

Negative Mode Checkout Procedure

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Note: The following is a checkout procedure for negative mode operation of the 6410 Triple Quad LC/MS. It is intended to be used for troubleshooting purposes. It is NOT a performance specification—as a result it should not be included in bids, tenders, and other sales documents as a condition of sale.

The methods and worklist are available on the <\\wscsfs01\Lsca\apgsup> server under \LCMS\G6410A QQQ\Negative Mode Checkout

Prepare the performance evaluation samples

Before you begin, be sure that you have the following:

- High-purity pesticide grade (or higher) Water (supplied by customer or Agilent p/n 8500-2236)
- High-purity pesticide grade (or higher) Acetonitrile (supplied by customer or Agilent p/n G2453-85050)
- 1 mL graduated pipette (p/n 9301-1423)
- 2 x 100 mL volumetric flasks (p/n 9301-1344)
- Approximately 250 mL of 50:50 Water:Acetonitrile (no modifiers)
- Electrospray Negative Ion Performance Standard, Agilent p/n G1946-85005 (customers would order product G2424A), 10 ng/μl of Acid Red 4 in 50:50 IPA/Water
- ZORBAX SB-18 3.5 μm, 2.1 x 30 mm (RR) (p/n 873700-902—the same column used for reserpine checkout upon installation)

The Negative Ion Performance Standard must be diluted to the concentration required for the Triple Quad system checkout. Use 50:50 water:acetonitrile.

Use the diluted samples within a day of dilution.

- Prior to analysis, confirm that the analytical system (including the HPLC) is thoroughly cleaned. If necessary, use the flushing procedure on page 46 of the Agilent 6410 Triple Quad LC/MS System Installation Guide (p/n G3335-90023).
- Always rinse the graduated pipettes and volumetric flasks thoroughly with deionized water before and between each use.
- Use polypropylene labware for preparing performance evaluation samples, since glass vessels introduce unacceptable levels of sodium.
- Always rinse the autosampler vials and caps with the solvent mix used for sample dilution before filling them with the negative ion performance standard. This minimizes any background that can be contributed by the vials and caps. The vials may be run uncapped if the septa are found to be a source of background contamination.

**6410 Triple Quad LC/MS
Electrospray G1948B Negative MRM Mode**

- 1** Transfer 1 mL of 10 ng/μl Acid Red 4 (p/n G1946-85005) to a 100 mL volumetric flask. Use a clean graduated pipette.
- 2** Dilute to the 100 mL mark with 50:50 water:acetonitrile solution. Transfer 1 mL of the first dilution to a 100 mL volumetric flask. Use a clean graduated pipette.
- 3** Dilute to the 100 mL mark with 50:50 water:acetonitrile. This provides the final 1 pg/μl of Acid Red 4 concentration required for checkout.
- 4** Transfer approximately 1 mL of the second dilution to an autosampler vial.
- 5** Store final and intermediate dilutions in a refrigerator for possible use later.

**Table 1 6410 Triple Quadrupole LC/MS Checkout
Summary, Negative Mode**

Table 1

Electrospray G1948B Negative Mode	
Sample	Acid Red 4 10 ng/μl in 50:50 water / IPA
Concentration after dilution	1 pg/μl
Injection volume	1 μl
Total sample amount injected	1 pg
Sample order number	G1946-85005
Solvents	50:50 isocratic 100 % Water (no modifiers) 100% Acetonitrile (no modifiers)
Method name	ESI Neg MRM Acid Red 4 Checkout.m
Checkout	20: 1 on height, peak-to-peak noise 0.7 to 1.1 minutes

Check out the 6410 Triple Quad sensitivity

1 Verify that the Collision Cell gas flow rate produces a high vacuum gauge reading in the range of 2.7 to 3.3 x 10⁻⁵ torr.

To view the high vacuum gauge reading, click the Cell tab in the Tune Context and check that the high vacuum gauge reading is within range. If not, see “To reset the Collision Cell gas flow rate” on page 59 of the Installation Guide (p/n G3335-90035).

2 Start the Agilent MassHunter Workstation software, change the Context to Tune, and start a negative mode Autotune.

After the autotune has completed, you may need to wait up to 30 minutes to allow for the calibrant solution to be pumped out of the Triple Quad. This minimizes any background signal attributable to the calibrant. In addition, you can sonicate the nebulizer in a small graduated cylinder filled with acetonitrile for 10 minutes.

3 Change the Context to Acquisition, click File > Load and then load the method ESI Neg MRM Acid Red 4 Checkout.m. The checkout method includes the following acquisition parameters:

- 1 µl injection
- isocratic from channels A and B at 0.4 mL/min.
- 1.6 minutes run time
- 350°C Drying gas temperature
- 12 L/min. Drying gas flow
- 60 psi nebulizer pressure
- 4000 V capillary voltage
- 200 msec dwell time
- 130 V fragmentor voltage
- 29 V collision cell energy
- 350 V Delta EMV
- Monitoring precursor ion 357.1 and product ion 170
- MS1 Resolution set to Wide and MS2 Resolution set to Unit

4 Edit the method to ensure that for channel A, the 100 % water solution is selected as the LC solvent. Ensure that for channel B, the 100 % acetonitrile solution is selected as the LC solvent.

5 Click File > Load and then load the method ESI Neg MS2 Scan.m.

6 Edit the method to ensure that for channel A, the 100 % water solution is selected as the LC solvent. Ensure that for channel B, the 100 % acetonitrile solution is selected as the LC solvent.

7 Place the vials into the LC autosampler.

- Position #1: An empty, uncapped vial
- Position #2: A vial containing the solvent used for dilution (this is the solvent blank)

- Position #3: A vial containing the Acid Red 4 sample (1 pg/μl)

8 From the File menu select Load and then load the worklist: ESI Neg MRM Acid Red 4 Checkout.wkl.

The worklist is set up to do one injection of the solvent blank using the ESI Neg MS2 Scan.m method in order to collect background ion data, then using the ESI Neg MRM Acid Red 4 Checkout.m method for the remaining runs, one injection of the empty vial, five injections of the solvent blank, and five injections of the Acid Red 4 sample.

9 Review the worklist to be sure that the method and data paths are correct and that the data file names given in the worklist are unique and have not already been acquired.

10 Run the worklist.

11 When the worklist is finished, calculate signal-to-noise for each injection.

a Load each solvent blank and Acid Red 4 sample data file into the Qualitative Analysis program.

b Generate MRM Chromatograms of the 357.1→170 transition.

c Integrate each Acid Red 4 peak, and select Calculate Signal to Noise.

d Calculate the signal-to-noise using Height.

e Under Noise Measurement, for Noise definition, select Peak-to-Peak. For Noise regions, type 0.7 – 1.1 minutes. (Make sure that the noise region does not include the Acid Red 4 peak. If the Acid Red 4 peak elutes within the default noise region, shift the noise region such that it is 0.45 to 0.90 minutes after the retention time of the Acid Red 4 peak.)

12 Generate a printout of each signal-to-noise calculation report for each solvent blank and Acid Red 4 injection. Include the chromatogram in the printout.

13 Open the Excel spreadsheet D:\MassHunter\Support\Checkout\Sensitivity Checkout Report.xls from the MassHunter Acquisition CD-ROM. Fill in the values to calculate the average signal to noise and save the spreadsheet.

The expected average signal-to-noise is greater than 20:1.

14 Generate a printout of the Excel signal-to-noise report.