Sample Formulations

This appendix provides instructions for preparing the tuning and calibration solutions.

Contents

The caffeine, MRFA, Ultramark 1621, and Reserpine needed to make the solutions are supplied with your chemical accessory kit. You can order replacement chemical accessory kits (P/N 97000-62042) from Thermo Fisher Scientific.

IMPORTANT Do not use plastic pipettes to prepare your tuning and calibration standards. Plastic products can release phthalates that can interfere with your analyses.

CAUTION Store and handle all chemicals in accordance with standard safety procedures. The Material Safety Data Sheets (MSDS) describing the chemicals being used are to be freely available to lab personnel for them to examine at any time. Material Safety Data Sheets (MSDS) provide summarized information on the hazard and toxicity of specific chemical compounds. MSDSs also provide information on the proper handling of compounds, first aid for accidental exposure, and procedures for the remedy of spills or leaks. Producers and suppliers of chemical compounds are required by law to provide their customers with the most current health and safety information in the form of an MSDS. Read the material safety data sheets for each chemical you use.

Potentially hazardous chemicals used in procedures throughout this manual include the following:

- Glacial acetic acid
- Acetonitrile
- Methanol
- Reserpine
- Formic Acid

Caffeine, MRFA, and Ultramark 1621 Stock Solutions

For tuning and calibrating the LTQ Series MS detector in the ESI mode, you use a calibration solution of caffeine, MRFA, and Ultramark 1621 in an acetonitrile / methanol / water solution containing 1% acetic acid. You prepare the ESI calibration solution from the following stock solutions described in this section:

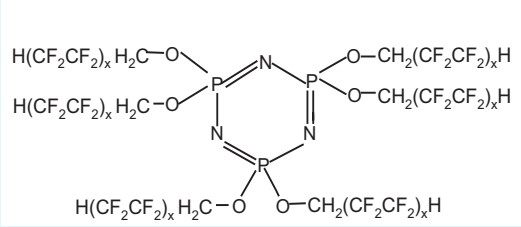
- "Caffeine Stock Solution," next section
- "MRFA Stock Solution" on page 131
- "Ultramark 1621 Stock Solution" on page 132

Note The API accessory kit contains vials of caffeine and MRFA. These compounds are available from Sigma Chemical Co. Sigma product number C6035 contains caffeine at a concentration of 1 mg/mL in methanol. Sigma product number M1170 is MFRA (MRFA=Met-Arg-Phe-Ala acetate salt). To order these compounds from Sigma, write or call:

Sigma Chemical Company
P. O. Box 14508
St. Louis, Missouri, USA 63178-9916
(800) 325-3010 (in the USA)
(905) 829-9500 (in Canada)
(314) 771-3750 (outside the USA or Canada)
www.sigmaaldrich.com

Note The API accessory kit contains a vial of Ultramark 1621 (neat liquid). This compound is available from Lancaster Synthesis.

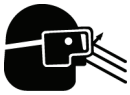
The structure of Ultramark 1621 is shown below (x is 1, 2, or3).



The Lancaster catalog number for Ultramark 1621 is 16698 (Ultramark 1621, Mass Spec Std.). To order this compound from Lancaster Synthesis, write or call:

Lancaster Synthesis, Inc.
P.O. Box 1000
Windham, NH USA 03087-9977
(603) 889-3306, (800)-238-2324 (in the USA & Canada)
+44 (0)1524 36101 (UK & International)
www.lancastersynthesis.com

CAUTION AVOID EXPOSURE TO POTENTIALLY HARMFUL MATERIALS. Always wear protective gloves when you use solvents or corrosives. Also contain waste streams, and use proper ventilation. Refer to your supplier's Material Safety Data Sheet (MSDS) for the proper handling of a particular solvent. Contain waste streams and use proper ventilation.



CAUTION AVOID EXPOSURE TO POTENTIALLY HARMFUL MATERIALS. Always wear safety glasses when you use solvents or corrosives. Also contain waste streams, and use proper ventilation. Refer to your supplier's Material Safety Data Sheet (MSDS) for the proper handling of a particular solvent. Contain waste streams and use proper ventilation.

Caffeine Stock Solution

A 1 mg/mL stock solution of caffeine in 100% methanol is provided with your LTQ Series MS detector. You can also order this solution through Sigma. The Sigma product number for this solution is C6035.

MRFA Stock Solution

❖ **To prepare a 3 mg/mL solution of MRFA in 50:50 methanol / water**

1. Using an analytical balance, weigh out 6 mg of the MFRA compound provided in the accessory kit.

   The L-methionyl-arginyl-phenylalanyl-alanine acetate•H2O (MRFA) provided in your accessory kit has an average molecular weight of 523.7 u.

2. Transfer the 6 mg quantity of MFRA to a vial.
3. Add 2.0 mL of 50:50 methanol / water to the vial.
4. Mix the solution (3 mg/mL) thoroughly.
5. Label the vial **MRFA Stock Solution 3 µg/mL**.

MRFA Diluted Stock Solution

❖ **To prepare a 0.1 µg/mL solution of MRFA in 50:50 methanol / water**

1. Transfer 100 µL of the 3 mg/mL MRFA stock solution to a vial.
2. Add 2.9 mL of 50:50 methanol / water to the vial.
3. Mix this solution (0.1 µg/mL) thoroughly.
4. Label the vial **Diluted MRFA Stock Solution 0.1 µg/mL**.

Ultramark 1621 Stock Solution

❖ **To prepare a 10 mL stock solution of 0.1% Ultramark 1621 in acetonitrile**

1. Using a syringe, transfer 25 µL of Ultramark 1621 to a 25 mL volumetric flask.
2. Fill the flask to volume with 100% acetonitrile.
3. Mix the solution thoroughly.
4. Transfer the solution to a vial and label the vial **Ultramark 1621 stock solution (1/1000 dilution)**.

ESI Calibration Solution: Caffeine, MRFA, Ultramark 1621

❖ **To prepare 10 mL of the calibration solution**

1. Pipet 200 µL of the stock solution of caffeine into a clean 10 mL volumetric flask.
2. Pipet 100 µL of the diluted MRFA stock solution (0.1 mg/mL) into the flask.
3. Pipet 100 µL of the stock solution of Ultramark 1621 into the flask.

   Note Use only glass pipets or stainless steel syringes when measuring glacial acetic acid. Using plastic pipet tips causes contamination of acid stock solutions that can introduce contaminants in the calibration solution.

4. Pipet 100 µL of glacial acetic acid into the flask.
5. Pipet 5mL of acetonitrile into the flask.
6. Bring the solution to volume with 50:50 methanol / water.
7. Mix the calibration solution thoroughly.
8. Transfer the solution to a clean, dry vial.
9. Label the vial **ESI Calibration Solution** and store it in a refrigerator at 2 to 8 °C until it is needed.

Reserpine

You use a 100 pg/µL solution of reserpine to tune the MS detector in the APCI mode. This section contains the following topics:

- "Preparing the Reserpine Stock Solution," next section
- "Preparing the Reserpine Tuning Solution" on page 133
- "Preparing the Reserpine Sample Solution" on page 134

Preparing the Reserpine Stock Solution

Ideally, you should prepare the stock solution or reserpine just before you use it. If you must store the solution, keep it in a refrigerator until you need it.

❖ **To prepare a stock solution of 1 µg/µL reserpine in 1% acetic acid-methanol**

1. Weigh out 10 mg of reserpine and transfer the sample to a 10 mL volumetric flask.

   The average molecular weight of reserpine is 608.7 u.

2. Fill the flask to volume with a solution of 1% acetic acid in methanol.
3. Mix the solution thoroughly.
4. Transfer the solution to a clean, dry, light-resistant vial.
5. Label this vial **Reserpine Stock Solution (1 µg/mL=1.64nmol/mL)**.

Preparing the Reserpine Tuning Solution

Ideally, you should prepare the reserpine tuning solution (100 pg/mL) just before you use it. If you must store the solution, keep it in a light resistant container in a refrigerator until you need it.

❖ **To prepare the reserpine tuning solution**

1. Pipet 100 µL of the reserpine stock solution (1 µg/µL) into a clean polypropylene microcentrifuge tube.
2. Add 900 µL of 1% acetic acid in 50:50 methanol / water to the tube.
3. Mix this solution (100 ng/µL=164 pmol/µL) thoroughly.
4. Transfer 10 µL of the 100 ng/µL solution to a clean polypropylene tube.
5. Add 990 µL of 1% acetic acid in 50:50 methanol / water to the tube.
6. Mix this solution (1 ng/µL=1.64pmol/µL) thoroughly.
7. Transfer 100 µL of the 1 ng/µL solution into a clean polypropylene tube.
8. Add 900 µL of 1% acetic acid in 50:50 methanol / water to the tube.
9. Mix this solution (100 pg/µL=164 fmol/µL) thoroughly.
10. Label the tube **Reserpine Tuning Solution (100 pg/mL***)*. If you are making a Reserpine Sample Solution, continue to "Preparing the Reserpine Sample Solution" on page 134.

Preparing the Reserpine Sample Solution

Ideally, you should prepare the reserpine sample solution (1 pg/mL) just before you use it. If you must store the solution, keep it in a light resistant container in a refrigerator until you need it. You can use this sample solution to perform the loop injection procedures in "Acquiring ESI/MS Data in the SIM Scan Type" on page 104 and "Acquiring APCI/MS Data in the SIM Scan Mode" on page 123.

❖ **To prepare the reserpine sample solution for the APCI mode**

1. Transfer 100 µL of the 100 pg/µL reserpine tuning solution to a clean polypropylene tube.
2. Add 900 µL of 1% acetic acid in 50:50 methanol / water to the tube.
3. Mix this solution (10 pg/µL=16.4 fmol/µL) thoroughly.
4. Transfer 100 µL of the 10 pg/µL solution into a clean polypropylene tube.
5. Add 900 µL of 1% acetic acid in 50:50 methanol / water to the tube.
6. Mix this solution (1 pg/µL, 1.64 fmol/µL) thoroughly.
7. Label the tube **Reserpine APCI Sample Solution (1 pg/mL)**.

High Mass Range Calibration Solution

The high mass range calibrant is a solution of 70 ng/µL polypropylene glycol (PPG) in a solvent of 65:35 methanol/10mM sodium acetate.

The high mass range calibration procedure is designed to work with a PPG that has an average molecular weight of approximately 2700 (M_n~2700), which is Aldrich product number 202347. PPG 2700 is a viscous liquid. To order this compound from Sigma-Aldrich, write or call:

Sigma Chemical Company
P. O. Box 14508
St. Louis, Missouri, USA 63178-9916
(800) 325-3010 (in the USA)
(905) 829-9500 (in Canada)
(314) 771-3750 (outside the USA or Canada)
www.sigmaaldrich.com

❖ To prepare the high mass range calibration solution

1. Weigh out 0.7 gm of PPG 2700.

   Tip Because PPG 2700 is a viscous liquid, use a glass pipette to transfer 0.7 gms of the liquid into a weigh boat, or weigh the liquid directly into a 20 mL (or larger) glass vial.

2. Transfer the PPG to a 20 mL glass vial.
3. Add 7 mL of HPLC-grade methanol to the vial.
4. Add 2.3 ml of HPLC-grade water to the vial.
5. To prepare a 100 mM solution of sodium acetate (NaOAc), do one of the following:
   - Dissolve 0.082 gm of anhydrous NaOAc in 10 mL of HPLC-grade water.
   - Dissolve 0.136 gm of NaOAc • $3H_2O$ in 10 mL of HPLC-grade water.
6. Add 0.7 mL of the 100 mM NaOAc solution to the PPG solution.
7. Label the vial 70 µg/mL PPG solution.
8. To prepare a 70 ng/mL PPG solution in 65:35 methanol/10mM sodium acetate
   a. Add 7 mL of HPLC-grade methanol to a clean vial.
   b. Add 2.3 ml of HPLC-grade water to the vial.
   c. Add 0.7 mL of the 100 mM NaOAc solution to the vial.
   d. Add 10 µL of the 70 µg/mL PPG solution to the vial and mix.
   e. Label the vial High Mass Calibration Solution: 70 pg/mL PPG.