[TECHNOLOGY BRIEF]

Using WinNonlin with UNIFI: Enhanced Export for the Metabolite Identification Application Solution

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GOAL

To demonstrate a simple workflow for the calculation of clearance PK values using WinNonlin from microsomal incubations acquired and processed using UNIFI® Scientific Information System.

BACKGROUND

Generating LC/MS data is an integral part of drug metabolism pharmacokinetic (DMPK) departments. How we convert this data to drive decisions is often the bottleneck as many different software tools are used in a typical DPMK lab. It is critical for LC/MS software to support easy transfer of results to a variety of third-party software packages in order to extract and provide key information to support drug discovery and development.

An example of this is to use LC/MS data to calculate pharmacokinetics parameters using industry-standard software packages such as Phoenix WinNonlin (Certara, St. Louis, MO, U.S.). Calculation of PK parameters are commonly performed to establish the viability of a drug candidate or series and help prioritization efforts. Electronic and customizable data transfer/integration between instrumentation software and third-party software platforms (WinNonlin, Spotfire, etc.) is the preferred approach and is essential for handling and processing large amounts of data with both accuracy and speed.

UNIFI supports completely customizable data transfer with a variety of third-party data packages. Enhanced export of tables supports communication with virtually any other calculation and statistical software package that support Microsoft Excel formats.

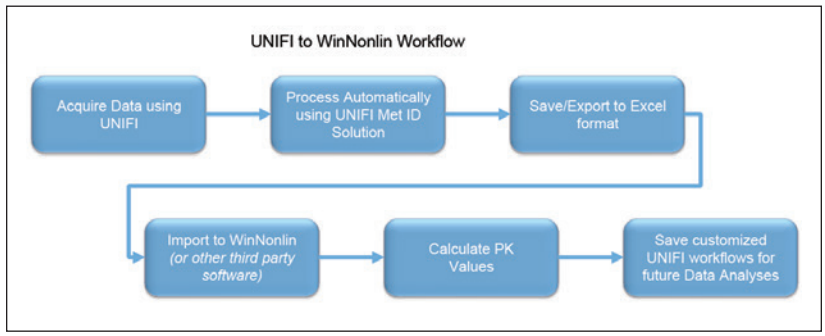


Figure 1. The workflow using UNIFI and WinNonlin software.

Enhanced export using UNIFI Software provides the following features:

- Direct export of tabular data (comprehensive Excel .xls support and limited support for open standard spectral .mzml formats).
- Full customization. Tables in UNIFI can be configured and saved to export exactly what the user needs. The export functionality can be tailored to include specific information, such as:
  - Specific columns and column order (such as response, response ratio, time, species, treatments, subject ID, etc.)
  - Custom columns (user customizable values)
  - Summary and custom calculations (support for built in UNIFI calculations such as max, min, average values, etc.)

- Entire processed datasets or filtered subsets data (such as separate tables for QCs, Unknowns, etc.)
- User defined filtering criteria. Flexibility in defining and customizing columns to be exported (i.e.: filter data performed in human species at a specific dose or time points).

The exported data can be brought into third-party packages without manual copying or transcription and the table layouts preferences can be saved within UNIFI. All of these features help to minimize errors and enable the more confident analysis of large data sets. Here, we provide a step-wise illustration of how to perform a calculation of microsomal clearance from UNIFI data acquired on Xevo® G2-S QTof using WinNonlin.

THE SOLUTION

Clozapine incubations were performed using human liver microsomes at 1 mg/mL protein concentration. Incubations were monitored for 2 hours to obtain a time-course study (aliquots withdrawn at 15-minute intervals). Samples were acquired and processed using an ACQUITY UPLC® I-Class System and a Xevo G2-S QTof mass spectrometer under UNIFI instrument control. Figure 2 shows a typical profile with parent drug, Clozapine, disappearing over the 2-hour time course as displayed in UNIFI. The data were exported out of UNIFI and imported into WinNonlin (version 6.3) for the calculation of clearance. The following steps illustrate the process.

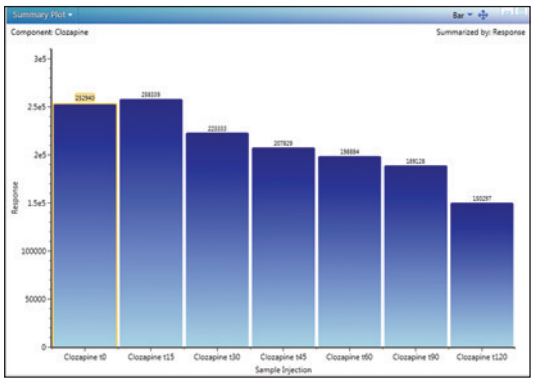
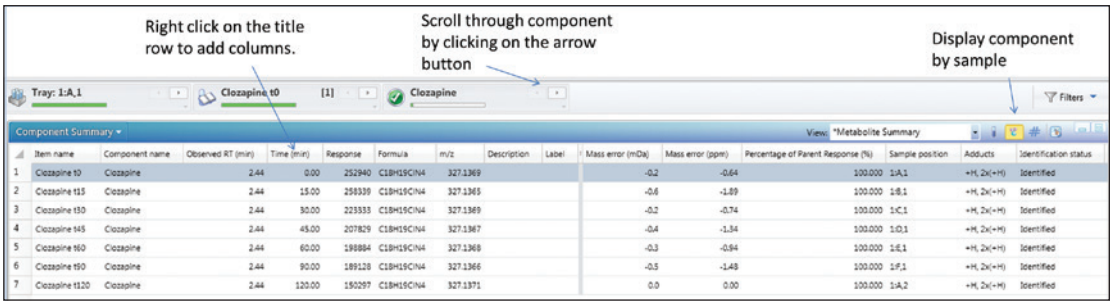


Figure 2. Profile (trend plot) of clozapine concentration over time.

Step 1: Customize UNIFI data for the export

High Resolution Mass Spectrometry (HRMS) data contains both full qualitative and quantitative information. For clearance calculations, only the parent compound information is desired. A table is arranged, filtered, and pivoted which shows the information exactly as we wish to export it (shown in Figure 3). If additional columns are needed for export or columns need to be removed, this is modified by right clicking on the title row and choosing Add (or Remove) Column. The add/remove column panel will appear allowing the user to modify the field displayed in the table (not shown).



	Item name	Component name	Observed RT (min)	Time (min)	Response	Formula	m/z	Description	Label	Mass error (mDa)	Mass error (ppm)	Percentage of Parent Response (%)	Sample position	Adducts	Identification status
1	Clozapine t0	Clozapine	2.44	0.00	252940	C18H19ClN4	327.1369			-0.2	-0.64	100.000	1:A,1	+H, 2x(+H)	Identified
2	Clozapine t15	Clozapine	2.44	15.00	258339	C18H19ClN4	327.1365			-0.6	-1.89	100.000	1:B,1	+H, 2x(+H)	Identified
3	Clozapine t30	Clozapine	2.44	30.00	223333	C18H19ClN4	327.1369			-0.2	-0.74	100.000	1:C,1	+H, 2x(+H)	Identified
4	Clozapine t45	Clozapine	2.44	45.00	207829	C18H19ClN4	327.1367			-0.4	-1.34	100.000	1:D,1	+H, 2x(+H)	Identified
5	Clozapine t60	Clozapine	2.44	60.00	198884	C18H19ClN4	327.1368			-0.3	-0.94	100.000	1:E,1	+H, 2x(+H)	Identified
6	Clozapine t90	Clozapine	2.44	90.00	189128	C18H19ClN4	327.1366			-0.5	-1.48	100.000	1:F,1	+H, 2x(+H)	Identified
7	Clozapine t120	Clozapine	2.44	120.00	150297	C18H19ClN4	327.1371			0.0	0.00	100.000	1:A,2	+H, 2x(+H)	Identified

Figure 3. Component summary data showing clozapine concentration changes over time (data shown is filtered using Identification Status = identified and Derived = no to display only the relevant information).

Step 2: Export UNIFI data

The data shown in the component summary table is then exported by choosing File > Export Filtered Result at the top right corner (not shown). A dialog box called Export Option appears (Figure 4). Choose Microsoft Excel as the file format and enter a filename and folder destination to complete the export. If desired, the data can also be exported using copy (by right-clicking within a table in UNIFI and choosing copy in the drop-down menu) and directly paste into other software packages with Excel-like functionality.

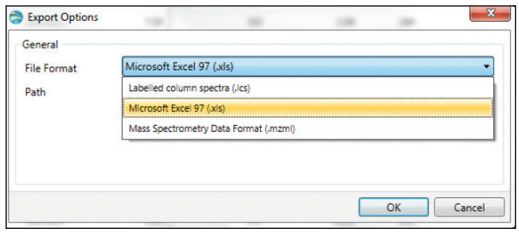


Figure 4. Export option panel displayed after choosing File, Export filtered result..

Step 3: Calculation of clearance value using WinNonlin

A simple workflow for clearance estimation is shown.

- Step 3A: Import the data into WinNonlin, navigate to the saved .xls file from Step 2 and import.
- Step 3B: Convert the response into Ln(response) for the first-order kinetics calculation using Data Wizard in WinNonlin as shown in Figure 5.

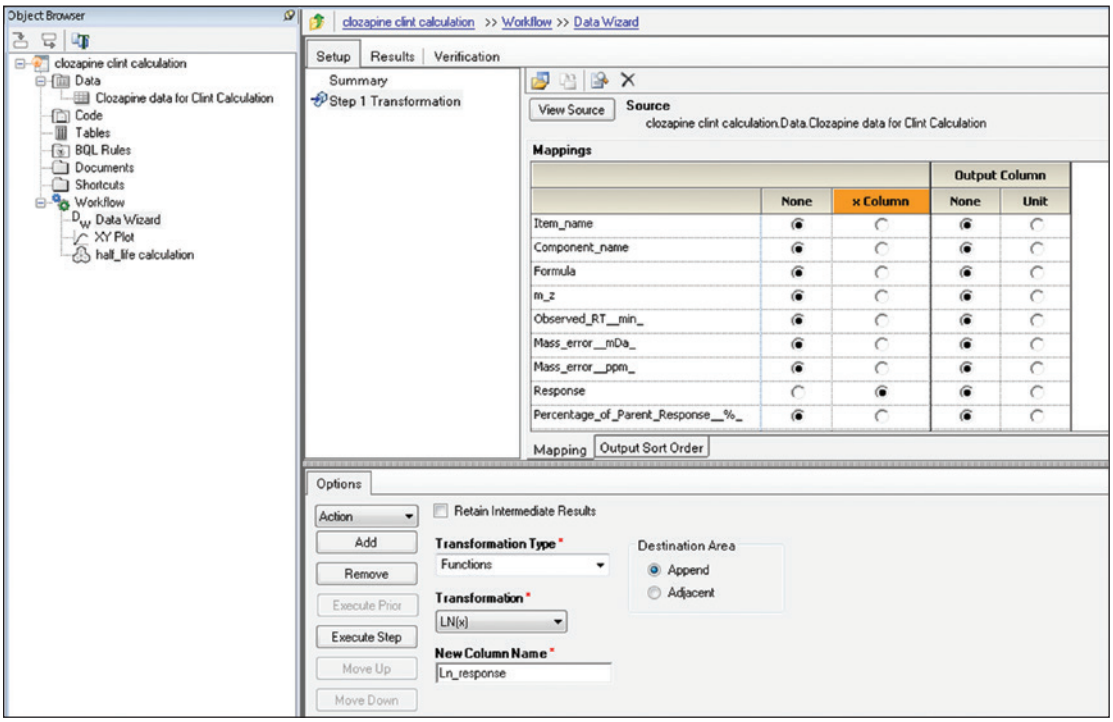


Figure 5. WinNonlin Data Wizard panel for the conversion of Response to Ln(response).

- Step 3C: Generate an XY Plot from the WinNonlin Data Wizard (Figure 5). The plot gives the user a first look of the data. A linear regression line can also be added to the plot.

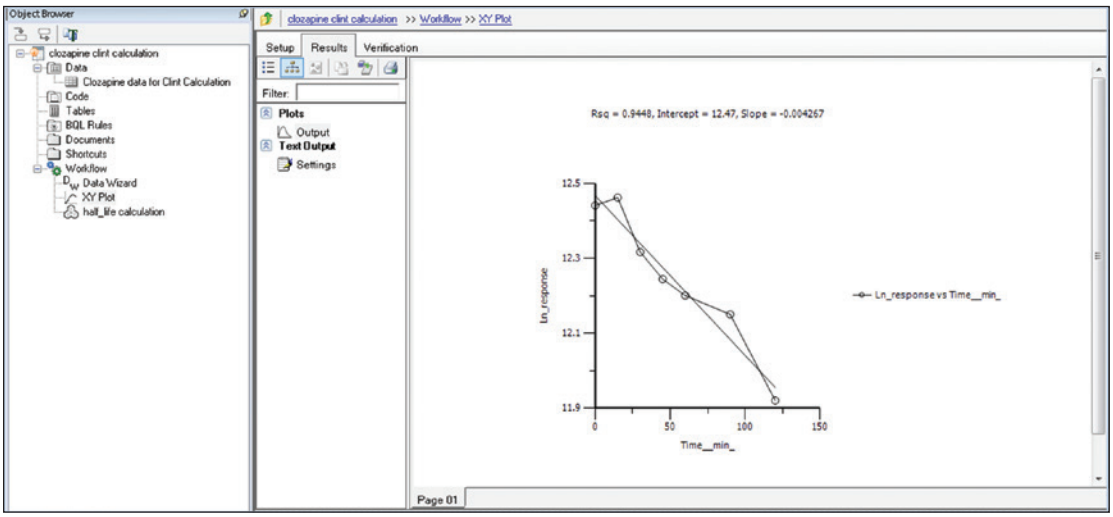


Figure 6. WinNonlin XY Plot panel where a plot of Ln(response) versus incubation time is displayed.

- Step 3D: The final step is to perform first-order kinetics calculation in order to obtain the clearance and $t_{1/2}$ values. The Phoenix Model setup panel is shown in Figure 7 and Figure 8. The calculation produced a $t_{1/2}$ of 162 ± 15 min and a Cl_{int} value of 4.3 ± 0.4 µL/min/mg. The clearance value is consistent with literature reported values of 6.2[1] and 5.02[2] µL/min/mg respectively.

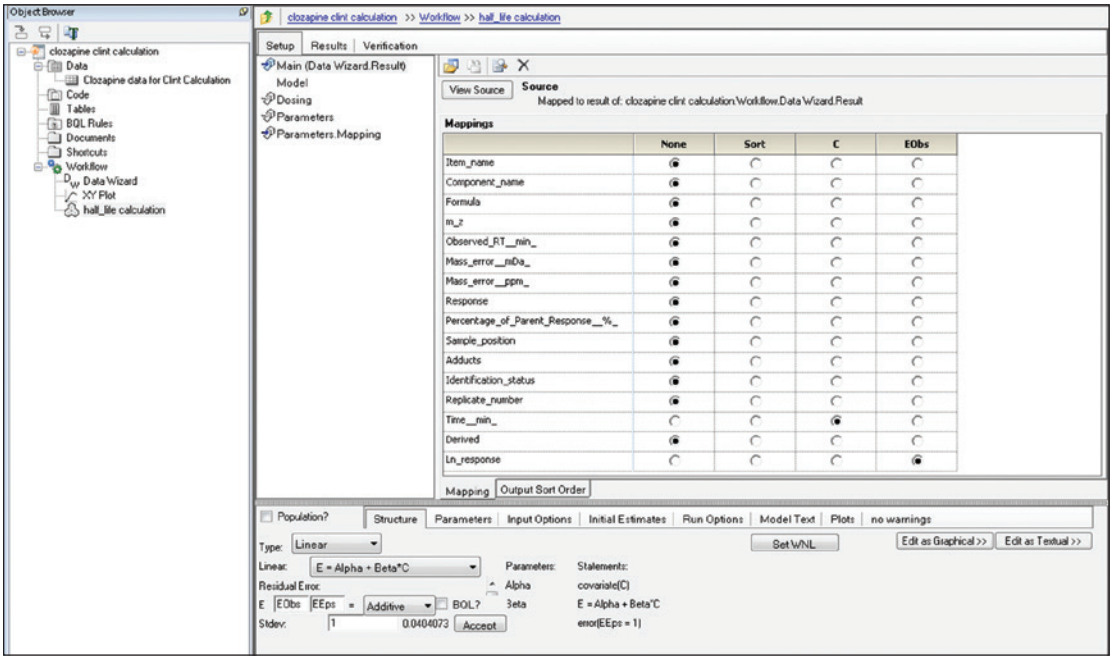


Figure 7. WinNonlin Phoenix Model Setup for linear fitting of time versus concentration.

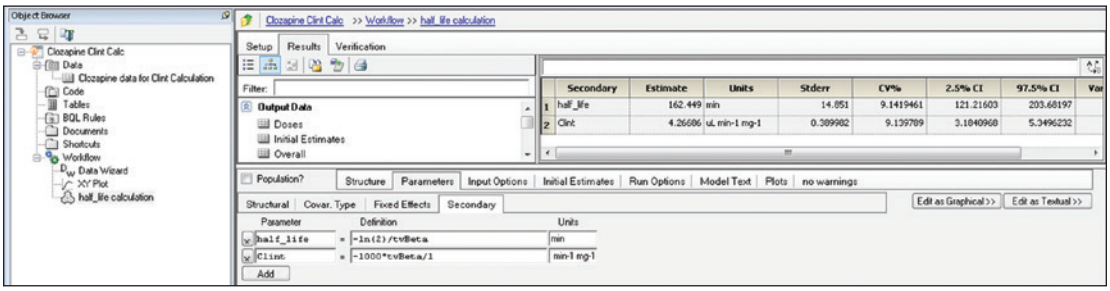


Figure 8. Calculated clearance, $t_{1/2}$, and associated statistical values from linear fitting of the data.

Finally, the above steps are captured by WinNonlin as a workflow shown in Figure 9. The workflow can be saved as a template and applied to future datasets.

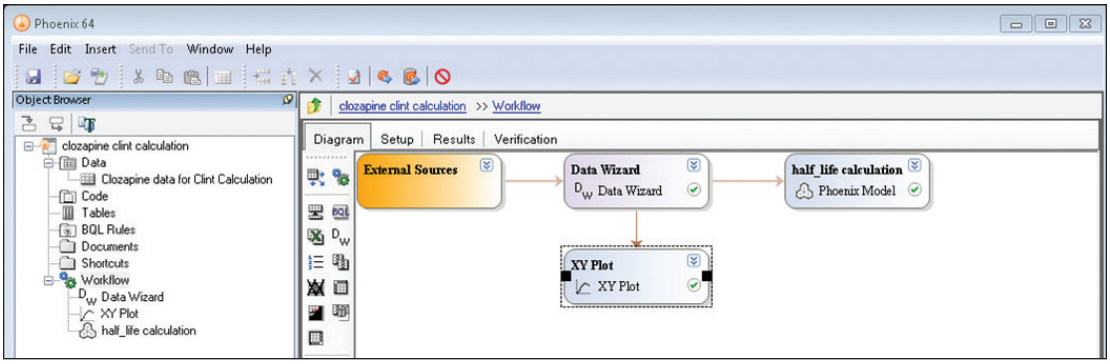


Figure 9. WinNonlin Workflow for the clearance calculation of clozapine data.

SUMMARY

Transferring data between software packages can be a bottleneck and is prone to errors if done manually. This technology brief illustrates that data acquired and automatically processed using UNIFI Software can be easily exported for calculations using third-party software solutions, providing both a simple and powerful solution for a DMPK department. By combining workflows in UNIFI and WinNonlin, the user can now easily import and process future datasets from UNIFI and then save these data views for future analyses. This is demonstrated with a calculation using WinNonlin for the estimation of clearance value of clozapine in human liver microsomes.

References:

1. Bonn B, Leandersson C, Fontaine F, Zamora I. Enhanced metabolite identification with MSE and a semi-automated software for structural elucidation, Rapid Comm. Mass. Spectr. 2010; 24: 3127–3138.

2. Obach R S, Prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: An examination of in vitro half-life approach and nonspecific binding to microsomes, Drug Metab. Dispos. 1999;27:1350-9.

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