A protocol to monitor performance of High-Flow LC-HRMS systems

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Abstract

Purpose: We have developed methods and acceptance criteria to use the Thermo Scientific™ Pierce™ Small Molecule System Suitability Standard (SMSS) for routine performance monitoring, system evaluation and troubleshooting of LC-MS systems, which contain Thermo Scientific™ Orbitrap Exploris™ 120 MS and the Thermo Scientific™ Orbitrap Exploris™ 240 MS.

Methods: Different amounts of the 10-fold diluted SMSS standard solution were loaded onto a C18 column. The sample was analyzed on various Orbitrap Exploris 120 mass spectrometers (OE120) using different methods. Two methods were specifically designed to evaluate sensitivity and MS2 performance. The third method can be used for system evaluation and troubleshooting.

Results: The methods were used to monitor the performance (sensitivity, MS2 performance, system evaluation) of more than 40 OE120 instruments using two different LC systems.

Introduction

Knowing liquid chromatography-mass spectrometry (LC-MS) instrument status prior to the analysis of unknown samples is of critical importance to data quality. While there are many protocols to monitor the status of individual components of LC-MS systems, a combined protocol, which tests the entire LC-MS system, is highly desirable. The Small Molecule System Suitability (SMSS) standard was specifically designed for this purpose. The standard contains 9 different compounds, ranging from *m/z* 75 to *m/z* 1222, that can be analyzed in positive and negative ion mode. A list of compounds in the SMSS standard is provided in Table (Tab. 1).

Tab. 1. List of compounds in SMSS

Positive Mode	Negative Mode
Atenolol	Methylmalonic acid
Atrazine	Rafoxanide
Flumetsulam	Ultramark
Glycine	Warfarin
Terfenadine	
Ultramark	
Warfarin	

We have developed three methods to use the SMSS for routine performance monitoring, system evaluation and troubleshooting on the Orbitrap Exploris Series 120/240 mass spectrometers.

Materials and methods

Sample Preparation

Equilibrate the SMSS vial to room temperature for 15 minutes. Vortex vial before sample preparation. Autosampler vials are supplied in Pierce Small Molecule Suitability Standard (A51740) for sample analysis. To prepare a 10-fold diluted sample, pipet 25 µL of the SMSS solution into 225 µL of 0.1 % formic acid in LC/MS-grade water to a final concentration of 4 ppb. Vortex the vial for mixing.

Methods

The SMSS solution was separated using a Thermo Scientific™ Hypersil GOLD™ C18 Selectivity LC Column (PN 25003-052130). The gradient is shown in Tab. 2.

Tab. 2. Gradient Conditions:
Solvent A: 0.1 % formic acid. Solvent B: methanol.

Time (min)	Flow (µL/min)	%B	Curve
0	500	1	5
0.5	500	1	5
3.1	500	98	5
4.5	500	98	5
4.6	500	1	5
5.5	500	1	5

Mass spectrometer detection was set up with heated electrospray using default source settings for 500 µL/min. Different MS methods were used for evaluation of sensitivity, MS2 performance and for system evaluation. MS acquisition settings can be found in the "System Evaluation" section in the method editor system templates tab (see Figure (Fig.) 1).

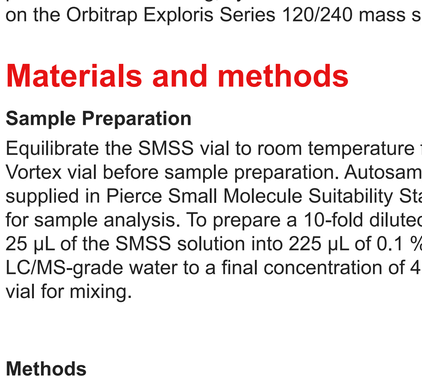

Fig. 1. Pre-built method templates are available on Exploris Series MS in ICSW 4.1.

Data Analysis

Thermo Scientific™ Chromeleon™ software/Thermo Scientific™ Xcalibur™ software as well as an in-house developed tool was used for data acquisition, processing, analysis and reporting.

Results

Sensitivity

Sensitivity was evaluated in positive ion mode using Full Scan and targeted SIM scans (template: OQ-Atrazine-IDL) with 0.1 µL injection volume. Example data for the reproducibility of Atrazine measurements at low concentrations on different OE120 instruments is shown in Fig. 2.

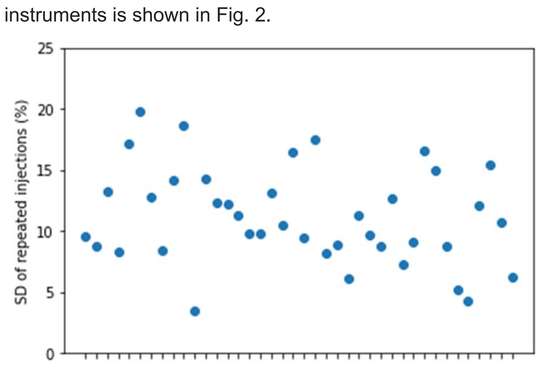

Fig. 2. Reproducibility of Atrazine measurements at low concentrations on different OE120 instruments (n = 40 - each dot corresponds to one instrument, 10 injections per instrument). Two different LC systems were used for testing. SD = standard deviation.

Based on these results, acceptance criteria have been agreed on: Relative standard deviation in percent (%RSD) of peak areas of 10 injections with 0.1 µL injection volume should be below 20 %.

MS2 Performance

MS2 performance was evaluated in negative ion mode using targeted MS2 scans (template: OQ-Warfarin-MS2) with 0.1 µL injection volume. Example data for the reproducibility of Warfarin measurements at low concentrations on different OE120

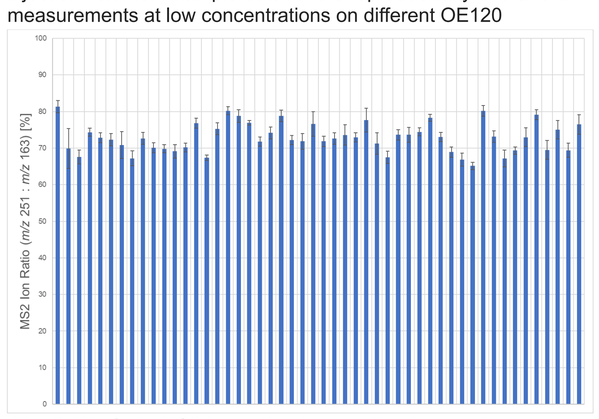

Fig. 3. Warfarin MS2 ion ratios in negative ion mode determined on 50 different OE120 instruments (n = 10). Mean +/-SD is shown, each bar corresponds to an individual instrument.

Based on these results, acceptance criteria shown in Tab. 3 have been agreed on.

Tab. 3. Ions used for evaluation of MS2 performance and acceptance criteria

Compound	Fragment Ion Ratio	Acceptance Range (%)
Warfarin	*m/z* 250.06372 : *m/z* 161.02449	50.0 – 100.0

System evaluation/troubleshooting

System evaluation/troubleshooting was performed in positive and negative ion mode using Full Scan and data dependent MS2 experiments (template: System Evaluation Small Molecule) with 5 µL injection volume. A typical LC-MS chromatogram is shown in Fig. 4.

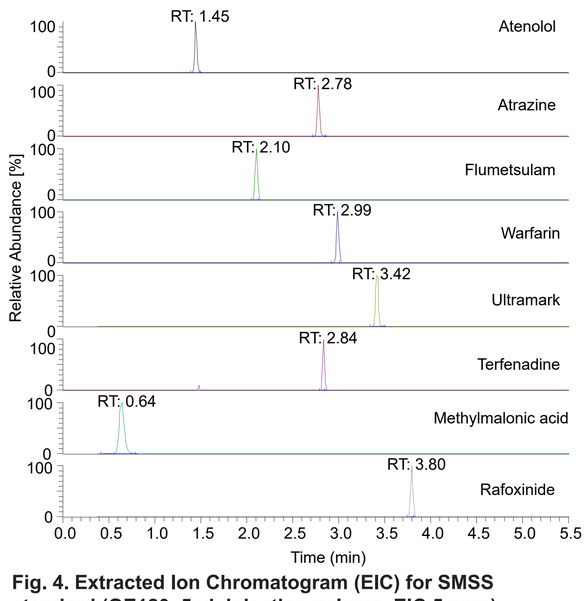

Fig. 4. Extracted Ion Chromatogram (EIC) for SMSS standard (OE120, 5 µL injection volume, EIC 5 ppm).

The sample was analyzed on 52 different OE120 instruments using the System Evaluation method using two different Thermo Scientific™ Vanquish™ LC systems. An overview on the obtained retention times is shown in Fig. 5 and Fig. 6. For retention times greater than 1 min, %RSD was below 2 % over a period of three months. The analysis was performed using different batches of the LC column, which is required for this test.

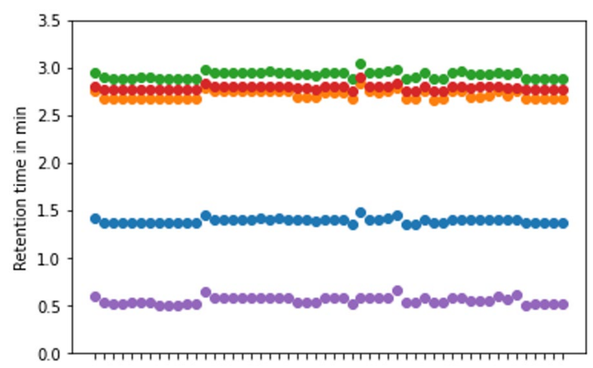

Fig. 5. Retention time stability of different analytes measured in positive ion mode (52 different OE120 instruments tested with 2 different LC systems; each dot corresponds to one instrument and shows mean value of 10 injections per instrument) for Atenolol (blue), Atrazine (red), Warfarin (green), Terfenadine (orange), and Methylmalonic acid (violet). Measurements were performed within a period of 3 months.

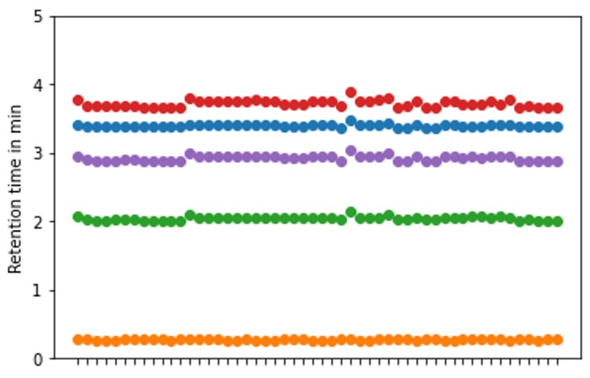

Fig. 6. Retention time stability of different analytes measured in negative ion mode (52 different OE120 instruments tested with 2 different LC systems; each dot corresponds to one instrument and shows mean value of 10 injections per instrument) for Ultramark (blue), Glycine (orange), Flumetsulam (green), Rafoxanide (red), and Warfarin (violet). All measurements were performed within a period of 3 months.

Based on these results, acceptance criteria have been agreed on: %RSD of retention times for 10 injections of 5 µL injection volume should be below 2 %.

A comparison of the obtained Warfarin peak areas in positive ion mode and Flumetsulam peak areas in negative ion mode of 52 different OE120 instruments is shown in Fig. 7 & 8. For Warfarin and Flumetsulam, inter-system variation was below 35 % and 36 %, respectively. Intra-system variation was below 5 %. For all compounds, inter-system variation was below 45 % and intra-system variation was below 10 %. Methylmalonic acid, Rafoxanide, Ultramark and Terfenadine were excluded from the inter-system variation analyses because measurements could not be performed within 6 h of sample preparation on all instruments.

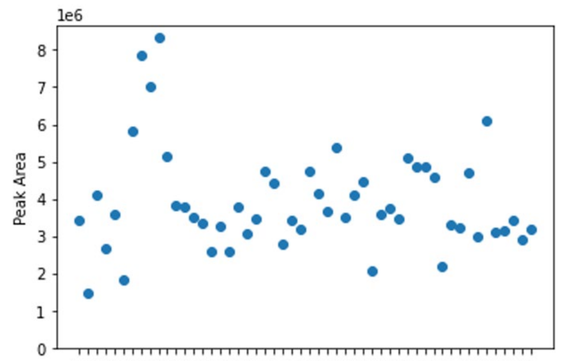

Fig. 7. Warfarin peak area reproducibility for 52 OE120 instruments (each dot corresponds to one instrument and shows mean value of 10 injections per instrument) in positive ion mode. Two different LC systems were used for the experiments.

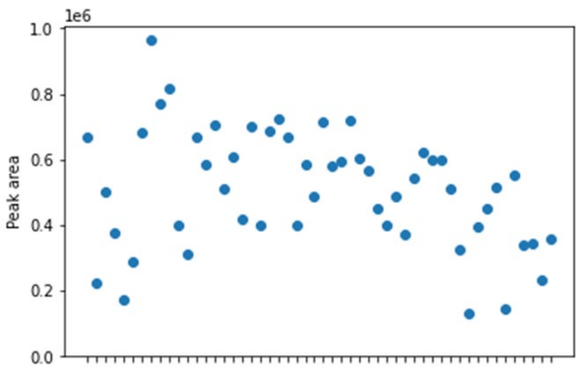

Fig. 8. Flumetsulam peak area reproducibility for 52 OE120 instruments (each dot corresponds to one instrument and shows mean value of 10 injections per instrument) in negative ion mode. Two different LC systems, were used for the experiments.

A comparison of the obtained Warfarin MS2 ion rations (*m/z* 251: *m/z* 163) in positive ion mode and Rafoxanide MS2 ion ratios (*m/z* 127: *m/z* 345) in negative ion mode is shown in Fig. 9 and Fig. 10. For Warfarin and Rafoxanide, inter-system variation was below 3 % and 12 %, respectively.

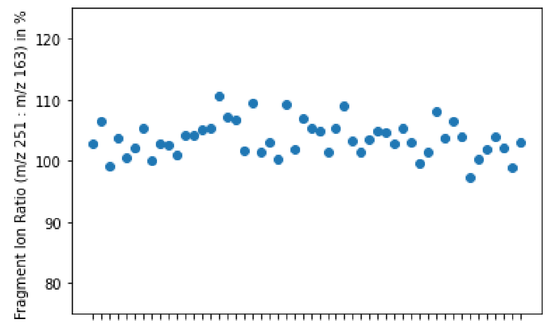

Fig. 9. Warfarin MS2 ion ratios for 52 OE120 instruments (each dot corresponds to one instrument and shows mean value of 10 injections per instrument) in positive ion mode. Two different LC systems were used for the experiments.

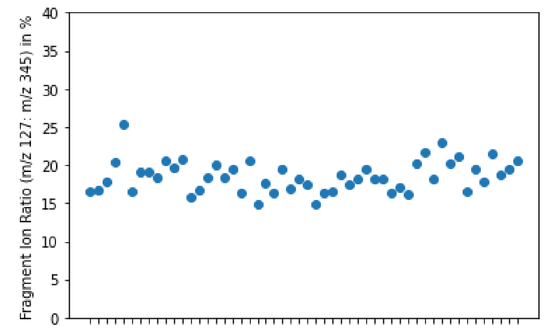

Fig. 10. Rafoxanide MS2 ion ratios for 52 OE120 instruments (each dot corresponds to one instrument and shows mean value of 10 injections per instrument) in negative ion mode. Two different LC systems were used for the experiments.

Troubleshooting

No MS2 is triggered: Check retention time settings in MS method.

Sensitivity and Reproducibility requirements not met: Repeat with new sample/ fresh solvents/ion transfer tube and check position of HESI source.

Conclusions

- SMSS is a ready-to-use solution for routine monitoring of LC-MS performance.
- Based on tests on at least 40 instruments, acceptance criteria were defined for various specifications that can be used to evaluate an LC-MS system prior to running unknown samples.
- Running the system evaluation test prior to analyzing unknown samples improves data quality and reduces waste.

Acknowledgements

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