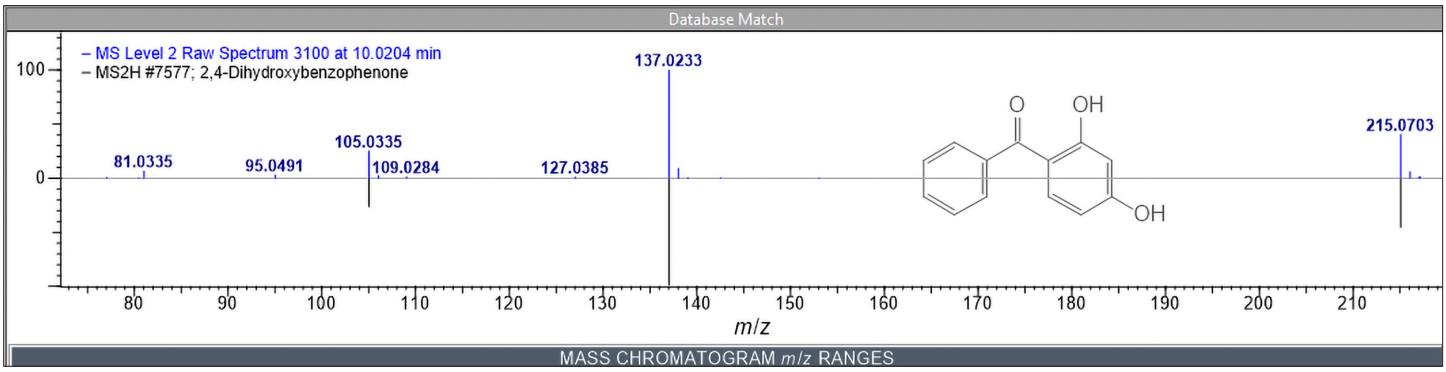


Figure 5. *The* ***Database Match*** *panel displays the Thermo Orbitrap spectrum matched against the NIST MS/MS library, confirming the same compound identification.*

The results for both data files were saved as an LC Expert file for future reference and comparison.

Conclusion

This study demonstrates both the automated and streamlined workflow capabilities of Wiley KnowItAll Analytical Edition and its effective application to data files from different LC-MS vendors. Despite very different data formats and chromatographic conditions between the two instruments, identical compound identification results were obtained for the same sample.

The vendor-neutral approach enabled seamless data processing where each file could be deconvoluted into component peaks and searched against the NIST MS/MS library using the same workflow. This capability provides a valuable solution for analytical laboratories seeking to increase productivity while

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. *National Institute of Standards and Technology (NIST)*. NIST / EPA / NIH Mass Spectral Library 2023: John Wiley and Sons, Inc.: Hoboken, NJ, 2023; https://sciencesolutions.wiley.com/solutions/technique/gc-ms/nist-epa-nih-mass-spectral-library/ (accessed 2025-03-19).

∗Subscription required to the NIST MS/MS Mass Spectral Libraries and LC Expert application.