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A simple method for monitoring LC-single quadrupole MS system performance

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Introduction

Due to both simplicity and specificity, liquid chromatography coupled to an entry level mass spectrometer is now an analytical technique widely used in analytical laboratories. Keeping a high level of instrument performance as well as maintaining instrument uptime are the basis of optimal lab productivity, efficiency, and ultimately, profitability. To ensure reliable and reproducible performance, a test method known as a quality control (QC) test is usually defined. As a complementary approach to instrument qualification, QC test methods are a great tool to evaluate potential measurement deviations whose origins can be numerous on an LC/MS system. This technical note presents a short test designed to monitor the LC and MS critical parameters.

Experimental

Reagents and consumables

- Methanol, Optima™ LC/MS Grade, Fisher Chemical™ (P/N 10031094)
- Acetonitrile, Optima™ LC/MS Grade, Fisher Chemical™ (P/N 10489553)
- Isopropanol, Optima™ LC/MS Grade, Fisher Chemical™ (P/N 10091304)
- Formic acid, Thermo Scientific™ Pierce™ LC-MS grade, 50 mL (P/N 85178)
- Thermo Scientific™ Hypersil GOLD™ HPLC column, 50 × 2.1 mm, 1.9 µm (P/N 25002-052130)
- Valine, Sigma-Aldrich, reagent grade, ≥98% purity

- Gallic acid, Sigma-Aldrich, 97.5–102.5% purity
- Caffeine, Sigma-Aldrich, ReagentPlus™, 99% purity
- Vanillin, Sigma-Aldrich, ReagentPlus™, 99% purity
- Deionized (DI) water, Type 1 reagent grade, 18 MΩ·cm resistivity

Sample preparation

Weigh 50 mg of each solid product (valine, gallic acid, caffeine, and vanillin) in a 500 mL bottle, add 249.5 mL DI water, 250 mL methanol, 500 µL formic acid, and mix thoroughly. Dissolve powders by stirring for at least 15 minutes at room temperature. The resulting concentration for each compound is 100 ng/µL. To evaluate linearity, this stock solution is diluted in methanol to obtain eight levels of dilution from 0.1 to 100 ng/µL.

Eluent preparation

- Solvent A: 0.1% v/v formic acid in water (in a 1 L bottle, add 1 mL of formic acid to 999 mL of DI water)
- Solvent B: Acetonitrile

LC and MS settings

A Thermo Scientific™ Vanquish™ Flex UHPLC system was used for this study. The complete setup is outlined below:

- System Base Vanquish Horizon / Flex (VF-S01-A-02)
- Dual Pump F (P/N VF-P32-A-01) equipped with 400 µL standard mixers
- Split Sampler FT (P/N VF-A10-A) with a 25 µL sample loop
- Column Compartment H (P/N VH-C10-A) equipped with active preheater

A Thermo Scientific™ ISQ™ EM Single Quadrupole Mass Spectrometer with dual HESI-APCI source was used for mass analysis.

Table 1a. LC conditions

Parameter	Value
LC column	Hypersil GOLD, 50 × 2.1 mm, 1.9 µm,
Mobile phase	A: 0.1% formic acid in water B: Acetonitrile
Flow rate	0.25 mL/min
Gradient	See Table 1b
Column oven	40 °C Forced Air mode with active preheater set to 40 °C
Injection volume	0.5 µL
Sampler wash solution	Methanol/acetonitrile/isopropanol/water 30/30/30/10 v/v
Rear seal wash solution	10% isopropanol in water v/v

Table 1b. Gradient settings

Time (min)	Flow rate (mL/min)	% A	%B
0	0.25	97	3
10	0.25	30	70
10	0.25	97	3
15	0.25	97	3

Table 2. MS settings

Parameter	Value
Vaporizer temperature	282 °C
Ion transfer tube temperature	300 °C
Source voltage, positive ions	3000 V
Source voltage, negative ions	-2000 V
Sheath gas pressure	49.9 psig
Aux gas pressure	5.7 psig
Sweep gas	0.5 psig
Method type	Component mode (see Table 3 for scan details) with Full Scan (*m/z* 50–1000 in positive mode)

Before a QC test, the system should be calibrated in positive and negative mode. Also, the sweep cone and transfer tube should be cleaned.

Software

- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS), version CM 7.3

Table 3. MS scan details

Analyte	Start time (min)	End time (min)	Mass	Ion polarity	Source CID voltage (V)
Valine	0	13	118.0	Positive	0
Caffeine	0	13	195.1	Positive	0
Gallic acid	0	13	169.1	Negative	0
Vanillin $[M+H]^+$	3	13	153.1	Positive	10
Vanillin $[M-H]^-$	3	13	151.1	Negative	10
Vanillin $[M+ACN+H]^+$	3	13	194.1	Positive	0

QC test results

Retention time

Many common products like antioxidants, amino acids, additives, and flavoring agents are often controlled and monitored in laboratories focused on food analysis. More specifically, caffeine, gallic acid, valine, and vanillin are compounds commonly analyzed in food contract testing labs. Figure 1 shows a chromatogram of the separation of these four compounds in fifteen minutes. Under these chromatographic conditions, valine is nearly unretained with a retention time of 0.65 minutes. Due to the gradient delay volume of approximatively 819 µL, gallic acid elutes isocratically under the initial conditions before the gradient reaches the column at 3.28 minutes. Caffeine and vanillin elute at 4.8 and 5.6 minutes, respectively. Retention times can be used to check the gradient delivery accuracy of the HPLC system. In our case, a dual low-pressure mixing gradient pump was used, and slight retention time adjustments are required when using a high-pressure mixing gradient pump. To ensure good retention time stability, a minimum of three blank runs should be performed before QC sample injections.

Batch-to-batch column variability represents a crucial parameter for QC testing of LC/MS systems. In this study we evaluated four different Hypersil GOLD column lots to check retention time reproducibility. Table 4 shows the average retention time and its standard deviation (n=6) for each compound. Low column-to-column variation demonstrates that the method is suitable for routine applications. Depending on the compound, detection was performed using positive or negative acquisition mode. Gallic acid was used to validate correct negative mode operation, whereas vanillin was used to check positive mode. Vanillin was also detected in negative mode, but at a much lower intensity. We also tracked the acetonitrile adduct of vanillin using a collision-induced dissociation (CID) voltage of 0.

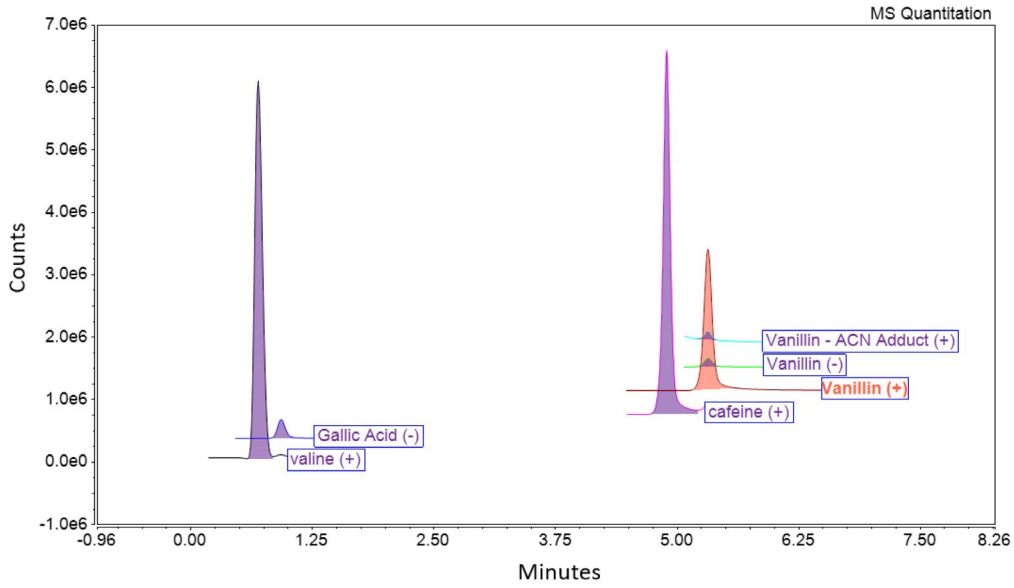

Figure 1. Extracted ion chromatograms of the four analytes obtained from 0.5 µL direct injection of a QC sample at 100 ng /µL

Table 4. Retention time (min) for each detected compound using four different column lots (#1–#4)

Compound	Lot # 1 S/N # 20071024	Lot # 2 S/N # 0796021X7	Lot # 3 S/N # 0701142X9	Lot # 4 S/N # 10904039		
	Retention time (min)				Average (min)	SD (min)
Valine	0.65	0.67	0.65	0.62	0.65	0.02
Gallic acid	0.89	0.91	0.93	0.91	0.91	0.02
Caffeine	4.97	4.86	5.05	5.05	4.98	0.09
Vanillin	5.72	5.20	5.73	5.86	5.63	0.29

MS response

Adjusting integration parameters in the processing method is critical for a good evaluation of the test criteria. The algorithm used in this QC test is Cobra. The tailing sensitivity factor was adjusted from 1% to 2% for vanillin (+) and gallic acid (-). Nine-point Gaussian smoothing was applied to all peaks (Figure 2).

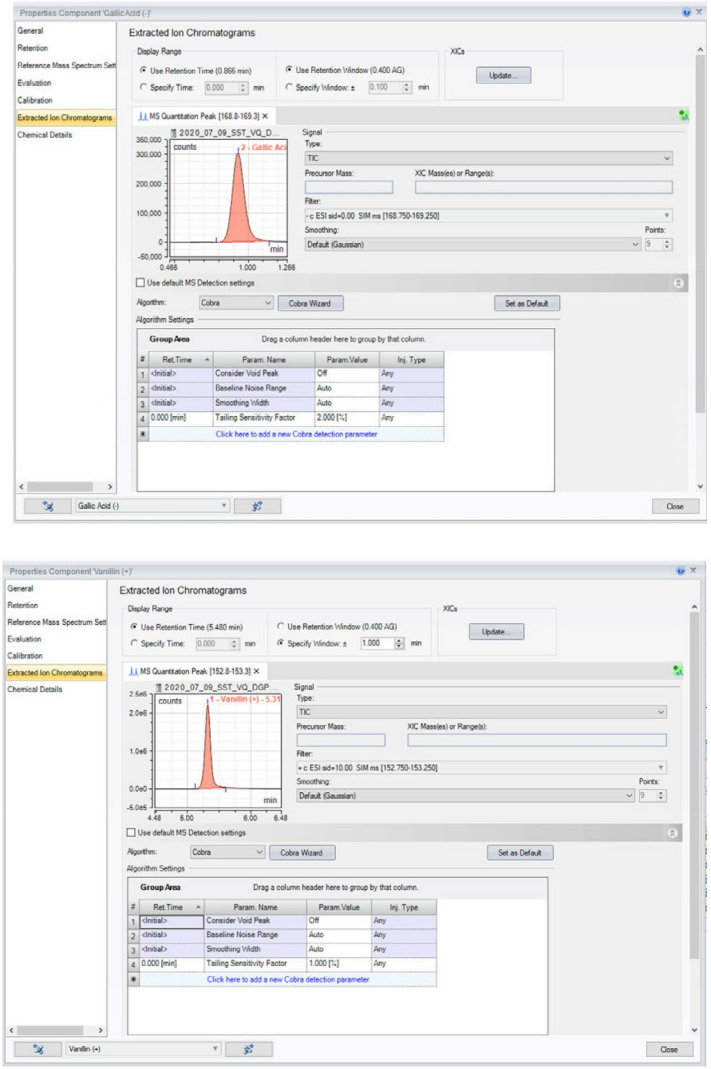

Figure 2. Integration parameters for gallic acid (-) and vanillin (+)

Figure 3 shows the peak shape for gallic acid (-) and vanillin (+) at 0.1 and 100 ng/µL. Due to the highest signal-to-noise ratio, peaks from 100 ng/µL samples were used to facilitate start and end peak determination, improving automated integration processes and saving operator time. This high concentration sample was chosen to monitor response during QC tests. Average peak areas for gallic acid (-) and vanillin (+) were 2.80e4 and 2.94e5 counts × min, respectively.

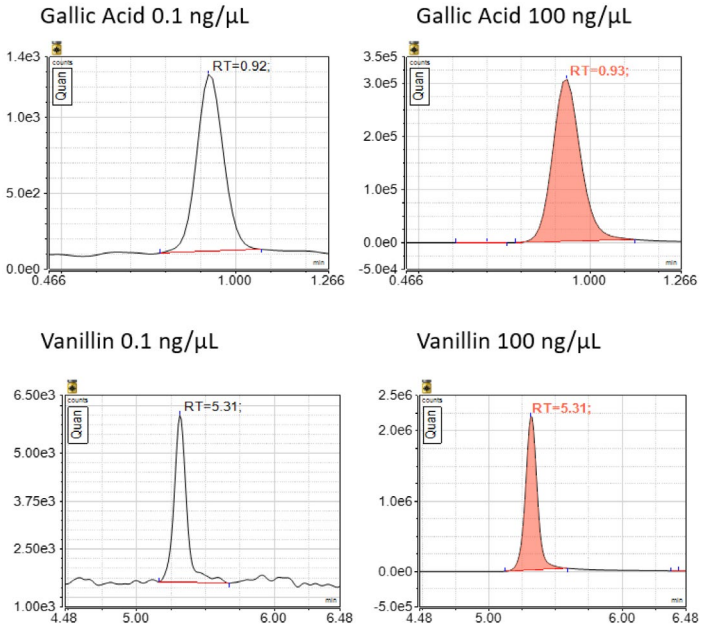

Figure 3. MS component view of calibration extrema points for gallic acid (-) and vanillin (+) measured in negative and positive mode, respectively

Method reproducibility and repeatability

Method reproducibility was assessed by comparing three technical replicates: three sample batches were injected on three consecutive days. Figure 4 illustrates the average peak area for gallic acid (-) and vanillin (+). Each average was calculated across 24 samples. Each analytical batch of 24 samples showed good area stability with relative measurement variability of ±15% for gallic acid (-) and vanillin (+). Repeatability was determined using six consecutive injections of a QC sample at 100 ng/µL. This experiment was repeated four times using four different columns (Lot #1 to #4). Table 5 reports results corresponding to vanillin (+). Relative standard deviation did not exceed 3.5%.

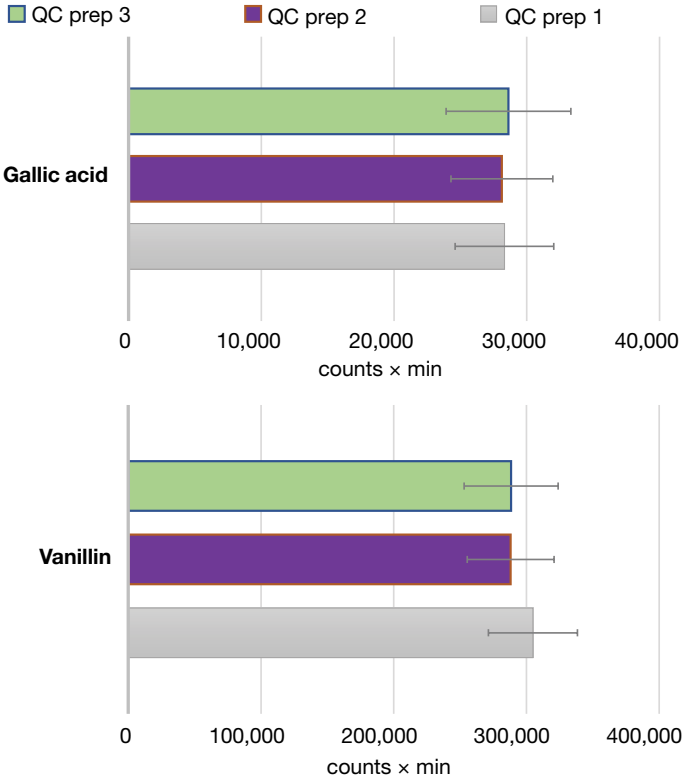

Figure 4. Reproducibility of MS peak areas of gallic acid (-) and vanillin (+) (average and standard deviation is based on 24 independent injections)

Table 5. Vanillin (+) peak areas corresponding to four injection batches (n=6)

	Column #1	Column #2	Column #3	Column #4
Sample #1	249432	324005	318060	288176
Sample #2	250286	328660	328080	285828
Sample #3	253665	326200	306633	287033
Sample #4	254641	328727	324030	284782
Sample #5	256247	317937	331747	296283
Sample #6	254692	305192	338762	288631
Average	253160	321786	324552	288455
SD	2701.22	9055.52	11221.84	4093.91
%RSD	1.07	2.81	3.46	1.42

A batch of 40 injections was acquired. Gallic acid (-) and vanillin (+) peaks were automatically detected and processed using the MS settings previously defined. The interactive chart visualization tool in Chromeleon 7 CDS helps to monitor results. Figure 5 shows the graphical representation of gallic acid peak areas for 40 consecutive injections. The analyst can define the lower limit for the peak area. In Figure 5, 2.00 e5 was selected as the lower limit.

Figure 5. Gallic acid (-) peak area trending (n=40) display using the Chromeleon CDS 7.3 interactive chart feature

Linearity

Due to higher response and better linearity in comparison with gallic acid, vanillin analyzed in positive mode was the best candidate for linearity check. System linearity was evaluated by generating a vanillin (+) calibration curve. Calibration curves were constructed using eight dilutions from 0.1 to 100 ng/µL. A linear calibration model was applied and the determination coefficient R^2 was 0.99991 (Figure 6).

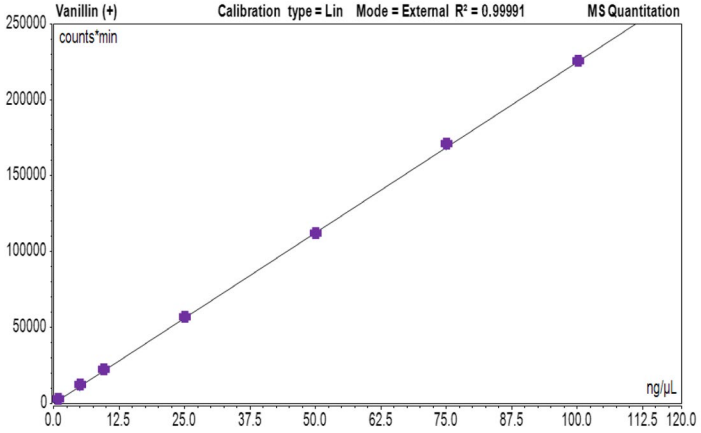

Figure 6. Calibration curve of vanillin measured in positive mode. Calibration was performed from 0.1 up to 100 ng/µL using diluted QC standard solution.

MS performance evaluation

Performing this short test regularly is an efficient tool to track performance over time, making it easier to identify potential issues early and to resolve them faster. To evaluate LC/MS system performance we injected six QC samples before and after a batch of unknowns and dirty samples. Figure 7 shows the critical decrease of gallic acid (-) peak area from 29,000 to 15,000 counts × min. Using this test, the operator can quantitatively evaluate instrument performance. The 50% decrease was mostly caused by sweep cone and transfer tube fouling. The ion transmission and resulting signal intensity were greatly reduced by deposited salts and other non-volatile compounds. Signal loss was eliminated with a simple cleaning of both parts and a system tune. After that, the average of gallic acid (-) peak area improved to 27,000 counts × min.

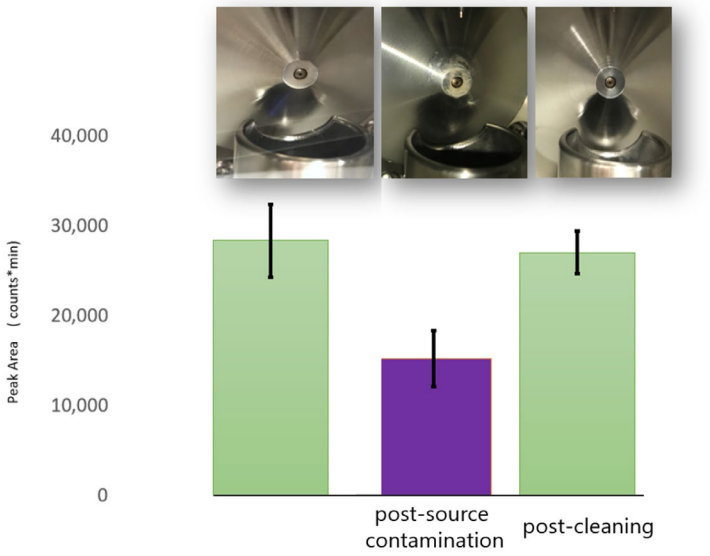

Figure 7. Impact of source contamination on gallic acid (-) peak area. Pictured are the sweep cone and ion transfer tube entrance at each step of the process.

Table 6. Evaluation of QC parameters

Checked parameters	How to evaluate?	Passed	Failed
Gradient precision	Check all retention times Valine: 0.2–1.2 min Gallic acid: 0.5–1.5 min Caffeine: 4.0–6.0 min Vanillin: 4.2–7.0 min	✓	
Organic solvent = acetonitrile	If vanillin adduct is detectable, test passed	✓	
Injection repeatability	% RSD area vanillin (+) <5%	✓	
Injection linearity	Coeff of determination for vanillin (+) >0.99	✓	
Check masses	Peaks detected using MS quantitation channel	✓	
Positive mode response	Vanillin 100 ng/µL (+) area >220,000 counts × min	✓	
Negative mode response	Gallic acid 100 ng/µL (-) area >20,000 counts × min	✓	

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Conclusions

This technical note describes a simple QC test for your LC-single quadrupole MS. Performing this QC test before sample batches can help ensure the system is working properly and, if necessary, preventive action can be taken. The laboratory thus maximizes productivity by improving instrument uptime.

Summary

Ideally such a QC test is performed immediately after system installation. As a result, reference values can be stored and compared with regularly acquired values. Depending on the system and requirements, target values can be slightly adjusted. Based on instrument sensitivity, the response of the QC test may be outside the linear dynamic range of the detector. If saturation is observed, the sample can be diluted 10-fold with methanol.

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