

Method validation based on ICH guidelines of a USP assay method of acetaminophen

Authors: Sylvia Grosse,¹ Shaun Quinn,²
Mauro De Pra,¹ Frank Steiner¹

¹Thermo Fisher Scientific,
Germering, Germany

²Thermo Fisher Scientific, Altrincham,
Great Britain

Keywords: Pharmacopoeia methods,
analytical procedure, acetaminophen, assay
method, HPLC, high-performance liquid
chromatography, Vanquish Core, Chromeleon,
eWorkflow, extension pack

Application benefits

- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) provides eWorkflow™ procedures for method validation based on International Council for Harmonisation (ICH) guidelines.
- Chromeleon eWorkflows are designed to deliver productivity and efficiency gains for method validation.

Goal

Demonstrate the ease of implementation and processing of a full method validation with Chromeleon CDS using eWorkflow procedures based on the ICH guidelines.

Introduction

Analytical procedures undergo method validation as a determination of their suitability to the intended analysis.



United States and European pharmacopoeia compendia contain guidelines on analytical procedure method validation.^{1,2} Additionally, ICH, Q2 (R1) guidelines describe the recommended validation criteria in great detail.³ They are as follows:

- Accuracy
- Precision (repeatability and intermediate precision)
- Specificity
- Detection and quantification limit
- Linearity and range
- Robustness

When performing method validation, laboratories typically create their own unique injection sequences, data processing steps, and reports. The creation of these optimized methods and reports is very complex and time-consuming. Quite often, the data can be exported manually or semi-automatically from the CDS to a data processing program (e.g., Microsoft® Excel®) for post-acquisition processing. This step is extremely error-prone and time-consuming.

The Chromeleon CDS offers an extension package for full method validation based on ICH guidelines. With predefined eWorkflow templates, the user can easily and quickly create a complete sequence because all associated files and methods are pre-set. This enables immediate method validation. The complete data processing can be done in Chromeleon CDS with predefined processing methods, which only require a few user-defined adjustments. Reporting is quick and easy because results are provided on a single sheet. Another important aspect is that unlike external programs, Chromeleon CDS is fully regulatory compliant.

In this application note, the United States Pharmacopeia (USP) <1225> assay method of acetaminophen⁴ is re-validated by using Chromeleon eWorkflows with integrated data evaluation and reporting. Also shown are the advantages of such an approach, including simple and quick implementation, and the resulting time savings.

Experimental

Chemicals

- Deionized water, 18.2 MΩ·cm resistivity or higher
- Fisher Scientific™ Methanol, Optima™ LC/MS grade (P/N A456-212)
- Fisher Scientific™ Sodium phosphate dibasic anhydrous (P/N BP332-500)
- Fisher Scientific™ Potassium dihydrogen orthophosphate for HPLC (P/N P/4806/50)
- Acetaminophen was purchased from a reputable vendor.

Equipment

- Vials (amber, 2 mL), Fisher Scientific (P/N 11545884)
- Snap Cap with Septum (Silicone/PTFE), Fisher Scientific (P/N 10547445)

Preparation of standards

The specification limit according to the USP assay method is 100 µg/mL (=100%).

A stock solution of the acetaminophen reference standard was prepared at 1 mg/mL in methanol.

See Table 1 for details on the standards used during the validation procedure.

Table 1. Standard solutions used during method validation with the corresponding eWorkflows; all standards are prepared in methanol.

Conc. [µg/mL]	% of Specification Limit	Chromeleon eWorkflow	Comments
0.02	0.02	ICH LOD LOQ (SN)	
0.05	0.05	ICH LOD LOQ (SN)	
25	25	ICH Linearity	
50	50	ICH Linearity	
70	70	ICH Accuracy	Three individual preparations
75	75	ICH Linearity	
100	100	ICH Linearity	Six individual preparations from analyst 1; six individual preparations from analyst 2
		ICH Specificity (RS)	
		ICH Accuracy	
		ICH Repeatability	
		ICH Intermediate Precision	
		ICH Robustness	
125	125	ICH Linearity	
150	150	ICH Linearity	
		ICH Specificity (PP)	
130	130	ICH Accuracy	Three individual preparations

Instrumentation

A Thermo Scientific™ Vanquish™ Core Quaternary HPLC system was used for the analysis:

- Thermo Scientific™ Vanquish™ System Base Core (P/N VC-S01-A)
- Thermo Scientific™ Vanquish™ Quaternary Pump C (P/N VC-P20-A)
- Thermo Scientific™ Vanquish™ Sampler CT (P/N VC-A12-A)
- Thermo Scientific™ Vanquish™ Column Compartment C (P/N VC-C10-A-03)
- Thermo Scientific™ Vanquish™ Diode Array Detector CG VC-D11-A with standard flow cell, 13 µL (P/N 6083.0510)

Table 2. Chromatographic conditions

Parameter	Value
Column	Thermo Scientific™ Hypersil GOLD™ C8, 100 x 4.6 mm, 3 μm (P/N 25203-104630)
Mobile phase	A: 1.7 g/L KH ₂ PO ₄ and 1.8 g/L Na ₂ HPO ₄ in water B: Methanol
Flow rate	1.0 mL/min
Gradient	Time (min) %B
	0.0 1
	3.0 1
	7.0 81
	7.1 1
12.2 1	
Mixer volume	350 + 50 μL
Column temp.	35 °C with passive pre-heater (forced air with fan speed 5)
Sampler temp.	8 °C
UV wavelength	230 nm
UV data collection rate	10 Hz
UV response time	0.05 s
Spectral scan	210–350 nm
Injection volume	5 μL

Data processing and software

The data acquisition, processing, and reporting was done with Chromeleon CDS, version 7.3 software, which included the extension package for ICH method validation.

Description of the workflow

The Chromeleon CDS offers an extension pack for ICH method validation. This package includes eWorkflow procedures with pre-populated injection lists, processing methods, and reports, as well as demo data and user manuals specific to each test (Figure 1).

After launching the eWorkflow procedure, the predefined injection list is displayed (Figure 2). The columns marked in blue can be changed manually, as appropriate, while columns B, F, H, and J are pre-filled reference fields that are required during data processing and reporting. Each test must have a value (e.g., Accuracy (End)) in column J for the last row in the sequence. This needs to be considered when adding rows at the end of the injection list so that the results are correctly reported and exported. It is recommended to insert rows before the last row of the injection list instead of appending them to the end.

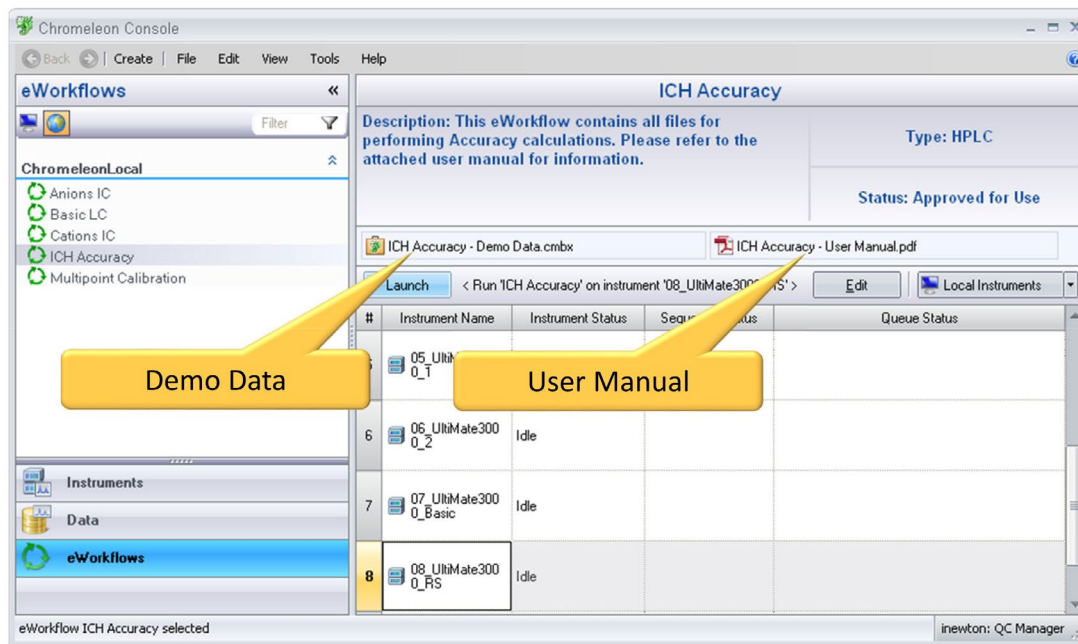


Figure 1. Example eWorkflow procedure in Chromeleon CDS extension pack for ICH accuracy. Each workflow contains demo data and a user manual that are test specific.

#	UV_VIS_↓	Name	Type	Position	Volume [μl]	Instrument Metho	Processing Metho	Status	Inject Time	Level	*TEST	*Range_Level [*Test_End
1		API 100% (Linearity Level 4 standard)	Calibration Standard	R:A5	5.00	Acetaminophen	ICH Accuracy	Finished	12/9/201...	01	Accuracy		
2		API 100% (Linearity Level 4 standard)	Calibration Standard	R:A5	5.00	Acetaminophen	ICH Accuracy	Finished	12/9/201...	01	Accuracy		
3		API 100% (Linearity Level 4 standard)	Calibration Standard	R:A5	5.00	Acetaminophen	ICH Accuracy	Finished	12/9/201...	01	Accuracy		
4		Accuracy_1	Check Standard	R:B5	5.00	Acetaminophen	ICH Accuracy	Finished	12/10/20...	06	Accuracy	70.0000	
5		Accuracy_1	Check Standard	R:B6	5.00	Acetaminophen	ICH Accuracy	Finished	12/10/20...	06	Accuracy	70.0000	
6		Accuracy_1	Check Standard	R:B7	5.00	Acetaminophen	ICH Accuracy	Finished	12/10/20...	06	Accuracy	70.0000	
7		Accuracy_2	Check Standard	R:C4	5.00	Acetaminophen	ICH Accuracy	Finished	12/10/20...	07	Accuracy	100.0000	
8		Accuracy_2	Check Standard	R:C5	5.00	Acetaminophen	ICH Accuracy	Finished	12/10/20...	07	Accuracy	100.0000	
9		Accuracy_2	Check Standard	R:C6	5.00	Acetaminophen	ICH Accuracy	Finished	12/10/20...	07	Accuracy	100.0000	
10		Accuracy_3	Check Standard	R:D1	5.00	Acetaminophen	ICH Accuracy	Finished	12/10/20...	08	Accuracy	130.0000	
11		Accuracy_3	Check Standard	R:D2	5.00	Acetaminophen	ICH Accuracy	Finished	12/10/20...	08	Accuracy	130.0000	
12		Accuracy_3	Check Standard	R:D3	5.00	Acetaminophen	ICH Accuracy	Finished	12/10/20...	08	Accuracy	130.0000	Accuracy (End)

Figure 2. Screenshot of the injection list for the accuracy workflow. Manual fields to be optionally updated are marked in blue. Reference fields for processing or reporting are marked in red.

After the measurements have been carried out, data evaluation is the next step. The processing method included in each workflow must be adapted manually. Details are shown in Figure 3. User defined adjustments on peak integration can be made under the tab “Detection”, either by running the automatic Chromeleon CDS peak integration algorithm, the Cobra Wizard, or by manual definition of criteria (A). Typically, the Cobra Wizard provides very good integration. Defining additional integration rules is only required during fine-tuning. Details about the target analytes, such as name and retention time window, as well

as the specific evaluation criteria, can be set under the tab “Component Table” (B). The desired recovery range, over which the validation must be successful, can be set as Lower_Limit and Upper_Limit for single values (SV) and average values (AV). In addition, the maximum allowed relative standard deviation (Max_RSD), with the number of decimal places (Max_RSD_DP), must be defined. For validation tests that require a calibration curve, the concentrations of the levels defined in the injection list (Figure 2, column G) are set under the tab “Component Table – Advanced” (C).

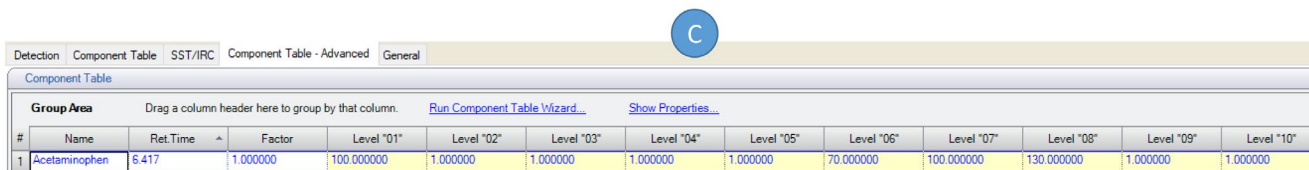
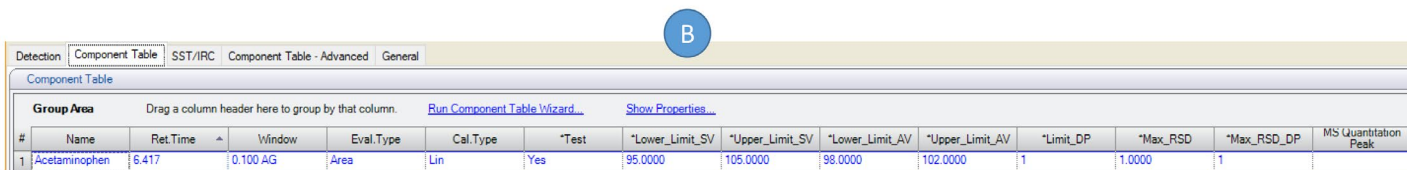
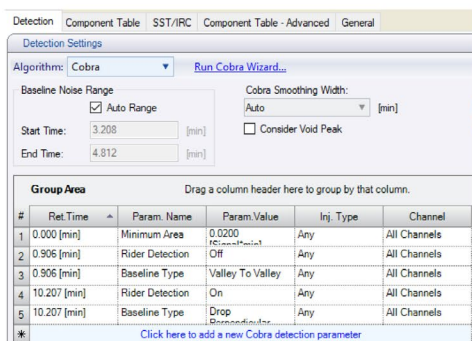


Figure 3. Important parameters and adjustments that can be carried out in the processing method. A: control of peak integration, B: details of target analytes and allowed validation limits, C: information on the concentration of calibration/check samples

After data processing, Chromeleon CDS reports are generated automatically. When this is performed outside of the CDS, it can be very tedious. Each validation report contains at least two sheets. One sheet shows the sequence details (Figure 4), and the other sheet shows the results (Figure 5).

The result page in Figure 5 summarizes the examined validation parameters (e.g., recovery) and shows, in addition to the individual values, a pass/fail result for a quick interpretation of the results obtained.

Validation Report: Accuracy				
Sequence Details				
Sample No.	Sample Name	Sequence Directory	Sequence Name	
1	API 100% (Linearity Level	00_Wrike\2017_STRAT_Projects\2017_STP_004_He	ICH Accuracy 101219094846	
2	API 100% (Linearity Level	00_Wrike\2017_STRAT_Projects\2017_STP_004_He	ICH Accuracy 101219094846	
3	API 100% (Linearity Level	00_Wrike\2017_STRAT_Projects\2017_STP_004_He	ICH Accuracy 101219094846	
4	Accuracy_1	00_Wrike\2017_STRAT_Projects\2017_STP_004_He	ICH Accuracy 101219094846	
5	Accuracy_1	00_Wrike\2017_STRAT_Projects\2017_STP_004_He	ICH Accuracy 101219094846	
6	Accuracy_1	00_Wrike\2017_STRAT_Projects\2017_STP_004_He	ICH Accuracy 101219094846	
7	Accuracy_2	00_Wrike\2017_STRAT_Projects\2017_STP_004_He	ICH Accuracy 101219094846	
8	Accuracy_2	00_Wrike\2017_STRAT_Projects\2017_STP_004_He	ICH Accuracy 101219094846	
9	Accuracy_2	00_Wrike\2017_STRAT_Projects\2017_STP_004_He	ICH Accuracy 101219094846	
10	Accuracy_3	00_Wrike\2017_STRAT_Projects\2017_STP_004_He	ICH Accuracy 101219094846	
11	Accuracy_3	00_Wrike\2017_STRAT_Projects\2017_STP_004_He	ICH Accuracy 101219094846	
12	Accuracy_3	00_Wrike\2017_STRAT_Projects\2017_STP_004_He	ICH Accuracy 101219094846	

Figure 4. Overview sheet of the accuracy report showing the sequence details

Accuracy										
Component Details										
Component Name	Acetaminophen									
Lower Limit (Single Values)	95.0000									
Upper Limit (Single Values)	105.0000									
Lower Limit (Average Values)	95.0000									
Upper Limit (Average Values)	105.0000									
Limit DP	1									
Maximum RSD	2.0000									
Maximum RSD DP	1									
Accuracy Results										
No.	Name	RANGE_LEVEL %	Expec. Amount	Calc. Amount	Recovery %	Average	RSD %	Pass/Fail (Recovery) (Single Values)	Pass/Fail (Recovery) (Average Values)	Pass/Fail (RSD)
			UV_VIS_1	UV_VIS_1	UV_VIS_1					
			Acetaminophen	Acetaminophen	Acetaminophen					
4	Accuracy_1	70.0000	70.0000	69.7603	99.6576			Pass		
5	Accuracy_1	70.0000	70.0000	70.2717	100.3881			Pass		
6	Accuracy_1	70.0000	70.0000	70.1363	100.1947	100.0801	0.3782	Pass	Pass	Pass
7	Accuracy_2	100.0000	100.0000	102.4724	102.4724			Pass		
8	Accuracy_2	100.0000	100.0000	101.6460	101.6460			Pass		
9	Accuracy_2	100.0000	100.0000	102.5287	102.5287	102.2157	0.4835	Pass	Pass	Pass
10	Accuracy_3	130.0000	130.0000	125.7911	96.7624			Pass		
11	Accuracy_3	130.0000	130.0000	125.1111	96.2393			Pass		
12	Accuracy_3	130.0000	130.0000	126.0048	96.9268	96.6428	0.3715	Pass	Pass	Pass
Tested Accuracy Range										
From 70% to 130%										

Figure 5. Reporting page with component details (A) and results (B). The pass/fail notification allows for a quick assessment of the obtained results.

For some of the tests (e.g., for peak purity or linearity) a graphical presentation of the results is included in the report (Figure 6). The linearity test was performed for the entire concentration range expected for the USP assay method for acetaminophen in the range of 25–150% of the specification limit. Some methods require two linearity

tests, one for the active pharmaceutical ingredient (API) and another one for the impurities, which normally must be detected in a much narrower and lower concentration range. Because of this, a second linearity workflow (ICH Linearity-2) is available, with a predefined concentration range of 0.1–1.5% of the specification limit.

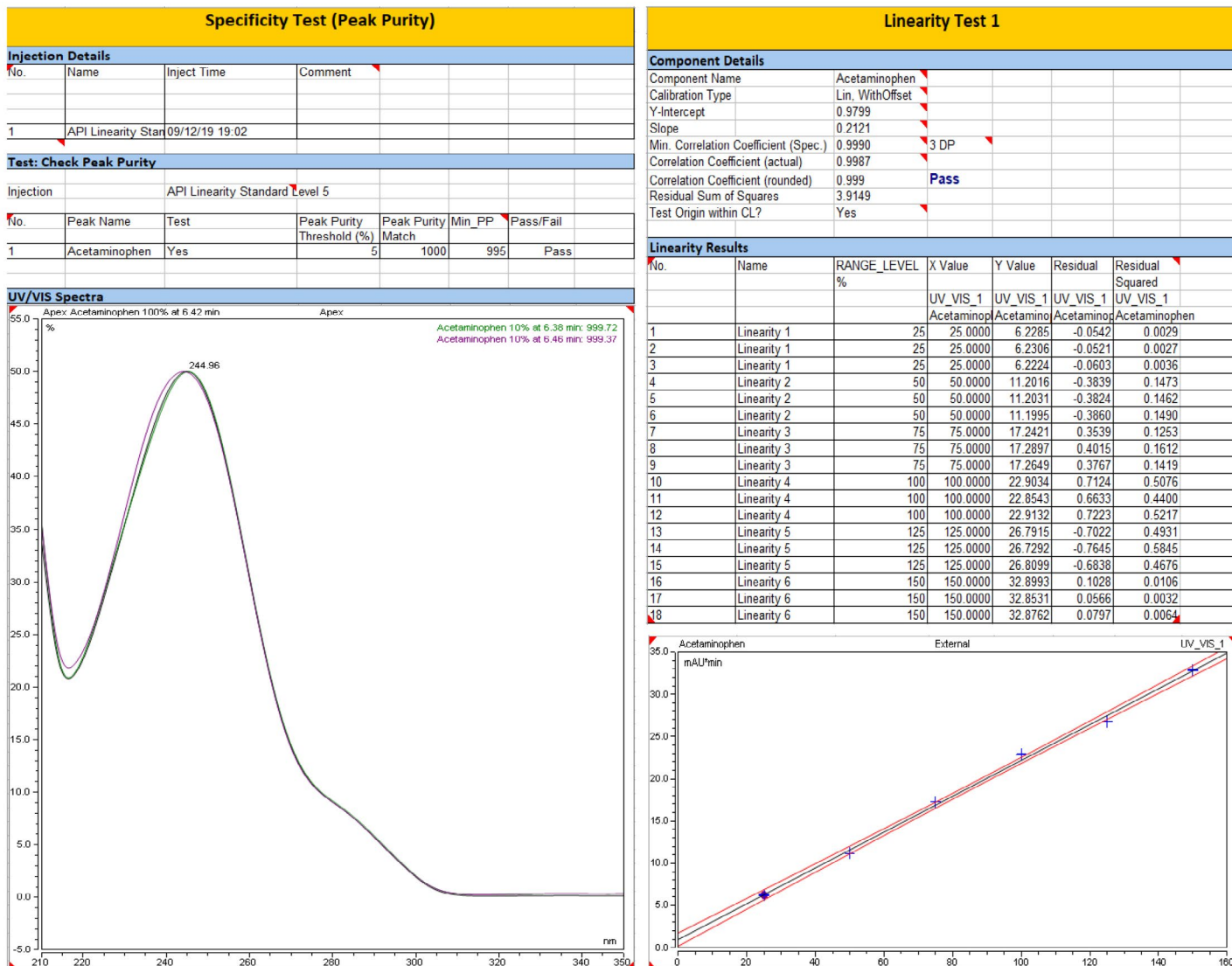


Figure 6. Report sheets with additional graphical data evaluation. Spectral details are shown for the peak purity report (left side), and calibration curve for linearity test (right side).

As a last step, Chromeleon CDS offers export of results in the most common file formats, as can be seen in Figure 7.

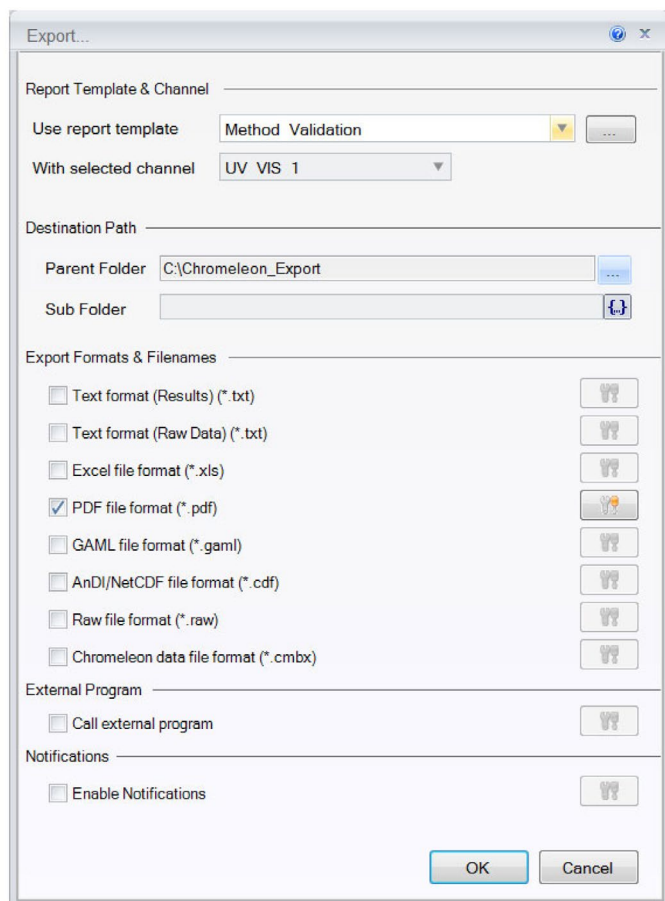


Figure 7. Result export options in the most common file formats

Individual reports of each test can be exported, but there is also one overall report using the query option in Chromeleon CDS. Details on how to combine reports can be found in each of the user manuals. The query option can also be used for report generation of intermediate precision and robustness because these tests require more than one sequence, and therefore should be combined into one report.

Results and discussion for acetaminophen validation

Method validation of an assay for acetaminophen was carried out for accuracy, precision, specificity, linearity,

and range as recommended by ICH, Q2 (R1) guidelines.³ This study also determined the limit of detection (LOD) and limit of quantification (LOQ) to demonstrate the ability to detect impurities contained in the sample at lower concentrations. A combined report for this application note can be found in the Thermo Scientific™ AppsLab Library⁵ in the download section of the application note.

For intermediate precision, the injection list was run twice, first for analyst 1 on day 1, and second for analyst 2 on day 2. Precision results from one analyst yielded a “pass” outcome while results from the other yielded a “fail” outcome. This is clearly reflected in the reports.

Robustness was first determined for temperature variations of ± 10 °C of the original method, and subsequently for flow rate with $\pm 10\%$ variation. A total of six sequences were run, three for each parameter.

Additionally, measurements on LOD and LOQ were performed to determine if the unknown impurities present in the sample can be accurately quantified with this method. The Chromeleon CDS extension pack offers three different options for the determination of LOD and LOQ; these options correspond with those outlined in the ICH guidelines. The three test options are based on signal-to-noise ratio (ICH LOD LOQ (SN)), the standard deviation of the blank (ICH LOD LOQ (Blank SD)), or the calibration curve (ICH LOD LOQ (Curve)). In this study, the LOD/LOQ measurements were performed based on the signal-to-noise ratio by comparing the signal of the API peak with a known low concentration (at the specification limit of the impurities described in the USP method) with the noise in the blank sample. The LOD and LOQ were determined as 13.66 ng/mL (0.014%), and 45.52 ng/mL (0.045%), respectively.

After complete validation, Chromeleon CDS eWorkflow procedures can save a tremendous amount of time compared to non-templated workflows and processes in which each sequence is set up and run individually and the data are evaluated manually in a separate spreadsheet program (Table 3).

Table 3. Estimated manual effort on each validation step

Validation step	Sequence		Data processing		Report of results	
	Non-templated	Chromeleon CDS templated	Non-templated	Chromeleon CDS templated	Non-templated	Chromeleon CDS templated
Manual Effort	High	Low	Very high	Low	Huge	Very low

Conclusion

The Chromeleon CDS extension pack for ICH validation:

- Significantly shortens data processing and reporting times
- Reduces susceptibility to errors
- Allows a quick and easy implementation of a full method validation with automated sequence generation, data evaluation, and reporting
- Reduces the time and effort to validate methods transferred to a new HPLC instrument
- Is fully regulatory compliant

References

1. European Directorate for the Quality of Medicines & HealthCare, Validation of Analytical Procedures, PA/PH/OMCL (13) 82 2R, **2014**.
2. United States Pharmacopeia, Chapter <1225>, Validation of Compendial Procedures.
3. International Conference on Harmonization (ICH), Q2 (R1), Guidance on Validation of Analytical Procedures: Text and Methodology, CPMP/ICH/381/95, **1995**.
4. United States Pharmacopeia USP40-NF35 S1, Acetaminophen assay method, The United States Pharmacopeia Convention, **2017**.
5. Thermo Scientific Appslab Library: <https://appslab.thermofisher.com/>

Find out more at thermofisher.com/chromeleon