Safety:
Wear safety goggles, lab coat, and nitrile gloves. Mixing and grinding of chemicals shall be performed in the hood.

Personnel responsible:
Lab

Test Method:
USP Monograph: Magnesium Sulfate, and General Chapter <291>

Purpose:
To verify that the selenium content of magnesium sulfate heptahydrate is below the USP limit of 0.003%.

Equipment:

- 2 25-mL Graduated Cylinder
- 2 100-mL Graduated Cylinder
- 2 250-mL Graduated Cylinder
- 3 150-mL Beakers (Labeled: 'Standard Solution', 'Reagent Blank', and 'Test Solution', Respectively)
- 50-mL Volumetric Flask with Stopper
- 100-mL Volumetric Flask with Stopper
- 200-mL Volumetric Flask with Stoppers
- 4 1000-mL Volumetric Flask with Stopper
- 5-mL Eppendorf Pipette and Tips
- 10-mL Eppendorf Pipette and Tips
- Mortar and Pestle
- Balance-Mettler Toledo X5105Du, B13929Z316
- Weigh Paper
- Spatula
- Water Bath-Boekel Grant GD120L
- 0.0-2.5 pH test strips-colorpHast
- 3 Low-actinic Separatory Funnels (Labeled: 'Standard Solution', 'Reagent Blank', and 'Test Solution', Respectively)
- 4 15-mL Centrifuge Tubes (Labeled: 'Standard Solution', 'Reagent Blank', 'Test Solution', and 'Water', Respectively)
- Centrifuge – Drucker 614B
- 10-mm Quartz Cell—Hach Co. 1-Q-10, Rectangular, Open Top, With Lid
- Laboratory Wipers
- UV-Vis Spectrophotometer—Hach Co. DR 5000

Reagents:

- DI H_2O
- Concentrated Nitric Acid
- Concentrated Ammonium Hydroxide
- Metallic Selenium Shot
- 2,3-Diaminonaphthalene (DAN)
- Hydroxylamine Hydrochloride
- 0.1 N Hydrochloric Acid
- Cyclohexane

Solutions Preparation:

Dilute Nitric Acid (1:2)— *NOTE: This solution is only used to prepare the Stock Solution.* Using a 100-mL graduated cylinder add 50 mL of concentrated nitric acid to 45 mL of DI H_2O in a 100-mL volumetric flask, dilute to volume, and mix.

Dilute Nitric Acid (1:30)— Using a 100-mL graduated cylinder add 34 mL of concentrated nitric acid to 450 mL of DI H_2O in a 1000-mL volumetric flask, dilute to volume, and mix.

Ammonium Hydroxide Solution (1:2)— Using a 100-mL graduated cylinder add 50 mL of concentrated ammonium hydroxide to 45 mL of DI H_2O in a 100-mL volumetric flask, dilute to volume, and mix. Pour this solution into a labeled dropper bottle.

Stock Solution— Using a mortar and pestle grind a piece of selenium shot into a powder. Weigh out 0.0400 g of the powder using the analytical balance and a weighing paper. Dissolve the powder in 100 mL of *Dilute Nitric Acid (1:2)* in a 1000-mL volumetric flask, warming gently in water bath if necessary to effect solution; add DI H_2O to volume, and mix. Pipet 5 mL of this solution into a 200-mL volumetric flask, add DI H_2O to volume, and mix. Each mL of the resulting solution contains the equivalent of 1 µg of selenium.

DAN Solution— Dissolve 0.0500 g of 2,3-diaminonaphthalene and 0.250 g of hydroxylamine hydrochloride in 0.1 N hydrochloric acid in a 50-mL volumetric flask, dilute to volume with 0.1 N hydrochloric acid, and mix. ***NOTE:*** **Prepare this solution fresh on the day of use**.

Standard Solution— Pipet 6 mL of Stock Solution into a labeled 150-mL beaker, and, using a 25-mL graduated cylinder, add 25 mL of *Dilute Nitric Acid (1:30)* and 25 mL of DI H_2O. Mix this solution.

Reagent Blank— Using a 25-mL graduated cylinder add of 25 mL of *Dilute Nitric Acid (1:30)* and 25 mL of DI H_2O to a labeled 150-mL beaker. Mix this solution.

Test Solution— Using a 25-mL graduated cylinder add of 25 mL of *Dilute Nitric Acid (1:30)* and 25 mL of DI H_2O to a labeled 150-mL beaker. Dissolve 0.200 g of magnesium sulfate sample (for liquid magnesium sulfate use 0.200 mL) in this solution and mix.

Procedure:

Treat the *Standard Solution*, *Reagent Blank*, and *Test Solution* concomitantly and in parallel, as follows:

1) Add *Ammonium Hydroxide Solution (1:2)* to each solution to adjust each to a pH of 2.0 ± 0.2. Check each solution with 0.0-2.5 pH test strips. (This can be done with an initial addition of 30 drops, and then pH testing after each additional drop).
2) Dilute each solution with DI H_2O to 60 mL, and transfer each to a labeled low-actinic separatory funnel with the aid of 10 mL of DI H_2O, adding the 10 mL of rinsings to each separatory funnel.
3) Add 0.200 g of hydroxylamine hydrochloride to the first separatory funnel, swirl to dissolve, immediately add 5.0 mL of DAN Solution, insert the stopper, and swirl to mix. Repeat this with the second and then the third separatory funnel.
4) Allow the solutions to stand at room temperature for 100 minutes.
5) Add 5.0 mL of cyclohexane to each separatory funnel, shake each vigorously for 2 minutes, and allow the layers to separate.
6) Discard the aqueous layers from each separatory funnel into an appropriate waste container.
7) Transfer the cyclohexane extracts into appropriately labeled centrifuge tubes. Place the *Standard Solution* and the *Reagent Blank* tubes opposite each other in the centrifuge.
8) Place the *Test Solution* centrifuge tube into a tarred beaker on the analytical balance and note the weight of the solution.
9) Add enough water to the 'water' centrifuge tube to match the weight of the *Test Solution* tube.
10) Place these two tubes opposite each other in the centrifuge along with the *Standard Solution* and the *Reagent Blank*.
11) Centrifuge the cyclohexane extracts at 5 minute intervals until any dispersed water (cloudiness) is removed.
12) While the extracts are in the centrifuge, turn on the UV-Vis spectrophotometer by flipping the switch on the back. The spectrophotometer will go through a series of diagnostic tests. ***Note:*** *Do not turn the instrument off and on in rapid succession. Always wait about 20 seconds before turning the instrument on again, otherwise the electronic and mechanical systems will be damaged. The instrument may be left on if more than one test is to be performed or powered down after a single test by flipping the switch on the back.*
13) Clean the 10-mm quartz cell with DI H_2O, then acetone, and dry with laboratory wipers.
14) On the UV-Vis spectrophotometer touch screen touch 'Wavelength Scan'.
15) When the 'Wavelength Scan' screen appears touch 'Options'. In the 'Options' menu touch 'λ'.
16) Set the range from 360 nm to 400 nm and the Step to 0.1 nm and touch 'OK'.
17) Touch 'Options'. In the 'Options' menu touch 'More…', then 'Scale & Units'.
18) Make sure 'Abs' and 'Auto' are selected, touch 'OK', then touch 'Return'.
19) Touch the stick figure icon and choose or create your operator ID.
20) Touch the beaker icon and choose 'Se_Standard' as the 'Sample ID'.
21) Decant 3-4 mL of the *Reagent Blank* cyclohexane extract into the 10-mm quartz cell, and put on the lid.
22) Open the cell compartment, place the quartz cell with *Reagent Blank* into the 10 mm square cell holder with the letter 'Q' facing the front, and close the cell compartment.

23) Touch 'Zero' on the touch screen and the spectrophotometer will perform a wavelength scan of the *Reagent Blank* to set a base level (zero) from which the other measurements will be performed.
24) Once the wavelength scan is finished open the cell compartment, remove the quartz cell, discard the *Reagent Blank* into an appropriate waste container, clean the quartz cell with acetone and dry with laboratory wipers.
25) Decant 3-4 mL of the *Standard Solution* cyclohexane extract into the 10-mm quartz cell, and put on the lid.
26) Place the quartz cell with *Standard Solution* into the 10 mm square cell holder with the letter 'Q' facing the front, and close the cell compartment.
27) Touch 'Read' on the touch screen and the spectrophotometer will perform a wavelength scan of the *Standard Solution*.
28) Once the wavelength scan is finished touch 'Options'. In the 'Options' menu touch the file folder icon and the Store Data list will be displayed.
29) Press 'Store' to save the current scan to the highlighted numbered line. A scan can be overwritten.
30) Open the cell compartment, remove the quartz cell, discard the *Standard Solution* into an appropriate waste container, clean the quartz cell with acetone and dry with laboratory wipers.
31) Touch the beaker icon and choose 'Se_Test' as the 'Sample ID'.
32) Decant 3-4 mL of the *Test Solution* cyclohexane extract into the 10-mm quartz cell, and put on the lid.
33) Place the quartz cell with *Test Solution* into the 10 mm square cell holder with the letter 'Q' facing the front, and close the cell compartment.
34) Touch 'Read' on the touch screen and the spectrophotometer will perform a wavelength scan of the *Test Solution*.
35) Once the wavelength scan is finished touch 'Options'. In the 'Options' menu touch the file folder icon and the Store Data list will be displayed.
36) Press 'Store' to save the current scan to the highlighted numbered line. A scan can be overwritten.
37) Open the cell compartment, remove the quartz cell, discard the *Test Solution* and all extra cyclohexane extracts into an appropriate waste container, clean the quartz cell with acetone and dry with laboratory wipers. Close the cell compartment and put away the quartz cell.
38) Touch 'Options' again. In the 'Options' menu touch 'Reference Off'. Select the 'Se_Standard' from earlier and touch 'Highlight Reference'. The 'Se_Standard' curve will be shown in grey and the 'Se_Test' curve will be shown in black.
39) Compare the absorbances of the 'Se_Standard' and the 'Se_Test' at the wavelength of maximum absorbance (about 380 nm). The following controls are used to do this:
    - The black and grey small boxes in the left upper corner switch between the 'Se_Standard' and the 'Se_Test' wavelength scans.
    - The curve icon switches between tracking and minimum/maximum cursor modes.
    - The arrow keys move the cursor and the wavelength/absorbance at that data point are displayed and highlighted.

    If the absorbance of the *Test Solution* ('Se_Test') is not greater than that of the *Standard Solution* ('Se_Standard') then the selenium content of the magnesium sulfate heptahydrate sample is below the USP limit of 0.003%.

Revision Number	Revision Date	Effective Date	Revision Author	Quality Approval	Production Approval	Revision Description
00	04/04/2012	04/04/2012	Stephen Ballew	Deborah Durbin	Jason Bumgarner	New Document