Technical guide | 004431



Automated system suitability testing with real-time decision making for carryover mitigation

Authors

Jingshu Guo, Kimy Young, Kirstin Faoro, Courtney Patterson, Kerry Hassell
Thermo Fisher Scientific,
San Jose, CA

Keywords

Chromeleon software, system suitability test/intelligent run control (SST/IRC), real-time data evaluation and decision making, intelligent and automated response triggering, analyte carryover mitigation, data integrity and quality assurance, AutoDilution/re-injection/ injection insertion

Highlights

- System suitability test/intelligent run control (SST/IRC) functionality enables real-time monitoring and evaluation of key operational and data-processing parameters. This capability allows laboratories to define specific thresholds and automatically respond to deviations, which prevents potential issues such as carryover and quantification inaccuracies.
- Consistent system performance is facilitated by SST/IRC, minimizing the risk of erroneous results and enhancing overall laboratory efficiency by automating routine checks and corrective actions. This leads to more reliable data, reduced downtime, and improved confidence in the analytical results.

Goal

Demonstrate the capabilities of SST/IRC in Thermo Scientific™ Chromeleon™ software to monitor and evaluate key operational and data processing results and make real-time decisions to safeguard the integrity and reliability of the dataset.

Introduction

In routine testing environments, including clinical and forensic toxicology laboratories, there is a clear trend towards the increasing use of liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the quantification of a wide variety of analytes in biological samples. This technique is utilized in various applications, including therapeutic drug monitoring, endocrinology, and drugs of abuse.[1] To ensure prolonged and reliable operation of a liquid chromatography-mass spectrometry (LC-MS) system, it is crucial to adhere to good laboratory practices, such as following standardized protocols for sample preparation, performing routine instrument maintenance, and regularly running system suitability test (SST) samples and quality control (QC) samples before submitting a large number of unknown samples or specimens. Several operational conditions and data processing results can be monitored and evaluated during or after data collection to safeguard the quality of the LC-MS dataset or to facilitate troubleshooting. These parameters include instrument feedback (e.g., LC backpressure), chromatographic behavior (e.g., peak shape and retention time), and processing results (e.g., mass accuracy, peak area, calculated amount, and ion ratios).

In addition, laboratories sometimes encounter situations that require immediate attention, and failure to react may lead to severe consequences. For example, unknown samples with analyte concentrations greatly exceeding the upper limit of quantification (ULOQ) may compromise the quantification accuracy of that sample and increase the risk of carryover to subsequent injections. To mitigate these issues, it is essential for LC-MS data acquisition software to incorporate intelligent features that allow users to define thresholds, evaluate each injection, and make real-time decisions if necessary. A typical sample sequence and the potential parameters that need to be monitored with different injection types are shown in Figure 1. In this technical guide, we will explain step by step how to set up the SST/IRC functionality in Chromeleon software to enable data evaluation and automated responses, thereby maintaining the integrity and reliability of the dataset and mitigating potential harmful effects such as carryover. This functionality can be applied to any type of Thermo Scientific™ mass spectrometer (MS), including the single-stage quadrupole Thermo Scientific™ ISQ™ MS, the triple-stage quadrupole Thermo Scientific™ TSQ™ MS, the Thermo Scientific™ ion trap MS, and the Thermo Scientific™ Orbitrap™ MS.

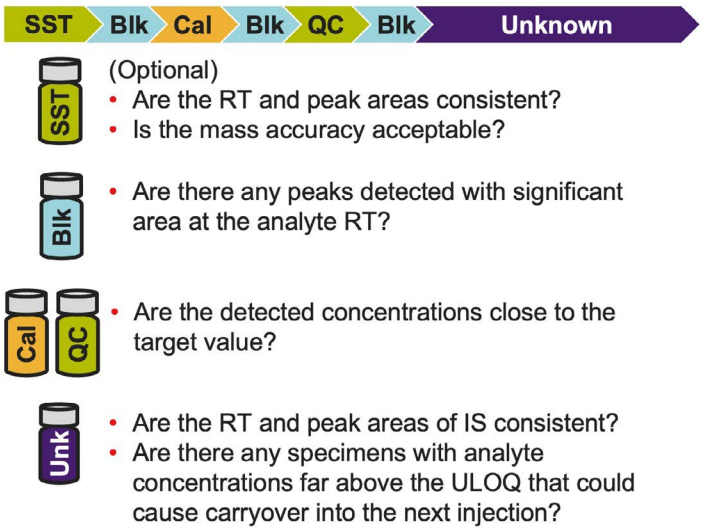


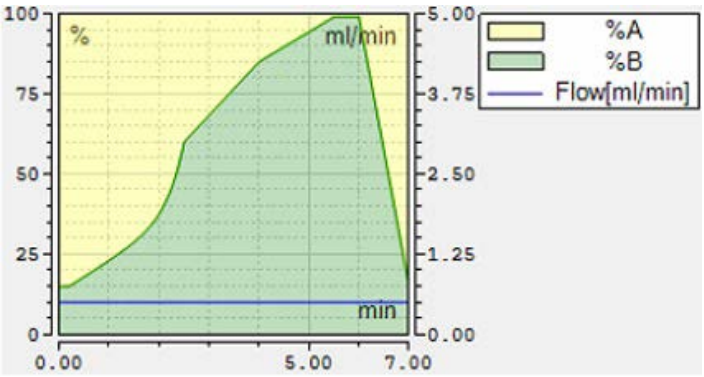
Figure 1. A representative LC-MS sequence used in a clinical and toxicology lab and some questions related to the quality of the dataset. SST: system suitability test; Blk: blank; Cal: calibration standards; QC: quality control; Unk: unknown.

Experimental

Samples and LC-MS parameters

Thirty-one drugs of abuse and their corresponding internal standards (IS) were mixed and spiked into negative human oral fluid to prepare for the eight samples (Cal1 to Cal8) at the analyte concentrations of 0.25, 0.5, 1, 5, 20, 100, 500, and 1,000 ng/mL. The IS concentration was constant at 125 ng/mL. The sample preparation using the in-tip dispersive solid-phase extraction of mixed-mode SCX/WAX chemistry is described in Thermo Fisher Scientific Technical Note **TN003851**.[2] A 1 µL sample was directly injected onto a Thermo Scientific™ Vanquish™ Flex UHPLC System coupled to a Thermo Scientific™ Orbitrap Exploris™ 120 Mass Spectrometer equipped with the Thermo Scientific™ OptaMax™ NG heated electrospray ionization (HESI) source. Analyte separation was achieved on a Thermo Scientific™ Accucore™ Biphenyl Reversed Phase HPLC Column (2.1 x 50 mm, 2.6 µm, Part No. 17826-052130) and the LC-MS parameters with data-dependent MS^2 ($ddMS^2$) acquisition mode, described in Thermo Fisher Scientific Technical Note **TN002363**[3] and Tables 1 and 2.[3] Five drugs with IS were randomly selected to demonstrate the SST/IRC functionality, but the same concept can be applied to any other analyte in the mix. The MS Component Table (Figure 2) shows the names, *m/z* values, retention time (RT), Calibration Standard, and Check Standard levels of the analyte and their internal standards.

Table 1. Chromatographic conditions used for the analyte separation.



Time (min)	Flow (µL/min)	%B	Curve
0.0	500	15	5
0.2	500	15	5
2.5	500	60	7
4.0	500	85	5
5.5	500	99	5
6.0	500	99	5
7.0	500	15	5
Column	Accucore biphenyl column (2.1 x 50 mm, 2.6 µm) at 40 °C		
Mobile phase A	0.1% formic acid in water		
Mobile phase B	0.1% formic acid in methanol		
Needle wash	50% isopropanol/acetonitrile		

Table 2. Ion source and MS parameters for ddMS² data acquisition using the Orbitrap Exploris 120 MS.

Thermo Scientific™ OptaMax™ Plus ion source properties	
Spray voltage (V)	(+) 3,500/(–) 2,000
Sheath/aux/sweep gas (Arb)	55/10/1
Ion transfer tube (ºC)	325
Vaporizer temp (ºC)	350
Chromatography peak width(s)	3
Lock mass correction	EASY-IC
Mode	RunStart
MS¹ parameters	
Orbitrap resolution	60,000
Scan range (*m/z*)	100–500
RF lens (%)	80
AGC target	Standard
Maximum injection time mode	Auto
Microscans	1
Data type	Profile
Polarity	Positive
Data-dependent parameters	
Intensity	1.0e5
Dynamic exclusion	Auto
Apex detection (%)	40
Top scans	4
Targeted mass	
Mass list and RT	Table 3
Mass tolerance (ppm)	5
ddMS²	
Isolation window (*m/z*)	1.5
Orbitrap resolution	15,000
First mass (*m/z*)	40
AGC target	Standard
Maximum injection time mode	Auto
Microscan	1
Data type	Profile

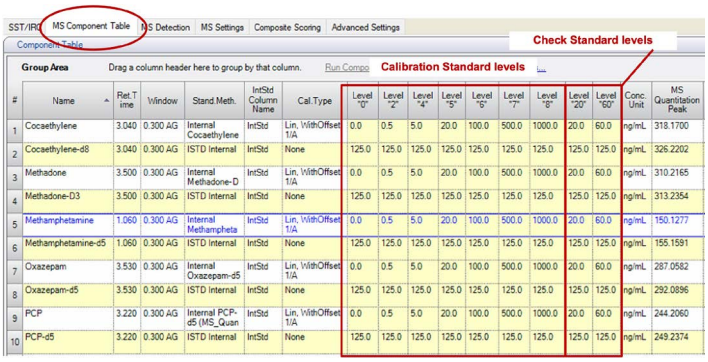


Figure 2. The MS Component Table showing the names, *m/z* values, RT, Calibration Standard, and Check Standard levels of the analyte and their internal standards used to demonstrate the SST/IRC capabilities in this technical guide.

Data analysis

Data acquisition and processing were performed using Chromeleon software version 7.3.2, with compatibility maintained for future versions. The calibration curves were generated using the samples Cal4 to Cal6 with concentrations of 5, 20, and 100 ng/mL, and the rest of the samples were used to evaluate the SST/IRC test cases in the Chromeleon software.

Results and discussion

Overview of SST/IRC and understanding the test case

The SST/IRC functionality in the Chromeleon Processing Method enables customers to define one or more test cases to determine whether certain operational conditions or data processing results required for a specific measurement are met, thereby triggering predefined actions accordingly. The test case is constructed with five parts (Figure 3). They can be restricted to specific injections, injection types, channels, and components:

- **Injection Condition** defines the set of injections to which the test case is applied during a sequence.
- **Evaluation** defines the test condition that is evaluated: what is tested, what value the result must be compared with, and where the test case result is to be returned (to Pass Actions or to Fail Actions) if the test condition cannot be evaluated. This is the main part of the test case.
- **Peak/Channel** defines the peaks (all components/analytes or a certain component) and channels (LC pressure, MS quantification) to which the test case is applied.
- **Pass Actions** or **Fail Actions** define the actions to be executed when the test case passes or fails, and what should happen if an action is unable to execute due to an error. Test cases can be employed for data review purposes only on the already-acquired raw files, under which conditions, no specific actions are selected for Pass Actions and Fail Actions.

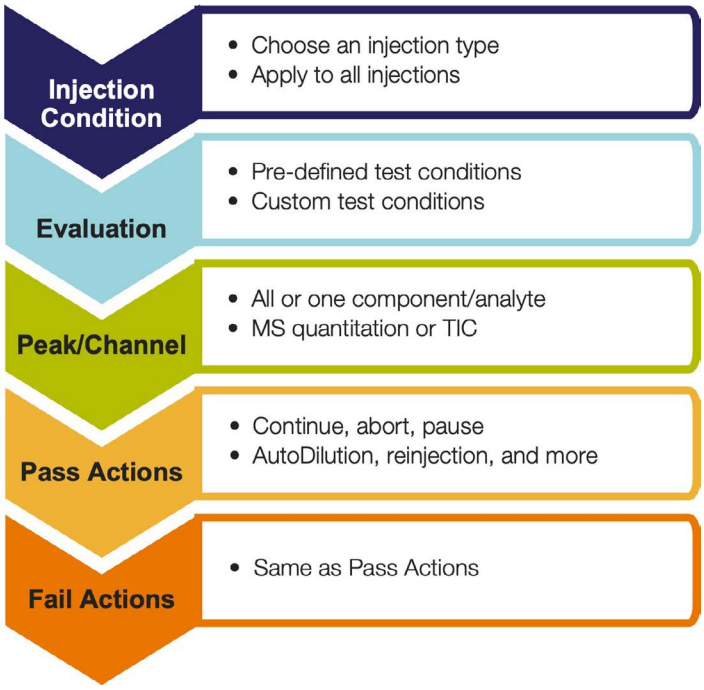


Figure 3. A test case is constructed of five parts.

Examples of the available options in each part of the test case are shown in Table 3. The setup of the test cases in SST/IRC starts from the Chromeleon Processing Method > SST/IRC Tab > Click here to add a new test case, then choose a pre-defined test case or a custom test case (Figure 4). There are a number of pre-defined test conditions available to choose from (Table 3). Figure 5 uses a pre-defined test case, "RSD of Peak Retention Times," to demonstrate each part of a test case to be used in the SST. Sections 3.2 and 3.3 will then explain in detail how to set up custom test cases for different injection type samples in the sequence.

Table 3. Example of test conditions and Pass or Fail Actions Chromeleon software SST/IRC can specify in the test cases.

Test case sections	Examples	
Injection Condition	• Apply to all injections • Injection type (choose from Unknown, Check Standards, Blank, etc.)	
Evaluation	Pre-defined test conditions: • Peak asymmetry • Peak resolution • RT • RSD of peak areas • RSD of RT • Signal/noise ratio • Theoretical plates	Custom (chromatographic behavior) test: • Tailing factor Custom (MS detection results) tests: • Mass accuracy • Peak area limit • RSD of internal standard peak area • RSD of internal standard RT
Peak/Channel	Component/peaks • All components • Component name (specify one component) Channels • TIC • Pressure • MS quantitation	
Pass Actions	• Continue to the next injection • Abort the sequence • Pause the sequence • Reinject the sample • Insert one or more injections • Perform an auto dilution (hardware dependent) • Run with a different instrument method	
Fail Actions	Same as Pass Actions	

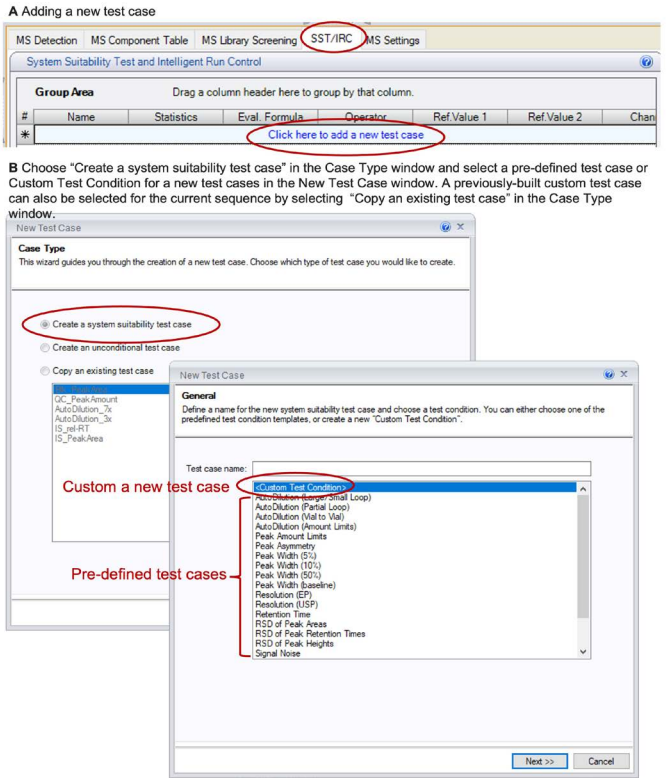


Figure 4. Setting up a test case in SST/IRC. (A) Adding a new test case by selecting "Click here to add a new test case." (B) Choose a pre-defined test case or a custom test case.

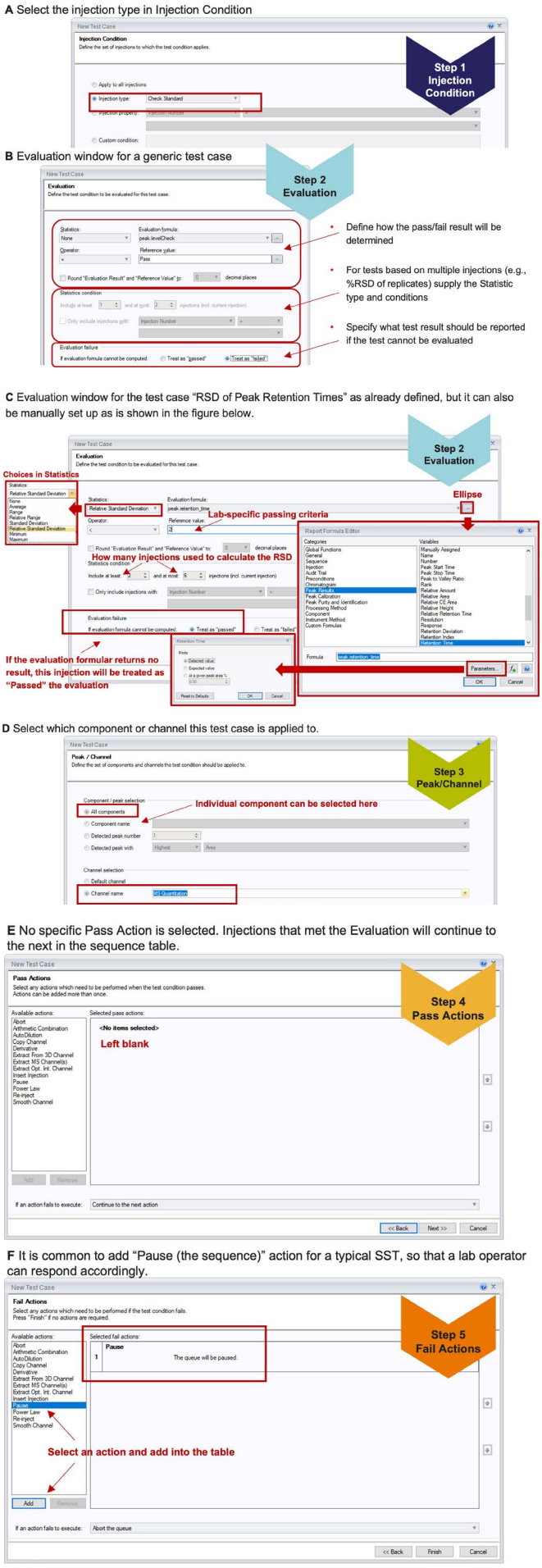


Figure 5. Setting up the pre-defined test case of "RSD of Peak Retention Times" for SST. (A) This test case is usually used for the Check Standards injection type during SST to ensure a consistent RT of the LC condition. (B) The Evaluation tab defines what is tested, what value the result must be compared with, and where the test case result is to be returned if the test condition cannot be evaluated. (C) Specifically, the test case "RSD of Peak Retention Times" evaluates the relative standard deviation of the detected peak retention time, which must be smaller than 2 from 2 (minimum) to 6 (maximum) injections to be considered as "Passed." It also defined that if the evaluation formula returns no result, this injection will be treated as "Passed" the evaluation and the corresponding Pass Actions will be applied. (D) This test case is applied to the MS quantification channel in all components/analytes in this method. (E and F) In a typical system suitability test, the Pass Actions is left blank and "Pause (the sequence)" is chosen for Fail Actions.

Establishing test cases for the SST (optional)

The purpose of the SST is to help ensure the LC-MS system is performing consistently and to expectations before submitting a large batch of samples containing real specimen. This test is optional and is usually executed by repeated injections of one sample, a synthetic standard in solvent, or a QC sample, and monitoring the analyte performance, such as peak area and RT.[4] Here, we injected the sample Cal5 seven times ("Cal5_SST_1" to "Cal5_SST_7") and chose the injection type Check Standard in the sequence table (Figure 6A). Three test cases were set up and evaluated (Figure 6B):

- "RSD of Peak Retention Times": evaluate the RT variation of all MS components in the injected Check Standard samples to be smaller than 2.0. The detailed explanation of each part of this test case is described in Figure 5.
  - » Inj.Condition "Check Standard"
  - » Statistics "Relative Standard Deviation," Eval.Formula "peak.retention_time" for Statistics Condition "at least 2 and at most 6 injections," Operator "<," and Ref.Value1 "2.0," N.A. (Evaluation failure, if evaluation formula cannot be computed, treated as "Passed")
  - » Channel "MS Quantification" and Peak "All Identified Peaks"
- "MassAccuracy": evaluate the mass accuracy in each injection for all components to be within ±5 ppm
  - » Inj.Condition "Check Standard"
  - » Statistics "None," Eval.Formula "peak.compScoring.massAccuracy," Operator "between" Ref.Value1 "-5" and Ref.Value2 "5"
  - » Channel "MS Quantification" and Peak "All Identified Peaks"

- “PeakArea”: evaluate the peak area in each injection, using PCP as an example, to be between 50,000 and 200,000
  - » Inj.Condition “Check Standard”
  - » Statistics “None” and Eval.Formula “peak.area,” Operator “between” Ref.Value1 “50,000” and Ref.Value2 “200,000”
  - » Channel “MS Quantification” and Peak “PCP”

“Evaluation failure, if evaluation formula cannot be computed, treated as Passed” is typically used for test cases. No specific Pass Action is usually employed and injections that met the evaluation will continue to the next in the sequence table. It is common to add “Pause (the sequence)” action for a typical SST and a lab operator can respond according to their lab operation procedure.

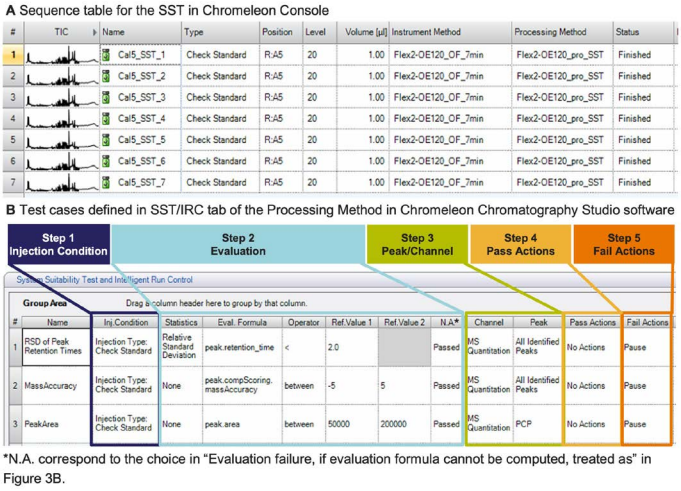


Figure 6. Setting up an SST sequence. (A) Sequence of seven injections of the Cal5 sample as Check Standard in the Chromeleon Console. (B) The three test cases used in the SST sequence shown in Chromeleon Chromatography Studio > Processing Method > SST/IRC tab. “RSD of Peak Retention Times” is calculated for at least two and at most six injections. Mass accuracy of all components (“All Identified Peaks”) and peak area of PCP were evaluated in each injection.

The test results are visualized in the Chromeleon Chromatography Studio in the customized Interactive Results SST table. Figure 7A shows the results for the first Cal5 sample (injection name “Cal5_SST_1”). According to the chosen Statistic Condition “at least 2 and at most 6 injections,” “RSD of Peak Retention Times” starts to compute from the second sample injection onward. Thus, Figure 7A only shows the test results for two test cases, “MassAccuracy” and “PeakArea.” The “Eval. Result” (column G) in the SST table corresponds to individual test case results: ppm values for “MassAccuracy” for all components, and area (counts*min) for “PeakArea” of PCP. Sample “Cal5_SST_1” passed both the test cases. Figure 7B shows the test results for the last Cal5 sample (injection name “Cal5_SST_7”). The “RSD of the Retention Time” was calculated based on the last six injections in the sequence table (Injection names “Cal5_SST_2” to “Cal5_SST_7”). Sample “Cal5_SST_7” passed all three test cases. No Pass Action is selected for the three test cases. Thus, injections that fulfill all three test cases in the evaluation, i.e., “Passed” in the “Test Result” (column E), will continue in the sequence table. The injections that failed any of the test cases, “Failed” in the “Test Result” column, will cause the sequence to pause, which allows the lab operator to decide to either inspect the LC-MS system or carry on to the subsequent injections.

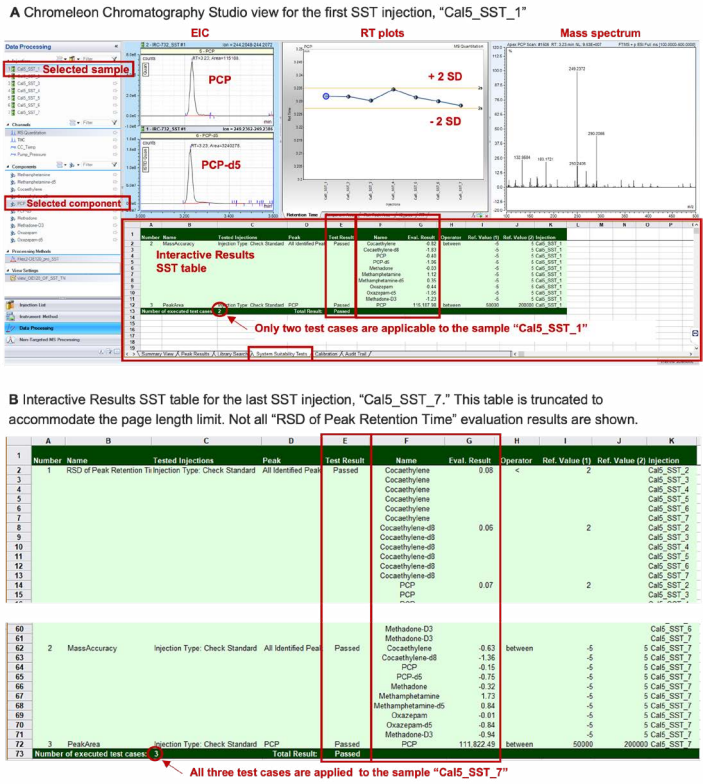
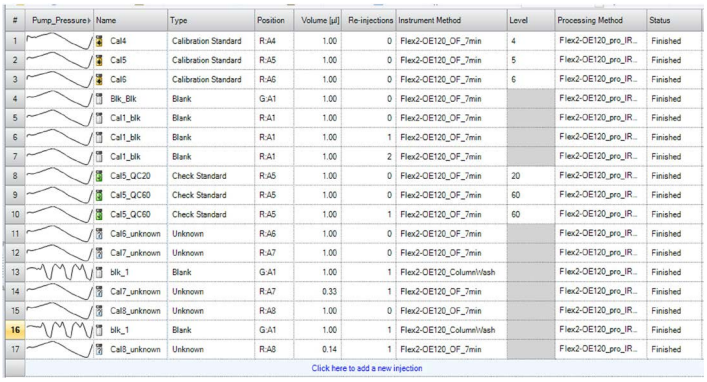


Figure 7. SST/IRC test results visualized in Chromeleon Chromatography Studio. (A) MS component PCP was selected as an example to show the extracted ion chromatogram (EIC) of its quantifying ion and that of its IS, the RT plots of all samples in the sequence (mean ±2 SD), and the mass spectrum at apex of the EIC. There is no “RSD of Peak Retention Time” computed for the first Cal5 sample injection “Cal5_SST_1.” The SST table of the Interactive Results only shows two test cases: the “MassAccuracy” of all components and the “PeakArea” of PCP. (B) Test results for all three test cases are shown in the SST table of the Interactive Results for the last Cal5 sample injection, “Cal5_SST_7.” “RSD of Peak Retention Time” was computed for the last six Cal5 sample injections in the sequence (“Cal5_SST_2” to “Cal5_SST_7”).

Setting up test cases for different injection types in a sequence table

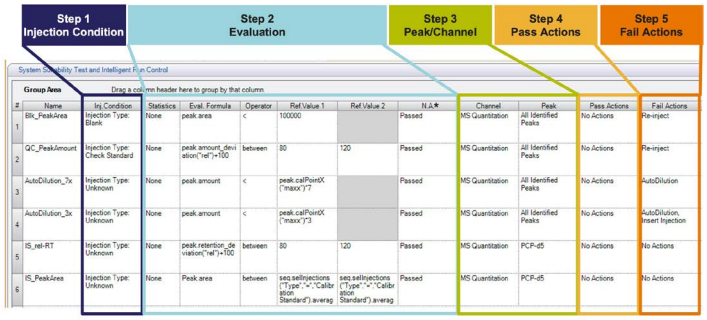
Six test cases for the commonly employed injection types, Blank, Check Standard, and Unknown, were set up in a sequence table (Figures 8 and 9). The calibration curves were generated using internal calibration and single injection of samples Cal4 to Cal6 at concentrations 5, 20, and 100 ng/mL (rows 1 to 3 in the sequence table, Figure 8). The EIC of PCP and its IS, PCP-D5, the calibration curve of PCP, the mass spectrum at the apex of the EIC, and MS Component Table are shown in Figure 10.



#	Pump_Pressure	Name	Type	Position	Volume [µl]	Re-injections	Instrument Method	Level	Processing Method	Status
1		Cal4	Calibration Standard	R:A4	1.00	0	Flex2-OE120_OF_7min	4	Flex2-OE120_pro_IR...	Finished
2		Cal5	Calibration Standard	R:A5	1.00	0	Flex2-OE120_OF_7min	5	Flex2-OE120_pro_IR...	Finished
3		Cal6	Calibration Standard	R:A6	1.00	0	Flex2-OE120_OF_7min	6	Flex2-OE120_pro_IR...	Finished
4		Blk_Blk	Blank	G:A1	1.00	0	Flex2-OE120_OF_7min		Flex2-OE120_pro_IR...	Finished
5		Cal1_blk	Blank	R:A1	1.00	0	Flex2-OE120_OF_7min		Flex2-OE120_pro_IR...	Finished
6		Cal1_blk	Blank	R:A1	1.00	1	Flex2-OE120_OF_7min		Flex2-OE120_pro_IR...	Finished
7		Cal1_blk	Blank	R:A1	1.00	2	Flex2-OE120_OF_7min		Flex2-OE120_pro_IR...	Finished
8		Cal5_QC20	Check Standard	R:A5	1.00	0	Flex2-OE120_OF_7min	20	Flex2-OE120_pro_IR...	Finished
9		Cal5_QC60	Check Standard	R:A5	1.00	0	Flex2-OE120_OF_7min	60	Flex2-OE120_pro_IR...	Finished
10		Cal5_QC60	Check Standard	R:A5	1.00	1	Flex2-OE120_OF_7min	60	Flex2-OE120_pro_IR...	Finished
11		Cal6_unknown	Unknown	R:A6	1.00	0	Flex2-OE120_OF_7min		Flex2-OE120_pro_IR...	Finished
12		Cal7_unknown	Unknown	R:A7	1.00	0	Flex2-OE120_OF_7min		Flex2-OE120_pro_IR...	Finished
13		blk_1	Blank	G:A1	1.00	1	Flex2-OE120_ColumnWash		Flex2-OE120_pro_IR...	Finished
14		Cal7_unknown	Unknown	R:A7	0.33	1	Flex2-OE120_OF_7min		Flex2-OE120_pro_IR...	Finished
15		Cal8_unknown	Unknown	R:A8	1.00	0	Flex2-OE120_OF_7min		Flex2-OE120_pro_IR...	Finished
16		blk_1	Blank	G:A1	1.00	1	Flex2-OE120_ColumnWash		Flex2-OE120_pro_IR...	Finished
17		Cal8_unknown	Unknown	R:A8	0.14	1	Flex2-OE120_OF_7min		Flex2-OE120_pro_IR...	Finished

Click here to add a new injection

Figure 8. Sequence table for test cases evaluation for injection types Blank, Check Standard, and Unknown.



*N.A. corresponds to the choice in "Evaluation failure, if evaluation formula cannot be computed, treated as" in Figure 3B.

Figure 9. The six test cases evaluated in the sequence table.

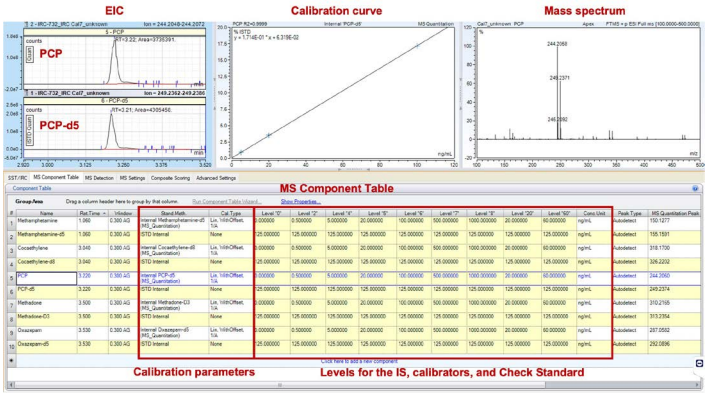


Figure 10. Chromeleon Chromatography Studio shows the MS Component Table and some of the processing results using PCP as an example: the stacked EIC of PCP and its IS, the calibration curve, and the mass spectrum at the EIC apex.

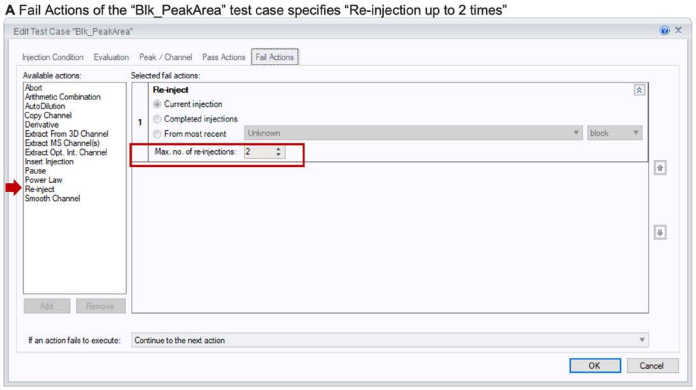
Blank samples

The test case, "Blk_PeakArea" was used to evaluate if significant peak area is detected at the RT of the components in the samples specified as blank (Figure 9). An arbitrary number of 100,000 was used as the peak area passing threshold. "Re-injection up to 2 times" is selected as the Fail Actions (Figure 11A).

- "BlkPeakArea":
  - » Inj.Condition "Blank"
  - » Statistics "None," Eval.Formula "peak.area," Operator "<" Ref.Value1 "100,000"
  - » Channel "MS Quantification" and Peak "All Identified Peaks"
  - » Pass Actions: No Actions
  - » Fail Actions: Re-inject (up to two)

A solvent blank sample, "Blk_Blk," and the Cal1 sample, "Cal1_blk," containing analytes with concentrations at 0.25 ng/mL and IS at 125 ng/mL, were used to test the Blank injection type (Figure 11B, rows 4 and 5, respectively). The test results of the two samples are reported in Figure 11C and Figure 11D. All detected peaks in sample "Blk_Blk" had the peak area less than 100,000 (Figure 11C), thus, passing the test case. In contrast, more than one peak in "Cal1_blk" was detected with peak areas higher than 100,000 (Figure 11D). This test result triggered automatic re-injection of the same sample up to two times (Figure 11B, rows 6 and 7). These two inserted samples were reflected by the number "1" and "2" in the column "Re-injections" in the sequence table. The repeated re-injection is used to confirm if the detected high peak areas are consistent or not. The consistently high peak areas in the presumptive blank sample indicate system contamination, while decreasing peak areas reflect potential analyte carryover from the preceding injections with high analyte concentrations.

A Fail Actions of the "Blk_PeakArea" test case specifies "Re-injection up to 2 times"



B Sequence table shown the injection of a solvent blank sample, "Blk_Blk" at position G:A1, and a sample containing analytes and IS, "Cal1_blk" at position R:A1. The inserted two re-injections were reflected as the numbers "1" and "2," in the column "Re-injections."

#	Pump_Pressure	Name	Type	Position	Volume [µl]	Re-injections	Instrument Method	Level	Processing Method	Status
4		blk_blk	Blank	G:A1	1.00	0	Flex2-OE120_OF_7min		Flex2-OE120_pro_IR...	Finished
5		Cal1_blk	Blank	R:A1	1.00	0	Flex2-OE120_OF_7min		Flex2-OE120_pro_IR...	Finished
6		Cal1_blk	Blank	R:A1	1.00	1	Flex2-OE120_OF_7min		Flex2-OE120_pro_IR...	Finished
7		Cal1_blk	Blank	R:A1	1.00	2	Flex2-OE120_OF_7min		Flex2-OE120_pro_IR...	Finished

C The SST table for the sample "Blk_Blk" (row 4 in the sequence table). All detected peaks had the peak area, in Eval. Result column, less than 100,000. Thus, this sample "Passed" the test case.

Tested Injections	Peak	Test Result	Name	Eval. Result	Operator	. Value (1)	ef. Value (2)	Injection
Injection Type: Blank	All Identified Peaks	Passed	Cocaethylene-d8	7,915.61	<	100000		Blk_Blk
			PCP-d5	2,751.52		100000		Blk_Blk
			ıthamphetamine	3,166.18		100000		Blk_Blk
			Oxazepam	26,919.61		100000		Blk_Blk
			Oxazepam-d5	5,340.21		100000		Blk_Blk
			Methadone-D3	8,316.83		100000		Blk_Blk
1		Total Result:	Passed					

D The SST table for the sample "Cal1_blk" (row 5 in the sequence table). At least one peak was detected with peak area more than 100,000. Thus, this sample "Failed" the test case, and two re-injections were inserted in the sequence table (rows 6 and 7).

Tested Injections	Peak	Test Result	Name	Eval. Result	Operator	. Value (1)	ef. Value (2)	Injection
Injection Type: Blank	All Identified Peaks	Failed	Cocaethylene	3,530.21	<	100000		Cal1_blk
			Cocaethylene-d8	7,387,257.56		100000		Cal1_blk
			PCP	1,365.87		100000		Cal1_blk
			PCP-d5	3,455,316.37		100000		Cal1_blk
			Methadone	2,488.14		100000		Cal1_blk
			Methamphetamin	1,947.78		100000		Cal1_blk
			ıthamphetamine	3,055,412.84		100000		Cal1_blk
			Oxazepam	22,374.75		100000		Cal1_blk
			Oxazepam-d5	4,369,026.37		100000		Cal1_blk
			Methadone-D3	9,096,970.50		100000		Cal1_blk
1		Total Result:	Failed					

Figure 11. Test case "Blk_PeakArea" used for injection type Blank.

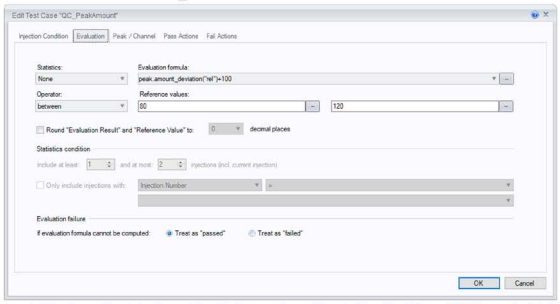
Check Standard samples

The calculated analyte concentrations in the QC samples, or Check Standard injection type termed in Chromeleon software, are expected to be within a certain range of the targeted values (Figures 9 and 12A). The test case, "QC_PeakAmount," is designed to evaluate if the relative analyte amount deviation is within 80–120% of the "Level 20" (20 ng/mL, defined in the MS Component Table in Figure 10) and "Level 60" (60 ng/mL) in the Check Standard injection type.

- "QC_PeakAmount"
  - » Inj.Condition "Check Standard"
  - » Statistics "None," Eval.Formula "peak.amount_deviation("rel")+100," Operator "between" Ref.Value1 "80" and Ref.Value2 "120"
  - » Channel "MS Quantification" and Peak "All Identified Peaks"
  - » Pass Actions: No Actions
  - » Fail Actions: Re-inject (one)

The IS had no internal calibration, thus, the test case would not compute for the IS peaks and will report as "n.a." in the result table. According to Figure 9 and Figure 12A, when evaluation cannot be computed, the result is treated as "Passed." Cal5 with the analyte concentration 20 ng/mL was injected and assigned as either Check Standard "Level 20" (target value 20 ng/mL) or "Level 60" (target value 60 ng/mL) to evaluate the test case (Figure 12B, rows 8 and 9). When Cal5 was tested for "Level 20" (injection name "Cal5_QC20"), the relative analyte amount deviations were within the 80–120% range or "n.a." (Figure 12C). Thus, this injection passed the test case, and the sequence table continued to the next sample. In contrast, when Cal5 sample was tested for the target value for "Level 60" (injection name "Cal5_QC60") the evaluation fell out of the reference range (Figure 12D) and triggered a re-injection of the sample (Figure 12B, row 10).

A The Evaluation tab for test case "QC_PeakAmount"



B Sequence table shows the injection of the Cal5 sample as Check Standard "Level 20" (sample "Cal5_QC20") or "Level 60" ("Cal5_QC60"). The inserted one re-injection was reflected as the number "1" in the column "Re-injections."

#	Pump_Pressure	Name	Type	Position	Volume [µl]	Re-injections	Instrument Method	Level	Processing Method	Status
8		Cal5_QC20	Check Standard	R:A5	1.00	0	Flex2-OE120_OF_7min	20	Flex2-OE120_pro_IR...	Finished
9		Cal5_QC60	Check Standard	R:A5	1.00	0	Flex2-OE120_OF_7min	60	Flex2-OE120_pro_IR...	Finished
10		Cal5_QC60	Check Standard	R:A5	1.00	1	Flex2-OE120_OF_7min	60	Flex2-OE120_pro_IR...	Finished

C The SST table for the sample "Cal_QC20" (row 8 in the sequence table). All detected analytes had the relative peak amount deviation, shown in the Eval. Result column, between 80 and 120. IS had no calibration. The test case would not compute for the IS peaks and will report as "n.a." According to Figure 8, "n.a" is treated as "Passed." Altogether, this sample passed the test case.

Tested Injections	Peak	Test Result	Name	Eval. Result	Operator	. Value (1)	ef. Value (2)	Injection
Injection Type: Check Standard	All Identified Peaks	NA -> Passed	Cocaethylene	101.06	between	80	120	Cal5_QC20
			Cocaethylene-d8	n.a.		80	120	Cal5_QC20
			PCP	96.59		80	120	Cal5_QC20
			PCP-d5	n.a.		80	120	Cal5_QC20
			Methadone	99.35		80	120	Cal5_QC20
			Methamphetamin	97.64		80	120	Cal5_QC20
			ıthamphetamine	n.a.		80	120	Cal5_QC20
			Oxazepam	102.71		80	120	Cal5_QC20
			Oxazepam-d5	n.a.		80	120	Cal5_QC20
			Methadone-D3	n.a.		80	120	Cal5_QC20
1		Total Result:	Passed					

D The SST table for the sample "Cal_QC60." The detected analytes had the relative peak amount deviation outside the 80–120 range. Thus, this sample failed the test case, and one re-injection was inserted in the sequence.

Tested Injections	Peak	Test Result	Name	Eval. Result	Operator	. Value (1)	ef. Value (2)	Injection
Injection Type: Check Standard	All Identified Peaks	Failed	Cocaethylene	34.18	between	80	120	Cal5_QC60
			Cocaethylene-d8	n.a.		80	120	Cal5_QC60
			PCP	33.53		80	120	Cal5_QC60
			PCP-d5	n.a.		80	120	Cal5_QC60
			Methadone	32.93		80	120	Cal5_QC60
			Methamphetamin	34.84		80	120	Cal5_QC60
			ıthamphetamine	n.a.		80	120	Cal5_QC60
			Oxazepam	33.69		80	120	Cal5_QC60
			Oxazepam-d5	n.a.		80	120	Cal5_QC60
			Methadone-D3	n.a.		80	120	Cal5_QC60
1		Total Result:	Failed					

Figure 12. Test case "QC_PeakAmount" is used for Check Standard injection type.

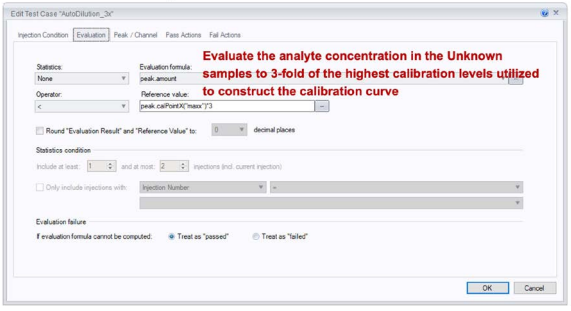
Unknown samples

Four test cases were evaluated in the Unknown samples. The two test cases of AutoDilution are the most important ones used to evaluate the calculated analyte concentrations and respond automatically when a specimen contains high analyte concentration that may cause carryover issues. In the test case “AutoDilution_3x,” the calculated analyte concentration in the Unknown samples is compared to 3-fold of the highest calibration level utilized to construct the calibration curve, which is 300 ng/mL (3 x Cal6 of 100 ng/mL) (Figure 13A). Similarly, test case “AutoDilution_7x” compared the calculated analyte concentrations to 7-fold of the highest calibration level, which is 700 ng/mL. To ensure the quantification accuracy and to mitigate the potential carryover, it is better to inject the Unknown sample, which has very high analyte concentrations, with a smaller injection volume. This is enabled by the AutoDilution function in Fail Actions, where either 1/3 injection volume (1/3 * 1 µL = 0.33 µL) or 1/7 injection volume (0.14 µL) was specified for samples failing either the “AutoDilution_3x” or “AutoDilution_7x” test case (Figure 13B).

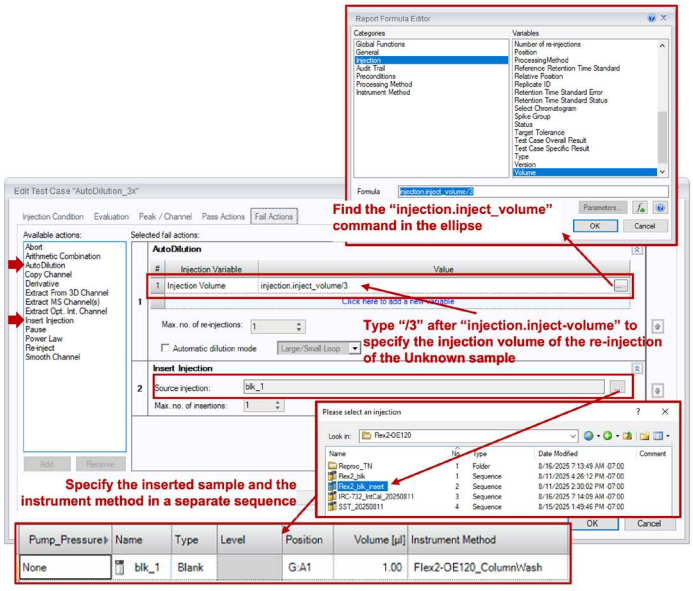
Ideally, the higher the analyte concentration, the smaller the injection volume. The Vanquish Flex UHPLC system autosampler is capable of reproducibly injecting 0.1 µL. The IS signal should also be high enough to compute the analyte concentration reliably. Thus, 1/7 (0.14 µL) was chosen as the smallest injection volume to balance the need for smaller sample volume re-injection and still have high enough IS response for reliable quantification. Prior to the re-injection/AutoDilution, a blank sample was inserted with a column wash method that rapidly ramps the organic and aqueous solvent for three cycles to remove potential carryover in the LC flow path (Figure 13C). This is enabled in the Insert Injection function in the Fail Actions and specified from a separately built sequence table containing the intended sample position and instrument method (Figure 13B).

- “AutoDilution_3x”:
  - » Inj.Condition “Unknown”
  - » Statistics “None,” Eval.Formula “peak.amount,” Operator “<” Ref.Value1 “peak.calPointX(“maxx”)*3”
  - » Channel “MS Quantification” and Peak “All Identified Peaks”
  - » Pass Actions: No Actions
  - » Fail Actions:
    - AutoDilution: Injection Variable “injection.inject_volume/3”
    - Insert Injection: source injection “blk_1” (referred from a separate sequence that runs a column wash method)
- “AutoDilution_7x”:
  - » Inj.Condition “Unknown”
  - » Statistics “None,” Eval.Formula “peak.amount,” Operator “<” Ref.Value1 “peak.calPointX(“maxx”)*7”
  - » Channel “MS Quantification” and Peak “All Identified Peaks”
  - » Pass Actions: No Actions
  - » Fail Actions:
    - AutoDilution: Injection Variable “injection.inject_volume/7”

A The Evaluation tab for “AutoDilution_3x”



B Fail Actions for “AutoDilution_3x” specifies the insertion of a blank sample with a column wash method (“Insert Injection” action), and re-injection of the same sample with 1/3 of the injection volume (“AutoDilution” action).



C Column wash method used for the inserted blank sample

LC conditions	
Flow rate	0.5 mL/min
Gradient, x3	15 to 99 %B, 0.5 min duration each
MS parameters	
HESI source	Same as Table 2
MS scan	*m/z* 100-500 at 60,000 resolution

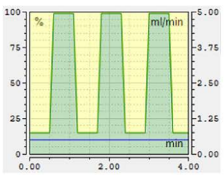


Figure 13. Test case “AutoDilution_3x” for Unknown injection type.

To evaluate the IS RT and peak area consistency, two test cases were employed, using PCP-d5 as an example (Figures 14 and 15). “IS_rel-RT” evaluated the relative RT deviation of PCP-d5 in Unknown samples to be between 80–120% (Figure 14). “IS_PeakArea” evaluates the peak area of PCP-d5 to be between 50–150% of those averaged from the Calibration Standards in the sequence table (Figure 15). We recommend employing these two test cases with no Fail Actions to not overcomplicate the SST/IRC process.

- “IS_rel-RT”:
  - » Inj.Condition “Unknown”
  - » Statistics “None,” Eval.Formula “peak.retention_deviation(“rel”)+100”), Operator “between” Ref.Value1 “80” and Ref.Value2 “120”
  - » Channel “MS Quantification” and Peak “PCP-d5”
  - » Pass Actions: No Actions
  - » Fail Actions: No Actions
- “IS_PeakArea”:
  - » Inj.Condition “Unknown”
  - » Statistics “None,” Eval.Formula “peak.area,” Operator “between” Ref.Value1 “seq.sellnjections(“Type,””=,””Calibration Standard”).average(“peak.area”)*0.5;” Ref.Value2 “seq.sellnjections(“Type,””=,””Calibration Standard”).average(“peak.area”)*1.5.”
  - » Channel “MS Quantification” and Peak “PCP-d5”
  - » Pass Actions: No Actions
  - » Fail Actions: No Actions

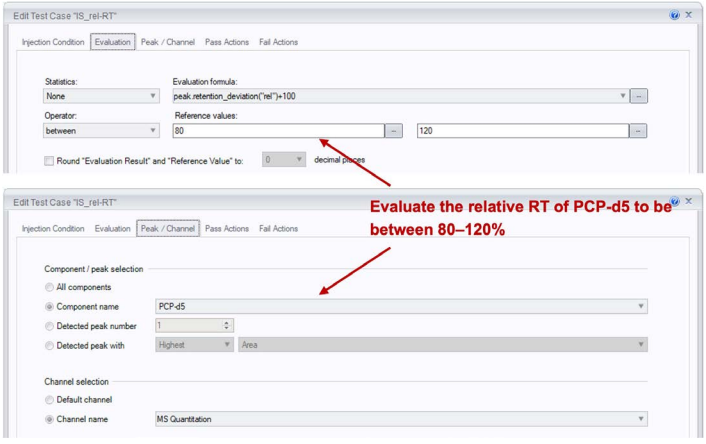


Figure 14. The Evaluation and Peak/Channel tabs for “IS_rel-RT.”

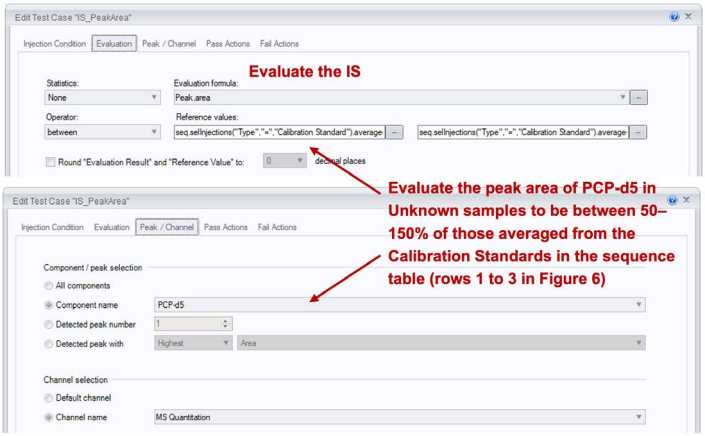


Figure 15. The Evaluation tab for “IS_PeakArea.”

Samples Cal6, Cal7, and Cal8 were used to evaluate the Unknown injection type (injection names “Cal6_unknown,” “Cal7_unknown,” and “Cal8_unknown” in rows 11, 12, and 15 in Figure 16A). Cal6 with analyte concentrations around 100 ng/mL passed the two AutoDilution test cases and the two test cases of PCP-d5 (Figure 16B), and the sequence table continued. Cal7 sample with analyte concentrations of about 500 ng/mL had at least one analyte concentration failing the “AutoDilution_3x” test case (Figure 13B). This triggered the automated insertion of a solvent blank injection with a column washing method followed by re-injection of the unknown sample with 1/3 injection volume (rows 13 and 14 in Figure 16A). Similarly, Cal8 had more than one analyte concentration over 700 ng/mL and failed both the “AutoDilution_3x” and “AutoDilution_7x” test cases. The sequence table automatically responded by inserting a solvent blank injection with a column washing method and re-injecting the unknown sample with 1/7 injection volume (rows 16 and 17 in Figure 16A). The column wash method rapidly ramped the organic and aqueous solvent for three cycles, and the employment of the method is reflected by the “wavy” pressure trace under the “Pump_Pressure” column in rows 13 and 16 of the injection list.

The blank insertion was only added to the “AutoDilution_3x” test case. The order of the two AutoDilution test cases was tested to ensure that only one blank sample was inserted. Specifically, if an unknown sample concentration exceeds the threshold defined in the “AutoDilution_7x,” a blank will not be added twice. In addition, for samples that fail both AutoDilution test cases, the 1/7 injection volume will overwrite the 1/3 injection volume.

A Sequence table showing the injection of the Cal6, Cal7, and Cal8 samples as Unknown type (rows 11, 12, and 15). Two injections were inserted for samples that failed the test cases: a solvent blank sample running with a column wash instrument method described in Figure 12C (rows 13 and 16), and re-injection of the same sample with 1/3 (0.33 µL) or 1/7 (0.14 µL) injection volumes (row 14 and 17).

#	Pump_Pressure	Name	Type	Position	Volume [µl]	Re-injections	Instrument Method	Level	Processing Method	Status
11		Cal6_unknown	Unknown	R:A6	1.00	0	Flex2-OE120_OF_7min		Flex2-OE120_pro_IR...	Finished
12		Cal7_unknown	Unknown	R:A7	1.00	0	Flex2-OE120_OF_7min		Flex2-OE120_pro_IR...	Finished
13		blk_1	Blank	G:A1	1.00	1	Flex2-OE120_ColumnWash		Flex2-OE120_pro_IR...	Finished
14		Cal7_unknown	Unknown	R:A7	0.33	1	Flex2-OE120_OF_7min		Flex2-OE120_pro_IR...	Finished
15		Cal8_unknown	Unknown	R:A8	1.00	0	Flex2-OE120_OF_7min		Flex2-OE120_pro_IR...	Finished
16		blk_1	Blank	G:A1	1.00	1	Flex2-OE120_ColumnWash		Flex2-OE120_pro_IR...	Finished
17		Cal8_unknown	Unknown	R:A8	0.14	1	Flex2-OE120_OF_7min		Flex2-OE120_pro_IR...	Finished

B The SST table for the sample "Cal6_unknown" (row 11 in the sequence table). All test cases passed the evaluation: detected analytes had the peak amount smaller than 3x of the highest calibration level (300 ng/mL); PCP-d5 relative peak retention time deviation is between 80 and 120%; PCP-d5 peak area is between 50–150% of those averaged from the calibration samples. Thus, this sample passed the test case.

Tested Injections	Peak	Test Result	Name	Eval. Result	Operator	Ref. Value (1)	Ref. Value (2)	Injection
Injection Type: Unknown	All Identified Peaks	NA -> Passed	Cocaethylene	101.93	<	peak.calPointX("maxx")*7		Cal6_unknown
			Cocaethylene-d	n.a.		peak.calPointX("maxx")*7		Cal6_unknown
			PCP	101.92		peak.calPointX("maxx")*7		Cal6_unknown
			PCP-d5	n.a.		peak.calPointX("maxx")*7		Cal6_unknown
			Methadone	100.42		peak.calPointX("maxx")*7		Cal6_unknown
			ethamphetamin	103.36		peak.calPointX("maxx")*7		Cal6_unknown
			thamphetamine	n.a.		peak.calPointX("maxx")*7		Cal6_unknown
			Oxazepam	101.35		peak.calPointX("maxx")*7		Cal6_unknown
			Oxazepam-d5	n.a.		peak.calPointX("maxx")*7		Cal6_unknown
			Methadone-D3	n.a.		peak.calPointX("maxx")*7		Cal6_unknown
Injection Type: Unknown	All Identified Peaks	NA -> Passed	Cocaethylene	101.93	<	peak.calPointX("maxx")*3		Cal6_unknown
			Cocaethylene-d	n.a.		peak.calPointX("maxx")*3		Cal6_unknown
			PCP	101.92		peak.calPointX("maxx")*3		Cal6_unknown
			PCP-d5	n.a.		peak.calPointX("maxx")*3		Cal6_unknown
			Methadone	100.42		peak.calPointX("maxx")*3		Cal6_unknown
			ethamphetamin	103.36		peak.calPointX("maxx")*3		Cal6_unknown
			thamphetamine	n.a.		peak.calPointX("maxx")*3		Cal6_unknown
			Oxazepam	101.35		peak.calPointX("maxx")*3		Cal6_unknown
			Oxazepam-d5	n.a.		peak.calPointX("maxx")*3		Cal6_unknown
			Methadone-D3	n.a.		peak.calPointX("maxx")*3		Cal6_unknown
Injection Type: Unknown	PCP-d5	Passed	PCP-d5	100.19	between	80	120	Cal6_unknown
Injection Type: Unknown	PCP-d5	Passed	PCP-d5	3,878,449.57	between	ard").average["peak.area"]*0.5:ige["peak.area"]*1.5		Cal6_unknown
4	Total Result:	Passed						

C The SST table for the sample "Cal7_unknown" (row 12 in the sequence table). At least one detected analytes had the peak amount larger than 3x but smaller than 7x of the highest calibration level. Thus, this sample failed the "AutoDilution_3x" test case. A blank sample with the column wash method was inserted (row 13), followed by a re-injection with 1/3 of the original injection volume (row 14).

Tested Injections	Peak	Test Result	Name	Eval. Result	Operator	Ref. Value (1)	Ref. Value (2)	Injection
Injection Type: Unknown	All Identified Peaks	NA -> Passed	Cocaethylene	506.57	<	peak.calPointX("maxx")*7		Cal7_unknown
			Cocaethylene-d	n.a.		peak.calPointX("maxx")*7		Cal7_unknown
			PCP	505.94		peak.calPointX("maxx")*7		Cal7_unknown
			PCP-d5	n.a.		peak.calPointX("maxx")*7		Cal7_unknown
			Methadone	507.85		peak.calPointX("maxx")*7		Cal7_unknown
			fethamphetamin	555.44		peak.calPointX("maxx")*7		Cal7_unknown
			thamphetamine	n.a.		peak.calPointX("maxx")*7		Cal7_unknown
			Oxazepam	498.87		peak.calPointX("maxx")*7		Cal7_unknown
			Oxazepam-d5	n.a.		peak.calPointX("maxx")*7		Cal7_unknown
			Methadone-D3	n.a.		peak.calPointX("maxx")*7		Cal7_unknown
Injection Type: Unknown	All Identified Peaks	Failed	Cocaethylene	506.57	<	peak.calPointX("maxx")*3		Cal7_unknown
			Cocaethylene-d	n.a.		peak.calPointX("maxx")*3		Cal7_unknown
			PCP	505.94		peak.calPointX("maxx")*3		Cal7_unknown
			PCP-d5	n.a.		peak.calPointX("maxx")*3		Cal7_unknown
			Methadone	507.85		peak.calPointX("maxx")*3		Cal7_unknown
			fethamphetamin	555.44		peak.calPointX("maxx")*3		Cal7_unknown
			thamphetamine	n.a.		peak.calPointX("maxx")*3		Cal7_unknown
			Oxazepam	498.87		peak.calPointX("maxx")*3		Cal7_unknown
			Oxazepam-d5	n.a.		peak.calPointX("maxx")*3		Cal7_unknown
			Methadone-D3	n.a.		peak.calPointX("maxx")*3		Cal7_unknown
Injection Type: Unknown	PCP-d5	Passed	PCP-d5	99.62	between	80	120	Cal7_unknown
Injection Type: Unknown	PCP-d5	Passed	PCP-d5	4,305,457.83	between	ard").average["peak.area"]*0.5:ige["peak.area"]*1.5		Cal7_unknown
4	Total Result:	Failed						

D The SST table for the sample "Cal8_unknown" (row 15 in the sequence table). At least one detected analyte had the peak amount larger than 7x of the highest calibration level. Thus, this sample failed both the "AutoDilution_3x" and "AutoDilution_7x" test cases. A blank sample with the column wash method was inserted (row 16), followed by a re-injection with 1/7 of the original injection volume (row 17).

Tested Injections	Peak	Test Result	Name	Eval. Result	Operator	Ref. Value (1)	Ref. Value (2)	Injection
Injection Type: Unknown	All Identified Peaks	Failed	Cocaethylene	902.75	<	peak.calPointX("maxx")*7		Cal8_unknown
			Cocaethylene-d	n.a.		peak.calPointX("maxx")*7		Cal8_unknown
			PCP	979.67		peak.calPointX("maxx")*7		Cal8_unknown
			PCP-d5	n.a.		peak.calPointX("maxx")*7		Cal8_unknown
			Methadone	979.23		peak.calPointX("maxx")*7		Cal8_unknown
			fethamphetamin	1,003.92		peak.calPointX("maxx")*7		Cal8_unknown
			thamphetamine	n.a.		peak.calPointX("maxx")*7		Cal8_unknown
			Oxazepam	955.91		peak.calPointX("maxx")*7		Cal8_unknown
			Oxazepam-d5	n.a.		peak.calPointX("maxx")*7		Cal8_unknown
			Methadone-D3	n.a.		peak.calPointX("maxx")*7		Cal8_unknown
Injection Type: Unknown	All Identified Peaks	Failed	Cocaethylene	902.75	<	peak.calPointX("maxx")*3		Cal8_unknown
			Cocaethylene-d	n.a.		peak.calPointX("maxx")*3		Cal8_unknown
			PCP	979.67		peak.calPointX("maxx")*3		Cal8_unknown
			PCP-d5	n.a.		peak.calPointX("maxx")*3		Cal8_unknown
			Methadone	979.23		peak.calPointX("maxx")*3		Cal8_unknown
			fethamphetamin	1,003.92		peak.calPointX("maxx")*3		Cal8_unknown
			thamphetamine	n.a.		peak.calPointX("maxx")*3		Cal8_unknown
			Oxazepam	955.91		peak.calPointX("maxx")*3		Cal8_unknown
			Oxazepam-d5	n.a.		peak.calPointX("maxx")*3		Cal8_unknown
			Methadone-D3	n.a.		peak.calPointX("maxx")*3		Cal8_unknown
Injection Type: Unknown	PCP-d5	Passed	PCP-d5	99.80	between	80	120	Cal8_unknown
Injection Type: Unknown	PCP-d5	Passed	PCP-d5	4,896,207.39	between	ard").average["peak.area"]*0.5:ige["peak.area"]*1.5		Cal8_unknown
4	Total Result:	Failed						

Figure 16. Sequence table and evaluation results for Unknown injection type.

Setting up an ion ratio test case for data review

In addition to the real-time monitoring, SST/IRC can set up test cases to use already-acquired data for reporting purposes in the data review process. For example, when quantifying an analyte using a selected reaction monitoring (SRM) or parallel reaction monitoring (PRM) method, the ion ratio between the peak area of the target (quantifying) ion and that of the confirming (qualifying) ion is crucial to confirm the identity of the analyte.[5] Ion ratios differentiate each analyte from co-eluting interfering compounds with similar SRM or PRM transitions. Consistent ion ratios across different samples indicate that the correct analyte is being measured, enhancing the specificity, reliability, and accuracy of the method. Deviations from expected ion ratios can signal issues such as instrument malfunction, sample contamination, or matrix effects. A typical ion ratio tolerance is 20% relative to that in the calibration samples.

A test case "Rel_IonRatio" was evaluated using a set of immunosuppressant (ISD) quantification results generated from an LC-triple quadrupole tandem MS (QqQ-MS/MS) method. Analytes tacrolimus, sirolimus, and everolimus used ascomycin as the IS for internal calibration, while cyclosporine A used cyclosporine D. To generate a 6-point calibration curve, 16 samples were injected as Unknown injection type (Figure 17A). The test case details are shown below and in Figure 17B.

- "Rel_IonRatio":
  - » Inj.Condition "Unknown"
  - » Statistics "None," Eval.Formula "(peak.confirmationRatio)/(seq.selInjections("Type,""=,""Calibration Standard").average["peak.confirmationRatio"])*100," Operator "between" Ref.Value1 "80," Ref.Value2 "120"
  - » Channel "MS Quantification" and Peak "All Identified Peaks"
  - » Pass Actions: No Actions
  - » Fail Actions: No Actions

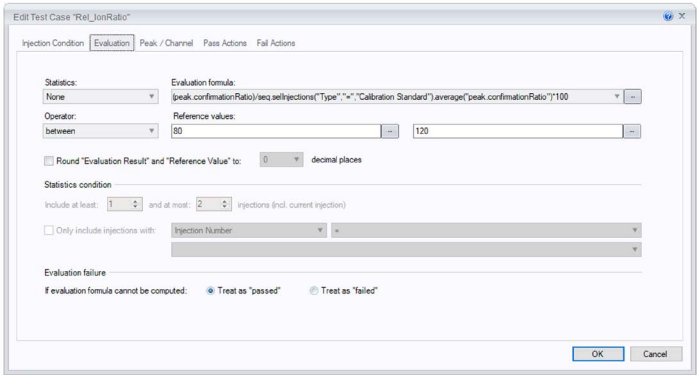
The Chromeleon Chromatography Studio view of the ISD quantification dataset processing results is shown in Figure 17C and Figure 17D. The SST table in the Interactive Results and relative ion ratio plots for all components across the 16 unknown samples were within ±20% of those averaged from the calibrators.

A Sequence table of the LC-QqQ-MS/MS data set used for the Rel_IonRatio test case

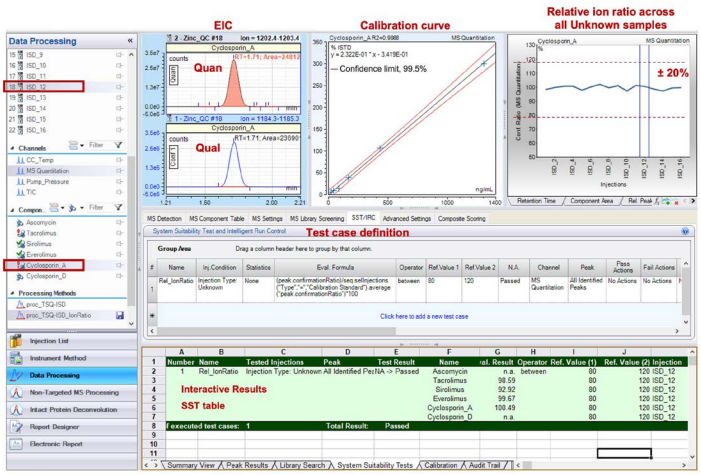
#	TIC	Name	Type	Level	Position	Volume [µL]	Instrument Method	Processing Method	Status
1		ISD_Cal1	Calibration Standard	1	G:A4	1.0000		proc_TSQ-ISD_IonRatio	Finished
2		ISD_Cal2	Calibration Standard	2	G:A5	1.0000		proc_TSQ-ISD_IonRatio	Finished
3		ISD_Cal3	Calibration Standard	3	G:A6	1.0000		proc_TSQ-ISD_IonRatio	Finished
4		ISD_Cal4	Calibration Standard	4	G:A7	1.0000		proc_TSQ-ISD_IonRatio	Finished
5		ISD_Cal5	Calibration Standard	5	G:A8	1.0000		proc_TSQ-ISD_IonRatio	Finished
6		ISD_Cal6	Calibration Standard	6	G:A9	1.0000		proc_TSQ-ISD_IonRatio	Finished
7		ISD_1	Unknown		G:B1	1.0000		proc_TSQ-ISD_IonRatio	Finished
8		ISD_2	Unknown		G:B3	1.0000		proc_TSQ-ISD_IonRatio	Finished
9		ISD_3	Unknown		G:B4	1.0000		proc_TSQ-ISD_IonRatio	Finished
10		ISD_4	Unknown		G:B5	1.0000		proc_TSQ-ISD_IonRatio	Finished
11		ISD_5	Unknown		G:B1	1.0000		proc_TSQ-ISD_IonRatio	Finished
12		ISD_6	Unknown		G:B3	1.0000		proc_TSQ-ISD_IonRatio	Finished
13		ISD_7	Unknown		G:B4	1.0000		proc_TSQ-ISD_IonRatio	Finished
14		ISD_8	Unknown		G:B5	1.0000		proc_TSQ-ISD_IonRatio	Finished
15		ISD_9	Unknown		G:B1	1.0000		proc_TSQ-ISD_IonRatio	Finished
16		ISD_10	Unknown		G:B3	1.0000		proc_TSQ-ISD_IonRatio	Finished
17		ISD_11	Unknown		G:B4	1.0000		proc_TSQ-ISD_IonRatio	Finished
18		ISD_12	Unknown		G:B5	1.0000		proc_TSQ-ISD_IonRatio	Finished
19		ISD_13	Unknown		G:B1	1.0000		proc_TSQ-ISD_IonRatio	Finished
20		ISD_14	Unknown		G:B3	1.0000		proc_TSQ-ISD_IonRatio	Finished
21		ISD_15	Unknown		G:B4	1.0000		proc_TSQ-ISD_IonRatio	Finished
22		ISD_16	Unknown		G:B5	1.0000		proc_TSQ-ISD_IonRatio	Finished

Click here to add a new injection

B The Evaluation tab for the test case "Rel_IonRatio"



C Chromeleon Chromatography Studio view of the ISD quantification dataset processing results using cyclosporin A and the unknown sample "ISD_12" as examples



D. Relative ion ratio plot in all unknown samples for tacrolimus, sirolimus, and everolimus

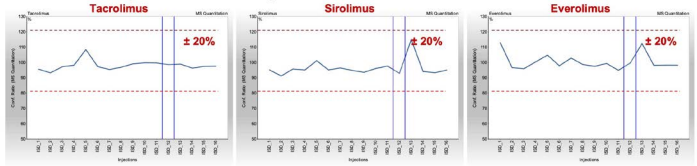


Figure 17. Evaluation of the test case "Rel_IonRatio" for a set of ISD quantification results generated using a LC-QqQ-MS/MS method. Analyte tacrolimus, sirolimus, and everolimus use ascomycin as the IS, while cyclosporine A uses cyclosporine D. (A) Sequence table consists of six calibration samples followed by 16 unknown sample injections. (B) The Evaluation tab for the test case "Rel_IonRatio." (C) Chromeleon Chromatography Studio view of the processed data, using cyclosporin A and the unknown sample "ISD_12" as examples: stacked EIC of the quantifying and qualifying ions, calibration curve, relative ion ratio across all unknown samples, test case setup, and the SST table in the Interactive Results. (D) The relative ion ratio plot in all unknown samples for tacrolimus, sirolimus, and everolimus was within ±20% of those averaged from the calibrators.

Conclusion

The SST/IRC capabilities in Chromeleon software provide a robust framework for maintaining the integrity and reliability of LC-MS datasets in routine testing laboratories, helping save precious samples and time for data review. By automating the evaluation of critical operational conditions and data processing results, Chromeleon software enables real-time decision-making to address potential issues promptly. This not only safeguards the quality of the data but also enhances laboratory efficiency and productivity. The ability to define and execute automated responses to test case outcomes helps ensure that the LC-MS system operates consistently and within expected parameters, ultimately leading to more accurate and reliable analytical results.

References

1. Seger, C., Salzmann, L. After another decade: LC-MS/MS became routine in clinical diagnostics. Clin Biochem. 2020, 82, 2–11. doi: 10.1016/j.clinbiochem.2020.03.004.
2. Patterson, C., Hassell, K. Quantitation of an oral fluid drug panel including THC using the new Stellar mass spectrometer. Thermo Fisher Scientific, Technical Note, TN003851, 2025.
3. Patterson, C., Tracy, M., et. al. Utilizing HRAM Orbitrap mass spectrometry to quantitate 106 drugs of abuse in urine. Thermo Fisher Scientific, Technical Note, TN002363, 2023.
4. Zipfell, P., Tontala, F. Performing automated system suitability testing and intelligent run control. Thermo Fisher Scientific, Technical Note, TN72823, 2018.
5. Clinical and Laboratory Standards Institute (CLSI). Liquid Chromatography–Mass Spectrometry Methods; Approved Guideline. CLSI document C62-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.

Find out more at **thermofisher.com/chromeleonms**

General Laboratory Equipment – Not For Diagnostic Procedures. © 2026 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manner that might infringe on the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details. **TG 004431-EN 0326**