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응용 자료

Streamlined Method Validation for Analysis of Formoterol Fumarate and Budesonide in Metered Dose Inhaler with Empower Method Validation Manager Software

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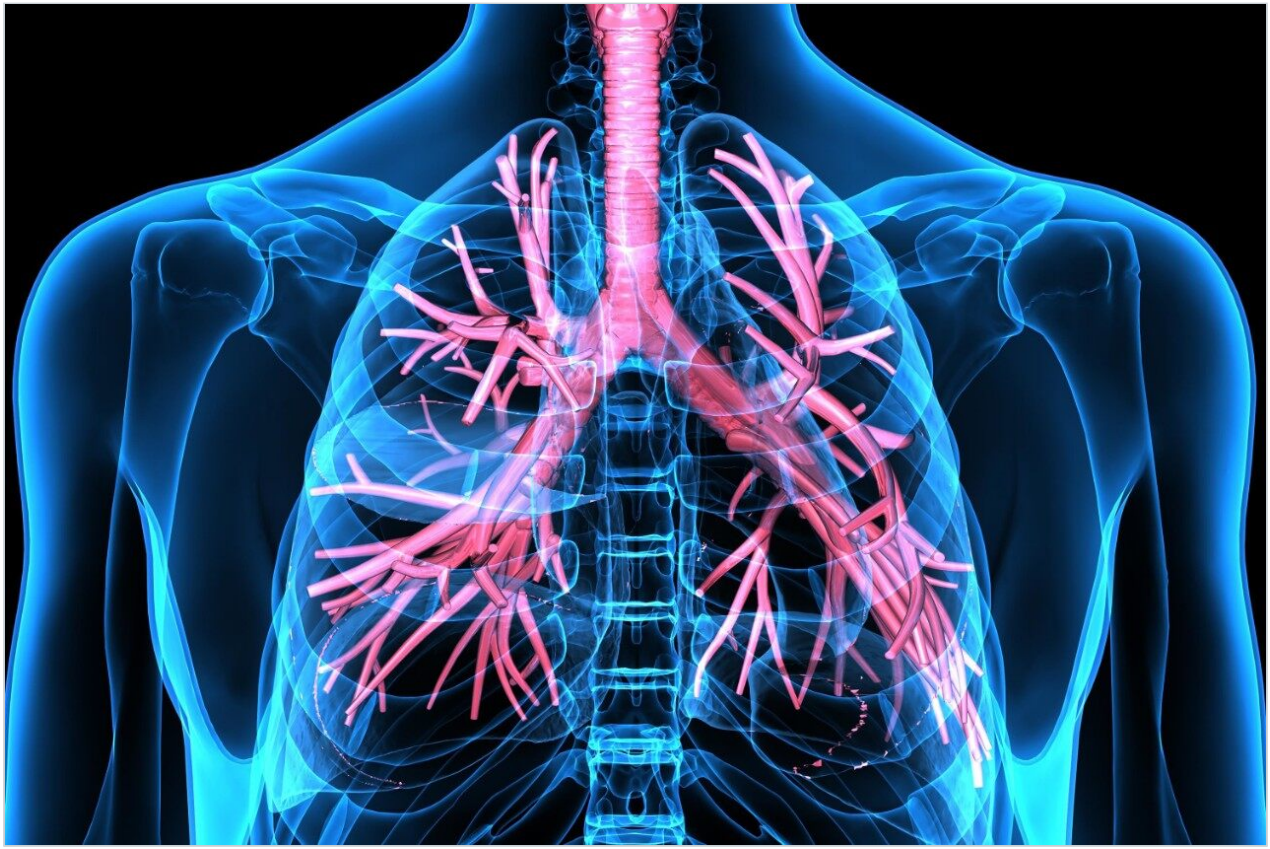

Abstract

This application note presents a validation of UPLC method for formoterol fumarate and budesonide active pharmaceutical ingredients using Empower Method Validation Manager (MVM).

The UPLC method for assay of formoterol fumarate and budesonide drugs was successfully validated for linearity, accuracy, repeatability, intermediate precision, specificity, and robustness. The validation results met the predefined performance requirements, demonstrating that the method is suitable for analysis of the active ingredients in the metered dose inhaler drug product.

Empower 3 MVM Software improved efficiency of the entire validation process and assured adherence to the validation requirements. Statistical evaluation of the data and generation of the validation results were performed within the software, which eliminated the need to export raw data to another software platform for analysis. During the study, Empower MVM tracked every step of the validation process, identifying steps and results that did not meet the defined validation requirements. Utilizing both UV and MS spectral data from an ACQUITY QDa Mass Detector increased confidence and assurance in determination of the chromatographic peak purity.

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Benefits

- Improves method validation workflows with Empower Method Validation Manager (MVM) Software
- Increases conformance of each validation steps with the validation requirements
- Assures compliance with regulations on data integrity, audit trails, data traceability, and electronic signature sign-off review and approvals

Introduction

Regulations and quality standards require analytical methods to be validated before implementation in quality control laboratory for routine testing to ensure quality and safety of the drug products.

The analytical method validation is a complex and demanding process by which laboratory studies are performed to demonstrate that the method generates consistent results with high accuracy and precision for

the intended use.[1,2,3] Validation studies must be executed following a protocol with a description of each validation characteristic and predetermined acceptance criteria. This will ensure compliance with the appropriate regulations and guidelines and assure that the method remains appropriate for testing of the drug products.

In addition, the second stage of the analytical procedure lifecycle approach described in the proposed USP General Chapter <1220>, states that the procedure should be qualified to confirm it generates reproducible values that meet the analytical target profile (ATP) criteria and remain appropriate for testing of the product.[4] The ATP is a set of performance characteristic requirements based on the intended use of the analytical method and may include criteria for accuracy, precision, specificity, and linearity. These method-specific performance attributes are consistent with traditional validation characteristics.

In this application note, we present validation of a UPLC method for formoterol fumarate and budesonide active pharmaceutical ingredients in a metered dose inhaler using Empower Method Validation Manager (MVM) software, an option for Empower 3 Chromatography Data Software. The metered dose inhaler containing formoterol fumarate and budesonide is commonly used for treatment of asthma and chronic obstructive pulmonary disease.[5] The UPLC method was validated for linearity, accuracy, repeatability, intermediate precision, specificity and robustness for analysis of the active ingredients. Employing Empower 3 MVM Software increased efficiency of the entire validation study and assured compliance to the validation requirements.

Experimental

Sample description

Standard solutions

Separate stock solutions were prepared by dissolving budesonide and formoterol fumarate in methanol at 1 mg/mL and 0.1 mg/mL concentration, respectively. The stock solutions were subsequently diluted with diluent (70:30 mobile phase A/acetonitrile) to make a working standard concentration (80/4.5 µg/mL budesonide and formoterol fumarate) and linearity standard solutions described in the study.

Budesonide and formoterol fumarate metered dose inhaler

Symbicort inhaler formulation containing 80/4.5 µg of budesonide and formoterol fumarate per actuation was prepared following a procedure described in the literature5 with two exceptions. Samples were diluted

with diluent containing a 70:30 mobile phase A/acetonitrile and filtered through 0.2-µm PTFE syringe filters prior analysis. The final working sample solution contained 80 µg/mL and 4.5 µg/mL of budesonide and formoterol fumarate, respectively.

LC conditions

LC system:	ACQUITY UPLC H-Class PLUS
Column:	ACQUITY UPLC BEH C_{18} 1.7 µm, 2.1 × 100 mm
Column temp.:	33 °C
Injection volume:	5.0 µL
Flow rate:	0.35 mL/min
Mobile phase A:	20 mM ammonium acetate, pH 8.2 adjusted with ammonium hydroxide
Mobile phase B:	Acetonitrile

Gradient

Step	Time (minutes)	Solvent A (%)	Solvent B (%)
1	Initial	95	5
2	2	95	5
3	27	40	60
4	28	40	60
5	28.1	95	5

Step	Time (minutes)	Solvent A (%)	Solvent B (%)
6	33	95	5

Purge/sample wash solvent:	70:30 water/acetonitrile
Seal wash:	90:10 water/acetonitrile
UV detector:	ACQUITY UPLC PDA, 210–400 nm (derived at 244 nm)

MS conditions

MS detector:	ACQUITY QDa (extended performance)
Ionization mode:	ESI+
Acquisition range:	150–475 *m/z*
Capillary voltage:	0.8 kV (pos/neg)
Cone voltage:	15 V
Data:	Centroid

Results and Discussion

UPLC method for formoterol, budesonide, and budesonide related compounds

The UPLC method validated in this study was developed using a software-assisted Quality by Design (QbD) approach.[6] An example of the UPLC chromatographic separation for formoterol, budesonide, and

budesonide-related compounds is shown in Figure 1.

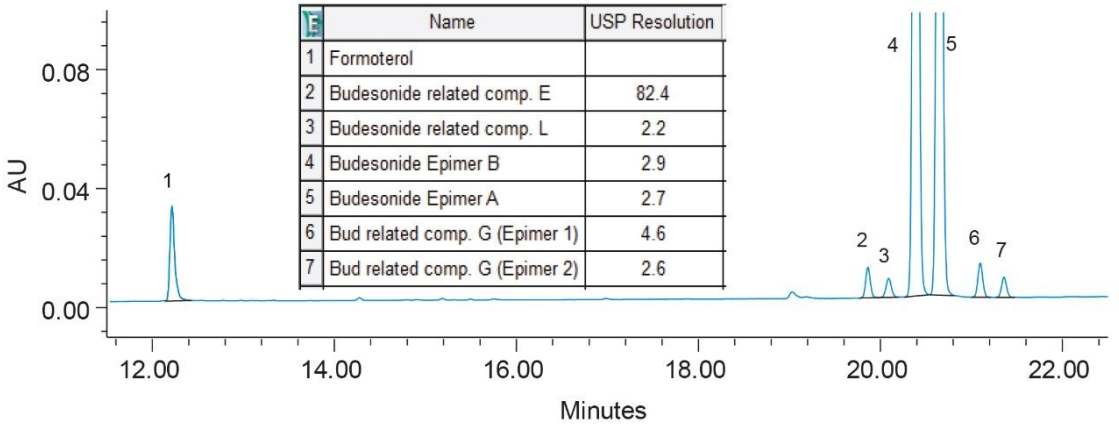


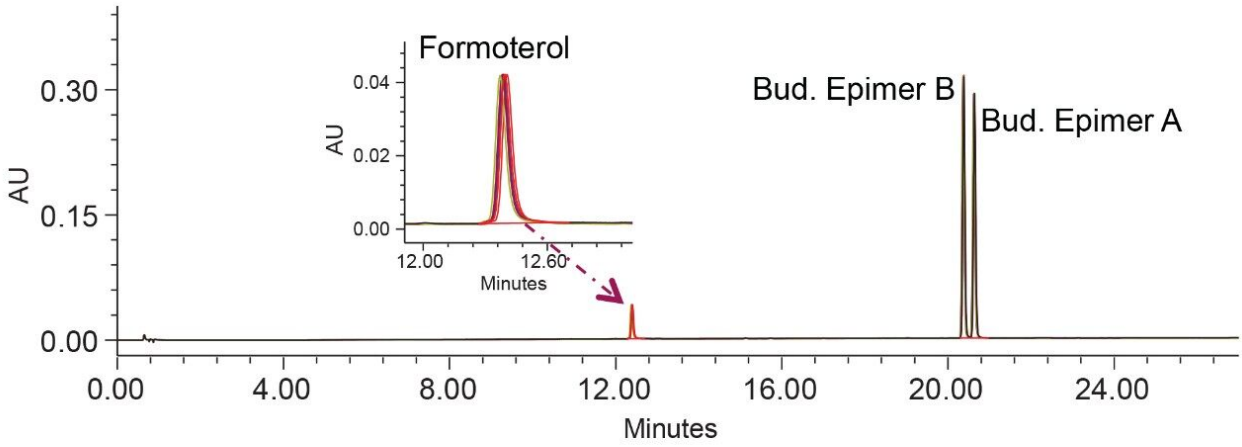
Figure 1. UPLC method for formoterol, budesonide, and budesonide related compounds. Working standard solution with related compounds at 0.5% level. UV at 244 nm.

Method validation

The purpose of this study is to demonstrate that the UPLC method is suitable for the determination of the active ingredients, formoterol and budesonide in metered dose inhaler combination dosage form. Therefore, the method was validated following the regulatory recommendations for quantitation of major components of bulk drug substances or active ingredients in finished pharmaceutical products.[2,3] The validation attributes included linearity, accuracy, repeatability, intermediate precision, specificity and robustness. System precision was evaluated for each chromatographic run using five replicate injections of the working standard sample, as recommended in the USP General Chapter <621> on Chromatography.[7] The system precision criteria included:

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

Example of system suitability determination (Figure 2) showed that the results were well below the acceptance criteria. A resolution mixture with formoterol and budesonide at the working level spiked with 0.5% of related compounds confirmed that the USP resolution between all the peaks was ≥2.0 (Figure 1).



System Suitability Report

Sample Set ID: 2544 Result Set Id: 2594

Processed Channel Descr.: PDA Ch1 244nm@4.8nm

	Peak Name	# inj.	Ave RT (min)	Ave USP Rs	Ave USP Peak Tailing	%RSD of Areas	% RSD of RT
1	Formoterol	5	12.388		1.4	0.42	0.10
2	Budesonide Epimer B	5	20.374	84.4	1.1	0.14	0.05
3	Budesonide Epimer A	5	20.632	2.7	1.1	0.12	0.05

Figure 2. System suitability results for five replicate injections of working standard sample. UV at 244 nm.

Using Empower 3 MVM Software streamlined the entire validation process, from creating a validation protocol to acquiring, analyzing, approving, and reporting validation data. During the study, Empower 3 MVM Software checked the adherence of each validation step against the requirements and flagged any results that did not meet the specifications (Figure 3). Built-in statistical calculations eliminated the need to export data to another software to generate validation results. Validation report templates available within the validation project allowed quick generation of summary reports for each validation test.

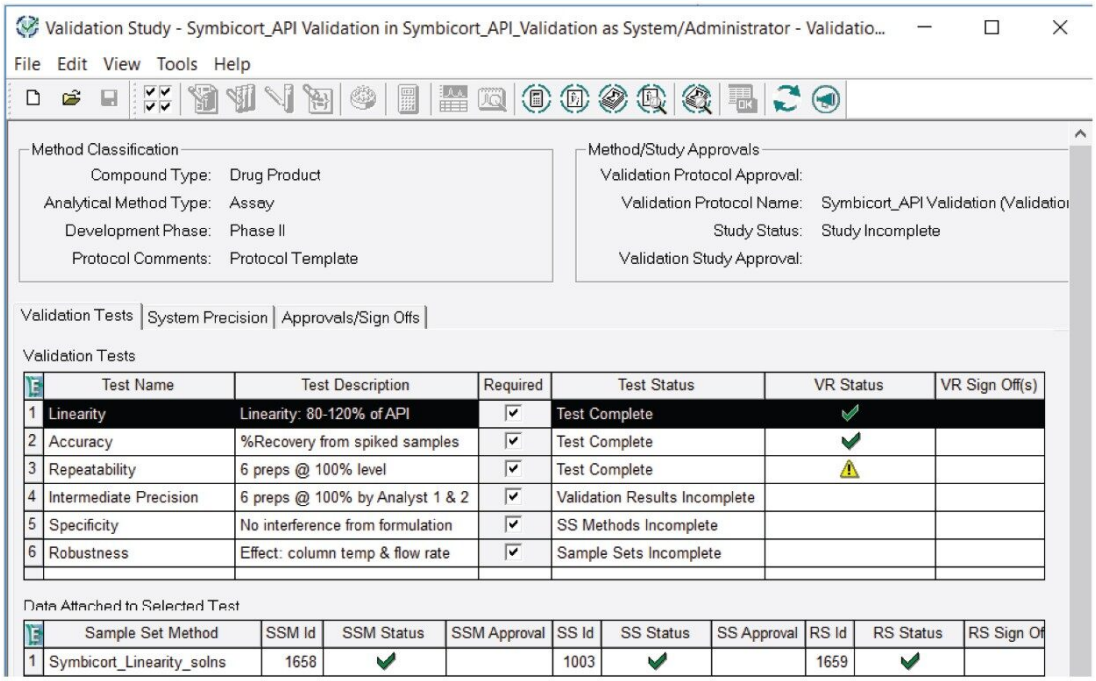


Figure 3. Example of the validation study with Empower 3 MVM Software. Conformance of each step against to the validation requirements is checked. Out-of-specification (OOS) results are flagged. Steps can be reviewed and approved via electronic signatures.

1. Linearity

Linearity was demonstrated by analyzing five different concentration levels for both the formoterol and budesonide ranging from 80 to 120% of the working sample concentration. Using Empower MVM Software, we calculated the regression equation and correlation coefficient for plot of average peak area against each drug concentration (Figure 4). Method exhibited an acceptable linearity, both analytes with correlation coefficients (R^2) ≥0.999.

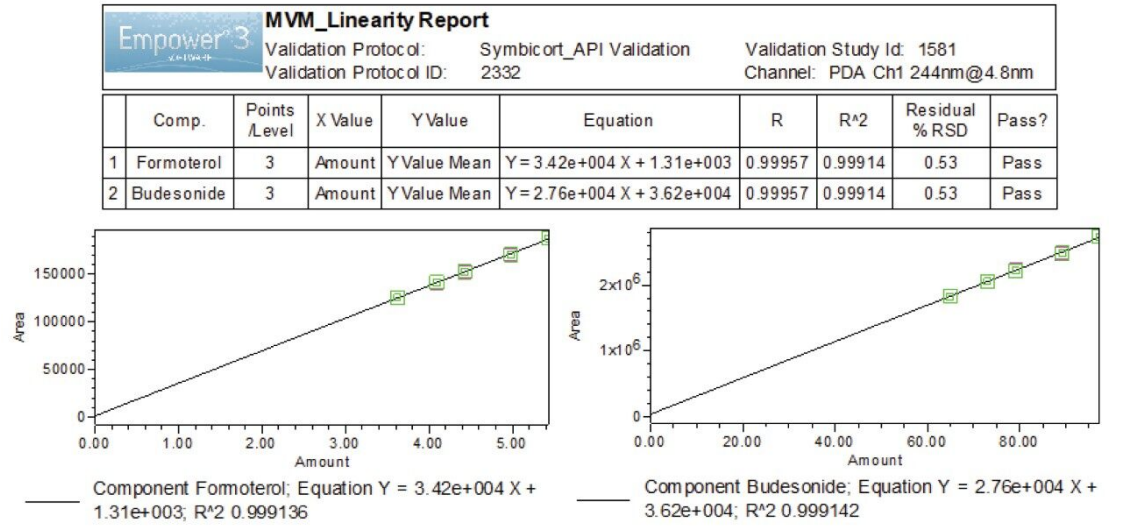


Figure 4. Method linearity for formoterol and budesonide generated by the Empower MVM Software.

2. Accuracy

Accuracy of the method was demonstrated by assessing recoveries of formoterol and budesonide drugs spiked to the Symbicort inhaler sample solutions at 80%, 100%, and 120% levels in triplicates. The average % recovery for the accuracy samples (Figure 5) ranged from 97.8 to 101.5% with %RSD ≤1.42%, which met the acceptance criteria of 95–105% and %RSD ≤5.0%.

Accuracy
Validation Protocol ID: 2525
Validation Study Id: 1581
Channel: PDA Ch1 244nm@4.8nm

Component: Budesonide Validation Result Id: 2521

	Component	Level	# Points per level	Average % Recovery at Level	% RSD of %Rec	Faults?
1	Budesonide	80%	3	99.8	1.34	No
2	Budesonide	100%	3	97.8	1.25	No
3	Budesonide	120%	3	101.5	1.42	No

Component: Formoterol Validation Result Id: 2520

	Component	Level	# Points per level	Average % Recovery at Level	% RSD of %Rec	Faults?
1	Formoterol	80%	3	100.6	1.35	No
2	Formoterol	100%	3	99.6	1.04	No
3	Formoterol	120%	3	100.3	1.17	No

Figure 5. Accuracy results at three different levels in triplicates generated by the Empower MVM Software.

3. Repeatability

To demonstrate repeatability, six separate preparations of Symbicort inhaler sample spiked with formoterol and budesonide at 100% level were examined for recovery. For analyst A, the average % recovery and %RSD of % recoveries for budesonide and formoterol met the specification of 100 ± 5.0% and %RSD ≤2.0%, respectively (Figure 6).

4. Intermediate precision

For intermediate precision, the second analyst prepared and analyzed six separate preparations of the Symbicort inhaler sample spiked at 100%. The results generated by analyst A and B met the criteria for recovery and %RSD (Figure 6).

Intermediate Precision
Validation Protocol ID: 2217
Validation Study Id: 1581
Channel: PDA Ch1 244nm@4.8nm

Component: Budesonide Validation Result Id: 2202

	Component	Experiment Group	Points	% Recovery	%RSD of %Rec.	Faults?
1	Budesonide	Analyst A	6	98.2	0.95	No
2	Budesonide	Analyst B	6	100.7	0.69	No

Component: Formoterol Validation Result Id: 2201

	Component	Experiment Group	Points	% Recovery	%RSD of %Rec.	Faults?
1	Formoterol	Analyst A	6	100.1	0.88	No
2	Formoterol	Analyst B	6	100.4	0.67	No

Figure 6. Repeatability (analyst A) and intermediate precision (analyst B) results generated using Empower MVM Software.

5. Specificity

Specificity demonstrates that the analytes can be separated and accurately measured in the presence of other components that may be expected to be present, such as impurities, degradation products, and matrix components. In addition, the spectral peak purity test is recommended to demonstrate that the chromatographic peak represents a single component or is spectrally homogenous.

The spectral purity of each active ingredient was demonstrated by spiking Symbicort inhaler sample formulation with related compounds. Both UV and MS spectral data were used to demonstrate that each active ingredient is not coeluting with other components in the sample (Figure 7). Peak table showed that the purity angle was below the threshold angle, confirming that each active ingredient is spectrally homogenous or representing one component (Figure 7A). The Mass Analysis Window from the Empower 3 Software with the purity spectrum showed spectral data across each peak – which is at the leading, apex, and trailing regions of the peak (Figure 7B). The top and bottom plots represent UV and MS spectrum, respectively. The mass spectrum showed presence of one mass (*m/z*) across each peak, specific for each active ingredient, which confirmed presence of a single component. Overall, utilizing both UV and MS spectra we demonstrated that our analytes are not subject to interference with any components of the formulation and/or the related compounds.

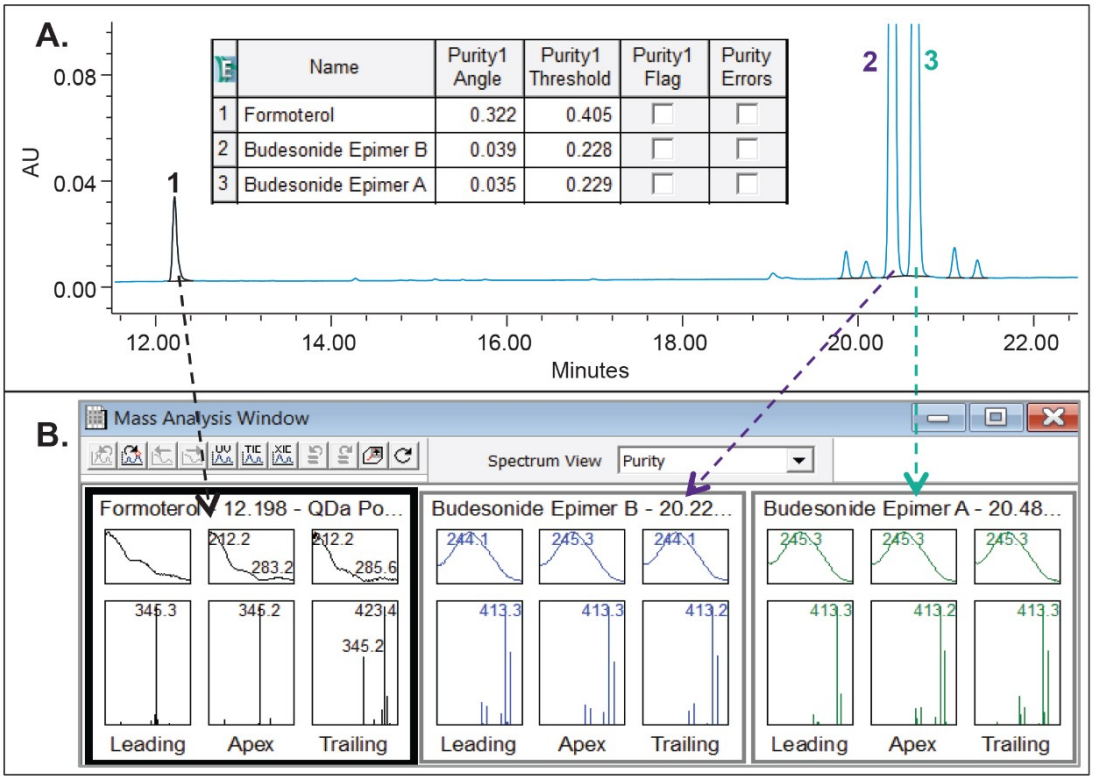


Figure 7. Peak purity (homogeneity) determination for formoterol and budesonise in Symbicort sample formulation spiked with related compounds. UV at 244 nm (A). Empower 3 Mass Analysis window with peak purity spectrum (B).

6. Robustness

For robustness, we studied the effect of changes in column temperature (33 ± 2.0 °C) and flow rate (0.35 ± 0.35 mL/min) on the USP resolution between peaks, with a goal of achieving a minimum USP resolution of ≥2.0. A full factorial experimental design in the Empower 3 MVM Software allowed us to investigate the interaction of the two factors and generated an experimental study design with combination of different chromatographic conditions (Figure 8). The robustness study showed that the USP resolution remains ≥2.0 across all conditions (Figure 9).

Robustness Parameters

Acquisition | Experiment Design | Processing and Acceptance Criteria

Experiment Design Table:

	Symbicort_Column_Temp	Symbicort_FlowRate_mLper_min
1	31 Degrees C	0.30
2	31 Degrees C	0.40
3	35 Degrees C	0.30
4	35 Degrees C	0.40

Figure 8. Design of experiment (DOE) with full factorial design.

Robustness Report

Validation Protocol: Symbicort_API Validation Validation Study Id: 1581

Validation Protocol ID: 2900 Channel: PDA Ch1 244nm@4.8nm

Assessed Field: USP Resolution

	Validation Result Id	Component	RT Mean (min)	Experiments	Assessed Field	Assessed Field Mean	LCL Assessed Field Mean	UCL Assessed Field Mean Mean	Pass /Fail
1	3075	Budesonide related comp. E	19.496	4	USP Resolution	79.75	73.05	86.45	Pass
2	3077	Budesonide related comp. L	19.731	4	USP Resolution	2.31	1.97	2.64	Pass
3	3071	Budesonide Epimer B	20.016	4	USP Resolution	2.82	1.98	3.67	Pass
4	3073	Budesonide Epimer A	20.272	4	USP Resolution	2.65	2.49	2.81	Pass
5	3079	Bud related comp. G (Epimer 1)	20.713	4	USP Resolution	4.48	4.30	4.66	Pass
6	3081	Bud related comp. G (Epimer 2)	20.968	4	USP Resolution	2.56	2.41	2.71	Pass

Figure 9. Robustness results. Resolution between peaks was ≥2.0.

Continued method performance verification

After implementation in the QC laboratory for routine use, method performance should be continuously monitored and verified to assure that it remains fit-for-purpose and meets the ATP goals throughout the method's lifecycle. Routine monitoring may include trend analysis of the system suitability data, tracking analytical results, out-of-specification, or other parameters as appropriate. Trending using control charts of the Empower 3 Software provides graphical representation of the method performance characteristics, which allows quick identification of any out-of-trend data or deviations from the performance goals.[8] As for example, monitoring the USP resolution between critical pair is important to assure acceptable separation is achieved for accurate quantitation (Figure 10).

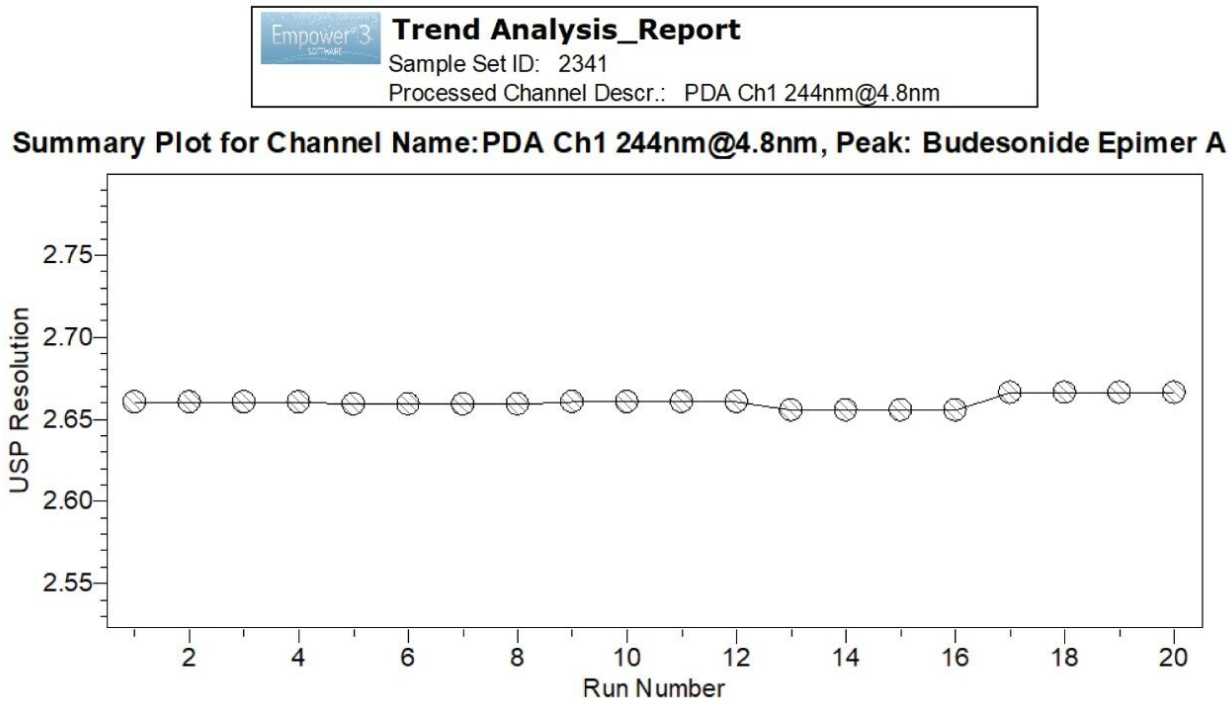


Figure 10. Trend analysis of USP resolution from method validation study.

Conclusion

The UPLC method for assay of formoterol fumarate and budesonide drugs was successfully validated for linearity, accuracy, repeatability, intermediate precision, specificity, and robustness. The validation results met the predefined performance requirements, demonstrating that the method is suitable for analysis of the active ingredients in the metered dose inhaler drug product.

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Finally, control charts of the Empower 3 Software enable quick trend analysis of the method performance characteristics, which helps to verify that the method continuously generates fit-for-purpose results.

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