Development of Small Molecule System Suitability Standard (SMSS) for routine monitoring of the performance of a liquid chromatography/mass spectrometry (LC-MS) system

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ABSTRACT

Purpose:
To develop a reference small molecule system suitability standard (SMSS), that contains highly defined, reproducible, and stable compounds for long-term system performance monitoring of triple quadrupole mass spectrometer LC-MS systems.

Methods:
A 1µL injection of a 10-fold diluted SMSS standard solution was separated by an analytical C18 LC column. A minimum of 2 SRM transitions for 9 different compounds of different hydrophobicity ranging from 75 to 1222 m/z in both positive and negative ionization mode were used for triple quadrupole mass spectrometer detection.

Results:
Our reference standard demonstrated excellent lot-to-lot consistency with robust stability from multiple lots over a year and half period. The SMSS can be used for instrument qualification and routine evaluation of LC-MS/MS system performance.

INTRODUCTION

System suitability testing (SST) is a critical part of analytical liquid chromatography mass spectrometry (LC-MS/MS) analysis. There are two different types of SSTs: evaluation of the mass spectrometer on its own, and evaluation of an integrated LC-MS system. To evaluate an MS instrument by itself, each MS instrument has its own calibration mixture to check mass accuracy, mass resolution and other parameters. However, there is a lack of standards available to assess and verify an integrated LC-MS system before the analysis of unknown samples. This may result in poor data quality and misleading data interpretation. Here, we have developed the Thermo Scientific™ Pierce™ Small Molecule System Suitability Standard (SMSS) to assess and verify multiple parameters of LC-MS system.

Figure 1. Thermo Scientific™ Pierce™ Small Molecule System Suitability Standard (SMSS) (PN: A51740) is provided as a 10-fold concentrated mixture with autosampler vials included.

MATERIALS AND METHODS

Sample Preparation

The Pierce™ Small Molecule Suitability Standard (SMSS, PN: A51740) was equilibrated to room temperature for 15 minutes and vortexed before sample dilution. To prepare a 10-fold diluted sample, 25 µL of the SMSS solution was pipetted into 225 µL of 0.1% formic acid in LC/MS-grade water to a final concentration of 4ppb. Autosampler vials supplied in SMSS kit was used for sample dilution. Diluted samples in autosampler vials were vortexed before in inject 1 µL for LC-MS analysis. Alternatively, 25 µL of the SMSS solution without dilution was transferred to an autosampler vial provided for analysis using high flow systems.

Test Method(s)

A 1µl sample of 10-fold diluted Pierce™ Small Molecule System Suitability standard was separated using a Thermo Scientific™ Hypersil GOLD™ C18 Selectivity LC Column (PN: 25002-052130) with a 1-98% gradient (A: 0.1% Formic Acid in LC/MS grade water, B: 100% LC/MS grade Methanol) at 0.5 mL/min on a Vanquish Binary Pump UHPLC. A Thermo Scientific™ TSQ Quantis™ Triple Quadrupole Mass Spectrometer (Catalog number: TSQ02-10001) with a heated electrospray source was used for detection with a minimum of 2 SRM transitions for 9 different compounds, ranging from 75 to 1222 m/z in both positive and negative ionization mode (Table 1).

Data Analysis

Thermo Scientific™ TraceFinder™ data analysis software was used for data acquisition, processing, analysis and reporting.

RESULTS

The Pierce™ small molecule system standard (SMSS) is mixture of 9 different compounds of different hydrophobicity ranging from 75 to 1222 m/z. This standard has been designed to run before other samples to verify that the LC/MS system is fit for sample analysis. Figure 2 shows an example chromatogram of SMSS run on Vanquish LC and TSQ Quantis Triple Quadruple instrument. To verify the system, we have specific requirements for SMSS related to a test method with predefined acceptance criteria such as peak shape (% peak symmetry) (Figure 3; left and middle graph), accuracy of different injection volumes (R^2) (Figure 3; right graph), minimum peak intensity, and precision RSD% (Table 2), and retention time (RT) difference between measured RT and expected RT (Table 3). If an SMSS run fails, then samples cannot be analyzed until the system is requalified for use. System requalification involves confirming the initial instrument hardware setup, operating method parameters, calibration column, solvents, and sample preparation before committing samples for analysis.

Table 1. SRM transitions for 9 compounds in both positive and negative ionization mode.

Compound	RT (min)	Polarity	Precursor (m/z)	Product (m/z)	CE (V)	RF Lens (V)
Glycine	0.25	Positive	76.039	30.179	9.42	39
Glycine	0.25	Positive	76.039	48.054	5.25	39
Methylmalonic Acid(-)	0.74	Negative	117.019	55.071	24.59	42
Methylmalonic Acid(-)	0.74	Negative	117.019	73.125	8.20	42
Atenolol	1.53	Positive	267.17	145.125	25.51	128
Atenolol	1.53	Positive	267.17	190.071	18.44	128
Flumetsulam	2.35	Positive	326.052	109.071	49.73	154
Flumetsulam	2.35	Positive	326.052	129.071	25.81	154
Atrazine	3.26	Positive	216.101	104	28.00	115
Atrazine	3.26	Positive	216.101	174.071	17.26	115
Terfenadine	3.42	Positive	472.321	57.125	40.34	184
Terfenadine	3.42	Positive	472.321	436.304	25.98	184
Warfarin	3.54	Positive	309.112	163.125	14.6	111
Warfarin	3.54	Positive	309.112	251.125	18.86	111
Warfarin (-)	3.55	Negative	307.098	161.071	18.27	153
Warfarin (-)	3.55	Negative	307.098	250.125	21.47	153
Ultramark	4.19	Positive	1221.991	990	55.00	250
Ultramark	4.19	Positive	1221.991	1090	54.07	250
Ultramark	4.19	Positive	1221.991	1221.991	5.00	250
Ultramark (-)	4.19	Negative	1265.982	906	31.12	250
Ultramark (-)	4.19	Negative	1265.982	1106	28.97	250
Ultramark (-)	4.19	Negative	1265.982	1266	5.00	250
Rafoxanide (-)	4.69	Negative	623.813	126.929	42.41	250
Rafoxanide (-)	4.69	Negative	623.813	344.875	31.75	250

Figure 2. Example LC-MS/MS chromatogram for nine compounds of SMSS (1 µL injection of 10-fold diluted SMSS: 25 µL SMSS in 225 µL 0.1% FA). The x-axis represents the retention time, and y-axis for the normalized peak intensity.
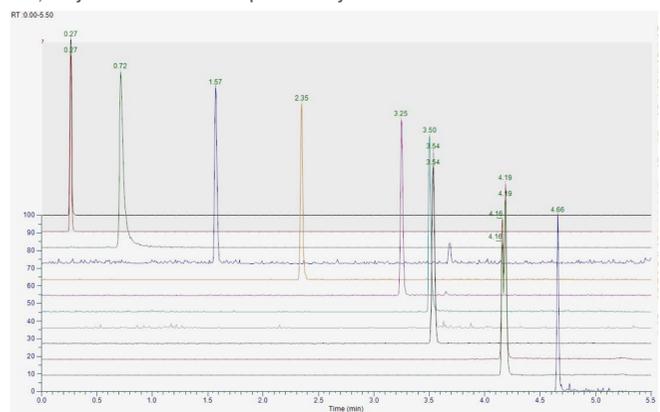

Figure 3. Examples of quantification ion and confirmatory product ion peaks for two SMSS compounds, Atrizine and Warfarin. Calibration curves for each compound at different injection volumes are shown (RT and peak intensity).
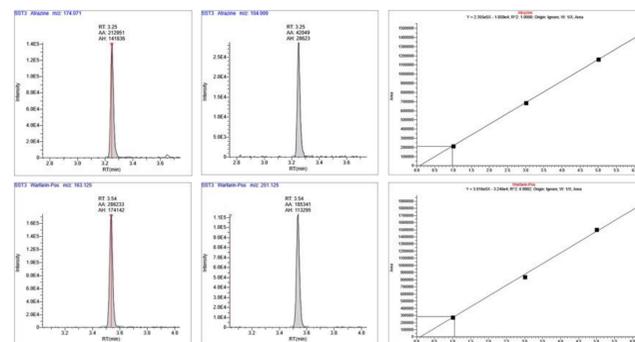

Table 2. Precession: RSD % value of average peak area of each compound

|	Glycine	Methylmalonic Acid (-)	Atenolol	Flumetsulam	Atrazine	Terfenadine	Warfarin (+)	Warfarin (-)	Ultramark (+)	Ultramark (-)	Rafoxanide (-)
SST1	516283	1030717	26522	36420	247778	189295	50907	205647	1352381	2172210	33404
SST2	509365	1045308	30893	34968	235018	194547	55700	210696	1332343	2080824	34371
SST3	522535	1045243	29918	35773	244470	180946	56093	218440	1315941	2107186	32632
SST4	501071	1001477	30081	37399	251878	196623	55558	208742	1300879	2153292	33382
SST5	513806	1044097	30107	34717	239005	199823	47798	209730	1283200	2078650	33097
SST6	500681	965786	28130	36702	245668	186166	50958	205530	1289903	2083096	27695
Ave	510624	1022105	29275	35996	243968	191233	52836	209798	1312441	2112543	32371
SD	8669	32338	1629	1038	6081	7508	3430	4734	26448	40672	2364
RSD(%)	1.7	3.2	5.6	2.9	2.5	3.7	6.5	2.3	2.0	1.9	7.3

Table 3. Retention Time Difference (RT Delta) Between Measured RT and Expected RT

	Glycine	Methylmalonic Acid (-)	Atenolol	Flumetsulam	Atrazine	Terfenadine	Warfarin (+)	Warfarin (-)	Ultramark (+)	Ultramark (-)	Rafoxanide (-)
SST1	-0.01	0.13	0.11	0.17	0.16	0.02	0.18	0.19	0.08	0.05	0.17
SST2	-0.01	0.12	0.11	0.17	0.17	0.03	0.19	0.19	0.08	0.05	0.17
SST3	-0.01	0.12	0.10	0.17	0.16	0.03	0.19	0.19	0.07	0.05	0.17
SST4	-0.01	0.12	0.11	0.17	0.17	0.03	0.19	0.19	0.08	0.05	0.17
SST5	-0.01	0.13	0.11	0.17	0.17	0.03	0.19	0.19	0.08	0.05	0.17
SST6	-0.01	0.12	0.11	0.17	0.17	0.03	0.19	0.19	0.08	0.05	0.17
Average RT Delta (min)	-0.01	0.12	0.11	0.17	0.17	0.03	0.19	0.19	0.08	0.05	0.17

To evaluate the robustness of lot-to-lot consistency, eight different full-scale lots (Lot#1 to Lot#8) were independently formulated by two different individuals during a year and a half period. We compared each lot in a pairwise fashion by using lot #3 as a median reference. The peak area ratios (each lot to lot #3) for all compounds were calculated. Our results show that the percent difference between different lots for all compounds was less than 10% except for Rafoxanide which was less than 15%, demonstrating the robustness of the formulation and manufacturing methods (Figure 4). Importantly, the precession (RSD%) of the differences between lots was less than 10%, demonstrating excellent lot-to-lot consistency.

Figure 4. Average Peak Area Ratio (each lot over lot#3) for nine compounds. Total of eight different lots were analyzed in a pair-wise fashion using lot#3 as a reference lot.
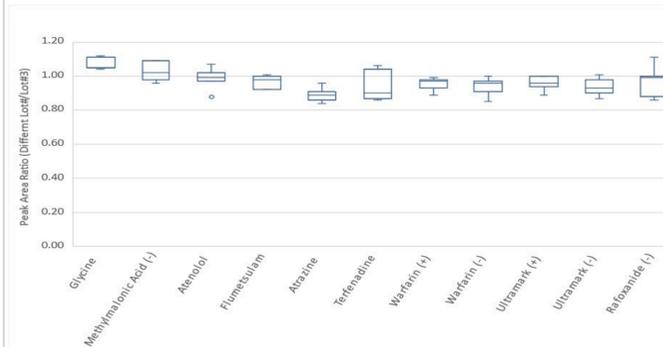
Next, we assessed the stability of the SMSS formulation at normal and reduced storage temperatures. Since the storage temperature for SMSS is 4°C, we evaluated real-time stability at 4°C. We selected lot #2 for real-time stability at 4°C compared to a reference sample of this lot stored at -80°C over time. To access potential changes in stability, we've prepared three heavy compounds mixture with known concentrations that were spiked in lot #2 sample to calculate the light-to-heavy ratio, thereby establishing the initial ratio at t=0. At t=0, the ratios for light over heavy Atazine-13C3, Methylmalonic acid-13C4, or Warfarin-d5 were 0.88, 0.91, and 0.86, respectively (Figure 6). At each real-time point, we measured the ratio to access potential changes at -80°C. At the one-year real-time stability point, the light-to-heavy ratios were less than 5% of each other, indicating that, at least, those three compounds are stable for a year at -80°C. Then we compared the -80°C-stored sample to the 4°C-stored sample to access real-time stability at 4°C. The data show that the level of all nine compounds stored at 4°C is very similar, if not identical, to the level at -80°C (Figure 5). This real-time stability data demonstrated the stability of SMSS for at least one year at 4°C.

Figure 5. Peak Area of each compound between -80°C- and 4°C-stored sample after one year. Blue and red box plot indicate positive and negative ionization mode, respectively.
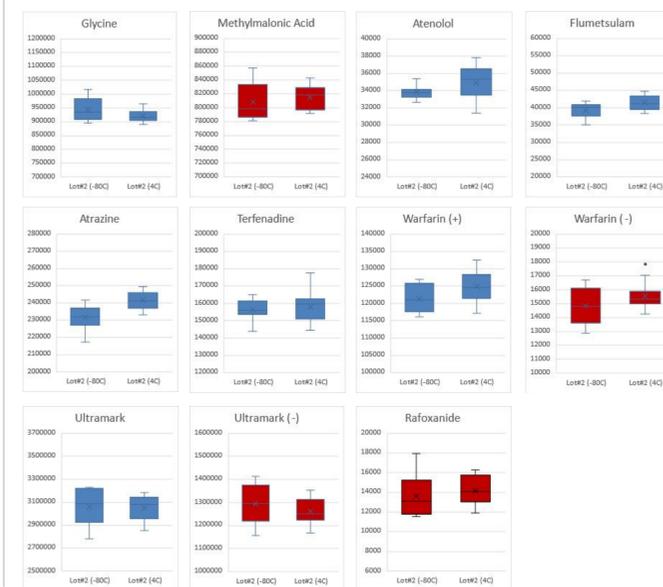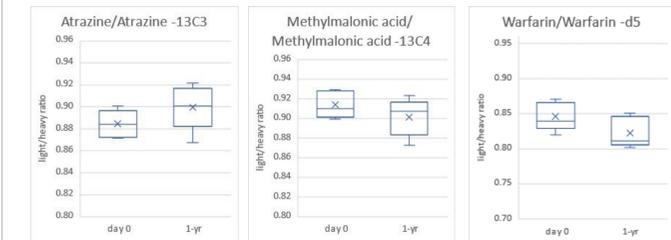

Figure 6. The light-to-heavy ratio of three available heavy compounds, Atazine-13C3, Methylmalonic acid-13C4, and Warfarin-d5. The heavy compound mixture was spiked in both lot #2 sample at day 0 and the sample at -80°C stored for a year.

Day-to-day reproducibility of the system suitability was accessed by setting up the assay on five different days. On each day of the assay, SMSS sample was diluted 10-fold by adding 25 µl SMSS into 225 µl 0.1% FA and injecting 1 µl into the system. Results show excellent day-to-day reproducibility for all compounds (<7% RSD) except Ultramark and Rafoxanide (<15% RSD) (Figure 7).

Figure 7. Day-to-Day Reproducibility: Peak Area Ratios in multiple days from SMSS sample freshly prepared in each day.
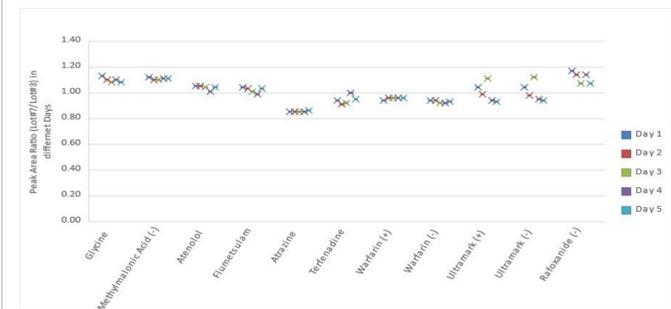
CONCLUSIONS

- SMSS is a ready-to-use solution for routine monitoring of LC-MS performance
- SMSS shows robust lot-to-lot consistency
- SMSS is stable for, at least, one year at 4°C

ACKNOWLEDGEMENTS

TRADEMARKS/LICENSING