

Smart solutions for high analyte concentrations and carryover in LC-MS monitoring

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Abstract

Purpose: Demonstrate the System Suitability Test / Intelligent Run Control (SST/IRC) functionality in Thermo Scientific™ Chromeleon™ software to automatically respond to samples with analyte concentrations exceeding the upper limit of quantification.

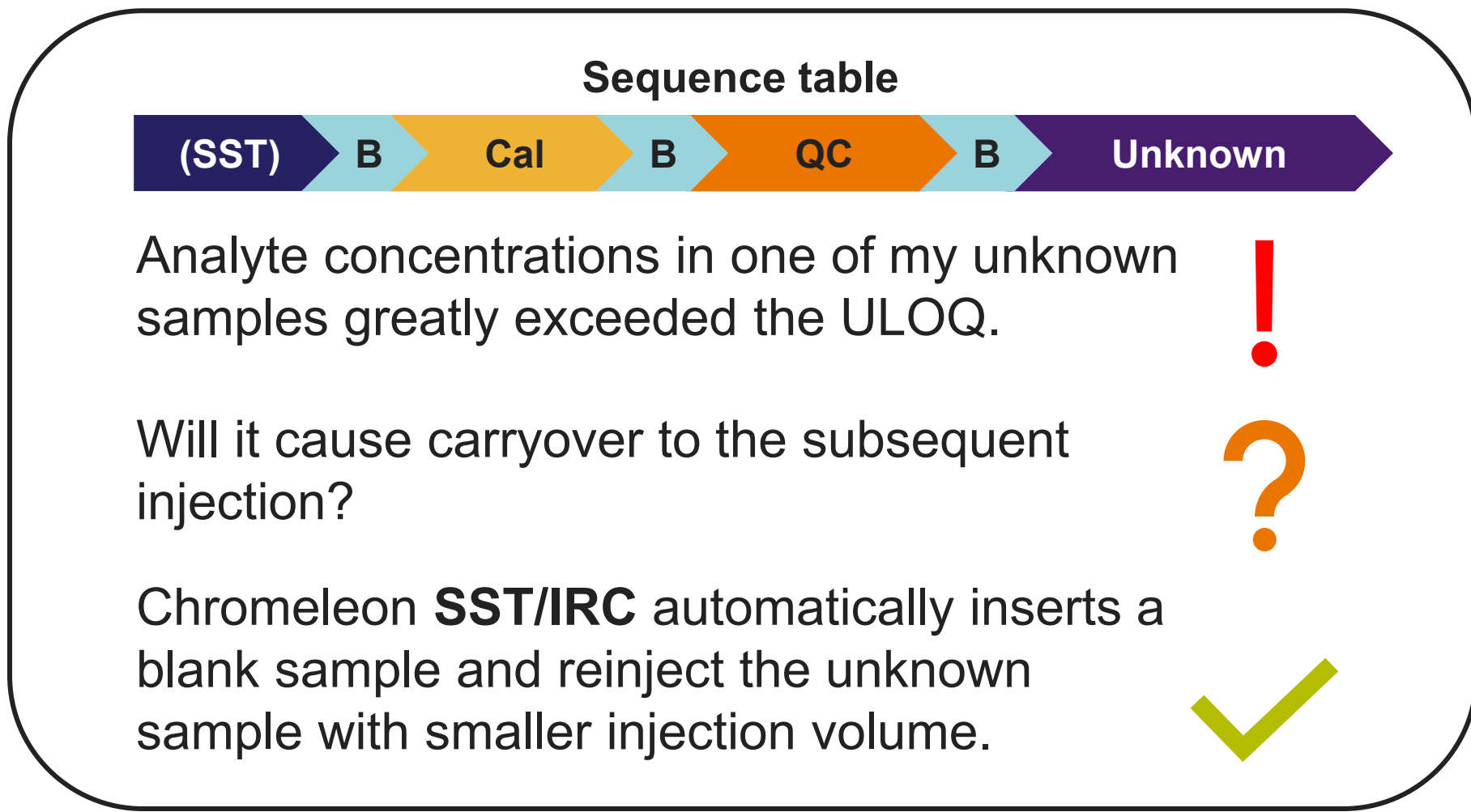
Methods: A panel of 31 drugs of abuse in oral fluids were measured on the Thermo Scientific™ Vanquish™ Flex UHPLC system coupled to the Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometry with data-dependent MS2 scan mode. Data was acquired and processed in Chromeleon software.

Results: This poster demonstrated the setup and utilization of the auto-dilution capability as an example in the SST/IRC functionality in Chromeleon software to monitor analyte quantification results in real-time and respond according. This intelligent feature ensures reliable quantification and preventing carryover in clinical toxicology applications.

Introduction

In clinical toxicology laboratories, routine quantification of drugs of abuse (DoA) in biological samples using validated liquid chromatography-mass spectrometry (LC-MS) methods often encounter samples with analyte concentrations exceeding the upper limit of quantification (Fig. 1). Such high concentrations may compromise the accuracy of quantification and increase the risk of carryover to subsequent injections. To mitigate these issues, it is essential for LC-MS data acquisition software to incorporate features that allow users to define thresholds, such as upper concentration limits, and evaluate each injection in real-time. This enables automated responses to prevent carryover and maintain the integrity and reliability of the dataset.

Figure 1. A typical sequence table used in the clinical and toxicology lab.



Materials and methods

A mixture of cocaethylene, oxazepam, methadone, methamphetamine, and phencyclidine (PCP), along with their corresponding internal standards, were spiked into negative human oral fluid (Table 1). A 1 µL mixture was directly injected onto a Thermo Scientific™ Vanquish™ Flex UHPLC system coupled to an Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer equipped with the Thermo Scientific™ OptaMax™ NG HESI source. The sample preparation using the DPX XTR™ tips with mixed mode SCX/WAX chemistry for INTip™ dispersive solid phase extraction (dSPE) from DPX Technologies and the LC conditions are described in the technical note TN003851.¹ The MS parameters using the data-dependent MS2 (ddMS²) acquisition method is listed in TN002363.² Data was acquired and processed in Chromeleon software (v 7.3.2 MUe).

The calibration curve was generated using the Cal-4, 5, and 6 samples with concentrations 5, 20, and 100 ng/mL. The samples Cal-7 and Cal-8 with the concentrations 500 and 1000 ng/mL were used to demonstrate the SST/IRC test cases “AutoDilution_3x” and “AutoDilution_7x” in the Unknown sample types.

Table 1. Spiked samples used to demonstrate SST/IRC.

Samples	Spiked conc. (ng/mL)	IS conc. (ng/mL)
Cal-4	5	125
Cal-5	20	
Cal-6	100	
Cal-7	500	
Cal-8	1000	

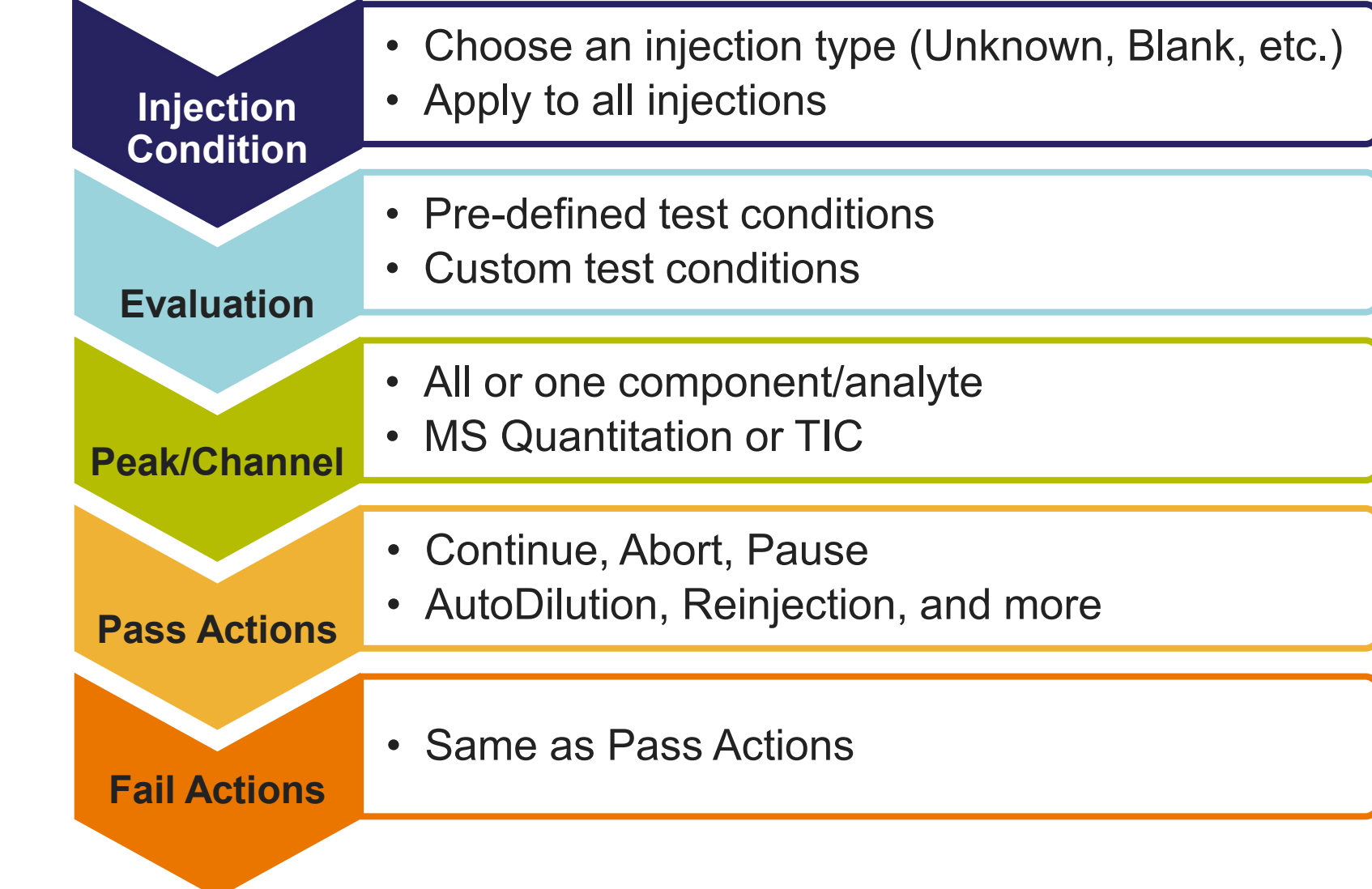
Results

SST/IRC test case overview

The SST/IRC functionality in a Chromeleon processing method enables customers to define one or more “test cases” to determine whether a certain operational conditions or processing results required for a specific measurement are met and performed pre-defined actions accordingly. The test case is constructed with five parts (Fig. 2). They can be restricted to specific injections, injection types, channels, and components. The main part of the test case is the Evaluation tab, which defines the test condition that is evaluated:

- What is tested; what value the result must be compared with
- where the test case result is to be returned (to Pass Actions or to Fail Actions) if the test condition cannot be evaluated.

Figure 2. Generic steps to set up the SST/IRC test cases.



Setting up test cases “AutoDilution_3x” and “AutoDilution_7x”

In the test case “AutoDilution_3x” (Fig. 3), the calculated analyte concentration (“peak.amount”) in the Unknown samples is compared to 3x the highest level in the calibration curve (3x Cal-6, 3x100 ng/mL = 300 ng/mL). “AutoDilution_3x” function in Fail Actions (Fig. 4) specifies a 1/3 injection volume for the re-injection of the sample, and the blank sample insertion running an instrument wash method prior to the sample re-injection. Similarly, “AutoDilution_7x” was set up to compare to 7x highest calibration level (700 ng/mL) and perform re-injection with 1/7 injection volume.

Test case results

Cal-6, 7 and 8 were injected as Unknown sample type to evaluate the test cases (sequence table is shown in Fig. 5).

- Row 12: “Cal6_unknown” has the calculated concentration at ~100 ng/mL. Both test cases passed the evaluation.
- Row 13: “Cal7_unknown” has the calculated concentration at ~500 ng/mL, failing AutoDilution_3x, but not AutoDilution_7x. Two injections were inserted: a solvent blank sample running with a column wash method (row 14), and re-injection of the same sample with 1/3 (0.33 µL) injection volumes (row 15).
- Row 16: “Cal8_unknown” has the calculated concentration at ~1000 ng/mL, failing both the test cases. Two injections were inserted: a solvent blank sample running with a column wash method (row 17), and re-injection of the same sample with 1/7 (0.14 µL) injection volumes (row 18).

Figure 3. Evaluation tab for the test case AutoDilute_3x.

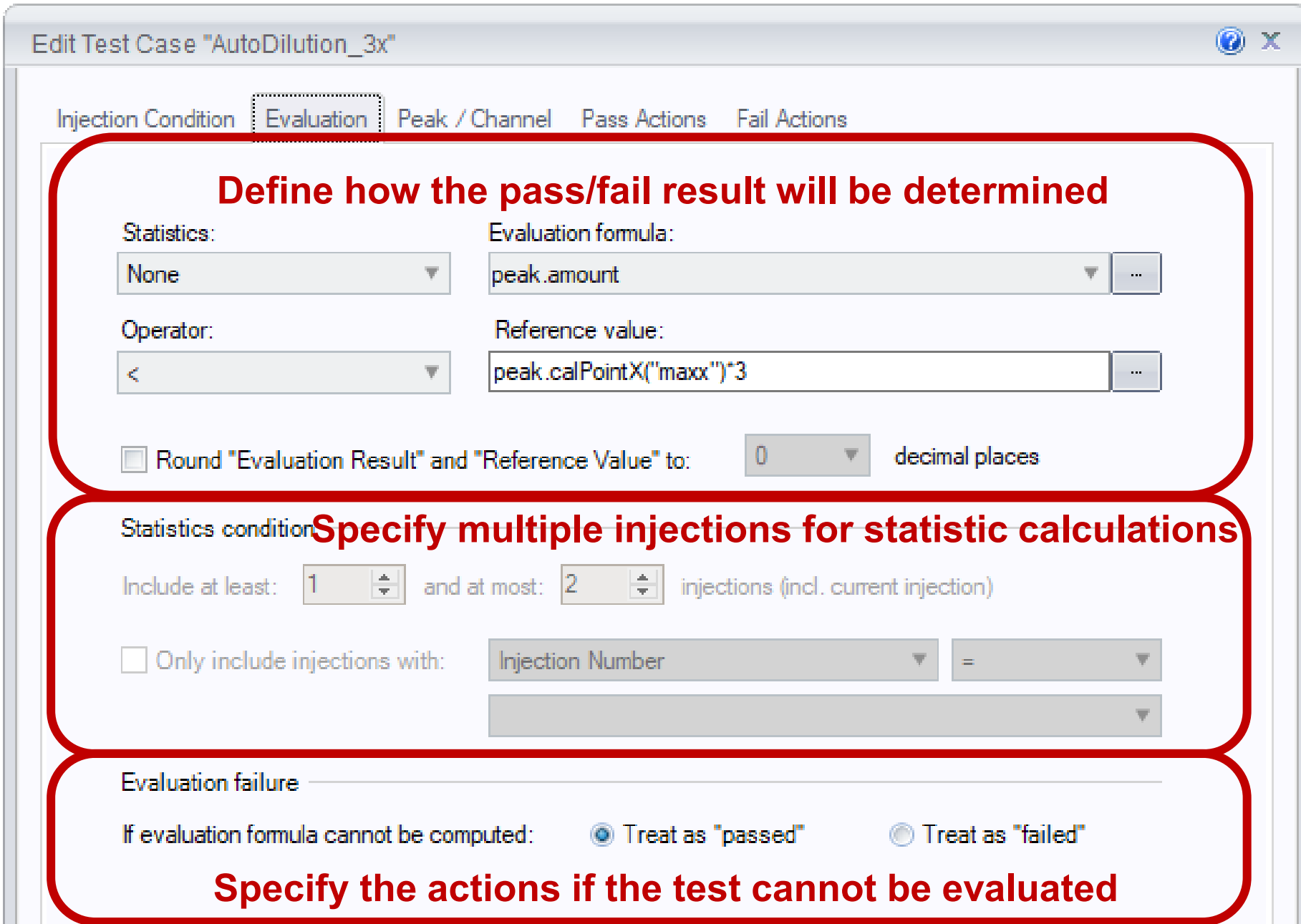


Figure 4. The Fail Actions of the test case AutoDilution_3x, which defined the insertion of the blank sample running with a column wash method and the re-injection of the unknown sample with 1/3 injection volume. The test case AutoDilution_7x can be set up similarly.

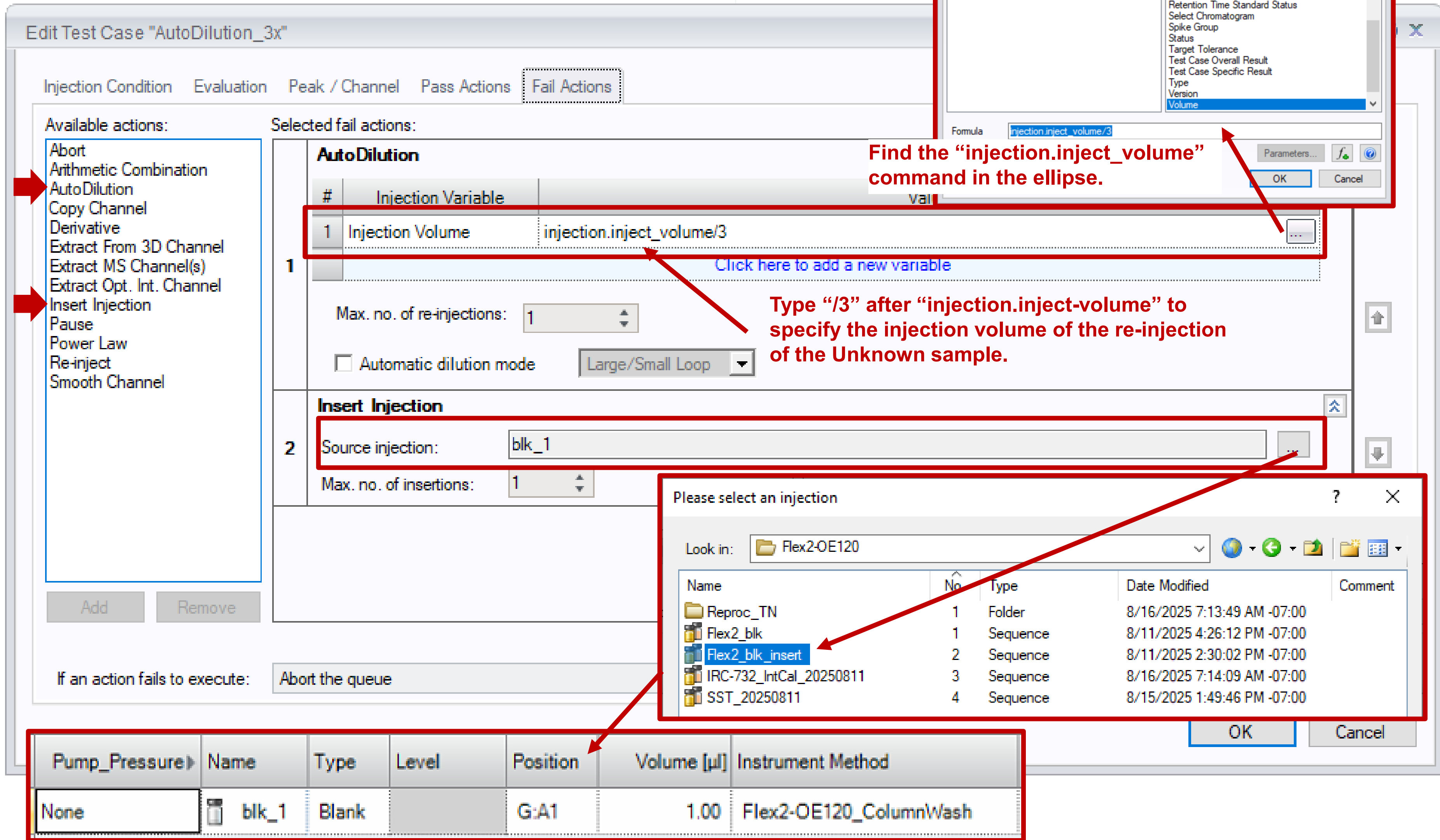


Figure 5. Sequence table shown the injection of the Cal6, Cal7, and Cal8 samples as Unknown injection type. The automatically inserted injections are reflected by the number “1” in the Re-injections column. The column wash method rapidly ramps the organic and aqueous solvent for three cycles to remove potential carryover in the LC flow path (rows 14 and 17). The 1/3 (row 15) or 1/7 (row 18) injection volumes decided by the test cases are shown in the corresponding re-injected samples.

#	Pump_Pressure>	Name	Type	Position	Volume [µl]	Re-injections	Instrument Method	AutoDilution_3x	AutoDilution_7x
12		Cal6_unknown	Unknown	R:A6	1.00	0	Flex2-OE120_OF_7min	Passed	Passed
13		Cal7_unknown	Unknown	R:A7	1.00	0	Flex2-OE120_OF_7min	Failed	Passed
14		blk_1	Blank	G:A1	1.00	1	Flex2-OE120_ColumnWash		
15		Cal7_unknown	Unknown	R:A7	0.33	1	Flex2-OE120_OF_7min		
16		Cal8_unknown	Unknown	R:A8	1.00	0	Flex2-OE120_OF_7min	Failed	Failed
17		blk_1	Blank	G:A1	1.00	1	Flex2-OE120_ColumnWash		
18		Cal8_unknown	Unknown	R:A8	0.14	1	Flex2-OE120_OF_7min		

Beyond AutoDilution test case

SST/IRC contains many pre-defined test cases that evaluate the chromatographic behavior and MS processing results in a sequence (Table 2). Many more can be customized based on users needs. Besides triggering real-time actions, some test cases can also be employed for reporting purpose only. This functionality can be applied to all Thermo Scientific mass spectrometers.

Table 2. Examples of test cases SST/IRC can evaluate and action upon.

Evaluation	Chromatographic behavior: <ul style="list-style-type: none"> Peak Asymmetry Tailing Factor Retention Time RSD of Peak Areas Signal / Noise Ratio MS detection results: <ul style="list-style-type: none"> Mass accuracy Peak area limit RSD of internal standard peak area Ion Ratios
Pass/Fail Actions	<ul style="list-style-type: none"> Continue to the next injection Abort the sequence Pause the sequence Reinject the sample Insert one or more injections Run with a different instrument method

Conclusions

This study successfully demonstrated the setup and utilization of two AutoDilution test cases in Chromeleon software to monitor analyte quantification results in real-time. Automatic responds were triggered by inserting a solvent blank injection with a column wash method followed by sample re-injection with a smaller injection volume. The SST/IRC allows users to select appropriate actions to adapt to their specific needs, ensuring reliable quantification and preventing carryover in clinical toxicology applications.

References

- Patterson, C., Hassell, K. Thermo Fisher Scientific, Technical Note, TN003851, 2025.
- Patterson, C., et. al. Thermo Fisher Scientific, Technical Note, TN002363, 2023.

General Laboratory Equipment – Not For Diagnostic Procedures.

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