TECHNICAL NOTE 73915

Complete workflow for determining the content of nevirapine and related impurities

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Goal

Guidance on generating an intelligent, automated workflow for batch-release analysis of an active pharmaceutical ingredient.

Introduction

In both research and development (R&D) and quality control (QC) pharmaceutical laboratories, determining the content of an active pharmaceutical ingredient (API) and its related impurities are typical analyses. While it is common to apply different methods for assay and impurity determination for one API, for instance in many pharmacopoeia monographs, in practice it is preferred to combine both analyses into one single run. Using a software assisted method development approach is hereby a very practical tool to speed up the method development process to save time and resources. After the final method is established, the next step is validation. The Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) offers an extension package with pre-defined eWorkflow™ templates for a full validation based on the International Council for Harmonisation (ICH) guidelines. This approach is described in a recently published application note in great detail.[1] Moreover, the Chromeleon software provides tangible strategies for routine data processing and reporting of batch analysis without the use of additional external programs (e.g., Microsoft® Excel®).

This technical note describes a comprehensive data analysis of a combined assay and impurity method for nevirapine, an antiretroviral drug used in HIV/AIDS therapy.[2] Three known related impurities are reported by the United States Pharmacopoeia (USP) monograph.[3] Before the actual analysis can be performed, a system suitability test (SST) must be carried out to demonstrate that the chromatographic system is suitable for the intended analysis. Typically, the data evaluation of the SST solution is a manual step for the analyst. In Chromeleon CDS a function called Intelligent Run Control (IRC) performs this task. Based on the result of the system suitability solution with regard to the individual criteria, the system reacts automatically to either continue with the next injection, re-inject the current solution, or abort the sequence. After all suitability criteria have been met successfully, the analysis of API and impurity samples can be performed. All results can be automatically reported in Chromeleon CDS and saved or exported in the desired format. Furthermore, an eWorkflow template is provided that contains a sequence as well as processing methods and report templates that can be easily adapted to the batch analysis of other APIs.

Experimental

Chemicals

- Thermo Scientific™ Barnstead™ GenPure™ xCAD Plus Ultrapure Water Purification System, deionized water, 18.2 MΩ·cm at 25 °C (P/N 50136149)
- Fisher Scientific™ Acetonitrile Optima™ LC/MS grade (P/N A955-212)
- Fisher Scientific™ Ammonium acetate, LC/MS grade (P/N A114-50)

Certified standards of the following were purchased from reputable vendors:

- Nevirapine
- 11-Ethyl-4-methyl-5,11-dihydro-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (Impurity A)
- 4-Methyl-5,11-dihydro-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (Impurity B)
- 4-Methyl-11-propyl-5,11-dihydro-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (Impurity C)

Preparation of standards

Two separate stock solutions of API nevirapine and stock solutions of the related impurities A and B were each prepared as listed in Table 1. For reasons of solubility, acetonitrile and mobile phase A (refer to Table 3) were added in a ratio of 1:20 (nevirapine solutions), 1:3 (impurity A solution) and 1:22 (impurity B solution). Firstly, the appropriate amount of acetonitrile was filled into the volumetric flask with the standard and sonicated for 10 minutes. Secondly, the mobile phase A was added until ¾ of the volume of the volumetric flask was reached and sonicated again for 10 minutes. After the solutions had cooled to room temperature, the volumetric flasks were filled to the respective volume with mobile phase A. Nevirapine solution I was used as the calibration standard for the assay method, Nevirapine solution II as the check standard. According to the USP monograph, the SST solution was prepared as 0.03 mg/mL nevirapine and impurity A, and 0.015 mg/mL impurity B by diluting the individual stock solutions with the appropriate volumes in mobile phase A.

Table 1. Preparation protocol of stock solutions for API nevirapine and related impurities A and B

	Nevirapine I (calibration standard assay)	Nevirapine II (check standard)	Impurity A	Impurity B
Weight [mg]	12.525	12.400	1.300	0.720
Volume [mL]	50	50	5	10
Concentration [µg/mL]	250.5	248.0	260.0	72.0

In addition, the nevirapine solution I was diluted to 0.48 µg/mL, which corresponds to the specification limit of 0.2% (Impurity STD). The solution was used as a calibration standard to determine the amount of impurities in the sample.

A limit of quantification (LOQ) solution (LOQ STD) was prepared by diluting the nevirapine solution I to 0.24 µg/mL, which corresponds to a specification limit of 0.1% for unknown impurities.

Preparation of sample

Two individual sample preparations were made at concentrations of 240 µg/mL using the procedure described for the nevirapine stock solutions I or II.

IRC test cases for system suitability and method performance criteria

The integrated IRC function in Chromeleon software is used to automatically check SST and method performance criteria and provide a pass or fail result followed by a subsequent action. This action can be "continue with next injection", "re-inject one or more times the current injection", or "abort the sequence". Five different IRC test cases were created as mentioned in Table 2. According to the general chapter <621> of USP monograph,[4] the relative standard deviation (RSD) of peak area for the API is calculated based on minimum five injections, and for the impurities based on minimum six injections due to different allowed acceptance limits. Six consecutive injections of Impurity STD and API I standards are used for the respective one-point calibration. In order to automate the calculations for assay and impurity determination, the weight and dilution factors are entered in the injection list as can be found in Table 2.

An additional IRC test case was implemented to duplicate the UV channel. In this way, two different calibration solutions of the same analyte can be used to quantify either the API or the impurities in the same processing method based on different concentrations for the one-point calibration.

Instrumentation

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

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

Table 2. Injection list with corresponding weight and dilution factors, and related IRC test case included in the processing methods.
Weight and dilution factors of 1.0000 means that there is no weight/dilution correction; the remaining dilution factors are calculated based on the theoretical dilution volume.

Injection #	Name	Weight [mg]	Dilution [mL]	IRC Test case
1	Matrix blank	1.0000	1.0000	Interferences/ carry-over
2	Matrix blank	1.0000	1.0000	Interferences/ carry-over
3	SST	1.0000	1.0000	Resolution USP
4	Matrix blank	1.0000	1.0000	Interferences/ carry-over
5	LOQ STD	1.0000	1.0000	Signal/noise ratio
6	Impurity STD	1.0000*	25,000.0000	
7	Impurity STD	1.0000*	25,000.0000	
8	Impurity STD	1.0000*	25,000.0000	
9	Impurity STD	1.0000*	25,000.0000	
10	Impurity STD	1.0000*	25,000.0000	
11	Impurity STD	1.0000*	25,000.0000	RSD of peak areas
12	API I	1.0000*	50.0000	
13	API I	1.0000*	50.0000	
14	API I	1.0000*	50.0000	
15	API I	1.0000*	50.0000	
16	API I	1.0000*	50.0000	RSD of peak areas
17	API I	1.0000*	50.0000	Peak asymmetry
18	Sample preparation 1	12.5750	50.0000	
19	Sample preparation 2	12.3750	50.0000	
20	Check standard API II	12.4000	50.0000	
21	SST	1.0000	1.0000	Resolution USP
22	Matrix blank	1.0000	1.0000	Interferences/ carry-over

*Weight of calibration standards are entered to the levels in the processing method.

The method was developed using ChromSwordAuto™ Developer software package, in which two columns were screened for their suitability. The Thermo Scientific™ Acclaim™ Polar Advantage II column was found to be ideal in terms of selectivity and run time. Afterwards the Chromeleon UHPLC speed-up tool was used to optimize the flow rate. The final method is shown in Table 3.

Table 3. Chromatographic conditions

Parameter	Value
Column	Acclaim Polar Advantage II, 150 x 3 mm, 3 µm (P/N 063705)
Mobile phase	A: 10 mM ammonium acetate, pH 5/ Acetonitrile (85/15; v/v) B: Acetonitrile
Flow rate	0.75 mL min^{-1}
Gradient	Time [min] %B 0.000 16 2.000 18 3.533 25 8.000 25 8.067 16 13.333 16
Column temperature	30 °C (with passive pre-heater), forced air mode with fan speed 5
Injection volume	5 µL
UV detector settings	Wavelength 240 nm, data collection rate 10 Hz, response time 0.5 s

Data processing and software

ChromSwordAuto 5 software 5.1.221.787 with the modules of ChromSwordAuto Developer and ReportViewer was used during the method development process.

Chromeleon software 7.3 was used for method optimization, data acquisition, processing, and reporting of the batch analysis.

Results and discussion

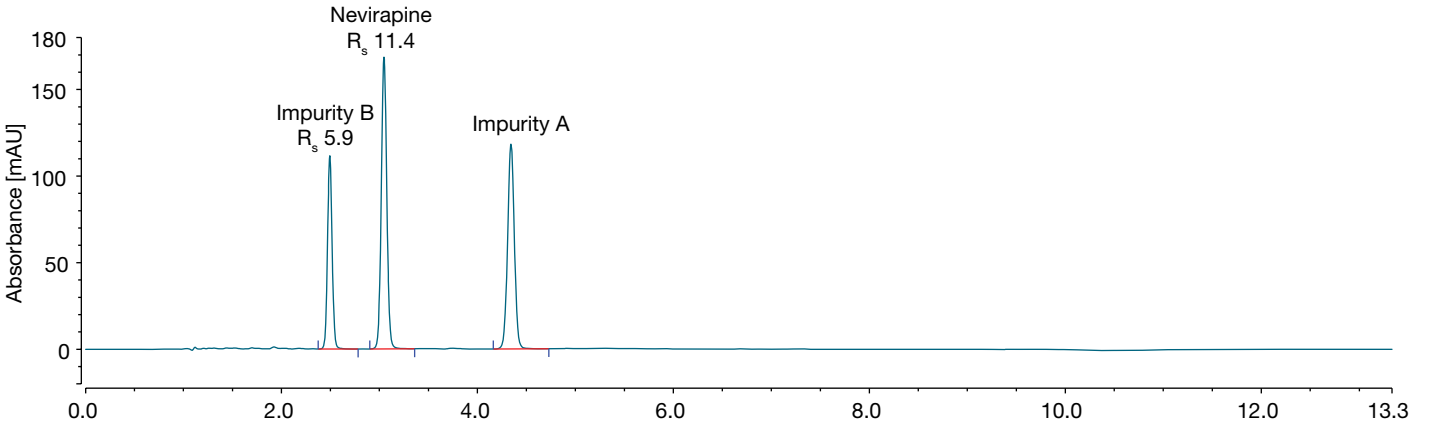
1) IRC test cases for system suitability and method performance

Two processing methods were created, one for SST and one for the batch analysis. The SST solution consisting of nevirapine and impurities A and B was evaluated according to the USP monograph[3] based on the resolution of impurity B and nevirapine peaks of not less than 5, and on nevirapine and impurity A peaks with not less than 7.4 (Figure 1).

The first IRC test case shown in Figure 1 for resolution between impurity B and nevirapine is described further in detail in Figure 2.

Chromeleon CDS offers a wizard that facilitates the creation of individual IRC test cases.[5] The “Evaluation” tab contains all pre-defined evaluation formulas included in the Chromeleon software that can be quickly chosen from the list, as detailed in the interactive result table section. In addition, various non-statistical or statistical reference criteria can be defined. Depending on the test case property, the specific IRC test case can be applied to certain injections from the injection list or limited to compounds.

All other test cases are generated in the same way with the respective criteria and actions. The full details of all test cases can be found in the Appendix, Figures 7–12.



System Suitability Test and Intelligent Run Control

Group Area — Drag a column header here to group by that column.

#	Name	Statistics	Eval. Formula	Operator	Ref.Value 1	Ref.Value 2	Channel	Peak	Inj.Condition	Pass Actions	Fail Actions	Result	SST Message	Rounding
1	Resolution (USP)	None	peak.resolution("usp","next")	>=	5		UV_VIS_1	Impurity B	injection.name = "SST"	No Actions	Re-inject	Passed		Off
2	Resolution (USP)	None	peak.resolution("usp","next")	>=	7.4		UV_VIS_1	Nevirapine	injection.name = "SST"	No Actions	Re-inject	Passed		Off

Figure 1. SST chromatogram with corresponding IRC test cases for resolution; with each “passed” result

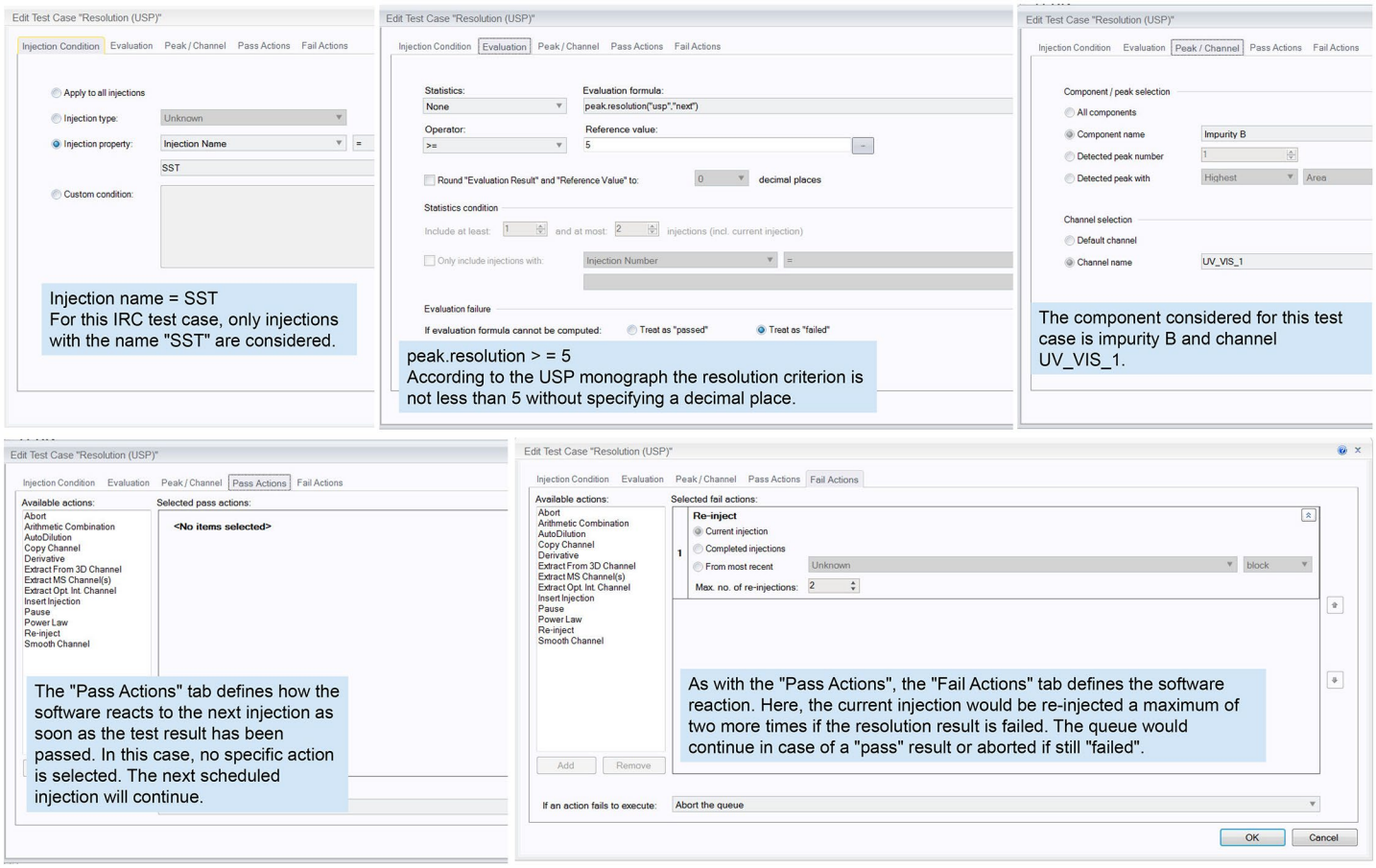


Figure 2. Example of one IRC test case for SST processing on resolution

To demonstrate the applicability of the procedure, two solutions of the injection list were manipulated to force fail results. First, the SST solution was exchanged with a different one containing more analytes than expected. To see how the software performs the programmed “fail actions”, the solution was reset prior to the last re-injection by the correct solution after the second injection was completed. Second, the vial position of the SST solution was entered to the injection of the blank solution to force Interferences/Carry-Over.

Figure 3 illustrates the original injection list (A) and the actual generated list (B), based on IRC test case results and programmed actions. The first and second SST solution did not meet the required resolution for nevirapine and impurity A of 7.4 and therefore, triggered the re-injections of the same solution. After replacing the SST solution with the correct one in the second re-injection, both resolution criteria were successfully met and the injection list continued to be processed with the blank run.

The “fail actions” for Interferences/Carry-Over have been programmed to be re-injected 3-times before aborting the sequence. All four injections failed, and the queue was automatically aborted after injection #9. Having this intelligence in the software, avoids sample and eluent wastage. The operator must first fix the issue before proceeding with the injection list. The corresponding entries can be found in the audit trail so that the automatically inserted injections can be tracked as required for a regulated environment (Figure 4). More details on data integrity regulations and the implementation in Chromeleon CDS can be found in a White Paper.[6]

A

#	UV_VIS_1	Name	Comment	Re-injections
1	None	Blank	Matrix Blank	0
2	None	Blank	Matrix Blank	0
3	None	SST		0
4	None	Blank	Matrix Blank	0
5	None	LOQ STD		0
6	None	Impurity STD		0
7	None	Impurity STD		0
8	None	Impurity STD		0
9	None	Impurity STD		0
10	None	Impurity STD		0
11	None	Impurity STD	impurity calibration	0
12	None	API I		0
13	None	API I		0
14	None	API I		0
15	None	API I		0
16	None	API I		0
17	None	API I	assay calibration	0
18	None	sample - preparation 1		0
19	None	sample - preparation 2		0
20	None	Check Standard API II		0
21	None	SST		0
22	None	blank	Matrix Blank	0

B

#	UV_VIS_1	Name	Comment	Re-injections
1		Blank	Matrix Blank	0
2		Blank	Matrix Blank	0
3		SST		0
4		SST		1
5		SST		2
6		Blank	Matrix Blank	0
7		Blank	Matrix Blank	1
8		Blank	Matrix Blank	2
9		Blank	Matrix Blank	3
10	None	LOQ STD		0
11	None	Impurity STD		0
12	None	Impurity STD		0
13	None	Impurity STD		0
14	None	Impurity STD		0
15	None	Impurity STD		0
16	None	Impurity STD	impurity calibration	0
17	None	API I		0
18	None	API I		0
19	None	API I		0
20	None	API I		0
21	None	API I		0
22	None	API I	assay calibration	0
23	None	sample - preparation 1		0
24	None	sample - preparation 2		0
25	None	Check Standard API II		0
26	None	SST		0
27	None	blank	Matrix Blank	0

Figure 3. Original injection list (A) and actual generated injection list (B). SST and blank solutions were manipulated to force "fail" results when checking the IRC criteria; software automatically reacts based on the actions entered in the IRC test case section without any user interaction

A

10/1/2020	8:52:44 AM +02:00			IRC Message for Test Case "Signal / Noise Ratio": Injection specification criteria has not been met
10/1/2020	8:52:44 AM +02:00			IRC Message for Test Case "RSD of Peak Areas": Injection specification criteria has not been met
10/1/2020	8:52:44 AM +02:00			IRC Message for Test Case "RSD of Peak Areas": Injection specification criteria has not been met
10/1/2020	8:52:44 AM +02:00			IRC Message for Test Case "Peak Asymmetry": Injection specification criteria has not been met
10/1/2020	8:52:44 AM +02:00			System Suitability Test: "Interferences/ Carry-Over", Failed
10/1/2020	8:52:44 AM +02:00			System Suitability Test: "Interferences/ Carry-Over", Failed
10/1/2020	8:52:44 AM +02:00			System Suitability Test: "Interferences/ Carry-Over", Failed
10/1/2020	8:52:44 AM +02:00			System Suitability Test: "Interferences/ Carry-Over", Failed
10/1/2020	8:52:44 AM +02:00			IRC Message for Test Case "Interferences/ Carry-Over": Successfully inserted previous injection to re-inject.
10/1/2020	8:52:44 AM +02:00			IRC Message for Test Case "UV channel duplicate": Pass Actions performed since the injection specification criteria is met
10/1/2020	8:52:44 AM +02:00			IRC Message for Test Case "UV channel duplicate": Copy Channel - channel "UV_VIS_1_COPY" created.
10/1/2020	8:52:46 AM +02:00			End of injection "Blank".

B

10/1/2020	9:06:36 AM +02:00			IRC Message for Test Case "Signal / Noise Ratio": Injection specification criteria has not been met
10/1/2020	9:06:36 AM +02:00			IRC Message for Test Case "RSD of Peak Areas": Injection specification criteria has not been met
10/1/2020	9:06:36 AM +02:00			IRC Message for Test Case "RSD of Peak Areas": Injection specification criteria has not been met
10/1/2020	9:06:36 AM +02:00			IRC Message for Test Case "Peak Asymmetry": Injection specification criteria has not been met
10/1/2020	9:06:37 AM +02:00			System Suitability Test: "Interferences/ Carry-Over", Failed
10/1/2020	9:06:37 AM +02:00			System Suitability Test: "Interferences/ Carry-Over", Failed
10/1/2020	9:06:37 AM +02:00			System Suitability Test: "Interferences/ Carry-Over", Failed
10/1/2020	9:06:37 AM +02:00			System Suitability Test: "Interferences/ Carry-Over", Failed
10/1/2020	9:06:37 AM +02:00			IRC Warning for Test Case "Interferences/ Carry-Over": Re-injection limit reached...
10/1/2020	9:06:37 AM +02:00			An abort error occurred during the post acquisition operations.
10/1/2020	9:06:37 AM +02:00			End of injection "Blank".

Figure 4. Excerpt of audit trail entries with (A) first blank injection and (B) last allowed re-injection; execution of IRC test cases and actions taken automatically (underlined in red)

2) Batch analysis

Two individual sample preparations were injected and processed regarding their content of API (assay). The acceptance criteria according to USP is 98.0% to 102.0%.

Data evaluation is always a critical and time-consuming step. Automating this task in the software helps the analyst to carry out the evaluation quickly and reliably. Only a few manual entries are required, such as the initial weights and dilutions, the concentration of the calibration standards and the information on the purity of the reference standard used.

Following, in more detail: The sample weight and the dilution volume are entered directly into the injection list as shown in Table 2 and are then automatically transferred to the processing method for data evaluation. The concentration of the calibration level, here in mg/mL, and the purity of the reference standard are entered in the component table of the processing method under “Level” or “Assay reference”, respectively. The eWorkflow template provided with this technical note contains the processing method for adapting to one´s own analysis.

In this study, the content of API is determined to be 99.2% for sample preparation 1 and 98.8% for sample preparation 2, which fulfils the criteria outlined in the monograph.

A more accurate quantification of known related impurities can be achieved by determining the relative response factors (RRF) of each compound, e.g., during method validation. Here, three to four different concentrations, starting at the LOQ level of the method, were analyzed and RRF values calculated. The values show no difference between the concentration levels (data not shown). Determined RRF values and the acceptance criteria on the amount [%] listed in the USP monograph for individual related impurities can be found in Table 4.

Table 4. Relative response factors and acceptance criteria of content for impurity method

Compound name	Relative response factor	Impurity content acceptance criteria, NMT* [%]
Nevirapine impurity B	0.89	0.2
Nevirapine	1.00	-
Nevirapine impurity A	1.25	0.2
Nevirapine impurity C	1.08	0.2
Any other individual unspecified impurity	1.00	0.1
Total impurities	-	0.6

*NMT = not more than

In this study, none of the known impurities could be detected in the sample. However, two unknown impurities were found. Both were below the LOQ level of the method and therefore do not need to be reported.

3) Reporting and eSignature

Documentation of the achieved results is of crucial importance for regulated pharmaceutical laboratories. Two report templates are included in the eWorkflow procedure of this technical note and enable a complete, clear summary of all relevant results for the individual method.

Customized print settings allow specific related information to be included in each electronic report. For example, the API report contains the results that relate only to the assay determination while the impurity report includes only relevant information on impurity method (Figure 5).

With one click, Chromeleon CDS generates an electronic report that contains the sequence results and is saved in the sequence. Once an electronic report has been electronically signed, the associated results cannot be changed (Figure 6).

Both report files of the results can be found in the Thermo Scientific™ AppsLab Library[7] in the download section of this technical note.

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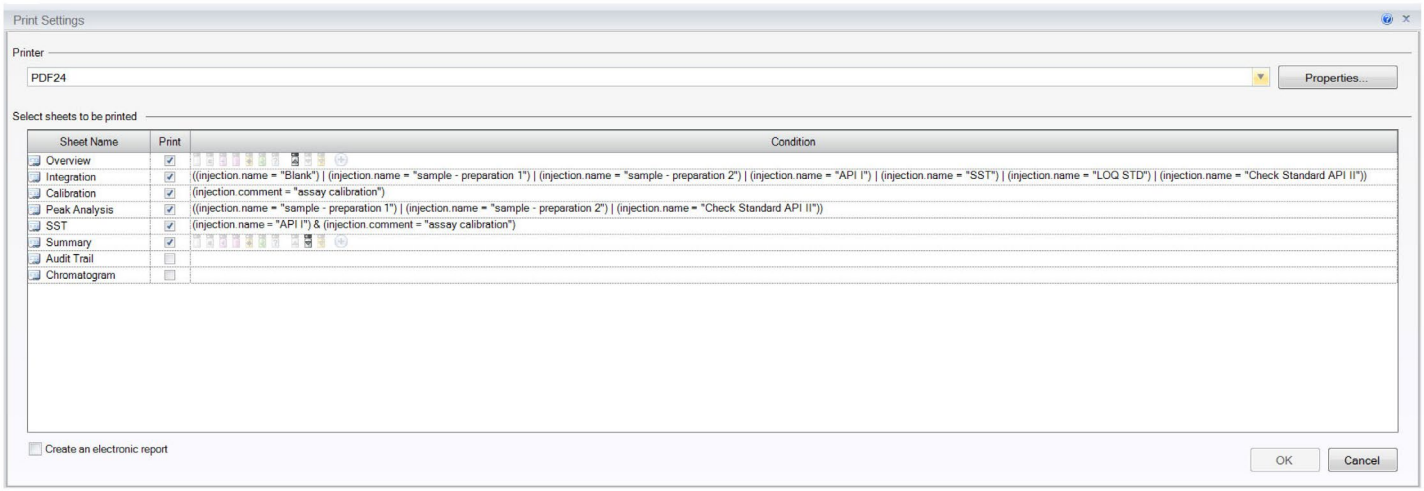

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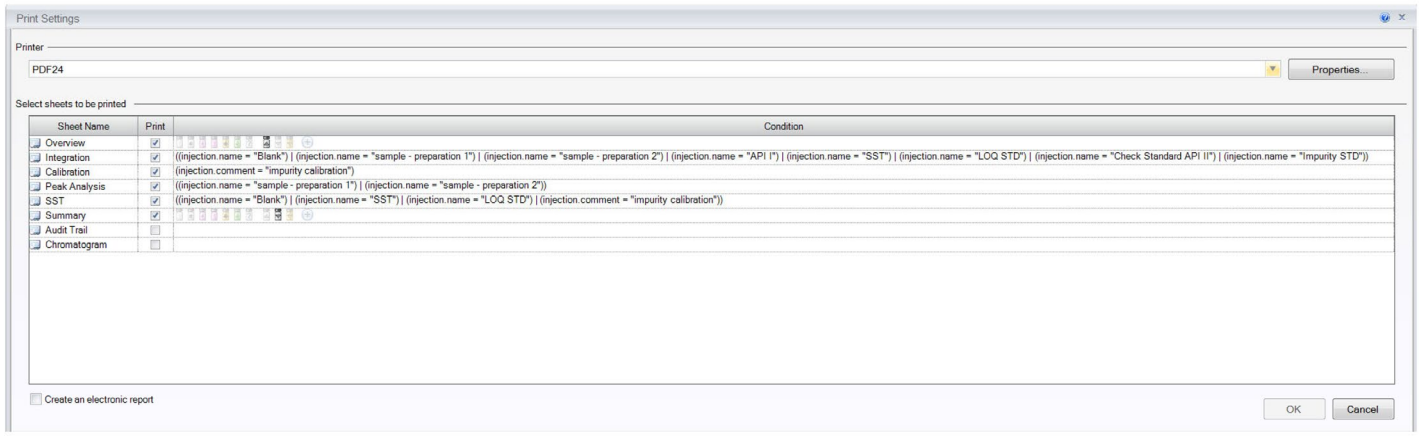
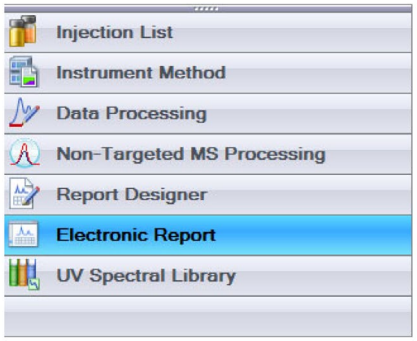


Figure 5. Customized print settings for each report template with (A) assay method and (B) impurity method

A



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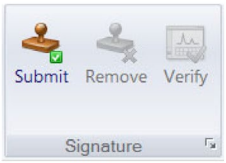

Figure 6. Navigation section (A) and electronic signature option (B) in the Chromeleon software

Conclusion

The presented Chromeleon eWorkflow procedure for a combined assay and impurity method contains the injection list along with related processing methods for SST, method performance, and data evaluation. Furthermore, the report templates are already customized to the specific information required based on regulatory guidelines.

- Intelligent run control in Chromeleon CDS enables automated monitoring of SST criteria and method performance to avoid unnecessary measurements if criteria fail.
- Automated data processing and reporting allows for a quick, reliable data evaluation and documentation, as it is required in the pharmaceutical regulated environment.

Acknowledgement

The authors are very thankful to Christiane Berkemeyer for her great support and the valuable insights into the regulated pharmaceutical environment.

References

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7. Thermo Scientific AppsLab Library: https://appslab.thermofisher.com/

Appendix: IRC test cases for SST and method performance

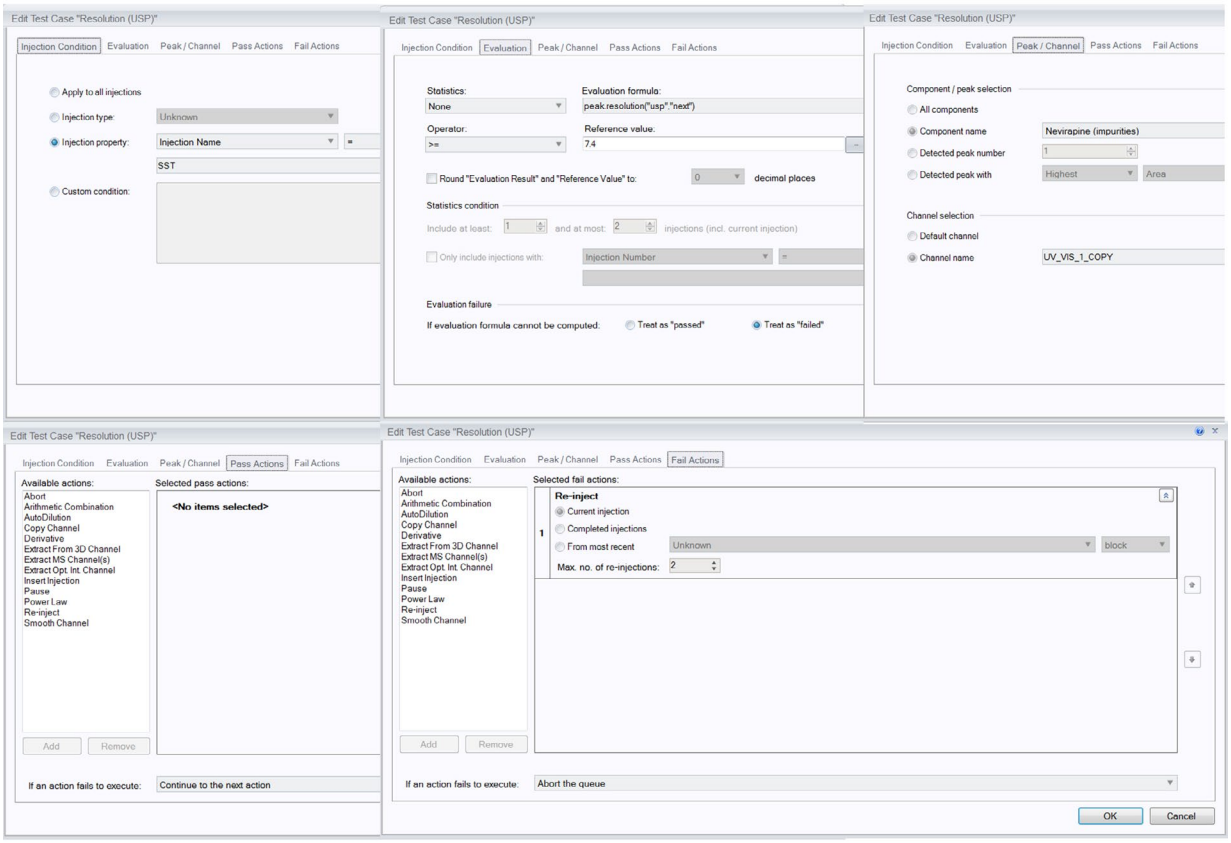

Figure 7. Resolution between nevirapine and impurity A on SST solution. Acceptance criteria is ≥ 7.4, if this fails re-injections are made a maximum of two more time.

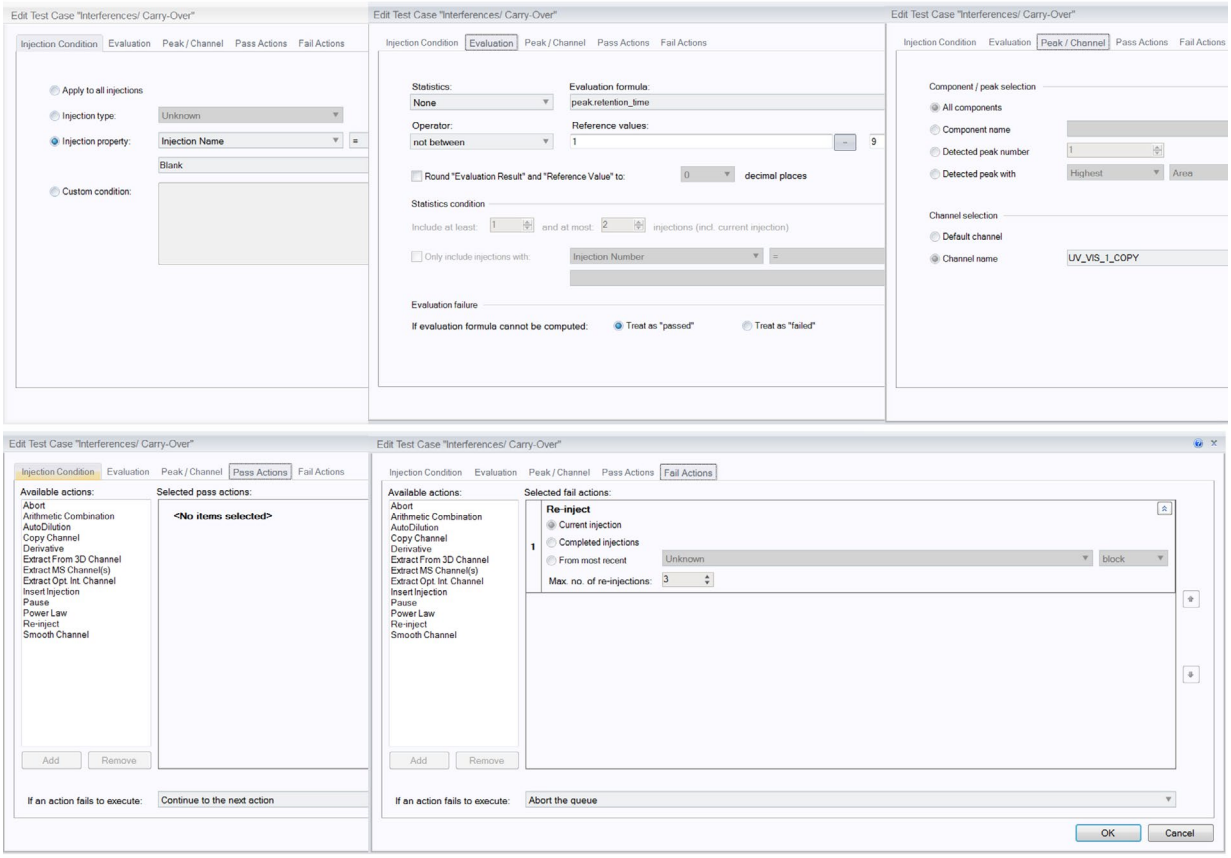

Figure 8. Interferences/Carry-Over on blank runs. Acceptance criteria is no peak detection between RT 1-9 minutes; if this fails, re-injections are made a maximum of three more times.

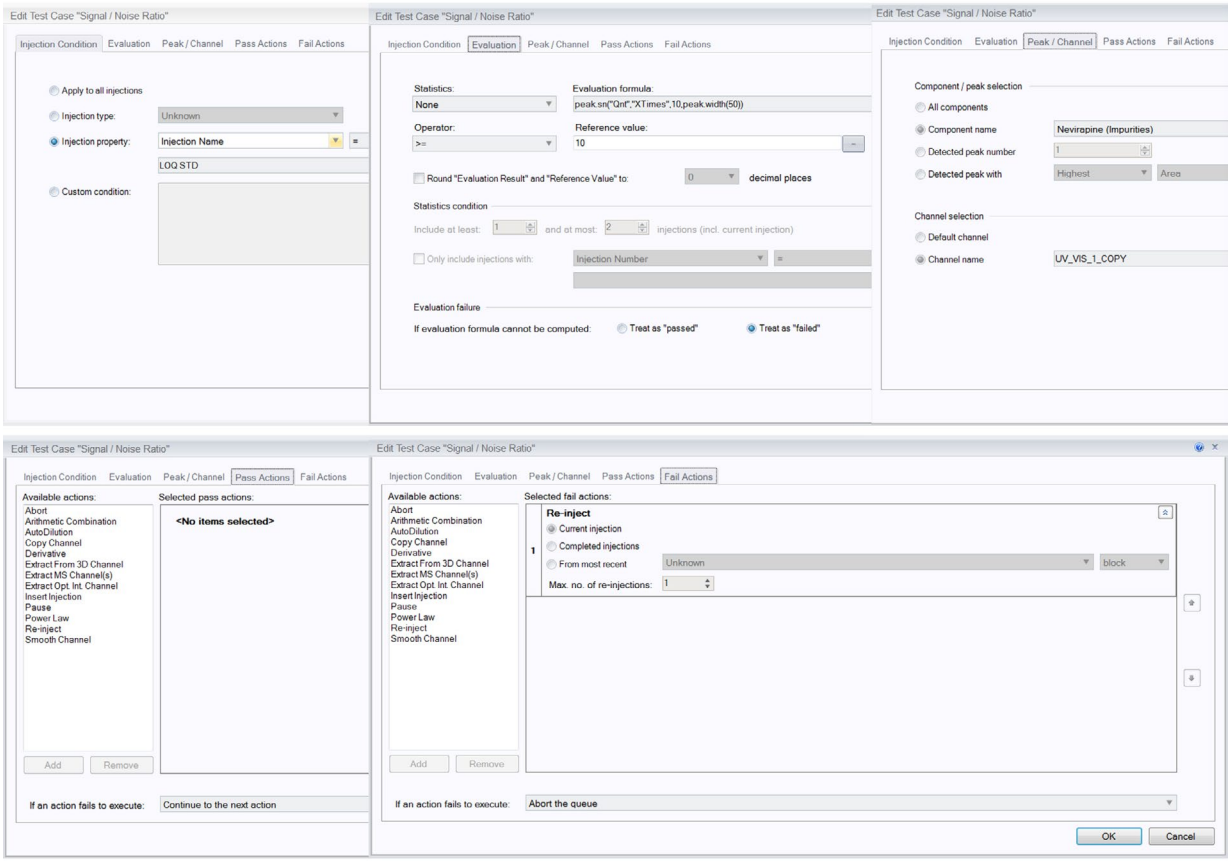

Figure 9. Signal to noise ratio (S/N) on LOQ solution. Acceptance criteria is a S/N ≥ 10, if this fails, re-injection is made a maximum of one more time.

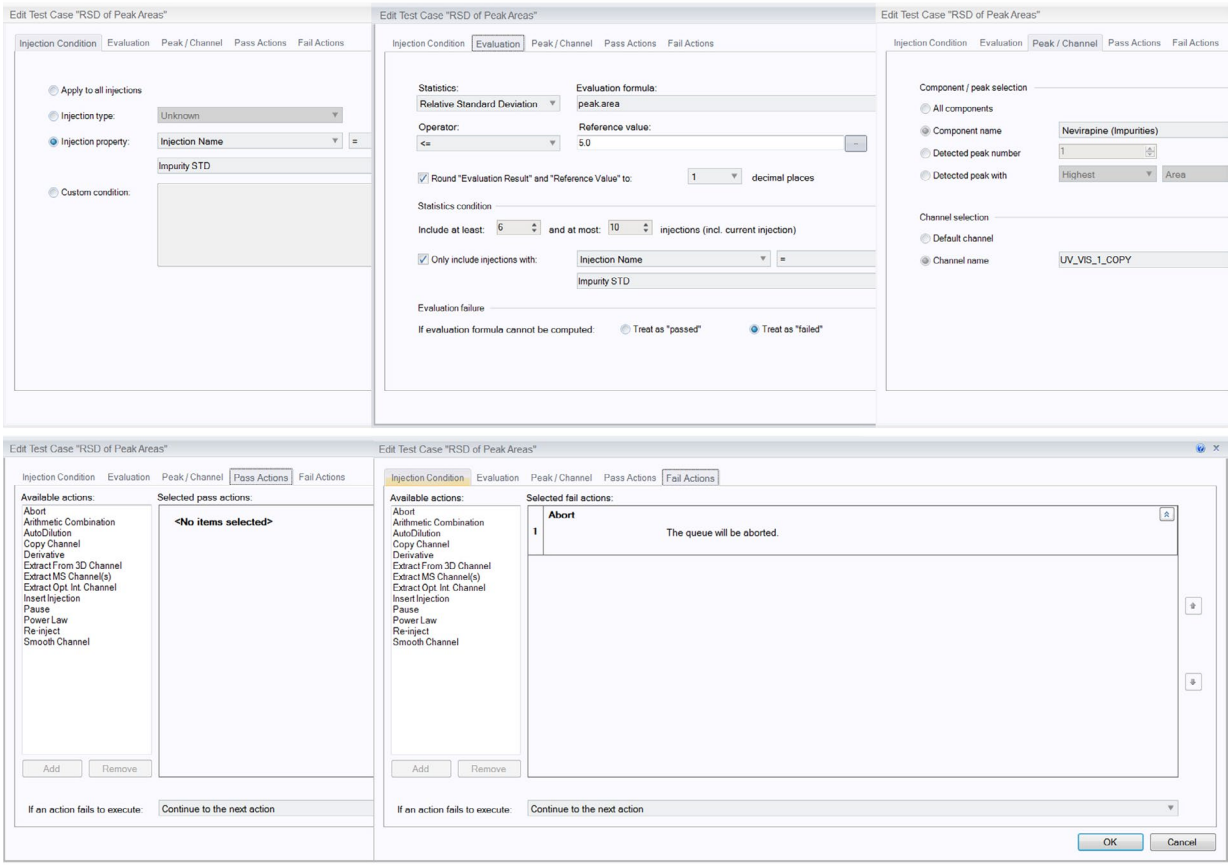

Figure 10. RSD area on impurity level. Acceptance criteria is ≤5.0 (one decimal place), with statistical condition of at least 6 out of 10 injections must be considered, if this fails, the queue is aborted.

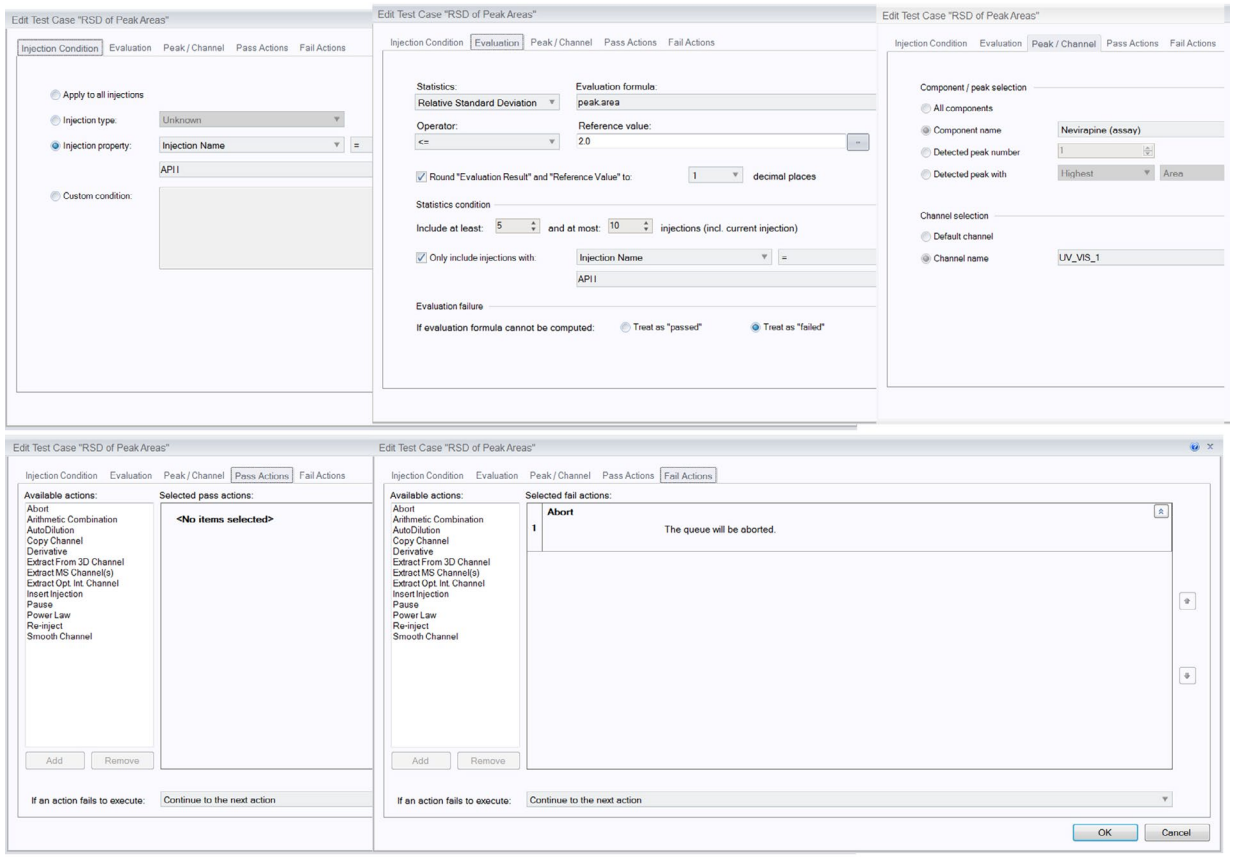

Figure 11. RSD area on API level. Acceptance criteria is ≤2.0 (one decimal place), with statistical condition of at least 5 out of 10 injections must be considered, if this fails, the queue is aborted.

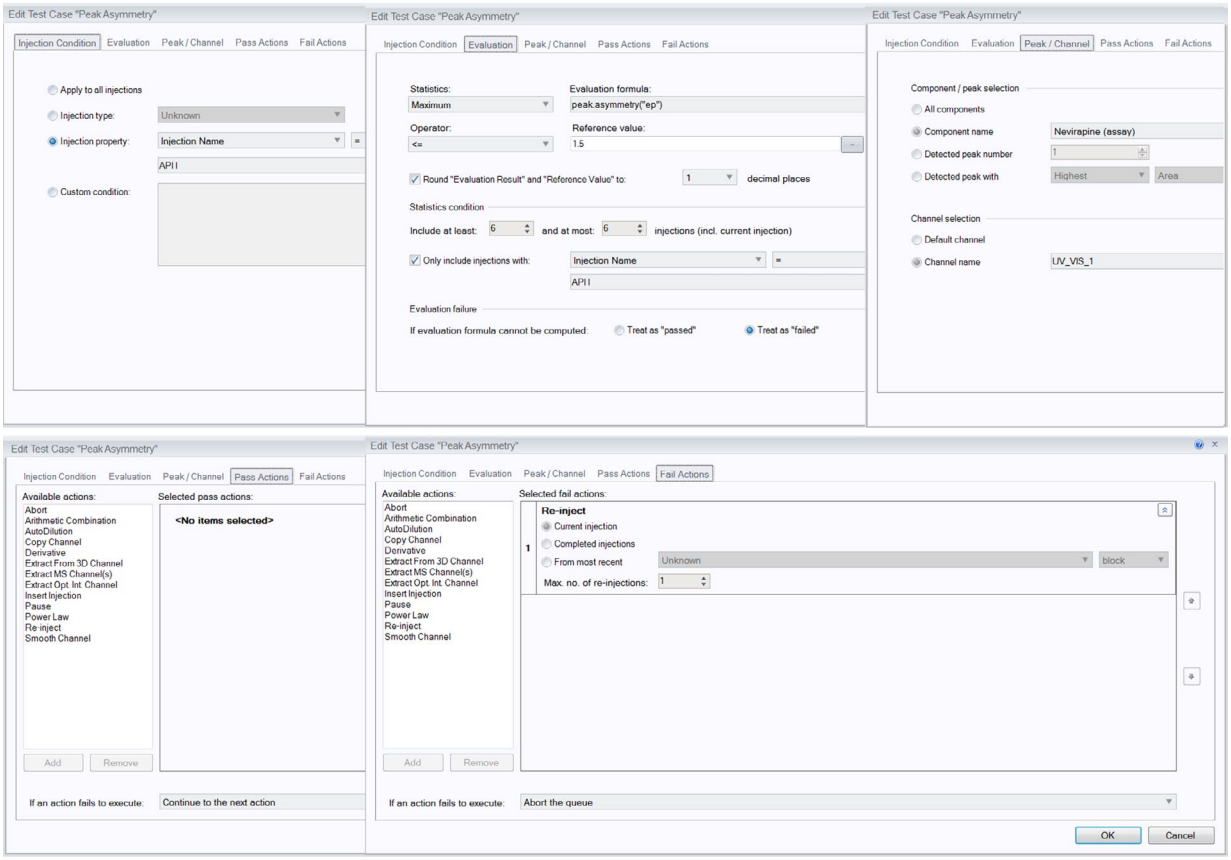

Figure 12. Peak asymmetry on API level. Acceptance criteria is ≤1.5 (one decimal place), if this fails, re-injection is made a maximum of one more time.

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