FORENSIC TOXICOLOGY LABORATORY
OFFICE OF CHIEF MEDICAL EXAMINER
CITY OF NEW YORK

BASIC ANALYTES CALIBRATORS AND CONTROLS

REAGENTS AND MATERIALS

Assorted laboratory glassware

50 mL volumetric flasks with stopper (nominal size: other sizes may be used with adjustments)

Methanol, acetonitrile, or other suitable organic solvent (ACS Grade)

Spatula

Weighing paper

Primary standards (purchased from a reputable commercial source)

Analytical balance

PREPARATION OF STOCK SOLUTIONS

1. The analytical balances are maintained and calibrated under contract with Atlantic Scale Co. quarterly. Records are maintained by the QA/QC supervisor.
2. Place a sheet of weighing paper on the pan of the balance and tare.
3. Weigh out the appropriate amount of primary standard for the concentration desired. (**See Table 1- Preparation of Stock Solution of 1000 mg/L**). Fill out the Preparation of Stock Solution form with all listed information. Assign a lot number to the solution being prepared. Lot number must indicate analyte content and be designated as a calibrator or control solution.
4. Transfer the weighed primary standard to a volumetric flask.
5. Rinse residual analyte from the weighing paper and into the volumetric with the correct organic solvent. Fill the volumetric with the organic solvent approximately half way.
6. Allow analyte to dissolve into the organic solvent. Once the analyte is dissolved, qs to mark.
7. Validate the stock solutions by diluting to 50 mg/L and injecting 1 µL onto the GC/MS (scan mode) and 5 µL onto the GC. Acceptance criteria are that the mass spectrum matches the library or reference spectra with a minimum match of 70 out of 100. All major ions must be present in the scan. There must be no additional peaks greater than 5% of total area in either chromatogram. If there are additional peaks, notify supervisor to determine if the criteria can be suspended for a given analyte.
8. Use validated stock solutions to prepare calibrator pools according to the instructions below. Validate the pool solutions by analyzing in triplicate by GC and GC/MS. The mean of the

triplicate analysis results for each component must be within ± 20 % of the weighed in amount.

9. To calculate the factor for analytes not listed in Table 1 use the following formula:

$$Factor = \frac{MW_{salt}}{MW_{free}}$$

Determine the amount of analyte to weigh by using the following formula:

$$Weight = \frac{factor}{1000} \times volume\ of\ solution\ made$$

The concentration of stock solutions is 1000 mg/L, unless otherwise specified.

10. Repeat the above process to make stock solutions of each analyte for use as controls. Ideally, a separate source, such as a different manufacturer or manufacturer's lot number should be used. When only one source of primary standard is available for an analyte, preparing two separate stock solutions from the same source is acceptable, one for calibrators and one for controls, prepared on different days or by different analysts.

PREPARATION OF MULTI-COMPONENT CALIBRATOR SOLUTIONS

A. 100 MG/L CALIBRATOR SOLUTION

Prepare a 100 mg/L calibrator solution containing each analyte indicated below for Screen, Cal Group 1 and Cal Group 2. You can achieve this by two independent processes or combination of the two. First process is to accurately weigh each analyte in its free form to a 50 mL volumetric flask. Second process is to add 5 mL of a 1000 mg/L calibrator solution of the needed analyte to the 50 mL volumetric flask. Once all analytes have been added, fill to mark with methanol and gently mix for 10 minutes. This will result in the desired 100 mg/L calibrator solution. Transfer to 20 mL Teflon-lined crimp vials, label with appropriate lot number, concentration, date prepared, expiration date and store frozen when not in use. The calibrator solution is stable frozen at -10^0 C or lower for at least one year.

B. 10 MG/L CALIBRATOR SOLUTION

Prepare a 10 mg/L calibrator solution containing each analyte indicated below for Screen, Cal Group 1 and Cal Group 2. You can achieve this by two independent processes or combination of the two. First process is to accurately weigh each analyte in its free form to a 50 mL volumetric flask. Second process is to add 5 mL of a 100 mg/L calibrator solution of the analyte to the 50 mL volumetric flask. Once all analytes have been added, fill to mark with methanol and gently mix for 10 minutes. This will result in the desired 10 mg/L calibrator solution. Transfer to 20 mL Teflon-lined crimp vials, label with appropriate lot number, concentration, date prepared, expiration date and store frozen when not in use. The calibrator solution is stable frozen at -10^0 C or lower for at least one year.

PREPARATION OF MULTI-COMPONENT CONTROL SOLUTIONS

A. 100 MG/L CONTROL SOLUTION

Prepare a 100 mg/L control solution containing each analyte indicated below for Screen, Control Group 1 and Control Group 2. You can achieve this by two independent processes or combination of the two. First process is to accurately weigh each analyte in its free form to a 50 mL volumetric flask. Second process is to add 5 mL of a 1000 mg/L control solution of the needed analyte to the 50 mL volumetric flask. Once all analytes have been added, fill to mark with methanol and gently mix for 10 minutes. This will result in the desired 100 mg/L ccontrol solution. Transfer to 20 mL Teflon-lined crimp vials, label with appropriate lot number, concentration, date prepared, expiration date and store frozen when not in use. The control solution is stable frozen at -10^0 C or lower for at least one year.

B. 10 MG/L CONTROL SOLUTION

Prepare a 10 mg/L control solution containing each analyte indicated below for Screen, Control Group 1 and Control Group 2. You can achieve this by two independent processes or combination of the two. First process is to accurately weigh each analyte in its free form to a 50 mL volumetric flask. Second process is to add 5 mL of a 100 mg/L control solution of the analyte to the 50 mL volumetric flask. Once all analytes have been added, fill to mark with methanol and gently mix for 10 minutes. This will result in the desired 10 mg/L control solution. Transfer to 20 mL Teflon-lined crimp vials, label with appropriate lot number, concentration, date prepared, expiration date and store frozen when not in use. The control solution is stable frozen at -10^0 C or lower for at least one year.

VALIDATION OF CALIBRATOR AND CONTROL SOLUTIONS

Prepare three (3) validation samples for each of the above solutions by fortifying 2 mL of certified negative matrix with the correct amount for each level. Extract, run in appropriate GC Quant Method and process each as samples. A lot is accepted if each component of the calibrator quantifies within ± 20 % of the target concentration. Prepare a new lot of calibrator or control as needed for each failed lot. This step it also applies to validation of internal standard.

PREPARATION OF CALIBRATOR – QUALITATIVE ANALYSIS

For qualitative analysis a single point calibrator will be fortified at a concentration of 1.0 mg/L. Calibrator solution is prepared by fortifying the appropriate amounts to 1 mL of certified negative matrix to achieve the proper concentrations.

CAL Screen Group

n-propylamphetamine	Ketamine	Diazepam

Amantadine	Caffeine	Nordiazepam
Meperidine	Methadone	Fentanyl
Ethylone	Amitriptyline	Zolpidem
Phencyclidine	Cocaine	Alprazolam
Lidocaine	Cocaethylene (EBE)	Buspirone

PREPARATION OF CALIBRATORS – QUANTITATIVE ANALYSIS

For quantitave analysis, a four point calibration curve will be fortified at 2.0 mg/L, 1.0 mg/L, 0.2 mg/L and 0.05 mg/L. Calibrator solutions are prepared by fortifiying the appropriate amounts into 2 mL of certified negative matrix to achieve the proper concentrations.

CAL Group 1 QUANT	***CAL Group 2 QUANT***
Meperidine	Bupropion
m-CPP	Diphenhydramine
Normeperidine	Doxylamine
Phencyclidine (PCP)	Tramadol
Ketamine	Chlorpheniramine
Levamisole	Dextromethorphan
Amitriptyline	Imipramine
Nortriptyline	Desipramine
Cyclobenzaprine	Zolpidem
Norcyclobenzaprine	Diltiazem
Citalopram	Verapamil
Midazolam	Norverapamil
Clozapine	Buspirone
Trazodone	

PREPARATION OF CALIBRATOR LEVEL- QUALITATIVE ANALYSIS

a. . Final concentration of 1.0 mg/L:

1.0 mg/L = 1.0 mg/1000 mL = 0.001 mg/ 1 mL = 1.0 µg/mL x 1 mL = 1 µg needed for 1.0 mL of negative matrix.

Thus, 10 µL of a 100 mg/L solution added to 1.0 mL of certified negative matrix will result in 1.0 mg/L calibrator.

PREPARATION OF CONTROL- QUALITATIVE ANALYSIS

a. Final concentration of 0.2 mg/L:

0.2 mg/L = 0.2 mg/1000 mL = 0.0002 mg/ 1 mL = 0.2 µg/mL x 1 mL = 0.2 µg needed for 1.0 mL of negative matrix.

Thus, 20 µL of a 10 mg/L solution added to 1.0 mL of certified negative matrix will result in 0.2 mg/L control.

b. External Control (Qualitative Analysis):

External control for qualitative analysis (screen) batches, are purchased from Quality Assurance Service Corp., Augusta, Georgia at a concentration of 2.0 mg/L.

The control is further diluted to 1:10 with certified negative blood to produce a concentration of 0.2 mg/L.

The external control will be validated prior to implementation.

The QAS external control may contain, but not limited to, the following analytes:

Methamphetamine	Ketamine	Codeine
Nicotine	Caffeine	Diazepam
Bupropion	Methadone	Nordiazepam
Meperidine	Amitriptyline	Fentanyl
Normeperidine	Nortriptyline	Flurazepam
Phencyclidine	Cocaine	Haloperidol
Lidocaine	EBE	Trazodone

PREPARATION OF CALIBRATOR LEVEL- QUANTITATIVE ANALYSIS

a. Final concentration of 2.0 mg/L:

2.0 mg/L = 2.0 mg/1000 mL = 0.002 mg/ 1 mL = 2.0 µg /mL x 2 mL = 4 µg needed for 2.0 mL of negative matrix.

Thus, 40 µL of a 100 mg/L solution added to 2.0 mL of negative matrix will result in 2.0 mg/L calibrator.

b. Final concentration of 1.0 mg/L:

1.0 mg/L = 1.0 mg/1000 mL = 0.001 mg/ 1 mL = 1.0 µg/mL x 2 mL = 2 µg needed for 2.0 mL of negative matrix.

Thus, 20 µL of a 100 mg/L solution added to 2.0 mL of certified negative matrix will result in 1.0 mg/L calibrator.

c. Final concentration of 0.2 mg/L:

0.2 mg/L = 0.2 mg/1000 mL = 0.0002 mg/ 1 mL = 0.2 µg/mL x 2 mL = 0.4 µg needed for 2.0 mL of negative matrix.

Thus, 40 µL of a 10 mg/L solution added to 2.0 mL of certified negative matrix will result in 0.2 mg/L calibrator.

d. Final concentration of 0.05 mg/L:

0.05 mg/L = 0.05 mg/1000 mL = 0.00005 mg/ 1 mL = 0.05 µg/mL x 2 mL = 0.1 µg needed for 2.0 mL of negative matrix.

Thus, 10 µL of a 10 mg/L solution added to 2.0 mL of certified negative matrix will result in 0.05 mg/L calibrator.

Repeat steps a, b, c and d above for Cal 2, Group 2 -2.0, Cal 2-1.0, Cal 2 - 0.2 and Cal 2 -0.05. In addition to Cal group 1 and Cal group 2, other analytes can be fortified in the same calibrator level as long as the analyte is well-resolved (better than base-line resolution) from all other analytes already present in the calibrator. An additional set of calibrators must be prepared as needed for analytes that are not well-resolved in group 1 and 2.

PREPARATION OF CONTROL LEVELS- QUANTITATIVE ANALYSIS

a. Final concentration of 1.0 mg/L:

1.0 mg/L = 1.0 mg/1000 mL = 0.001 mg/ 1 mL = 1.0 µg/mL x 2 mL = 2 µg needed for 2.0 mL of negative matrix.

Thus, 20 µL of a 100 mg/L solution added to 2.0 mL of certified negative matrix will result in 1.0 mg/L control.

b. Final concentration of 0.5 mg/L:

0.5 mg/L = 0.5 mg/1000 mL = 0.0005 mg/ 1 mL = 0.5 µg/mL x 2 mL = 1 µg needed for 2.0 mL of negative matrix.

Thus, 10 µL of a 100 mg/L solution added to 2.0 mL of certified negative matrix will result in 0.5 mg/L control.

c. Final concentration of 0.025 mg/L:

0.025 mg/L = 0.025 mg/1000 mL = 0.000025 mg/ 1 mL = 0.025 µg/mL x 2 mL = 0.05 µg needed for 2.0 mL of negative matrix.

Thus, 5 µL of a 10 mg/L solution added to 2.0 mL of certified negative matrix will result in 0.025 mg/L control.

Repeat steps a,b, and c above for Control 2, Group 2 -1.0, Control 2 - 0.5 and Control 2 -0.025. Additional analytes can be fortified to Control group 1 and Control group 2, as long as the analyte is well-resolved (better than base-line resolution) from all other analytes already present in the control. An additional set of controls must be prepared as needed for analytes that are not well-resolved in group 1 and 2.

INTERNAL STANDARD

Methapyrilene 1000 mg/L solution

Weigh 0.1140 g methapyrilene HCl in a 100 mL volumetric flask. Fill to mark with methanol. Assign a lot number, affix a label that includes concentration, date prepared, expiration date, preparer's initials and store solution frozen at -10^0 C or lower. The solution is stable for at least one year.

Methapyrilene 50 mg/L solution

Using a volumetric pipet, pipet 10 mL of 1000 mg/L to a 200 mL volumetric flask. Fill to mark with methanol. Assign a lot number, affix a label that includes concentration, date prepared, expiration date, preparer's initials and store solution frozen at -10^0 C or lower. The solution is stable for at least one year.

Promazine 100 mg/L solution is used as the internal standard in cases where methadone and methadone metabolite are detected in the screening procedure, as the major methadone metabolite is poorly resolved from methapyrilene. Weigh 0.0056 g of promazine HCl in a 50 mL volumetric flask to produce a solution of 100 mg/L. Fill to mark with methanol. Assign a lot number, affix a label that includes concentration, date prepared, expiration date, preparer's initials and store solution frozen at -10^0 C or lower. The solution is stable for at least one year.

In rare instances, both methapyrilene and promazine are unsuitable as the internal standard. In these cases, another analyte such (e.g. asenapine and loxapine) will be chosen as the internal standard. These exceptions will be noted on the sequence list.

The internal standard working solution is stored in a 20 mL glass vial sealed with a Teflon-lined stopper and a crimped aluminum seal until put in use. Internal standards will be validated prior to implementation.

ADDITIONAL CALIBRATORS AND CONTROLS

Calibrators

Calibrators must contain each analyte detected in the case specimens following the initial screening. If there are analytes present in any case in the batch that are not in Groups 1 or 2, then additional calibrators must be prepared. Additional analytes may be added to calibrator group 1 or group 2, as long as they are well-resolved (better than base-line resolution) from all other analytes already present in that calibrator. Should poor resolution occur, prepare a set of four tubes containing certified negative matrix fortified with necessary analytes to achieve a final concentration of 2.0 mg/L, 1.0 mg/L, 0.2 mg/L and 0.05 mg/L.

Follow the steps for **PREPARATION OF CALIBRATOR LEVEL- Quantitative Analysis** section to prepare the additional calibrators.

Controls

Quantitative analysis for each analyte detected in the initial screen must be performed along with high and low controls for that analyte. If there are analytes present in a case that are not in group 1 or group 2, then additional controls must be prepared. Additional analytes may be added to a control, as long as they are well-resolved (better than base-line resolution) from analytes already present in the control. Should poor resolution occur, prepare a set of three

tubes containing certified negative matrix fortified with the necessary analytes to achieve a final concentration of 1.0 mg/L, 0.5 mg/L and 0.025mg/L.

Follow the steps for **PREPARATION OF CONTROL LEVEL- Quantitative Analysis** section to prepare the additional controls.

TABLE 1: Preparation of Stock Solution of 1000 mg/L (based on hydrochloride salt)

Analyte	*MW free base*	*MW salt*	*Factor*	*Weight of salt for 100 mL*	*Weight of salt for 50 mL*
Amitriptyline	277.39	313.84	1.1314	0.1131	0.0566
Bupropion	239.74	276.20	1.1521	0.1152	0.0576
Buspirone	385.51	422.00	1.0947	0.1095	0.0547
m-cpp *or* 1-(3-chlorophenyl)-piperazine)	196.68	233.14	1.1850	0.1185	0.0592
Chlorpheniramine	274.80	390.80	1.4221	0.1422	0.0711
Citalopram	324.40	405.30	1.2494	0.1250	0.0625
Cyclobenzaprine	275.40	311.90	1.1325	0.1132	0.0566
Dextromethorphan	271.41	352.31	1.2981	0.1298	0.0649
Desipramine	266.37	302.82	1.1368	0.1137	0.0568
Diazepam	284.76	284.76	1.0000	0.1000	0.0500
Diltiazem	414.53	450.98	1.0880	0.1088	0.0544
Diphenhydramine	255.35	291.80	1.1427	0.1143	0.0571
Doxylamine	270.38	388.38	1.4364	0.1436	0.0718
Haloperidol	375.88	375.88	1.0000	0.1000	0.0500
Hydroxyzine	374.92	447.80	1.1944	0.1194	0.0597
Imipramine	280.40	316.85	1.1300	0.1130	0.0565
Ketamine	237.74	274.19	1.1533	0.1153	0.0577
Levamisole	204.29	240.76	1.1786	0.1178	0.0589
Lidocaine	234.38	234.38	1.0000	0.1000	0.0500

Meperidine	247.35	283.80	1.1474	0.1147	0.0574
Methadone	309.45	345.90	1.1178	0.1118	0.0559
Midazolam	325.77			0.1000	0.0500
Nicotine	162.20	507.40	3.1280	0.3128	0.0156
Norcyclobenzaprine	260.35			0.1000	0.0500
Normeperidine	233.31	269.77	1.1560	0.1156	0.0578
Nortriptyline	263.37	299.85	1.1385	0.1139	0.0569
Norverapamil	440.10	477.05	1.0840	0.1084	0.0542
Paroxetine	329.37	374.87	1.1381	0.1138	0.0570
Phencyclidine (PCP)	243.38	279.83	1.1498	0.1150	0.0575
Tramadol	263.40	299.84	1.1383	0.1138	0.5692
Trazodone	371.87	408.30	1.0980	0.1098	0.0549
Verapamil	454.59	491.04	1.0802	0.1080	0.0540
Zolpidem	307.40	764.88	1.2441	0.1244	0.0622

Note: *Since some analytes are unstable with light, once prepared, stored in an aluminum wrapped 20 mL glass vial.*

REVISION HISTORY

Ver 03.08.2013	1. Revision history implemented.
Ver 08.31.2015	1. Added preparation for calibrator and control solution at 100 mg/L and 10 mg/L. Added new calibration points. Updated the list of analytes in each group.