Small Molecule System Suitability (Evaluation) Test for LC-MS/MS

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ABSTRACT

Quality Control Standard for High-Flow LC-MS Workflows

Purpose: Quick, fast and easy evaluation to assess performance of LC-MS/MS systems.

Methods: LC-MS/MS analysis of a pre-prepared standard solution, is injected 10 times using a simple and fast binary gradient. Automated data processing and reporting allows multiple aspects of the entire LC-MS/MS system to be evaluated and assessed.

Results: Performance and acceptance criteria are assessed for each module of the LC-MS/MS system. LC pump and LC column performance were monitored by assessing RT precision and column back-pressure. Autosampler precision assessed by replicates of very low injection volume and accuracy by injecting variable injection volumes. Mass spectrometer performance evaluated by achieving a minimum intensity threshold and maintaining precision (RSD < 10%) for all compounds in both positive and negative ionization mode. The automatically generated report confirms when systems are at expected performance.

INTRODUCTION

For any analytical laboratory utilizing liquid chromatography with tandem mass spectrometry (LC-MS/MS), determining whether the entire analytical system is working to an expected performance level can be of vital importance. Any method used to do this, must be simple to set-up, quick to run and have pre-defined acceptance criteria with a report automatically generated confirming that the system is performing as expected. The methodology should test all modules (*e.g.,* LC pump, autosampler, and mass spectrometer) of the entire LC-MS/MS system. Specifically designed for this purpose, the commercially available Thermo Scientific™ Pierce™ Small Molecule System Suitability Standard (SMSS) was used to assess the performance of LC-MS/MS systems.
SMSS Part number: A51740

MATERIALS AND METHODS

Sample Preparation

25µL of the SMSS standard was transferred into a reduced volume, silanized HPLC vial, supplied with the SMSS kit

Test Method(s)

LC method: 5-minute binary gradient (Figure 1) on a Thermo Scientific™ Vanquish™ Flex or Horizon Binary UHPLC system equipped with temperature-controlled autosampler and column compartment.

Analysis: 10 replicate injections of 0.1µL SMSS standard separated on a 2.1 x 50mm Thermo Scientific™ Hypersil GOLD™ column. Mass Spectrometer detection: Heated electrospray with a minimum of 2 SRM transitions for 9 different compounds, ranging from m/z 75 to 1222 in both positive and negative ionization mode (Figure 2) on Thermo Scientific™ TSQ Altis™ Plus, TSQ Quantis™ Plus or TSQ Fortis™ Plus triple quadrupole mass spectrometers.

Figure 1. Binary LC-gradient gradient using mobile phases of water with 0.1% formic acid and methanol

No	Time	Flow [ml/min]	%B	Curve
1	0.000	Run		
2	0.000	0.500	1.0	5
3	3.600	0.500	98.0	5
4	4.500	0.500	98.0	5
5	4.600	0.500	1.0	5
6	*New Row*			
7	5.500	Stop Run		

Figure 2. Compounds and ionization mode included SMSS standard..

Positive mode	Negative mode
Atenolol	Methylmalonic acid
Atrazine	Raltoxanide
Flumetsulam	Ultramark
Glycine	Warfarin
Terfenadine	
Ultramark	
Warfarin	

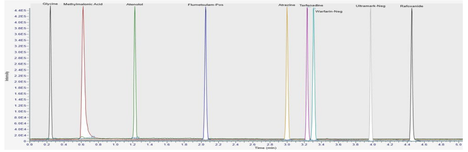
Data Analysis: Data acquisition, processing, analysis and reporting were performed using Thermo Scientific™ TraceFinder™ or Chromeleon™ data analysis software.

RESULTS

Components easily detected with low injection volume

Evaluating Peak response and precision, across the chromatographic gradient, in both positive and negative modes in less than 5 minutes, using a very low 0.1 µL injection volume (Figure 3)

Figure 3. Normalized LC-MS/MS chromatogram of all components with 0.1µL injection volume



Report based on 10 replicate injections assessing peak area response and precision

A summary report is automatically generated (Figure 4). Pass criteria are evaluated based on peak area (specific to each TSQ model), reproducibility across the mass range and linearity of the autosampler. Pass indicates the entire LC-MS/MS system is performing as expected.

Figure 4. Summary report evaluating entire LC-MS/MS system.

TSQ Triple Quadrupole LC-MS/MS System Evaluation

Batch Name: SMSSTSQ-A-30112-23FEB2022
Date of Last Acquisition: 23/02/2022 15:55
Instrument: TSQ Altis Plus

	Peak Response	Peak Precision	Peak Linearity
	PASS	PASS	PASS

	Atenolol	Atrazine	Flumetsulam-Pos	Glycine	MMA	Raltoxanide	Terfenadine	Ultramark-Neg	Ultramark-Pos	Warfarin-Neg	Warfarin-Pos
SMSS_RSD_01	2.76E+05	2.01E+06	4.78E+05	2.19E+07	6.34E+06	6.43E+05	2.05E+06	4.77E+05	8.83E+04	1.59E+05	9.92E+05
SMSS_RSD_02	2.72E+05	2.03E+06	4.76E+05	2.18E+07	6.33E+06	6.37E+05	2.04E+06	4.73E+05	8.79E+04	1.67E+05	1.02E+06
SMSS_RSD_03	2.80E+05	2.07E+06	4.97E+05	2.21E+07	6.42E+06	6.73E+05	2.04E+06	4.63E+05	8.99E+04	1.65E+05	1.01E+06
SMSS_RSD_04	2.98E+05	2.00E+06	4.61E+05	2.10E+07	6.17E+06	6.30E+05	1.98E+06	4.41E+05	9.22E+04	1.56E+05	9.73E+05
SMSS_RSD_05	2.95E+05	2.10E+06	4.96E+05	2.20E+07	6.33E+06	6.69E+05	2.05E+06	4.59E+05	9.32E+04	1.72E+05	1.06E+06
SMSS_RSD_06	2.90E+05	1.99E+06	4.54E+05	2.15E+07	5.97E+06	6.16E+05	1.97E+06	4.45E+05	8.97E+04	1.65E+05	9.76E+05
SMSS_RSD_07	3.09E+05	2.04E+06	4.89E+05	2.16E+07	6.28E+06	6.77E+05	2.03E+06	4.57E+05	9.21E+04	1.67E+05	1.02E+06
SMSS_RSD_08	2.86E+05	1.98E+06	4.63E+05	2.08E+07	5.90E+06	6.45E+05	1.93E+06	4.45E+05	8.72E+04	1.62E+05	9.61E+05
SMSS_RSD_09	3.18E+05	2.09E+06	5.02E+05	2.20E+07	6.35E+06	6.77E+05	2.07E+06	4.49E+05	1.04E+05	1.69E+05	1.03E+06
SMSS_RSD_10	3.31E+05	2.07E+06	4.81E+05	2.19E+07	6.36E+06	6.63E+05	2.01E+06	4.31E+05	9.38E+04	1.71E+05	9.81E+05
Ave Response	2.95E+05	2.04E+06	4.80E+05	2.17E+07	6.24E+06	6.53E+05	2.02E+06	4.54E+05	9.19E+04	1.65E+05	1.00E+06
% RSD	6.4	2.1	3.5	2.1	2.8	3.3	2.2	3.1	5.3	3.2	3.1
Linearity R^2	not tested	0.9994	0.9996	not tested	not tested	0.9955	0.9994	0.9979	0.9975	0.9996	0.9990

Autosampler evaluation

As well as %RSD based on 10 replicate injections, plotting peak areas of variable injection volumes, a linear response demonstrates the accuracy of the autosampler. (Figure 5)

UHPLC Pump performance evaluation

Overlay of 10 replicate pump pressures evaluate the reproducibility of the gradient and therefore the precision of the UHPLC pumps. Retention time precision again highlights the precision of the LC system. (Figure 6)

Figure 5. Linear response of 0.1, 0.2, 0.3, 0.4 and 0.5 µL injections volumes

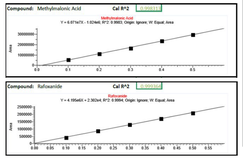
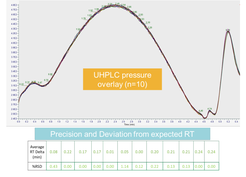


Figure 6. Pump pressure overlay for 10 replicate injections and precision of Retention time for SMSS analytes.



RESULTS

Carryover of the autosampler

Comparing the difference between a blank injection at the start of the sequence, a blank injected immediately after the highest amount of SMSS (0.5 µL injection) and the ratio to the SMSS standard (Figure 7) the % carryover of the LC system can be evaluated (Table 1)

Figure 7. Overlay of initial blank, 0.5 µL injection of SMSS and blank injected immediately afterwards, for Flumetsulam (left) and Warfarin (right)

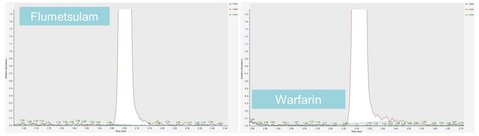


Table 1. Calculated Carryover %

	Flumetsulam-Pos	Warfarin-Pos
Blank_03	2.70E+02	2.48E+02
SMSS_Linearity_05	2.61E+06	3.04E+06
Blank_04	3.45E+02	4.61E+02
% Carryover	<0.01	<0.01

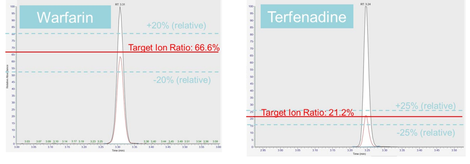
Ion Ratios evaluates consistency of Q2 collision cell and transmission of product ions

Ion ratios can be calculated and checked to see whether they are within limits defined by EU Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (2002/657/EC) (Figure 8). Ion ratio plot of Warfarin and Terfenadine, showing the relative limits based on the relative intensity of the confirming ion (Figure 9)

Figure 8. Ion Ratio limits as defined by 2002/657/EC

Relative intensity (% of base peak)	LC-MS, LC-MS^n (relative)
> 50 %	± 20 %
> 20 % to 50 %	± 25 %
> 10 % to 20 %	± 30 %
≤ 10 %	± 50 %

Figure 9. Ion Ratios plot for Warfarin (left) and Terfenadine (right)

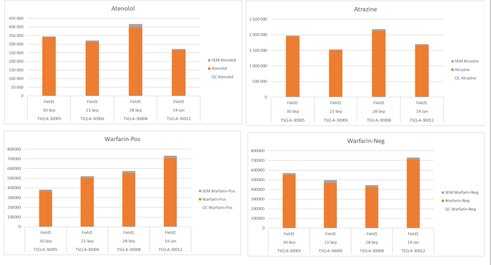


Instrument Performance Tracking

Instrument Performance can easily be tracked either between instruments or to monitor the performance over time.

A SMSS Evaluation software tool has been developed to easily allow the SMSS data to be visualized. The automatically generated report from the SMSS analysis, can simply be imported into SMSS Evaluation and the results visualized (Figure 10). Orange bars represent the average peak area for the 10 replicates. Grey bar is the Standard Error of the Mean (SEM) for the 10 replicates.

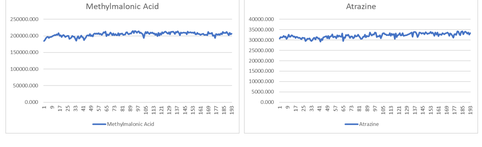
Figure 10. SMSS Evaluation of Atenolol, Atrazine and Warfarin (pos & neg)



Instrument Performance for long term robustness

Although the standard SMSS evaluation is based on 10 replicates, because only 0.1 µL is injected on column, just 25 µL of the SMSS standard can be used to evaluate performance for 200 consecutive injections, ~16 hours (Figure 11)

Figure 11. Long term SMSS Evaluation of Methylmalonic acid (neg) and Atrazine (pos)



Discussion

Holistic Evaluation of System Performance

The SMSS Evaluation test is quick and easy to set-up and implement. No sample preparation or dilution is required. Tests multiple facets of the entire system, ensuring LC, MS, and software are all functioning as a fully integrated system. The automated sample processing and report demonstrate system performance, for users of any experience level, to give confidence that the LC-MS/MS system is performing at an expected level.

Additional tools are available to allow long term tracking of instrument performance, as well as intra- and inter-lab comparisons.

CONCLUSIONS

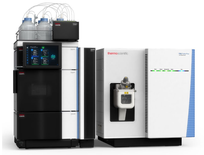
- SMSS test is quick and easy
- SMSS tests response and precision
- SMSS tests pump, autosampler and oven
- SMSS monitors multiple chemistries in both pos/neg
- SMSS tests hardware and software integrity
- SMSS can be implemented for ongoing system evaluation

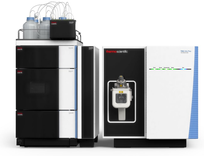
ACKNOWLEDGEMENTS

We would like to thank the team at Pierce for their support and collaboration.

TRADEMARKS/LICENSING

PO66144-EN0422S





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