SOP-010 Calibrator and Control Standard Preparation

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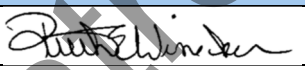
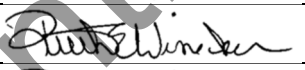
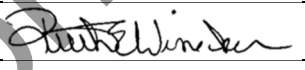
SOP Name: Calibrator and Control Standard Preparation		SOP #: 010
North Carolina Office of the Chief Medical Examiner Toxicology Laboratory	**Revision:**	**Revision Date/Initials:**
	5.3.2 – Updated controlled substance usage instructions 5.1.1, 5.1.2 – Updated storage locations 5.1.2.1 – Added Lipomed 5.1.4.1 – Updated ampule usage instructions 5.5 – Added working standard prep. section. 6.3 – Added Dilution Table	MSF – 10/7/2016 MSF – 05/24/2017

Approving Authority Name	Approving Authority Signature	Approval Date
Ruth E. Winecker, Ph.D.		04/07/2015
Ruth E. Winecker, Ph.D.		06/10/2016
Ruth E. Winecker, Ph.D.		08/29/2017

SOP-010 Calibrator and Control Standard Preparation

1. Principle

1.1. This method is designed to allow the user to prepare solutions used to spike calibrators and controls during extraction procedures.

2. Specimens

2.1. N/A

3. Reagents and Materials

3.1. Volumetric flasks; Class A, borosilicate glass

3.2. Stoppers; borosilicate glass

3.3. Volumetric pipettes; Class A, borosilicate glass

3.4. Pipette pump; 5 mL or equivalent

3.5. Methanol; HPLC grade

3.6. Acetonitrile; HPLC grade

3.7. Chloroform; HPLC grade

3.8. Deionized Water; HPLC grade

3.9. Certified drug standard; 1 mL ampule (Cerilliant/Alltech-Grace or equivalent)

3.10. Stock drug standard; 98%+ pure; powder

3.11. Pasteur Pipettes

3.12. Pipette Bulb; 2mL

3.13. 12 x 75mm test tubes; borosilicate glass

3.14. Weighing boat or paper

3.15. Small spatula

4. Instrumentation and Equipment

4.1. Analytical balance

5. Procedure

5.1. Calibrator or control (QC) prepared from certified 1mL ampule or stock solution (v/v).

5.1.1. Certified reference standards (ampules) are located in R2-2603. Their storage location within the refrigerator can be found using the "standard locate sheet" stored on top of the safe in room 2603.

5.1.2. In-house prepared stock solutions are stored in R1-2601. Their reference numbers can be found with the Microsoft Access "Standards2003" or "qc check standards 2003" databases located in S:\toxicology\analyst\access\toxdata.

5.1.2.1. Note: Cerilliant standards should be used for calibrators and Lipomed or Alltech/Grace standards for controls (QC) if possible. Calibrators and controls (same analyte) shall be made from reference standards or material with different lot numbers when possible.

5.1.3. Before using, check ampule/stock solution label to verify:

5.1.3.1. Drug name

5.1.3.2. Concentration

5.1.3.3. Solvent

5.1.3.4. Expiration Date

5.1.4. Determine the initial volume and final volume needed to achieve desired concentration (see Calculations section). Assemble, clean (rinse three times with solvent to be used), and air-dry the appropriate volumetric pipette(s) and volumetric flask(s).

5.1.4.1. Note: Alltech/Grace ampules:due to inconsistent filling volume by the manufacturer the full volume of an Alltech/Grace ampule (~1 mL) should not be used to prepare calibrators and controls. If 0.9 mL or more of solution is needed, more than one ampule should be used. Other manufacturers overfill their ampules allowing a full 1ml to be measured and used.

5.1.5. Determine the appropriate solvent to be used (typically same as ampule label).

5.1.5.1. Note: When preparing a mixed drug solution (multiple analytes), if any of the ampules are prepared in acetonitrile, acetonitrile should be used to dilute to the final volume, otherwise, methanol is typically used.

5.1.6. Make an entry in the appropriate log book ("Standards" log book for calibrators or "Controls" log book for QC's). The entry should include:

5.1.6.1. Standard number

5.1.6.2. Analyte(s)

5.1.6.3. Final concentration

5.1.6.4. Manufacturer and lot number of stock

5.1.6.5. Concentration of stock

5.1.6.6. Amount used of stock

5.1.6.7. Final volume (QS)

5.1.6.8. Dilution solvent

5.1.6.9. Dilution solvent manufacturer and lot number

5.1.6.10. Analyst initials

5.1.6.11. Date prepared

5.1.6.12. Date expired (typically 1 year)

5.1.7. Open an ampule/stock solution to be diluted and transfer contents to a 12 x 75mm test tube. (Stock solution does not need to be transferred).

5.1.8. Using a volumetric pipette attached to a pipette pump, draw the solution into the pipette so that the level is approximately 1 inch above the target volume hash mark.

5.1.9. Adjust the volume down so that the bottom of the meniscus is even with the hash mark, remove the tip from the solution, and draw up air (~$^1/_2$ inch).

5.1.10. Transfer the solution from the volumetric pipette into the volumetric flask by depressing the release lever on the side of the pipette pump. When the level has dropped to the tip of the pipette, count to ten, touch the tip to the inside of the flask, and remove.

5.1.10.1. Note: DO NOT expel the last of the solution from the volumetric pipette into the flask.

5.1.11. Using a Pasteur pipette, transfer dilution solvent into the volumetric flask to bring the bottom of the meniscus up to the hash mark.

5.1.12. Place the stopper into the flask and invert at least three times to mix.

5.1.13. Transfer the standard solution from the volumetric flask into a 16 X 100mm screw-top test tube labeled with the standard number, analytes present, and concentration, and cap.

5.1.14. Enter the standard info into the appropriate database (Standard /Control). Print a label, and attach to the test tube containing the newly made standard with packing tape.

5.1.14.1. Label should contain the following info:

5.1.14.1.1. Standard #

5.1.14.1.2. Analyte(s)

5.1.14.1.3. Concentration

5.1.14.1.4. Solvent

5.1.14.1.5. Preparer's initials

5.1.14.1.6. Expiration date

5.1.15. Place the test tube in the appropriate rack in R4.

5.1.16. Clean all glassware by rinsing a minimum of three times with methanol, dry, and put away.

5.2. Internal standard (IS) prepared from certified 1mL ampule (v/v).

5.2.1. Follow procedure outlined in steps 5.1.1 – 5.1.6, no volumetric pipette is needed.

5.2.2. Open an ampule to be diluted and, with a Pasteur pipette, transfer contents to the appropriate volumetric flask

5.2.3. With a Pasteur pipette, transfer approximately 1 mL of dilution solvent into the empty ampule to rinse. Transfer this solution into the volumetric flask.

5.2.4. Follow procedure outlined in steps 5.1.11– 5.1.16.

5.3. Calibrator or control (QC) prepared from stock powder (w/v).

5.3.1. Stock powders are stored in 4 boxes located in room 2603.

5.3.2. Controlled substances are located in the safe in room 2603. (See a senior chemist for access to the safe).

5.3.2.1. Anyone accessing the safe and using a controlled substance to prepare a standard must enter the following on the Controlled Substance Usage Log:

5.3.2.1.1. Date
5.3.2.1.2. Analyst name
5.3.2.1.3. Drug name
5.3.2.1.4. Manufacturer
5.3.2.1.5. Lot number
5.3.2.1.6. Amount used

5.3.3. Determine the weight of stock needed to obtain the desired final concentration (typically 1mg/mL). (See Calculations section).

5.3.4. Determine in which solvent the drug best dissolves and is most stable, e.g. methanol, acetonitrile, chloroform, DI water. This information can be found in *Clarke's Analysis of Drugs and Poisons* or an internet search. It is also helpful to reference the Standard/Control log books to see how the standard had been prepared in the past.

5.3.5. Check the calibration of the analytical balance AB135-S (QA-QC Manual).

5.3.6. Place a weighing boat or paper on the balance and tare.

5.3.7. Using a small spatula, place a small amount of stock drug powder onto the tared weighing boat/paper. Repeat until the predetermined weight is achieved.

5.3.8. Transfer the weighed stock into the appropriate volumetric flask. Using a Pasteur pipette, rinse any remaining powder from the weighing boat/paper into the flask using the predetermined solvent.

5.3.9. Follow procedure outlined in steps 5.1.11– 5.1.16.

5.3.10. Make an entry in the appropriate log book as outlined is step 5.1.6.

5.4. Volatiles calibrator or control (QC) prepared from stock liquid (w/v).

5.4.1. Determine the weight of stock needed to obtain the desired final concentration (See Calculations section).

5.4.2. Add a small amount of DiH_2O to the appropriately sized volumetric flask.

5.4.3. Place flask onto precision balance and tare.

5.4.4. With a Pasteur pipette, add liquid stock drop wise until predetermined weight is achieved.

5.4.4.1. Repeat with other compounds if necessary.

5.4.5. Bring volumetric flask up to volume with DiH_2O.

5.4.6. Follow procedure outlined in steps 5.1.11– 5.1.16.

5.4.7. Make an entry in the appropriate log book as outlined in step 5.1.6.

5.5. Preparation of working standards/internal standards.

5.5.1. Often, to achieve a calibrator or control concentration that is appropriate for use in postmortem toxicological analysis, the stock solutions prepared in the steps above need to be further diluted.

5.5.1.1. To determine the required standard concentration(s) for a particular assay, refer to the appropriate standard and control worksheet.

5.5.1.2. Using the techniques described in steps 5.1, dilute the stock solution to the desired concentration.

5.5.1.3. Note – multiple dilutions (serial dilutions) may be required to achieve the desired concentration(s).

6. Calculations

6.1. Concentration (dilution of solutions)

6.1.1. $C_i \times V_i = C_f \times V_f$

6.1.1.1. C_i = initial concentration

6.1.1.2. V_i = initial volume

6.1.1.3. C_f = final concentration

6.1.1.4. V_f = final volume

6.2. Stock powder mass adjusted for salt

6.2.1. (molecular weight (drug + salt)/ molecular weight (drug)) x final volume = mass to be weighed.

6.3.

Dilution Table		
Dilution	Volume Added Stock Standard (mL)	Final Volume (mL)
1:5	2	10
1:10	1	10
1:20	0.5	10
1:50	0.2	10
1:100	0.1	10
1:5	1	5
1:10	0.5	5
1:20	1	20
1:50	1	50
1:100	1	100

7. References

7.1. Best Practices for Volumetric Measurement, RTI On Demand Presentation, Jeri D. Ropero-Miller, Ph.D., D-ABFT, https://www.forensiced.org/training/fsg.cfm