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

Yun Alelyunas, Paul Rainville, and Mark Wrona; Waters Corporation

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.

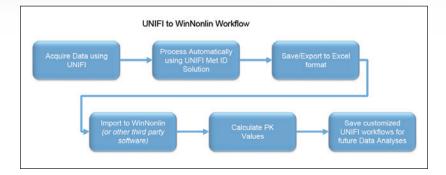


Figure 1. The workflow using UNIFI and WinNonlin software.

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.

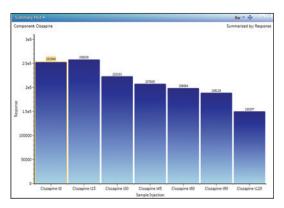


Figure 2. Profile (trend plot) of clozapine concentration over time.

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).

		0	Right click on the title row to add columns.			Scroll through component by clicking on the arrow button							Display compo by sample		
	Tray: 1:A,1		Clozapine	t0	[1] · •	Cloz	apine								Tritters
Co	mponent Sumn	nary 🕶										View: *Metabolite !	Summary		: # 🕞 📼
4	Item name	Component name	Observed RT (min)	Time (min)	Response	Formula	m/z	Description	Label	1 Mass error (mDa)	Mass error (ppm)	Percentage of Parent Response (%)	Sample position	Adducts	Identification statu
1	Clozapine t0	Clozapine	2.44	0.00	252940	C18H19CIN4	327.1369			-0.2	-0.64	100.000	1:41	+H, 2x(+H)	Identified
2	Ciozapine t15	Ciozapine	2.44	15.00	258339	C18H19CIN4	327.1365			-0.6	-1.89	100.000	161	+H, 2x(+H)	Identified
3	Clozapine t30	Clozapine	2.44	30.00	223333	C18H19CIN4	327.1369			-0.2	-0.74	100.000	14,1	+H, 2x(+H)	Identified
4	Clozapine 145	Clozapine	2.44	45.00	207829	C18H19CIN4	327.1367			-0,4	-1.34	103.000	10,1	+H, 2x(+H)	Identified
5	Ciozapine t60	Clozapine	2.44	60.00	198884	C18H19CIN4	327.1368			-0.3	-0.94	100.000	15,1	+H, 2x(+H)	Identified
	Clozapine t90	Clozapine	2.44	90.00	189128	C18H19CIN4	327.1366			-0.5	-1.43	100.000	19,1	+H, 2x(+H)	Identified
6	Cictabilie (30														

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.

Seneral File Format	Microsoft Excel 97 (.xls)
Path	Labelled column spectra (.ics)
	Microsoft Excel 97 (.xis)
	Mass Spectrometry Data Format (.mzml)

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.

Object Browser Ø	clozapine clint calculation >>	Vorkflow >> Data Wizard									
	Setup Results Verification	n									
⊡- clozapine clint calculation	Summary										
Clozapine data for Clint Calculation Code Ultrables Stables Stables	PStep 1 Transformation	View Source clozapine clint calcula	tion.Data.Clo	apine data for Clini	nt Calculation						
- Documents					Output	Column					
Shortcuts Workflow			None	x Column	None	Unit					
D _w Data Wizard		Item_name	(6	0	· · ·						
- XY Plot - B half_life calculation		Component_name	6	0		0					
w		Formula	۲	0		0					
		m_z	6	C	6	C					
		Observed_RTmin_	(0	(0					
		Mass_errormDa_	•	0		0					
	Mass_errorppm		0	(6	0						
		Response	C	۲	•	0					
		Percentage_of_Parent_Response%_	(ē	0		0					
		Mapping Output Sort Order	Mapping Output Sort Order								
	Action	ntermediate Results Nation Type Destination Area									

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.

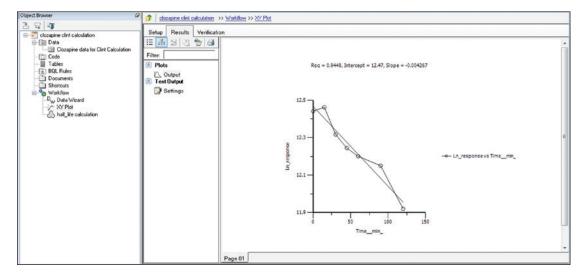


Figure 6. WinNonlin XY Plot panel where a plot of Ln(response) versus incubation time is displayed.

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 6.2¹ and 5.02² µL/min/mg respectively.

Generation	Setup Results Verification										
e 🗇 Data	Hain (Data Wizard Result)	Wew Source Mapped to result of: clocapine clinit calculation Workflow:Data Woard Result Mappings									
Clozapine data for Clint Calculation Code Tables SOL Rules	Model & Dosing & Parameters										
Documents Shortcuts	Parameters.Mapping		None	Sort	с	EObs					
- % Workflow		Item_name	6	0	C	0					
D., Data Wizard		Component_name	C	0							
A half_life calculation		Formula	6	0	C	0					
CO INSTITUTION	1	m_z	(0	0	0					
		Observed_RTmin_	6	0	С	0					
		Mass_errormDa_	(0	C	0					
		Mass_errorppm_	6	C	С	C					
		Response	۲	0	C	0					
		Percentage_of_Parent_Response%_	6	C	C	C					
		Sample_position	۲	0	C	C					
		Adducts	6	C	С	C					
	1	Identification_status	6	0	C	0					
		Replicate_number	6	C	С	0					
		Timemin_	C	0	6	0					
		Derived	(0	C	0					
		Ln_response	0	0	C	6					
		Mapping Output Sort Order									
	Population? Structure	no warnings									
	Population? Structure Parameters Input Options Initial Estimates Run Options Model Text Plots no warnings Type: Linear Linear Linear E-Alpha + Beta*C Abha covariate(C)										
	E E0bs EEps = Additive Stdev: 1 0.040	BOL? 3eta E = Alpha + B 4073 Accept enor[EEps = 1									

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

Clozapine Clint C	alc >> Workflow >> ha	I life calculation																	
H 🚠 🔄 🍋	2 3		JГ								25								
Filter:				Secondary	Estimate	Units	Stderr	CV%	2.5% CI	97.5% CI	Var								
Output Data			1	half_life	162.449	min	14.851	9.1419461	121.21603	203.68197									
Doses			2	Clint	4.26686	uL min-1 mg-1	0.309982	9.139789	3.1040968	5.3496232									
Unitial Estimates								- >											
Population?	Structure Param	eters Input Options	In	itial Estimates	Run Options I	Nodel Text Plo	ts no warnings			0968 5.3496232									
Structural Covar.	Type Fixed Effects	Secondary						Edi	2.5% CI 97.5% CI V 121.21603 203.68197 3.1840968 5.3496232	>									
Parameter	Definition			Units															
whalf_life	= -ln(2)/tvBeta			min															
Clint	= -1000*ryBet.a/		_	min-1 mg-1															
	Setup Results E das A Construction of the set of the s	Setup Results Verification Image: Setup Setup Setup Setup Fate: Image: Setup Setup Setup Fate: Image: Setup Setup Setup Image: Setup Dose: Image: Setup Setup Image: Setup Cover: Type Fixed Effects Parameter Structural Cover: Type Fixed Effects Definition Value Definition Definition Setup Setup	Betup Results Verification Filer: Betup Results Verification Filer: Output Data Dores Invisit Erimates Overall Posulation? Structure Parameters Input Options Structural Cover.Type Fixed Effects Secondary Pasaneter Pasaneter Definition Verification verification	Betup Results Verification Betup Results Verification Filter.	Betup Results Verification Betup Results Verification Image: Secondary Image: Secondary Prize: Image: Secondary Image: Secondary Image: Secondary Population? Structure Parameter Input Options Image: Structure Parameters Image: Structure Definition Structural Covar. Type Passeter Definition Units Image: Secondary	Secondari un car. Wentication Entry Results Verification Entry Secondary Estimate Filter: Indial Estimates Indial Estimates Ordpat Data Indial Estimates Indial Estimates Overall Verification Indial Estimates Produktion? Structure Parameter Input Options Indial Estimates Verifications Produktion? Structure Parameter Produktion? Structure Verifications Parameter Input Options Indial Estimates Parameter Input Options Units Vinal Lovar. Type Fixed Effects Secondary Units Verification Units	Secondary Estimate Betup Results Verification Image: Secondary Estimate Image: Secondary Image: Secondary Estimate Image: Secondary Image: Secondary Estimates Image: Secondary Image: Secondary Secondary Estimates Image: Secondary Secondary Estimates Image: Secondary Secondary Image: Secondary Population? Structure Parameters Input Options Image: Secondary Parameters Input Options Image: Secondary Parameters Definion Units Units Image: Secondary Definion Units Vinat: 116 Secondary Units	Secondary Estimate Image: Structure Secondary Ender Structure Parameters Input Options Initial Estimates Overall Structure Parameters Input Options Initial Estimates Secondary Parameters Input Options Initial Estimates Secondary	Setup Result Verification Image: State in the setup	Secondary Links Workshow Secondary Betup Results Verification Image: Secondary Secondary Estimate Image: Secondary Estimate Units Stderr Image: Secondary Estimate Units Stderr Image: Secondary Estimate Units Stderr Image: Secondary Estimate Image: Secondary Statemate Image: Secondary Estimate Image: Secondary Estimate Image: Secondary Estimates Image: Secondary Estimates Image: Secondary Estimates Image: Secondary Estimates Image: Secondary Estimates Image: Secondary Estimates Pasender Defension Units Units Estimates Image: Secondary Units Units Estimates Estimates	Secondary Letter Without 7 (secondary 7) Betup Result Verification Image: Secondary Secondary Estimate Units Stderr CV% 2.5% CL 97.5% CL Image: Secondary Secondary Estimate Units Stderr CV% 2.5% CL 97.5% CL Image: Secondary Estimate Image: Secondary Estimate Image: Secondary 14.14551 9.1419461 121.21503 203.46197 Image: Image: Secondary Edit as Graphical>> Edit as Graphical>><								

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

TECHNOLOGY BRIEF

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.

Phoenix 64	
File Edit Insert Send To Window Help	
🚽 🐸 🤣 👗 🖻 🛍 🛗 🛱 🛱	
Object Browser 🔗	plozapine clint calculation >> Workflow
2 G 🗤	
E-2 clozapine clint calculation	Diagram Setup Results Verification
Data Docta Do	External Sources S Data Wizard S half Life calculation
- Code	Data Wizard 🕥 🖓 Phoenix Model 🔗
Tables	
BQL Rules Documents	Ma P.,.
- Shortcuts - % Workflow	I T T
- Workflow	🗱 🔟
D _w Data Wizard	
half_life calculation	

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.
- 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.

Waters



Waters, The Science of What's Possible, ACQUITY UPLC, Xevo, and UNIFI are registered trademarks of Waters Corporation. All other trademarks are the property of their respective owners.

©2014 Waters Corporation. Produced in the U.S.A. February 2014 720004947EN TC-PDF Waters Corporation 34 Maple Street Milford, MA 01757 U.S.A. T: 1 508 478 2000 F: 1 508 872 1990 www.waters.com