

MORSE LABORATORIES, INC.

DETERMINATION OF TRIFLUMIZOLE IN CROPS

Analytical Method# Meth-115, Revision #3

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DETERMINATION OF TRIFLUMIZOLE IN CROPS

Reasons for Revision:

1. To add alternate GC conditions.
2. To clarify quality control for SPE cartridges.
3. To add provisions for hop and nutmeat samples.

1.0 PRINCIPLE

Residues of triflumizole and its aniline-containing metabolites are converted to FA-1-1 (4-chloro-2-trifluoromethylaniline) by acidic and alkaline reflux, followed by distillation. The distillate is acidified then extracted with hexane. The hexane extract then undergoes a silica solid phase extraction (SPE) purification. Detection and quantitation are conducted by a gas chromatograph equipped with a mass selective detector. The LOQ (limit of quantitation) is 0.01 ppm for most matrices; 0.02 ppm for hops and 0.05 ppm for nutmeats.

2.0 EQUIVALENCE STATEMENT

During the conduct of this analysis, comparable apparatus, solvents, glassware, and techniques (such as sample extract evaporation) may be substituted for those described in this method, except where specifically noted otherwise. In the event a substituted piece of equipment or technique is used, its use will be documented in the study records.

3.0 APPARATUS AND EQUIPMENT

Gas Chromatograph: Hewlett-Packard (HP) gas chromatograph Model 5890A equipped with a HP 5970B mass selective detector, a HP 7673 autosampler, and a HP G1701AA MS ChemStation

Column: *Primary:*
25 M × 0.25 mm i.d. fused silica column crossbonded with 0.50 μm film thickness Rtx-200, (manufacturer: Restek)

Alternate:
10 M × 0.18 mm i.d. fused silica column crossbonded with 0.4 μm film thickness Rtx-200, (manufacturer: Restek)

Balances: Analytical balance capable of weighing to ± 0.1 mg

Top-loading balance capable of weighing to ± 0.1 g

Distillation still:	Glass, 19 inch. (DuPont Glass Shop, Wilmington, DE) See Figure 1
Evaporators:	N-Evap Laboratory Sample Evaporator Model 115 attached to a nitrogen source (Organomation Associates, South Berlin, MA) Rotary evaporator equipped with a Dewar condenser (Buchler Instruments, Labconco, Lenexa, KS)
Evaporation flasks:	Glass, flat bottom, 500 mL
Reaction flasks:	Glass, 1 liter, 29/42 ground glass fittings, heavy walled, flat bottom
Volumetric flasks:	Glass; 200, 100, 50 and 25mL
Funnels:	Glass, 75 mm diameter
Separatory funnels:	Glass, 250 mL
Graduated cylinders:	Glass, assorted sizes
Graduated mixing cylinders:	Glass; 1000 and 100 mL
Heating mantles:	1 liter capacity
Qorpak jar:	Glass, 8 oz., with Teflon [®] -lined lids (Qorpak, Pittsburgh, PA)
Solid Phase Extraction Apparatus:	Vac Elut SPS 24 (Varian Analytical Instruments, Sunnyvale, CA)
Teflon [®] sleeves:	for 29/42 ground glass joints
Test (culture) tubes:	Glass, 13×100 mm, with Teflon [®] lined screw caps
Transformers:	(individual) Glas-Col, PL-312, minitrol
Vortex mixer:	VWR Vortexer 2 (Scientific Industries, Inc., Bohemia, N.Y.)
Assorted laboratory glassware	

4.0 REAGENTS AND MATERIALS

Acetone:	Pesticide grade
Analytical standards:	Triflumizole, analytical grade FA-1-1, analytical grade FD-1-1, analytical grade FD-2-1, analytical grade FM-5-1, analytical grade FM-6-1, analytical grade FM-8-1, analytical grade
Antifoam:	30% Silicone Defoamer (Dow Corning, Midland, MI)
1-decanol:	"Resi-analyzed" (J.T. Baker Chemical Company, Phillipsburg, NJ)
Glacial Acetic Acid:	Reagent grade
Glass Wool	
Ethyl Acetate:	Pesticide grade
Hexane:	Pesticide grade
Hydrochloric Acid:	Reagent grade
Sodium Acetate:	Crystal, trihydrate (J.T. Baker Chemical Co., Phillipsburg, NJ)
Sodium Sulfate:	Analytical grade, anhydrous granular, #8024 (Mallinckrodt, St. Louis, MO)
Sodium Hydroxide:	Pellets, reagent grade
Solid Phase Extraction Tubes:	Silica (SI) Bond Elut LRC, 10cc/500mg (Varian Analytical Instruments, Sunnyvale, CA)
PTFE (Teflon®) Boiling Stones:	Chemware®, Norton Performance Plastics, (VWR Scientific Products, Bridgeport, NJ)
Water	Morse Laboratories, Inc. Polymetrics DI water system

4.1 Reagents and Materials to be Prepared

- 4.1.1 Diluted Antifoam: To a 100-mL graduated mixing cylinder, add 40 mL antifoam. Bring to 100 mL with deionized water. Mix well.
- 4.2.2 20% NaOH Solution: Place 200 g of NaOH pellets into a 1000-mL graduated mixing cylinder. Bring to 1000 mL with deionized water. Mix well.
- 4.2.3 0.1N HCl: To a 200-mL volumetric flask, add ~100 mL of deionized water, then add 1.65 mL concentrated HCl. Mix. Bring to 200 mL final volume with deionized water. Mix well.
- 4.2.4 10% 1-decanol: Place 10.0 g of 1-decanol in a 100-mL volumetric flask. Fill to mark with hexane. Mix well.

5.0 STANDARD PREPARATION

Note: Prepare all analytes as individual solutions.

5.1 Stock Standard Solutions

25.0 mg (corrected for purity) of triflumizole (or metabolite) analytical standard is accurately weighed, quantitatively transferred to a 25-mL volumetric flask, and brought to volume with acetone to make a stock standard solution having a concentration of 1000 $\mu\text{g/mL}$. This solution is to be stored at 1 to 8°C when not in use.

Note: Because it is difficult to weigh liquid analytical standards to specific predetermined values, they are weighed to $\pm 10\%$ of the target value. When calculated, the actual concentration of the stock solution produced is expressed to four significant figures. Appropriate adjustments in the preparation of subsequent dilutions can be made in order to produce concentrations that are more manageable to work with (i.e., where a 1 to 10 dilution of the stock solution may result in a solution having a concentration of 109 $\mu\text{g/mL}$, adjustments in that dilution can be made resulting in a concentration of 100 $\mu\text{g/mL}$).

5.2 Fortification Solutions

Typically the following concentrations of triflumazole (or metabolite) standard solutions are prepared. They are stored at 1 to 8°C when not in use.

100 $\mu\text{g/mL}$: Transfer 5.0 mL of 1000 $\mu\text{g/mL}$ stock standard solution to a 50-mL volumetric flask. Bring to volume in acetone. Mix well.

10 $\mu\text{g/mL}$: Transfer 5.0 mL of 100 $\mu\text{g/mL}$ standard solution to a 50-mL volumetric flask. Bring to volume in acetone. Mix well.

1 $\mu\text{g/mL}$: Transfer 5.0 mL of 10 $\mu\text{g/mL}$ standard solution to a 50-mL volumetric flask. Bring to volume in acetone. Mix well.

5.3 GC (Calibration) Standard Solutions

Typically the following concentrations of *FA-1-1* GC standard solutions are prepared. They are stored at 1 to 8°C when not in use.

0.006 $\mu\text{g/mL}$: Transfer 150 μL of 1.0 $\mu\text{g/mL}$ standard solution to a 25-mL volumetric flask. Bring to volume in ethyl acetate. Mix well.

0.02 $\mu\text{g/mL}$: Transfer 50 μL of 10 $\mu\text{g/mL}$ standard solution to a 25-mL volumetric flask. Bring to volume in ethyl acetate. Mix well.

0.05 $\mu\text{g/mL}$: Transfer 125 μL of 10 $\mu\text{g/mL}$ standard solution to a 25-mL volumetric flask. Bring to volume in ethyl acetate. Mix well.

0.10 $\mu\text{g/mL}$: Transfer 250 μL of 10 $\mu\text{g/mL}$ standard solution to a 25-mL volumetric flask. Bring to volume in ethyl acetate. Mix well.

6.0 **SAMPLE FORTIFICATION**

1. Weigh appropriate amount of sample (50.0 g for moist and watery samples, 10.0 g for dry samples and 5.0 g for hops and nutmeats) into a one-liter reaction flask.
2. Fortify the sample with the appropriate amount of standard solution.
3. Proceed with Step 7.1.1.1 or 7.1.2.1 as appropriate.

7.0 **SAMPLE EXTRACTION**

7.1 REFLUX IN ACID

7.1.1 Moist and watery samples

1. Weigh 50.0 g of sample into a one-liter reaction flask. As applicable, fortify spikes at this point. Add 100 mL deionized (DI) water and swirl to mix.
2. Add 8.8 mL of concentrated glacial acetic acid, 40 g of sodium acetate trihydrate and swirl to mix.
3. Add 5 mL of diluted antifoam and 5-6 PTFE boiling stones. Swirl to mix.

4. Attach the reaction flask to the reflux condenser of the distillation still which is supplied with dry-ice cooled water using a Teflon[®] sleeve at the connection. Make sure the stopcock on the reflux-distillation channel is closed prior to start of reflux.
5. Heat flask under reflux conditions for 90 minutes.

Note: Adjust the transformer setting to "high" until the reaction mixture just comes to a boil. Then reduce the setting to establish the reflux point in the lower two bulbs of the condenser. The 90 minute timing of reflux starts when boiling commences.

7.1.2 Dry samples (including hops and nutmeats)

1. Weigh 10.0 g of sample into a one-liter reaction flask (5.0 g for hops and nutmeats). As applicable, fortify spikes at this point. Add 150 mL deionized (DI) water and swirl to mix.
2. Add 8.8 mL of concentrated glacial acetic acid, 40 g of sodium acetate trihydrate and swirl to mix.
3. Add 5 mL of diluted antifoam and 5-6 PTFE boiling stones. Swirl to mix.
4. Attach the reaction flask to the reflux condenser of the distillation still which is supplied with dry-ice cooled water using a Teflon[®] sleeve at the connection. Make sure the stopcock on the reflux-distillation channel is closed prior to start of reflux.
5. Heat flask under reflux conditions for 90 minutes.

Note: Adjust the transformer setting to "high" until the reaction mixture just comes to a boil. Then reduce the setting to establish the reflux point in the lower two bulbs of the condenser. The 90 minute timing of reflux starts when boiling commences.

7.2 REFLUX IN BASE

7.2.1 Moist and watery samples

1. Following the 90 minute acid reflux, and while still refluxing, add 50 mL 20% NaOH solution through the reflux condenser. Add base slowly so the sample does not foam up into the condenser.
2. Rinse the condenser with ~10 mL deionized water, allowing the rinsate to mix with the sample.
3. Heat under the same reflux conditions as discussed in Step 7.1.1.5, for 90 minutes.

7.2.2 Dry samples (including hops and nutmeats)

1. Following the 90 minute acid reflux, and while still refluxing, add 50 mL 20% NaOH solution through the reflux condenser. Add base slowly so the sample does not foam up into the condenser.
2. Rinse the condenser with ~10 mL deionized water, allowing the rinsate to mix with the sample.
3. Heat under the same reflux conditions as discussed in Step 7.1.2.5, for 90 minutes.

7.3 DISTILLATION (All samples)

1. Just prior to the completion of the 90 minute base reflux, place a 250-mL beaker containing 50 mL of hexane under the distillation condenser. Position condenser tip under the hexane and seal gap between beaker wall and condenser with aluminum foil.
2. On completion of the base reflux, open stopcock on the reflux-distillation channel to permit distillation to proceed.
3. Adjust transformer setting to produce an efficient, steady distillation at a rate of ~1 mL/min.
4. Distill ~100 mL. The volume of solution in the receiving beaker should be ~150 mL (50 mL hexane + 100 mL distillate).
5. Rinse the condenser with 10 mL deionized water and collect the rinsate in the receiving beaker.
6. Quantitatively transfer contents of the beaker to an 8-oz Qorpak jar to permit chilling of the sample and refrigerated storage if necessary. This is an acceptable stopping point. See Appendix II for handling of alkaline waste.

8.0 **SOLVENT PARTITION**

1. Quantitatively transfer the chilled sample from the 8-oz Qorpak jar to a 250-mL separatory funnel.
2. Add 10 mL 0.1N HCl.
3. Shake for ~1 minute, then allow layers to separate.
4. Drain the aqueous layer into the same Qorpak jar from Step 8.0.1. Drain the hexane layer through a funnel containing a glass wool plug layered with ~50 g sodium sulfate

into a 500-mL evaporation flask. Rinse the sodium sulfate with ~10 mL of hexane and collect rinsate in the same flask.

5. Transfer the aqueous layer back into the 250-mL separatory funnel and re-extract with another 50 mL portion of hexane.
6. Discard the aqueous layer and drain the hexane layer through the sodium sulfate and into the 500-mL evaporation flask from Step 8.0.4. Rinse the sodium sulfate with ~10 mL hexane and collect rinsate in the same flask.
7. Add 0.2 mL of 10% 1-decanol to the combined extract and concentrate the volume to ≥ 3 mL using a rotary evaporator at $\leq 33^{\circ}\text{C}$. Maintain the vacuum at ≤ 25 " Hg.
8. Quantitatively transfer the concentrated extract to a 13×100 mm test tube calibrated at 5.0 mL. Adjust volume of the extract to 5.0 mL with hexane. Mix. Proceed with silica solid phase extraction (SPE) cleanup.

9.0 SILICA SPE CLEANUP

- Notes: 1) Check or calibrate the SPE cartridges prior to use in order to ensure optimum method performance. In general, check one cartridge per lot number. This assessment should be conducted well in advance of needing the cartridges for sample analysis. Recovery of $>90\%$ is desired to ensure that a lot is suitable for use. The analyses are conducted on a reagent spike basis.

See Appendix I for detailed instructions on assessment of the SPE cartridges.

- 2) Prior to use of each cartridge, tap on countertop to settle packing, then push frit down against packing to eliminate any gap.
1. Set up Vac Elut system and support apparatus and proceed with Silica SPE cleanup.
2. Using vacuum, prewash the SPE cartridge with 5 mL hexane. Allow the hexane to drain to approximately 1 mm above the packing. Discard this prewash.
3. For **moist and watery samples**, transfer 1.0 mL of the sample extract from Step 8.0.8 (using a 1.0-mL graduated pipet) to the SPE cartridge. Using vacuum, allow the solvent to drain to approximately 1 mm above the packing. Discard this loading solvent.

For **dry samples and hops**, transfer the entire 5.0 mL of the sample extract from Step 8.0.8 (using a pasteur pipet) to the SPE cartridge. Rinse and vortex mix the original sample tube two times with 1.0 mL hexane. Transfer each rinse to the cartridge. Using vacuum, allow the solvent to drain to approximately 1 mm above the packing. Discard this loading solvent.

For **nutmeats**, transfer 2.0 mL of the sample extract from Step 8.0.8 (using a 2.0-mL graduated pipet) to the SPE cartridge. Using vacuum, allow the solvent to drain to approximately 1 mm above the packing. Discard this loading solvent.

4. Using vacuum, wash the cartridge with 8 mL of hexane and allow to drain to approximately 1 mm above the packing. Discard this wash.
5. Elute FA-1-1 from the cartridge with 5 mL of ethyl acetate using vacuum (adjusting the vacuum so that distinct droplets of solvent emerge from the cartridge) into a 13 × 100-mm screw-cap culture tube calibrated at 5.0 mL. Allow the cartridge to drain to dryness.
6. Adjust volume of extract to 5.0 mL with ethyl acetate or blow down gently with nitrogen if necessary. Mix. Submit to GC analysis. The resulting sample concentration is:

Watery and dry samples: 1 mL = 2.0 g

Hops: 1 mL = 1.0 g

Nutmeats: 1 mL = 0.4 g

10.0 GAS CHROMATOGRAPHIC ANALYSIS

Note: The column and conditions stated in the method have been satisfactory for the matrix being analyzed. The specific column packing/coating, carrier gas, column temperature and flow rate listed are typical conditions for this analysis. Specific conditions used will be noted on each chromatographic run and will not otherwise be documented.

10.1 Operating Conditions

Instrument: Hewlett-Packard (HP) gas chromatograph Model 5890A equipped with a HP 5970B mass selective detector, a HP 7673 autosampler, and a HP G1701AA MS Chemstation.

Primary column and conditions:

Column: 25 M × 0.25 mm i.d. fused silica column crossbonded with 0.5 μm film thickness Rtx-200, (manufacturer: Restek)

Inlet liner: 4 mm i.d. gooseneck splitless liner packed with Carbo Frit™ (manufacturer: Restek)

Injection Volume: 2 μL

Carrier Gas: Helium

Column Head
Pressure: 8 psi

Purge Flow
Timing: on at 0.80 minutes

Tuning: Prior to analysis, the instrument is tuned manually for ion m/e 219.

Ion Monitored: FA-1-1 m/e 175, 195.

Dwell Time: 45 msec

Temperatures: Injector: 240°C

GC/MSD
Transfer line: 295°C

Column: Initial: 65°C, hold 1.00 minute
Rate: 20°C/min.
Final: 285°C, hold 4.00 minutes

Retention Time: approximately 7.2 minutes

Alternate column and conditions:

Column: 10 M × 0.18 mm i.d. fused silica column crossbonded with 0.4 μm film thickness Rtx-200, (manufacturer: Restek)

Inlet liner: 4 mm i.d. gooseneck splitless liner packed with Carbo Frit™ (manufacturer: Restek)

Injection Volume: 2 μL

Carrier Gas: Helium

Column flow: 1.0 mL/min. constant flow

Purge Flow
Timing: on at 0.80 minutes

Tuning: Prior to analysis, the instrument is tuned manually for ion m/e 219.

Ion Monitored: FA-1-1 m/e 175, 195.

Dwell Time: 50 msec

$$ppm = \mu\text{g/mL FA-1-1} \times \frac{FV \text{ (mL)}}{\text{samp. wt. (g)}} \times \frac{\text{dist. (mL)}}{\text{dist. aliq (mL)}} \times \frac{\text{part. ext. (mL)}}{\text{part. ext. aliq (mL)}} \times MWCF \times GC \text{ dil. fact.}$$

where:

$\mu\text{g/mL FA-1-1}$	=	$\mu\text{g/mL}$ of FA-1-1 found
FV (mL)	=	volume of final extract submitted to GC
samp. wt. (g)	=	gram weight of sample extracted
dist.(mL)	=	volume of distillate collected
dist. aliq (mL)	=	volume of distillate taken through solvent partition
part. ext. (mL)	=	volume of extract resulting from solvent partition and submitted to SPE cleanup step
part. ext. aliq (mL)	=	volume of solvent partition extract taken through SPE cleanup
MWCF	=	molecular weight conversion factor for converting FA-1-1 to the compound analyzed
Conversion Factors:		
Triflumizole	= 1.77	FD-1-1 = 1.51
FM-5-1	= 1.65	FD-2-1 = 1.30
FM-6-1	= 1.51	FA-1-1 = 1.00
FM-8-1	= 1.29	
GC dil. fact.	=	dilution of sample extract required to produce an analyte response bracketed by standards

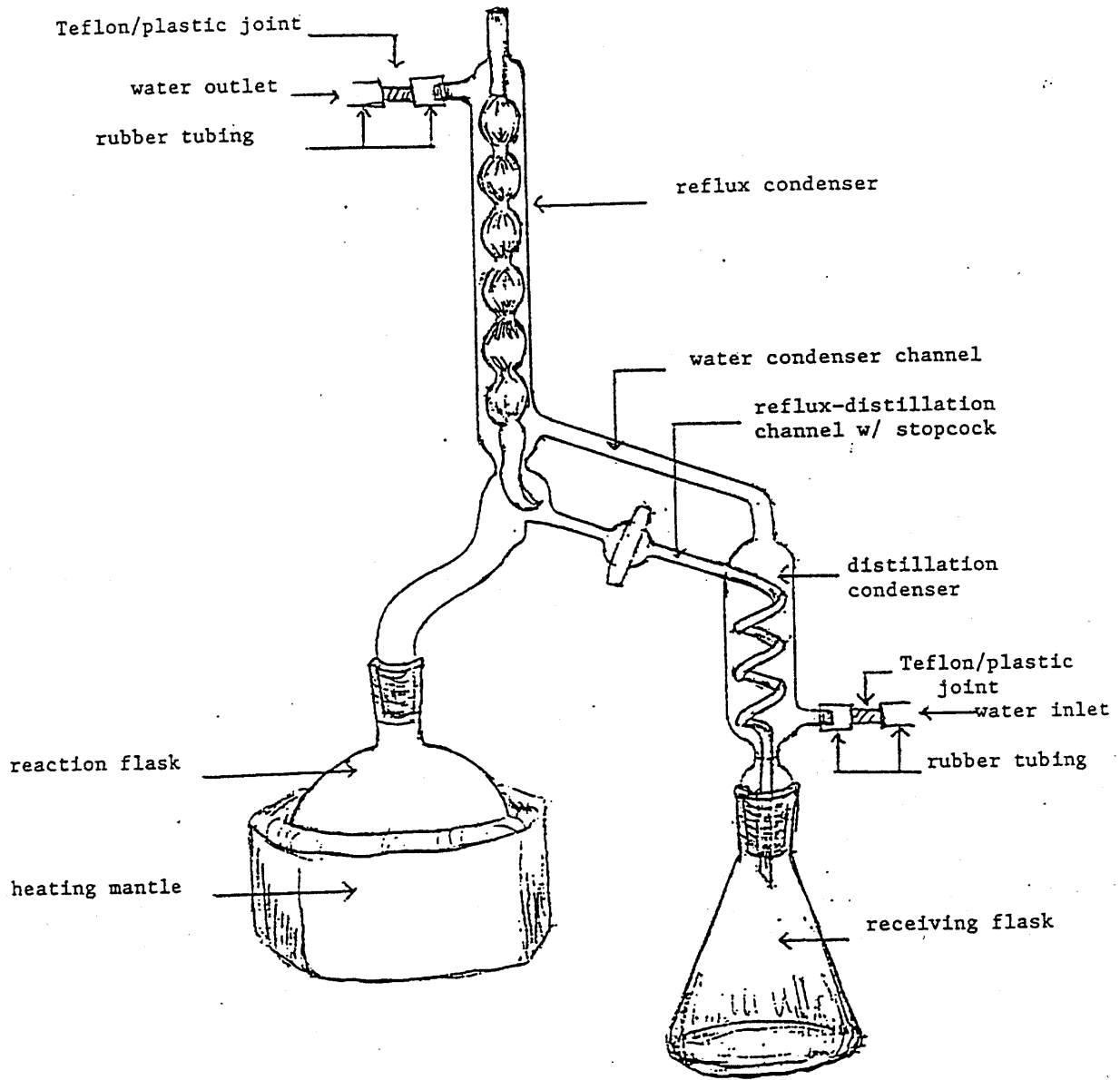
2. The percent recovery for fortified control samples is calculated as follows:

$$\% \text{ Rec.} = \frac{\text{ppm found in fortified control (spike)} - \text{ppm found in control}}{\text{fortification level (ppm) added}} \times 100$$

12.0 REFERENCES

1. Uniroyal Method: CRM-3-96, "Analytical Method for the Determination of Triflumizole Residues in Crops"
2. Morse Laboratories, Inc. SOP# Meth-65, Revision #3, "Determination of Substituted Urea Herbicides, Linuron, Diuron, and their Metabolites in Soil and Agricultural Products", 08/93
3. Morse Laboratories, Inc. Modifications dated 3/22/96 to Uniroyal Method: CRM-3-96

Method author: Gary L. Westberg



Distillation Still
Figure 1

APPENDIX I

Quality Control for SPE Cartridges

Quality Control for SPE Cartridges

Silica SPE Cartridge

Add 100 μL of 10 $\mu\text{g}/\text{mL}$ FA-1-1 standard (in acetone) to a 13 \times 100 mm test tube. Add 0.2 mL of 10% 1-decanol, evaporate just to dryness with nitrogen, then add 5.0 mL of hexane. Follow steps 1 through 6 of section 9.0 in the procedure, using dry sample provision. Dilute resulting solution 1 to 4 with ethyl acetate. Final concentration is 0.05 $\mu\text{g}/\text{mL}$.

APPENDIX II

Handling of Alkaline Waste

Handling of Alkaline Waste

The spent alkaline reaction mixture produced by this procedure is transferred from the reaction flasks with appropriate rinsing of the flasks (~200 mL tap water per 400 mL reaction mixture) to a one-gallon Nalgene bottle. This resulting solution is considered alkaline waste and requires neutralization. Its strength is ~1%.

Using an elementary neutralization unit, add acid (H_2SO_4) or acid waste at appropriate strength until pH is between 6-8. Once the waste solution is neutralized, it can be discarded through the sewer.

The neutralization and discard of the above alkaline waste will be conducted by the appropriate laboratory personnel.