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Xevo G2-S/XS QTof LC-MS^E QC - UNIFI

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BENEFITS

This document describes a SST (System suitability test). Routinely using this test will allow you to monitor system performance.

Critical parameters can be judged from comparison data like

- Solvent background level
- Stability of Retention time
- Mass Accuracy

WATERS SOLUTIONS

Xevo G2-XS QTof

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KEYWORDS

Xevo G2-S/XS QTof, UNIFI, System Suitability Test, LCMS-QC

INTRODUCTION

This document is designed to help you test the complete LC-MS system prior to routine use. This test is designed so that stringent quality criteria (mass accuracy, RT stability, peak separation and solvent background) are assessed using a Waters certified standard solution (LCMS QCRM) in a 5 minute run. The method enables screening for 9 substances using the mass and retention time values as quality criteria. The method can also display and identify matched fragments, to further demonstrate the system's screening capabilities.

Waters recommends that you routinely perform this or a similar test to maintain the highest levels of confidence in your data and to assess the status of your instrument.

Checklist

- Check solvents and run ACQUITY LC system start-up to prime LC system and wash syringes
- Create a new analysis
- Go to initial conditions
- Run 3 Blanks and 6 LC-MS QC reference standard samples
- Review the report and ensure system meets performance criteria

Standards

Waters Part# 186006963 LCMS QC Reference Standard 1x 500 µL vial

LC conditions

Column:	2.1 x 50mm ACQUITY BEH C18 1.7 µm
Column temp.:	50 °C
Sample temp.:	10 °C
Inj. volume:	1 µL
Flow rate:	500 µL/min.
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in acetonitrile
Wash	0.1% formic acid in acetonitrile
Purge	0.1% formic acid in water
Seal wash	90:10 Water / methanol
Gradient	See Table

MS conditions

System	Xevo G2-S/XS QTof
Ionisation mode	ESI^+ (ESI^-)
Acquisition mode	MS^E, sensitivity mode
Acquisition range	*m/z* 50-1000
Capillary voltage	0.80 kV (< 2.0 kV)*
Collision energy	20-40 eV ramping
Total run time	5 min

EXPERIMENTAL

LCMS QC Analysis parameters

Before you run the SST for the first time, import the analysis method, report template, sample list, custom fields, and library. Refer to the KCS article WKB92598 "How to import the system suitability test materials for Vion IMS QTof?". Refer to *Table 1* for the Reference Compound details in order of elution.

Setup the LC system to the recommended LC conditions.

Use the following LC gradient for the analysis:

Time (min)	Flow rate (ml/min)	%A	%B	Curve
0.00	0.500	95	5	Initial
0.50	0.500	95	5	6
1.50	0.500	75	25	6
2.00	0.500	75	25	6
3.50	0.500	30	70	6
3.90	0.500	30	70	6
4.00	0.500	95	5	6

Preparation of LCMS QC reference standard and blank sample

Prepare the lock mass and the calibration standard. Details for preparing the lock mass and the calibration solution can be found in KCS articles WKB92649 and WKB2458.

Run the Xevo G2-XS QTof LockSpray check (or LockSpray setup) and Calibration check (or Calibration). Ensure that fluidics bottle B contains sufficient lock mass solution.

Prime the LC system using the system start-up function.

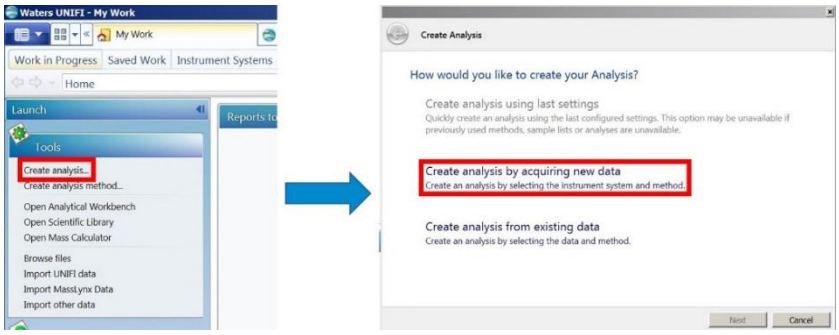
Dilute 1:10 the LCMS QC reference standard in 95:5 water:acetonitrile + 0.1% formic acid. Prepare a blank sample with 95:5 water:acetonitrile + 0.1% formic acid.

Component	Formula	Exact Mass $[M+H]^+$	Exact Mass $[M-H]^-$	Concentration µg/mL
Sulfaguanidine	$C_7H_{10}N_4O_2S$	215.0597	213.0452	0.5
Acetaminophen	$C_8H_9NO_2$	152.0706	150.0561	1
Caffeine	$C_8H_{10}N_4O_2$	195.0877	n/a	0.15
Val-Tyr-Val	$C_{19}H_{29}N_3O_5$	380.2180	378.2034	0.25
Leucine Enkephalin	$C_{28}H_{37}N_5O_7$	556.2766	554.2620	0.25
Sulfadimethoxine	$C_{12}H_{14}N_4O_4S$	311.0809	309.0663	0.1
Verapamil	$C_{27}H_{38}N_2O_4$	455.2904	n/a	0.02
Reserpine	$C_{33}H_{40}N_2O_9$	609.2807	607.2661	0.06
Terfenadine	$C_{32}H_{41}NO_2$	472.3210	n/a	0.02

Table 1: LCMS QC mix content in order of elution

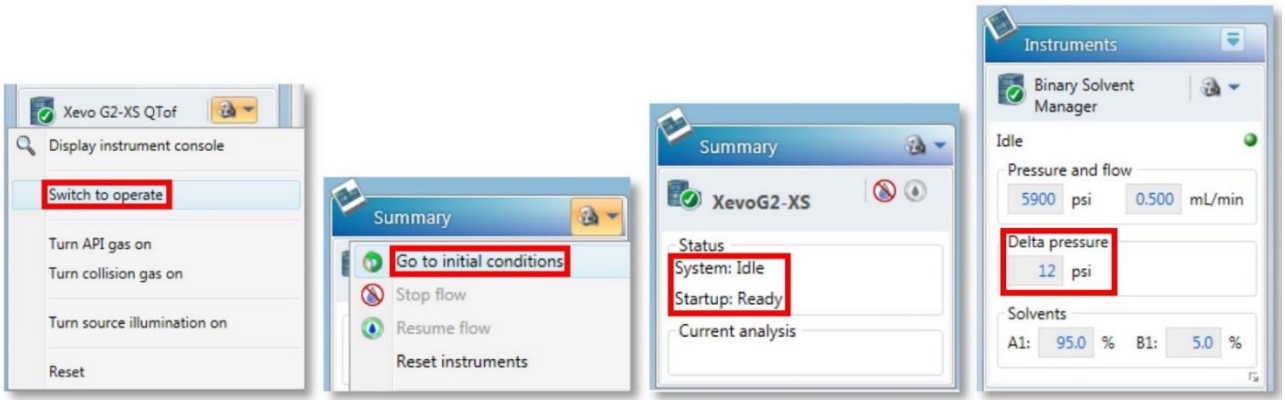
DATA ACQUISITION

From the My Work pane, click **Create analysis**, and then select **Create analysis by acquiring new data**.



Browse for the sample list (Xevo LC-MS QC SST) and analysis method (Xevo LC-HDMSE QC SST), specifying an analysis name and description.

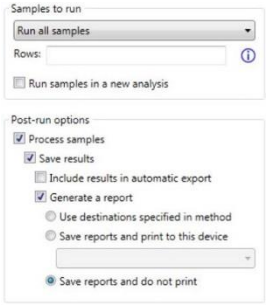
From the newly created analysis tab, open the console pane by clicking the green tick on bottom right. Switch the MS to operate. From the summary menu, select *"Go to initial conditions"*. The system should display *"Idle"*, and the status *"Ready"*. Wait for the Binary Solvent Manager delta pressure to be ≤ 20 PSI (≤1.37 bar). as shown in the figure below.



If necessary, amend the sample list vial position for the blank and the LCMS QC reference standard.

Click on the green start button to initiate the analysis.

Select *"Run all samples"* and make sure the options are ticked as depicted in the figure below



When the analysis is complete, click the **Report** tab and evaluate the results against the criteria defined in the "Results Evaluation" section. The report will use the following custom fields.

Name	Description	Formula
Average Peak Width in seconds	Average Chromatographic Peak Width in seconds of QC sample type	AVG(Chromatographic width,Sample type="QC")*60
RMS ppm error	RMS ppm error of QC sample type	(SUM(Mass error^2,Sample type="QC")/COUNT(Mass error,Sample type="QC"))^0.5

RESULTS EVALUATION

After you have run the test, evaluate the results against the specifications in the table below:

Criteria	Specitication
Mass accuracy	Better than 2 ppm RMS across all injections for all nine compounds
Retention time stability	Less than 3 seconds (0.05 minutes) RMS for the replicates
Peak width	Less than 3 seconds (0.05 minutes), measured as FWHM
BPI baseline level (solvent background)	Below 5% when evaluating the signal as XIC of the detected components in the "2: HD TOF MS" function (see figure 1 below).

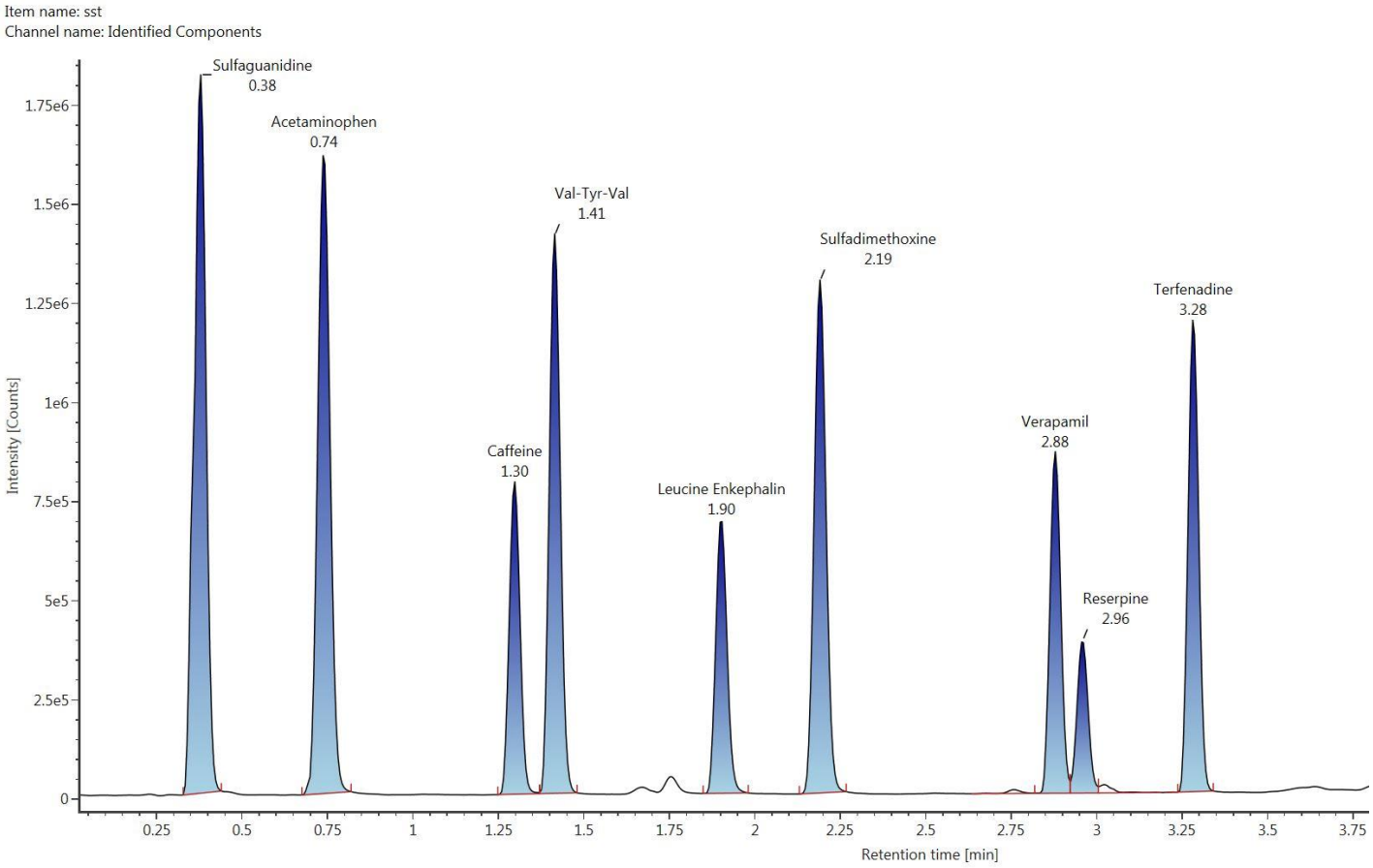


Figure 1: Typical Xevo G2-S/XS QTof LC-MS^E system suitability test chromatogram

If the mass accuracy acceptance criteria are not met, it is required to rerun the instrument MS setup. In case of poor chromatographic performance, prepare fresh mobile phases and inspect the LC flow path for potential leaks or blockages. Rerun the SST.

The SST can be run in negative ion mode by changing the ionisation polarity to negative ionisation as indicated in the Experimental section. The targeted substances in the purpose tab must be re-imported to update the negative ion masses as indicated in table 1. Note that caffeine, verapamil, and terfenadine are not detected in Negative Ion mode.