# Chromeleon CDS workflow for bioanalysis

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# Introduction

Bioanalytical studies are a fundamental part of the drug development process. They involve the quantitative measurement of compounds of interest and/or their associated metabolites in biological samples. Tandem quadrupole mass spectrometry coupled with highperformance liquid chromatography (HPLC-MS/MS) is primarily used for compound detection.

Developing sensitive and selective assays remains a significant challenge for today's scientists, as the industry strives to keep pace with increasing demands for a robust methodology that will ensure proper dosage, efficacy and safety of any therapeutic product. Alongside this are the ever-evolving regulatory guidelines and intensified demands on data integrity in this market space, which means that the modern-day Chromatography Data System is coming under close scrutiny throughout every stage of the bioanalysis workflow where traceability, accuracy, and



integrity of the data is vital. Thermo Scientific<sup>™</sup> Chromeleon<sup>™</sup> 7 Chromatography Data System (CDS) can address these needs by providing an end-to-end solution from samples to results, interfaced to a Laboratory Information Management System (LIMS) that is designed for bioanalysis.

#### One software package

Analysis often involves multiple software platforms for Chromatography and Mass Spectrometry, but Chromeleon CDS provides a single software solution that is scalable, from a local standalone workstation to a multi-site enterprise system, with built-in features to help users meet compliance requirements. Chromeleon software provides robust and reliable control of the HPLC and MS with integrated MS/MS functionality for data acquisition and processing. This technical note highlights the key features of the Chromeleon CDS workflow for bioanalysis.



## Compliance

Ensuring that a potential therapeutic product is safe for use means adhering to regulations outlined by regulatory bodies such as the U.S. Food and Drug Administration (FDA) and the Medicines and Healthcare products Regulatory Agency (MHRA). With Chromeleon CDS, you can satisfy regulatory requirements without sacrificing efficiency by using the integrated security system, audit trails, and version management tools.

## **Data integrity**

Data integrity applies equally to paper and electronic data. However, electronic data is deemed more secure since it reduces human oversight, is more difficult to manipulate or change, with such changes being easier to detect. There is a definite drive, even in society in general, to use technology to automate and mitigate many of these issues. Chromeleon CDS can help you overcome the challenges faced when implementing technical controls in the laboratory, as it is a robust system that provides an outstanding set of access controls and security features that complement mass spectrometry and bioanalysis.

The Chromeleon Administration Console provides a comprehensive yet simple interface (Figure 1) with complete system configuration (Figure 2), flexible user management (Figure 3) and secure access control (Figure 4), which incorporates electronic signatures (Figure 5) for compliance with 21 CFR Part 11 requirements to aid the implementation of a paperless environment.



Figure 1. The Central Administration Console allows easy implementation and management of technical controls.

License Manager (localhost) Scheduler Global Policies User Database	E Save Policies Repor	Privileged Actions									
eWorkflow Tags	🖾 User Mode	Settings Standard Con	nments								
Domain Resources Local Machine	Host Name Resolution		Select All No								
	<ul> <li>Injection Locking</li> <li>Remote Data Vaults</li> </ul>		Action	Requires Authorization	Requires Comment	Default Comment	ŀ				
	Instrument Data	+ Instrument Configura	ation								
	Detection Algorithm Ve	Instrument Method									
	User Templates Locati	+ Instrument Audit Tra	il								
	UI Customizations	Data Vault									
	Privileged Actions	+ Folder									
	Email Configuration	Query									
	License	+ Sequence					=				
	🖾 Raw Data	Injection									
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		<ul> <li>Report Template</li> </ul>									
		<ul> <li>Electronic Report</li> </ul>									
		<ul> <li>View Settings</li> </ul>									
		<ul> <li>Attachment</li> </ul>									
		<ul> <li>Administration Globe</li> </ul>	al Policies								
		+ Administration User	Policies								

Figure 2. System configuration with Global Policies. Privileged Actions can enforce when, and how, passwords and comments must be applied when users make changes.



Figure 3. User Management in Chromeleon CDS: Privileges define what users can and cannot do.

Back 🔘 Create	File Edit	View	Tools	Help				
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۵ 🚖	Filter	Y	N	ame	Туре	Date	Modified	Comme
BA DV			🛅 Seque	nces	Folder	02-Jul-19 10:2	22:42 AM +01:00	
Approved Sequences	iences							
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S Quality Control							×××	
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o John Smith							^	
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Add User or Gro	up Role Nan	ne			Allow		Deny	
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Figure 5. Showing electronic signatures. Once signed further modifications are not permitted and the sequence can only be reviewed by a user with the associated privilege.

Figure 4. Access Groups can be used to control access to specific Data Vaults, folders within Data Vaults, and instruments.

## Audit trail review

With a comprehensive set of audit trails covering data, instruments, user management and system administration, Chromeleon CDS provides traceability, records historical changes for data reconstruction, while making audit trail review simple and less onerous.

The software records versions for data objects (Processing Method, Instrument Method, Report Template etc.), while the "Show Changes" feature provides a quick and easy comparison of object versions with a simple side-by-side display featuring icons that clearly flag insertions, deletions, and changes (Figure 6). At any point, a current version may be restored to a previous version as long as versioning has been enabled.



Version	Date / Tim	e	Operator	Client Computer				Visualization Options
	02/12/200913:02:38 02/12/200913:03:44	SQuinn SQuinn		L-GERSQUINN L-GERSQUINN				<ul> <li>Complete</li> <li>Only Changes</li> </ul>
Prop	erty Name		Value	version 1			Value version	2
Comment nstrument Server nstrument	L	JltiMate3000 GERSQUINN 15_UltiMate3000_1				UltiMate3000 L-GERSQUINN 05_UltiMate3000_1		Change
		Version 1					Version 2	
Time	Symbol	V	alue	Comment	Time	Symbol		Comment
🔺 💈 Initial Time	Instrument Setup			)	🔺 💈 Initial Time	Instrument Setup		
	Sampler.InjectMode Sampler.PumpDevice Sampler.SyncWithPump Pump.MaximumFlowRar		mirř]			Sampler.InjectMode Sampler.PumePonce Samplor.syncWithPump Pump.MaximumFlowRampDown	Normal "Pump" On 1.000 [ml/mir²]	
8	Pump.%B	.Equate		"Acetonitrile"		Pump.%A.Equate	1	"0.1M Phosphate Buffer"
8	Pump.%C.Equate Pump.Pressure.LowerLin	''Methano mit 0 [bar]			8	Pump.Pressure.LowerLimit Pump.Pressure.UpperLimit Pump.%B.Equate	0 [bar] 400 [bar] ''Acetonitrile''	
	Pump.F	Pressure.Upperl	.imit	400 [bar]	8	UV.UV_VIS_1.Wavelength	280.0 (nm)	
0.00	Inject reparation				▷ 0.00 ▷ 0.00	Inject Preparation		
> 0.00	Inject Start Run				D 0.00 ⊿ 🖁 0.00	Inject Start Run		
a g 0.00	UV_VV_VIS_1.AcqOn				+	UV.UV_VIS_1.AcqOn Pump.Pump Pressure.AcqOn		
a 🔮 0.00	Run				⊿ 🔮 0.00	Run		
<b>8</b> ▲ <b>8</b> 8.00	Pump.Flow	1.000 [ml/	min]		<b>8</b> ▲ <b>8</b> 8.00	Pump.Flow	2.000 (ml/min)	
8	Pump.Flow	1.000 [ml/	min]		8	Pump.Flow	2.000 [ml/min]	
4 💈 8.00	Stop Run				4 🖁 8.00	Stop Run		
	UV.UV_VIS_1.AcqOff					Pump.Pump_F	Pressure.AcqOff	
			Deletio					Addition

Figure 6. Illustrating the "Show Changes" feature within Chromeleon CDS.

Going one step further Chromeleon CDS provides a visual comparison by opening selected items from the data audit trail in a corresponding Chromeleon Studio window (Figure 7), thus any changes made can be clearly verified during the review process. To maintain the integrity of the data, all objects remain read-only except for the View Settings. It's also possible to open up several Chromeleon Studio windows with different versions (Figure 8).

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	Report	章 Show Ch	anges 🎽	9 Restore	Studio	Include S	Subfolde	ers from		Ŧ
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	9 A Closant	el Q Chrom	atogram	7	21-May-19	20:58:54 PN	+01:00	Peter Zipfell	Changed	
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	5 A Closant	elQ 💿	Studio			0:50:49 PN	+01:00	Peter Zipfell	Changed	
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Figure 7. Illustrating the first stage of a visual audit trail review.



Figure 8. Showing a visual comparison when reviewing audit trails. Note how changes to the sequence are not permitted—shown by the highlighted yellow bar.

#### **Compliant mass spectrometry tuning**

Typically the tune application resides as a separate component from the software that's in place for instrument control and data acquisition. Compliance requirements in bioanalysis demand that all points of access are controlled, and that even the tune files are safely stored and audited accordingly. That's why such files are securely stored within Chromeleon CDS.

The ability to perform MS manual tune or calibration solely from an ePanel (Figure 9) within Chromeleon software can also be privilege controlled (Execute On-Demand MS Manual Tune/Calibration). This ensures that control is exercised on a permission basis, with all activities being recorded in the audit trails. It is then not possible for a user to open up the tune application outside of Chromeleon CDS, preventing inappropriate access.

#### Working with your data

It's important that a CDS can handle large datasets without compromising on productivity, whether processing or simply reviewing the data. With Chromeleon CDS, there are a number of tools and features that complement the bioanalysis workflow, with simplicity and efficiency at the heart of its design. SmartLink provides users with pre-defined component-centric templates that enable users to choose which injections to include (Figure 10) and the associated orientation of the plot layout. This allows for an easy comparison of the data, identifying trends straight forward and recognizing data discrepancies, such as missing peaks uncomplicated (Figure 11).



Figure 11. Calibration Standard Quantitation lons across a sequence for a specific component per injection. Notice the un-integrated peak. In addition, there may also be instances where a user needs to make injection specific changes to the detection settings, possibly due to chromatographic interferences or a poor injection. Using this approach, it can be possible to reduce the need for manual integration. Accessing the MS detection tab (Figure 12) found in the Chromeleon Processing Method allows a user to apply changes to a specific injection or all injections.

# **QC** terminology

Quality Control Samples play a vital role to ensure consistency, validity, and reliability of results. They should be analyzed in every single assay to demonstrate robustness and acceptability. It's important that they are easy to define (Figure 13) and that clear indicators of pass or fail are available (Figure 14). Chromeleon CDS provides a comprehensive collection of features for both Calibration and Quality Control Samples that enable users to review the results quickly and effortlessly.

	etection			
Component: Te	estosterone		▼ ►	
KIC: M	S Quantitation F	Peak [289.20 / 97.00-97	.00, 109.00-109. 🔻 🔶	
Use Processing Methor	d (applied for al	ll injections)		
Use MS default detection	tion settings			
Use XIC specific determine	ection settings			
Use Injection specific d	etection setting	s		
Detection Settings				
Detection Algorithm:	Genesis	•		
Algorithm Settings				
	0.0	[0.0999.0]	Peak S/N cutoff:	50.0
		[0.0999.0]	Peak S/N cutoff: Rise percentage (%):	50.0 0.1
S/N threshold:		[0.0999.0]		
S/N threshold:			Rise percentage (%):	0.1
S/N threshold: Enable valley detection Expected width (sec):			Rise percentage (%): Valley depth:	0.1
Expected width (sec): Constrain peak width	0.00	[0.00999.00]	Rise percentage (%): Valley depth: Calculate noise as:	0.1 1.0 RMS

Figure 12. MS Detection settings can be applied to a whole sequence or specific injection.

Level "QC1"	Tolerand	:e[%]	Leve	l "QC2"	Tolerance [%	] Lev	el "QC3"	Tolerar	nce [%]		Figure 13. Ea	asy to apply	tolerance levels.
5.000000	15.00 [%]		10.000	000	15.00 [%]	50.00	0000	15.00 [%	15.00 [%]		0	,,	
5.000000	15.00 [%]		10.000	000	15.00 [%]	50.00	0000	15.00 [%	5]				
5.000000	15.00 [%]		10.000	000	15.00 [%]	50.00	0000	15.00 [%]					
5.000000	15.00 [%]		10.000	000	15.00 [%]	50.00	0000	15.00 [%]					
5.000000	15.00 [%]		10.000	000	15.00 [%]	50.00	0000	15.00 [%	5]	•	Figure 14 C	loar nass/fai	results with
5.000000	15.00 [%]		10.000	000	15.00 [%]	50.00	0000	15.00 [%	5]		•	plot indicator	
		Injecti	on Deta	ils (QC San	nples)			:		<u> </u>			
5.000000	15.00 [%]	No.	Name	Theo. Amt	Amount	Amount	Analyte F	eak Area	ISTD P	eak Area	Area Ratio	Level Check	Record Modified
				ng/mL	ng/mL	% Diff	coun	ts*min	coun	ts*min			?
5.000000	15.00 [%]		QC 1	5.0	4.8	-4.24	9	26	15	558	0.59	Pass	No
CALCHER PRODUCT		25	QC 2	10.0	8.1	-19.10		79		773	1.00	Fail	No
		26	QC 3	50.0	48.4	-3.17		04		516	6.01	Pass	No
		89	QC 1	5.0	5.3	5.74		94		516	0.66	Pass	No
		90	QC 2	10.0	10.1	0.66	19	965	15	573	1.25	Pass	No



91 QC 3

154 QC 1

156 QC 3

155 QC 2 50.0

5.0

10.0

50.0

46.2

5.1

9.8

52.0

-7.62

2.69

-2.42

4.04

#### Plot indicators

9055

1047

1856

9667

Check standard/QC sample point within its tolerance limits.

1580

1644

1533

1498

Check standard/QC sample point within its tolerance limits (when it is the selected injection).

5.73

0.64

1.21

6.45

Pass

Pass

Pass

Pass

No

No

No

No

- $\perp$  Level tolerance line with check standard/QC sample point within its tolerance limits.
- Check standard/QC sample point outside its tolerance limits (outlier).

Level tolerance line when check standard/QC sample point is outside its tolerance limits.

#### Pre-defined report template for bioanalysis

Of course, data processing inevitably ends with a report output of some sort and for that the software has a built-in report template (Figure 15). With a spreadsheetbased approach, the template is highly customizable with predefined calculations that are common for bioanalysis.

#### Scalable infrastructure for mass spectrometry

Mass spectrometry data acquisition and control has often been standalone, and data is often stored locally or on a file share with access only from the instrument computer. With a networked, or enterprise CDS, it is possible to extend the capabilities for bioanalysis and provide the security and reliability that's needed when it matters most.

Delivering these capabilities across a network brings a unique set of challenges. The sheer volume of data may pose a problem, however Chromeleon CDS has been designed and optimized to overcome these challenges and brings the following benefits to the bioanalysis workflow:

Category	Thermo S	cientific Templates	T	
Bioan	alysis	Default MS Report		
	Protein volution		-	Injection Overview      A      A



- Scalable architecture from local standalone to multi-site enterprise provides a seamless solution that can grow alongside any business expansion
- Remote Instrument and data access means there is no need to be in the lab to view data or even to control the mass spectrometer
- Centralized data storage with a relational database at the heart of the application provides security and peace of mind
- Tools for network failure protection ensure that data is not lost in the event of a network outage

eWorkflow Wizard

- Built-in archiving capabilities provide flexibility and easy management of the data
- Data consistency checks to ensure data are tamper-protected

#### Bringing it all together—eWorkflow procedures for MS

Streamlining workflows in any bioanalysis laboratory is never an easy task, however with sequences often tied to a Standard Operating Procedure (SOP) they follow a repetitive trend for analysis and structure that remains the same. eWorkflow<sup>™</sup> procedures provides an ideal solution, where the process of sequence creation can be automated, guiding the operator through a minimal number of choices needed to create a complete, correct sequence with predefined files and a well-defined structure (Figure 16). Using an eWorkflow simplifies sequence creation and minimizes the amount of training required, allowing users with little training to run routine MS analyses without error, saving valuable time and resources.

	© uuence Preview		<b>&gt;</b>	•	Rack View	-	Number of sample 1 Sampler start pos R:A1 Run sequence	[19] ition:     [R:A1SY:3]	
#	Chromatogram	Name	Туре	Level	Position	Volume [µl]	Instrument Method	Processing Method	Status
25	None	📱 QC 2	QC Sample	QC2	R:C1	1.00	Bionanalysis Instr	Bioanalysis Proces_	Idle
26	None	📱 QC 3	QC Sample	QC3	R:C2	1.00	Bionanalysis Instr	Bioanalysis Proces_	Idle
.0			Unknown		R:C3	1.00	Bionanalysis Instr	Bioanalysis Proces_	Idle
-	None	🖥 Blank							
27	None None	8 Blank 8 Blank	Unknown		R:C4	1.00	Bionanalysis Instr	Bioanalysis Proces	Idle
27 28			Unknown Unknown		R:C4 R:C5	1.00 1.00	Bionanalysis Instr Bionanalysis Instr	Bioanalysis Proces Bioanalysis Proces	Idle Idle
.7 18	None	🖥 Blank							
17 18 19	None None None	7 Blank 7 Sample-Day1Hours0	Unknown		R:C5	1.00	Bionanalysis Instr	Bioanalysis Proces_	Idle
17 18 19 10	None None None	Blank       Sample-Day1Hours0       Sample-Day1Hours2	Unknown Unknown		R:C5 R:C6	1.00 1.00	Bionanalysis Instr Bionanalysis Instr	Bioanalysis Proces_ Bioanalysis Proces_	Idle Idle
27 28 29 30 31 32	None None None None	Blank       Sample-Day1Hours0       Sample-Day1Hours2       Sample-Day1Hours6	Unknown Unknown Unknown		R:C5 R:C6 R:C7	1.00 1.00 1.00	Bionanalysis Instr Bionanalysis Instr Bionanalysis Instr	Bioanalysis Proces Bioanalysis Proces Bioanalysis Proces	Idle Idle Idle

Figure 16. Starting an eWorkflow—select how many samples you want to analyze and the sequence is populated from predefined rules and templates.



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# A leading combination from an industry accepted data management system together with a gold standard CDS platform

The need to ensure accuracy and consistency throughout the data life-cycle has never been more important for bioanalytical studies. Thermo Scientific<sup>™</sup> Watson<sup>™</sup> LIMS software is built for bioanalysis with its extensive options to manage compliance throughout an entire workflow. From study management and design, samples can be tracked, analyzed, and reported using pharmacokinetic and toxicokinetic calculations available in Watson LIMS software.

Harmonizing and automating the complete bioanalysis workflow is achieved thanks to the out-of-the-box Chromeleon CDS and Watson LIMS Gateway (Figure 17). Utilizing eWorkflow procedures, data from an analytical run can be sent from Watson LIMS to create a sequence in Chromeleon CDS with the resulting data from the processed sequence being imported back into a Watson study.

Instrument Parameters	
Parameter	Value
Please select a Chromeleon Instrument	U3000 on UKALT-9F3ZVZ1
Please select a Chromeleon eWorkflow	UKALT-8MTMRQ2\72SR5Vault\Watson-Demo
Please select a sample start position	RA1
	PA2         ▲           PA3         A           PA4         PA5           PA5         PA6           PA6         PA6           PA7         ₹
S Enter Chromeleon User Name	And Password – ×
Wats	son Gateway for Chromeleon
User Name: Ter	stUser3
Password:	Change
Role: Ful	I_NoModifyNotification V
	Log on Cancel Help

Figure 17. Watson Gateway for Chromeleon CDS-easy sequence creation.



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