[APPLICATION NOTE]

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Increasing Efficiency of Method Validation for Metoclopramide HCl and Related Substances with Empower 3 MVM Software

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APPLICATION BENEFITS

- Automated method validation workflow
- Reduced time to complete the steps required to test and document a validated method
- Compliance with regulations on data security, different user privileges, audit trails, data traceability, and electronic signature sign-off requirements

WATERS SOLUTIONS

ACQUITY UPLC® H-Class System

Empower® 3
Chromatography Data Software

Empower 3
Method Validation Manager (MVM)

ACQUITY UPLC Columns

ACQUITY® QDa® Detector

KEY WORDS

UPLC, method validation, metoclopramide HCl, sub-2-µm particles, mass detection, ApexTrack™ peak integration

INTRODUCTION

Method validation, which demonstrates that a method is suitable for its intended purpose,[1,2] is an important regulatory requirement for pharmaceutical organizations and their supporting contract partners. A compliant laboratory must provide documented evidence and assurance that the analytical method used for testing a drug product's identity, quality, purity, and potency generates accurate and reliable results.

The validation process of an analytical method is a complex and demanding activity, consisting of many time-consuming steps. Some of these steps include creation of validation protocols, experimental work, reviewing and processing data, performing calculations, approving, and final reporting. Since some of these steps are prone to errors, a well-organized plan is essential for successful validation of an analytical method and to ensure that the appropriate regulations and guidelines are being followed.

Once validation is executed, adherence to the validation plan and specification is a critical compliance requirement. Any validation results not meeting the specifications must be clearly identified and addressed during the validation process. Raw and processed data must be appropriately stored and traceable by providing data security, audit trails, and automatic data documentation required for reviews and audits.

In this application note, we present validation of a UPLC® method for metoclopramide HCl and related substances using Empower 3 Method Validation Manager (MVM), an option for Empower 3 Chromatography Data Software.

We show how Empower 3 MVM tracked every step of the method validation process, identifying the steps and data that did not meet defined validation requirements.

Overall, we demonstrate that Empower 3 MVM automates the method validation workflow within a single software environment, reducing time and ensuring conformance to the validation requirements and acceptance criteria defined in the protocol.

EXPERIMENTAL

Method conditions

LC system:	ACQUITY UPLC H-Class
Column:	ACQUITY UPLC CSH C_{18}, 2.1 x 50 mm, 1.7 µm (Waters p/n 186005296)
Column temp.:	45 °C
Injection volume:	1.0 µL
Flow rate:	0.6 mL/min
Solvent A:	125 mM Formic acid in water
Solvent B:	Water
Solvent C:	Methanol
Separation:	Gradient

Step	Time (minutes)	Solvent A (%)	Solvent B (%)	Solvent C (%)
1	Initial	10	85.0	5.0
2	5.0	10	30.0	60.0
3	5.5	10	30.0	60.0
4	5.6	10	85.0	5.0
5	7.5	10	85.0	5.0

Purge/Sample wash:	50:50 water/methanol
Seal wash:	90:10 water/acetonitrile
UV detector:	ACQUITY UPLC PDA
UV setting:	210-400 nm (derived at 270 nm)
Mass detector:	ACQUITY QDa (Extended Performance)
Ionization mode:	ESI+, ESI-
Acquisition range:	100 – 440 *m/z*
Sampling rate:	10 pts/sec
Data:	Centroid
System control, Data acquisition, and analysis:	Empower 3 FR2 CDS Software

RESULTS AND DISCUSSION

UPLC method for metoclopramide HCl and related substances

The UPLC method validated in this study was developed using a systematic method development protocol.[3] An example of the UPLC chromatographic method for metoclopramide and related compounds is shown in Figure 1.

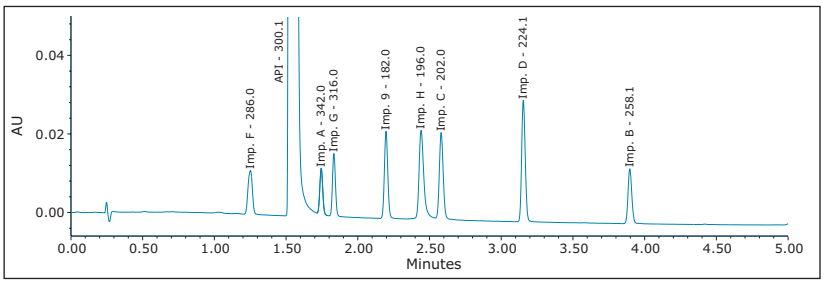


Figure 1. UPLC method for metoclopramide and related substances with UV at 270 nm.

About Empower 3 MVM

Empower 3 Method Validation Manager (MVM) is software that automates the validation process and enables efficient validation of chromatographic methods to ensure compliance to the validation requirements defined by the analytical laboratory. As shown in Figure 2, a validation workflow consists of many steps. A validation protocol (Figure 3) is created and used to execute the study. Once executed, Empower 3 MVM checks data for adherence with the validation requirements and flags any results that do not meet specifications. Validation results can be displayed in a report using validation report templates specific for each test available in Empower MVM software. The report templates can be customized as needed.

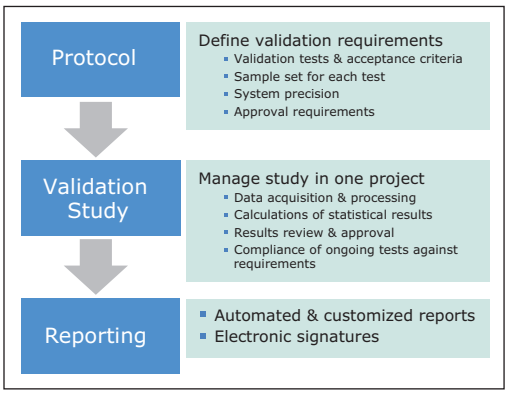
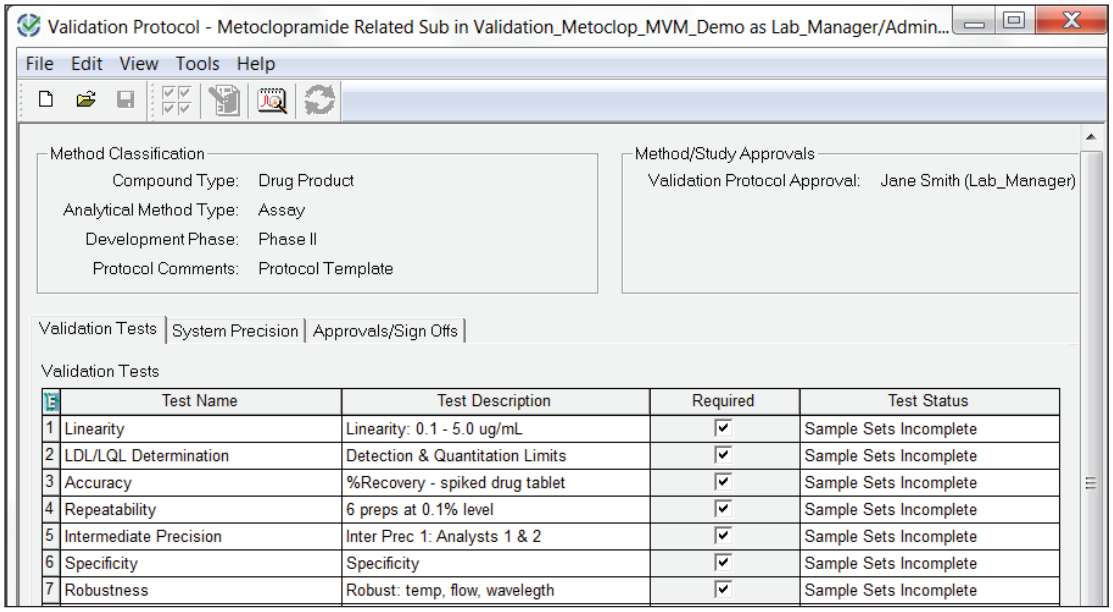


Figure 2. Workflow for validation of chromatographic methods with Empower 3 MVM.



	Test Name	Test Description	Required	Test Status
1	Linearity	Linearity: 0.1 - 5.0 ug/mL	☑	Sample Sets Incomplete
2	LDL/LQL Determination	Detection & Quantitation Limits	☑	Sample Sets Incomplete
3	Accuracy	%Recovery - spiked drug tablet	☑	Sample Sets Incomplete
4	Repeatability	6 preps at 0.1% level	☑	Sample Sets Incomplete
5	Intermediate Precision	Inter Prec 1: Analysts 1 & 2	☑	Sample Sets Incomplete
6	Specificity	Specificity	☑	Sample Sets Incomplete
7	Robustness	Robust: temp, flow, wavelegth	☑	Sample Sets Incomplete

Figure 3. Validation protocol method created within Empower 3 MVM project. The validation tests, acceptance criteria for each validation test, and requirements for approval are defined in the validation protocol method. The validation protocol is approved by a lab manager via electronic signature.

Method validation

We used an established validation protocol method approved by a lab manager (via electronic signatures) to execute the validation of our UPLC method for related substances of metoclopramide HCl in drug tablet formulation. The validation tests included linearity, detection and quantitation limits, accuracy, repeatability, intermediate precision, specificity, and robustness. System precision was evaluated for each validation test using five replicate injections of the sample, as recommended in the USP General Chapter <621> on Chromatography.[4] The system precision criteria include:

- %RSD of retention times: ≤1.0%
- %RSD of peak areas: ≤2.0%
- USP resolution: ≥1.5
- Peak tailing: ≤1.5

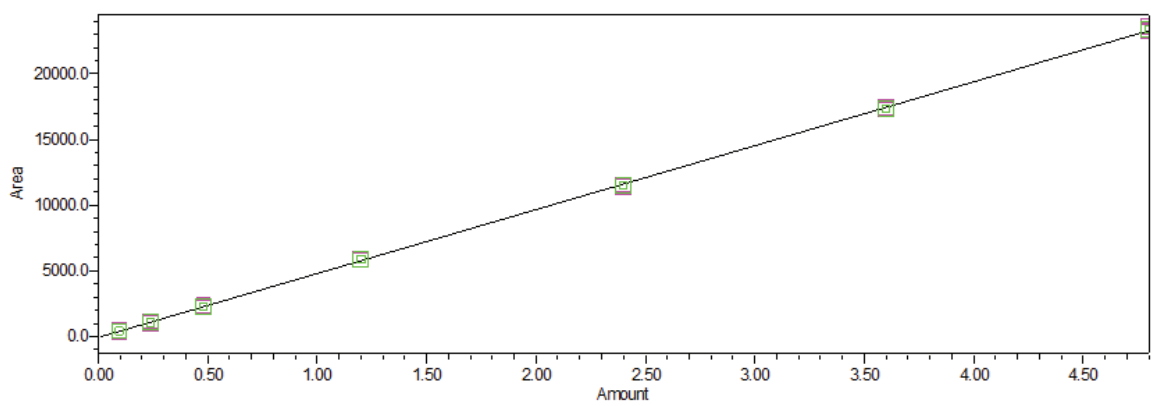
1. Linearity

Method linearity for related substances was evaluated by analyzing seven concentrations of standard solutions ranging from 0.1 to 5.0 µg/mL. These concentrations corresponded to 0.02, 0.05, 0.1, 0.25, 0.5, 0.75, and 1.0% of the metoclopramide HCl target concentration of 0.5 mg/mL. We used Empower 3 MVM to calculate regression equation and correlation coefficients for a plot of average peak areas against the concentrations. Method linearity results generated by the software are displayed in Figure 4. The method shows linear relationship between the peak areas and concentrations for all related compounds with the correlation coefficients (r^2) greater than 0.999.

Validation_Linearity Report

Validation Protocol: Impurities_Meth_Val_Study Validation Study Id: 1299
Validation Protocol ID: 22180 Channel: PDA 270

	Comp.	Points /Level	X Value	X Value Units	Y Value	Equation	R	R^2	Intercept	Residual % RSD	Pass?
1	Imp. F	3	Amount	ug/mL	Response	Y = 4.87e+003 X - 7.11e+001	0.99990	0.99980	-71	1.39	Pass
2	Imp. A	3	Amount	ug/mL	Response	Y = 3.24e+003 X + 1.73e+002	0.99987	0.99975	173	1.51	Pass
3	Imp. G	3	Amount	ug/mL	Response	Y = 4.40e+003 X - 1.81e+001	0.99989	0.99978	-18	1.43	Pass
4	Imp. 9	3	Amount	ug/mL	Response	Y = 7.31e+003 X - 2.45e+001	0.99988	0.99976	-25	1.52	Pass
5	Imp. H	3	Amount	ug/mL	Response	Y = 9.01e+003 X - 2.23e+002	0.99988	0.99976	-223	1.52	Pass
6	Imp. C	3	Amount	ug/mL	Response	Y = 6.08e+003 X - 3.62e+001	0.99991	0.99981	-36	1.33	Pass
7	Imp. D	3	Amount	ug/mL	Response	Y = 9.04e+003 X - 1.06e+001	0.99992	0.99984	-11	1.22	Pass
8	Imp. B	3	Amount	ug/mL	Response	Y = 4.68e+003 X + 2.23e+001	0.99987	0.99974	22	1.58	Pass



Component Imp. F; Equation Y = 4.87e+003 X - 7.11e+001; R^2 0.999799

Figure 4. Method linearity results for metoclopramide related substances generated by Empower 3 MVM.

2. Detection and quantitation limits

We determined the lowest detection and quantitation limits (LDL and LQL) based on the USP signal-to-noise criteria of 3:1 and 10:1, respectively. In addition to signal-to-noise, other methods for LDL and LQL determination are available within Empower 3 MVM, such as based on linearity curve residual standard deviation and linearity curve intercept standard deviation.

As shown in Figure 5, the LDL and LQL for related substances ranged from 0.03 to 0.07 µg/mL and from 0.1 to 0.2 µg/mL, respectively.

Validation_LDL_LQL

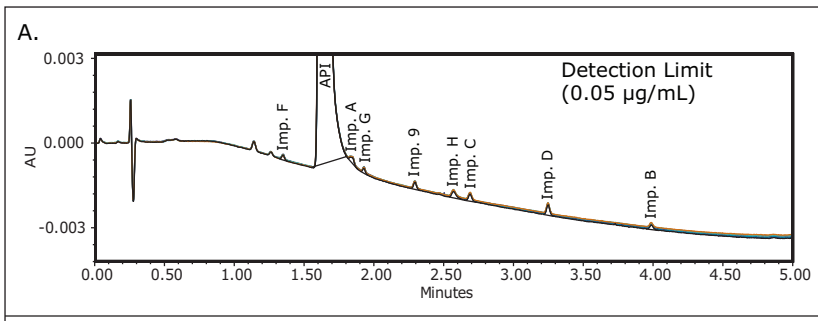
Validation Protocol: Impurities_Meth_Val_Study Validation Study Id: 1299
Validation Protocol ID: 22841 Channel: PDA 270

LDL and LQL Determination

	Component	RT Mean (min)	LDL/LQL Method	Signal/Noise Type	Lowest Detection Limit	Lowest Quantitation Limit	Units
1	Imp. F	1.305	Signal to Noise	USP s/n	0.0563	0.1706	ug/mL
2	Imp. A	1.791	Signal to Noise	USP s/n	0.0732	0.2219	ug/mL
3	Imp. G	1.889	Signal to Noise	USP s/n	0.0534	0.1618	ug/mL
4	Imp. 9	2.241	Signal to Noise	USP s/n	0.0376	0.1140	ug/mL
5	Imp. H	2.479	Signal to Noise	USP s/n	0.0436	0.1321	ug/mL
6	Imp. C	2.627	Signal to Noise	USP s/n	0.0504	0.1528	ug/mL
7	Imp. D	3.178	Signal to Noise	USP s/n	0.0312	0.0945	ug/mL
8	Imp. B	3.907	Signal to Noise	USP s/n	0.0657	0.1991	ug/mL

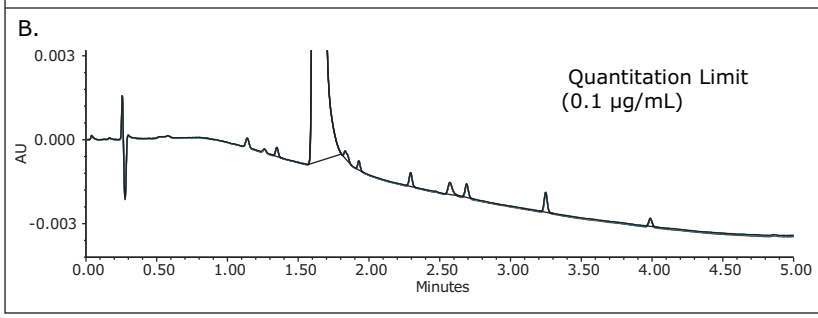
Figure 5. Lowest detection and quantitation concentrations determined using USP signal-to-noise criteria.

We then validated the results by analyzing six replicate injections of the solutions prepared near the LDL (0.05 µg/mL) and LQL (0.1 µg/mL) to verify the performance, Figure 6. The LDL and LQL replicates tested in this study exceeded the USP signal-to-noise criteria



System Suitability
Sample Set ID: 8149
Result Set ID: Result Set Id 8518
Channel Name: PDA Ch1 270nm@4.8nm
Sample: 0.05 ug/mL soln

	Name	# of Inj.	%RSD RT	%RSD Peak Areas	Ave US P s/n
1	Imp. F	6	0.06	7.34	9.5
2	Imp. A	6	0.05	2.50	11.6
3	Imp. G	6	0.06	4.69	9.6
4	Imp. 9	6	0.03	4.52	14.4
5	Imp. H	6	0.03	4.37	12.9
6	Imp. C	6	0.03	1.93	14.1
7	Imp. D	6	0.02	2.85	21.1
8	Imp. B	6	0.03	5.05	8.3



System Suitability
Sample Set ID: 8149
Result Set ID: Result Set Id 8670
Channel Name: PDA Ch1 270nm@4.8nm
Sample: 0.1 ug/mL soln

	Name	# of Inj.	%RSD RT	%RSD Peak Areas	Ave US P s/n
1	Imp. F	6	0.06	3.07	15.6
2	Imp. A	6	0.04	3.38	12.6
3	Imp. G	6	0.03	1.47	15.9
4	Imp. 9	6	0.03	3.35	24.0
5	Imp. H	6	0.03	3.65	21.3
6	Imp. C	6	0.03	2.70	23.9
7	Imp. D	6	0.03	2.34	34.8
8	Imp. B	6	0.02	3.07	13.9

Figure 6. Overlay of six replicate injections of LDL and LQL solutions. The ApexTrack enabled consistent integration of all the peaks.

A. All components exceeded signal-to-noise criteria of 3:1 for detection.

B. All components exceeded signal-to-noise criteria of 10:1 for quantitation.

3. Accuracy

The accuracy of an analytical method includes quantitative determination of impurities in the presence of sample matrix components. Accuracy of our method was assessed by analyzing triplicate preparations of the drug tablet solutions spiked with related substances at 0.1, 0.5, and 1.0% levels in the presence of metoclopramide HCl concentration of 0.5 mg/mL. Accuracy results are summarized in Figure 7. The % recovery for all nine determinations ranged from 97 to 101% with %RSD ≤4.21%, which passes the acceptance criteria of 90–110% and %RSD ≤10%, respectively.

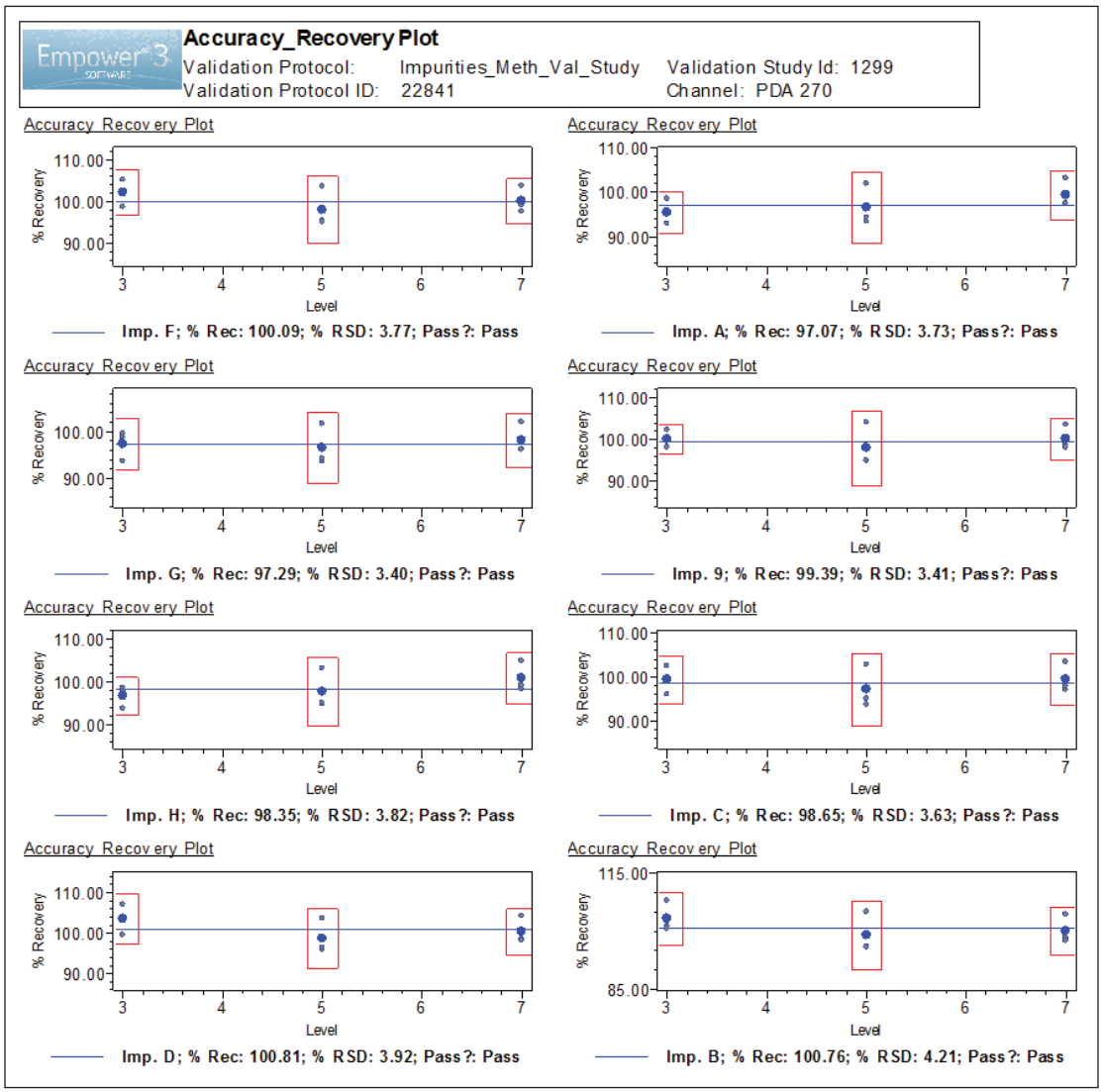


Figure 7. Accuracy results generated by Empower 3 MVM. Summary of 9 determination, 3 preparation at each levels: 0.1%, 0.5%, and 1.0%.

4. Repeatability

Method repeatability is a quantitative analysis of impurities from six independent preparations of the drug product by a single analyst. Repeatability of our method was demonstrated by spiking drug tablet sample solution with related substances at 0.1% level with respect to the metoclopramide HCl (API) concentration of 0.5 mg/mL. Repeatability results generated by analyst A (Figure 8) met the criteria for % recovery of 100 ±10% and %RSD ≤10%.

5. Intermediate precision

Intermediate precision was evaluated by a different analyst, on a different day, using different instrument and column. Summary of results from six individual spiked drug tablet sample preparations generated by analysts A and B are shown in Figure 8. Overall, the intermediate precision results met the criteria for % recovery of 100 ±10% and %RSD ≤10%.

Intermediate Precision_Validation Results
Validation Protocol: Impurities_Meth_Val_Study Validation Study Id: 1299
Validation Protocol ID:11903 Channel: PDA Ch1 270nm@4.8nm, PDA

Component: Imp. A Validation Result Id: 9208

	Component	RT Mean (min)	Experiment Group	Points	%Recovery Mean	Std. Dev.	%RSD of %Recovery
1	Imp. A	1.792	Analyst A	6	94.8	2.31	2.44
2	Imp. A	1.825	Analyst B	6	96.6	2.05	2.13

Component: Imp. G Validation Result Id: 9209

	Component	RT Mean (min)	Experiment Group	Points	%Recovery Mean	Std. Dev.	%RSD of %Recovery
1	Imp. G	1.890	Analyst A	6	97.3	3.45	3.54
2	Imp. G	1.922	Analyst B	6	96.8	1.60	1.66

Component: Imp. 9 Validation Result Id: 9210

	Component	RT Mean (min)	Experiment Group	Points	%Recovery Mean	Std. Dev.	%RSD of %Recovery
1	Imp. 9	2.248	Analyst A	6	99.8	2.21	2.21
2	Imp. 9	2.305	Analyst B	6	98.8	1.17	1.18

Component: Imp. F Validation Result Id: 9285

	Component	RT Mean (min)	Experiment Group	Points	%Recovery Mean	Std. Dev.	%RSD of %Recovery
1	Imp. F	1.311	Analyst A	6	101.4	2.62	2.58
2	Imp. F	1.343	Analyst B	6	101.8	1.40	1.38

Component: Imp. C Validation Result Id: 9212

	Component	RT Mean (min)	Experiment Group	Points	%Recovery Mean	Std. Dev.	%RSD of %Recovery
1	Imp. C	2.636	Analyst A	6	99.5	2.05	2.06
2	Imp. C	2.696	Analyst B	6	96.9	0.89	0.92

Component: Imp. D Validation Result Id: 9213

	Component	RT Mean (min)	Experiment Group	Points	%Recovery Mean	Std. Dev.	%RSD of %Recovery
1	Imp. D	3.186	Analyst A	6	102.4	3.28	3.20
2	Imp. D	3.254	Analyst B	6	97.4	1.15	1.18

Component: Imp. B Validation Result Id: 9214

	Component	RT Mean (min)	Experiment Group	Points	%Recovery Mean	Std. Dev.	%RSD of %Recovery
1	Imp. B	3.914	Analyst A	6	102.2	3.35	3.28
2	Imp. B	3.996	Analyst B	6	100.0	2.21	2.22

Component: Imp. H Validation Result Id: 9211

	Component	RT Mean (min)	Experiment Group	Points	%Recovery Mean	Std. Dev.	%RSD of %Recovery
1	Imp. H	2.489	Analyst A	6	96.5	2.57	2.66
2	Imp. H	2.574	Analyst B	6	102.0	1.46	1.43

Figure 8. Repeatability (analyst A) and intermediate precision (analysts A and B) results generated by Empower 3 MVM.

6. Specificity

For the impurity test, specificity demonstrates that impurities can be separated and accurately measured in the presence of the sample matrix. This is typically done by spiking a drug substance or drug product with appropriate levels of impurities. In addition to demonstrating robust and reliable separation, it is important to identify that the desired components are not subject to interference with other species present in the sample. The UV peak purity determination is often used to show homogeneity of the chromatographic peak.

Specificity of our method was demonstrated by spiking drug tablet samples containing 0.5 mg/mL of metoclopramide HCl with related substances at 0.1% level. Accuracy and repeatability results show acceptable recoveries of each related substance. To demonstrate that the related substances are not coeluting with other components of the sample matrix, we used UV in conjunction with the MS spectral data as shown in Figure 9. Peak homogeneity was assessed using UV peak purity plot (Figure 9B). The peak purity angle is below the threshold angle, indicating the Impurity A peak is spectrally homogeneous. The mass spectral data provided additional information at the leading, apex, and tailing regions of the peak to confirm that only one mass is detected under the UV peak. The MS spectrum (Figure 9C) at the leading and tailing edge of the peak indicates the presence of an ion with mass of 342.0 m/z, which is specific to Impurity A. Overall, the UV peak purity plot and the MS spectrum shows that Impurity A is not coeluting with other peaks.

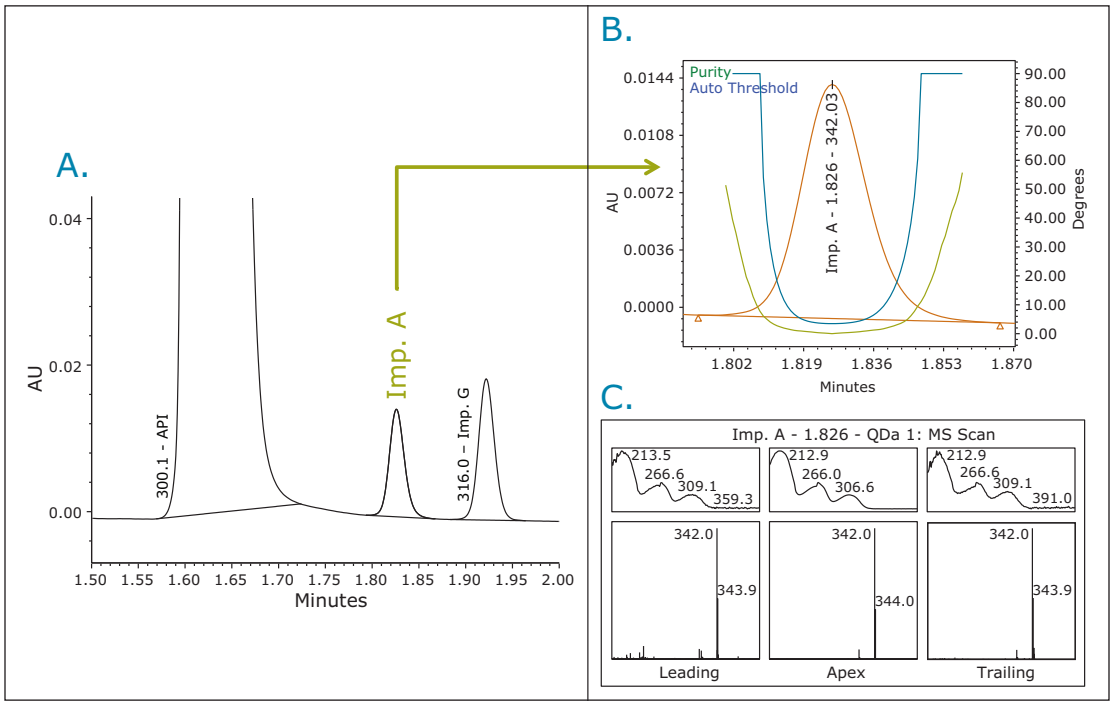


Figure 9. Peak homogeneity determination for specificity.
A. Accuracy sample with UV at 270 nm.
B. Peak purity plot of Impurity A.
C. UV and Mass profile of an Impurity A at the leading, apex, and tailing edge of the peak.

7. Robustness

Robustness is determined by the ability of the method to remain unaffected by the changes of chromatographic parameters. The parameters assessed in our study included:

- Column temperature: 45 ± 2.0 °C
- Flow rate: 0.6 ± 0.05 mL/min
- Wavelength: 270 ± 2 nm

Robustness was performed using a full factorial experimental design to investigate combination of different instrument conditions on the resolution between all the peaks, with a goal of achieving a minimum resolution of ≥2.0 for each peak. The robustness results in Figure 10 showed that the method met the criteria for resolution.

Validation_Robustness_Report

Validation Protocol: Impurities_Meth_Val_Study Validation Study Id: 1299
Validation Protocol ID: 22535 Channel: PDA Ch1 270nm@4.8nm

Assessed Field: USP Resolution

	Validation Result Id	Component	RT Mean (min)	Experiments	Assessed Field	Mean Rs	Lower Limit of Mean Rs	Upper Limit of Mean Rs	Pass /Fail
1	17451	API	1.617	8	USP Resolution	4.8	4.7	4.9	Pass
2	17487	Imp. A	1.825	8	USP Resolution	4.0	3.9	4.2	Pass
3	17489	Imp. G	1.923	8	USP Resolution	2.8	2.4	3.1	Pass
4	17491	Imp. 9	2.312	8	USP Resolution	10.3	10.1	10.5	Pass
5	17493	Imp. H	2.597	8	USP Resolution	6.2	5.5	7.0	Pass
6	17495	Imp. C	2.712	8	USP Resolution	2.4	2.0	2.8	Pass
7	17497	Imp. D	3.267	8	USP Resolution	12.9	12.3	13.4	Pass
8	17499	Imp. B	4.006	8	USP Resolution	17.1	16.6	17.6	Pass

Figure 10. Robustness results. Resolution for each component was ≥2.0.

CONCLUSIONS

We successfully validated the UPLC method for related substances of metoclopramide HCl for linearity, detection and quantitation limits, accuracy, repeatability, intermediate precision, specificity, and robustness using Empower 3 MVM. Validation results showed that the method is linear, accurate, repeatable, precise, specific, and robust for all related substances tested in this study. The quantitation limit of all related compounds was below the reporting threshold of 0.1% or 0.5 µg/mL.

Empower 3 MVM software streamlined the entire validation process, from creating a validation protocol method to acquiring, reviewing, analyzing, approving, and reporting validation data. The updated tool/message center provided status of each validation test in a study and whether the results met the acceptance criteria, and flagged any out-of-specification results. Using ApexTrack for automated peak integration enabled consistent integration of all peaks during the validation process. Using the ACQUITY QDa Detector for mass detection in conjunction with UV detection enabled quick determination of peak purity using mass and UV spectral data. Finally, the validation results and validation study were reviewed and approved via electronic signatures.

Empower 3 MVM is compliant-ready software that can be easily adapted by any analytical laboratory to improve efficiency of the chromatographic method validation process and to ensure conformance to validation requirements.

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