PhenX Toolkit

Data Collection Worksheet

Please Note: The Data Collection Worksheet (DCW) is a tool to aid integration of a PhenX protocol into a study. The PhenX DCW is not designed to be a data collection instrument. Investigators will need to decide the best way to collect data for the PhenX protocol in their study. Variables captured in the DCW, along with variable names and unique PhenX variable identifiers, are included in the PhenX Data Dictionary (DD) files.

Solutions

1. Preparation of Methyl Tertiary Butyl Ether (MTBE) extraction solution (containing quinoline)

a. Determine the desired number of 4 L bottles of MTBE needed, based on the expected use of the method.
b. In a small amber bottle, weigh 500 mg ± 10 mg of quinoline for every 4 L bottle of MTBE to be used.
c. If a single 4 L MTBE bottle is needed, remove approximately 100 ml of MTBE from the bottle, add the quinoline directly to the 4 L bottle, rinse the amber bottle with the 100 ml of MTBE, and pour the rinse back into the 4 L bottle. Shake the bottle to mix well.
d. For more than one bottle of MTBE, remove approximately 100 ml of MTBE, pour the number of desired bottles into a 12 L carboy, and then add the quinolone. Rinse the amber bottle as above, and pour the rinse back into the carboy. Shake the carboy to mix well.
e. Assign a solution ID "MTBE-mmddyy-#," where mmddyy is the date prepared and # is a sequential number for lots made on the same day.
f. Record the solution ID, component information, weight of the quinoline, and the analyst initials on TL-METHOD.035.F01, MBTE Solution Preparation Log.

2. Preparation of 2N NaOH

a. 2N NaOH is typically stored in its original container with a bottle top dispenser attached.
b. If a secondary container is necessary for the bottle top dispenser, assign a refill ID "NaOH-mmddyy-#," where mmddyy is the date prepared and # is a sequential number for multiple refills on the same day.
c. When refilling the secondary container, record the NaOH lot# and date received, the manufacturer’s expiration date, the expiration date for the refill, and the analyst initials on TL-METHOD.035.F02, Ancillary Solution Preparation Log.

3. Preparation of 50% Methanol

a. 50% methanol is stored in a bottle with sealed lid. The solution is prepared by adding equal volumes of methanol and water to the bottle. Volumes may be measured with a graduated cylinder.
b. Assign a refill ID "Me50-mmddyy-#," where mmddyy is the date prepared and # is a sequential number for multiple refills on the same day.
c. When refilling the container, record the MeOH lot# and date received, the manufacturer's expiration date, the expiration date for the refill, and the analyst initials on TL-METHOD.035.F02, Ancillary Solution Preparation Log.

Standards

1. Nicotine analytical standards

a. Nicotine stock solution (0.16 mg/ml)
    i. Add 8.20 g ± 0.20 g of nicotine to 50 ml of isopropanol.
    ii. Assign a solution ID "NIC-mmddyy-#," where mmddyy is the date prepared and # is a sequential number for lots made on the same day.
    iii. Record the solution ID, component information, weight of nicotine, prepared solution expiration date, and the analyst initials on TL-METHOD.035.F02, Nicotine Stock Preparation Log.
    iv. Store the stock solution in amber glassware in an ultralow freezer (target 70°C, or at least ≤ -20°C).
b. Nicotine calibration standards
    i. Select the desired volume of standard to be prepared based on the expected use.
    ii. Add the appropriate volume of the nicotine stock solution detailed in the table below, and mix well.

    Note: The calculations below are an example based on a stock standard concentration of 0.162432 µg/ml. The values calculated using TL-METHOD.035.F06, Nicotine Calibration Standard Preparation, may vary, depending on the actual concentration of the prepared stock standard.

Nicotine Analytical Standard	Stock Added per 10 ml Solvent (µl)	Standard Concentration (µg/ml)	Nicotine Added to Sample (mg) in 400 µl spike
Nic-1	15	0.24	0.10
Nic-2	75	1.22	0.49
Nic-3	225	3.65	1.46
Nic-4	600	9.74	3.90
Nic-5	1,150	18.68	7.47

Nic-6	1,850	30.04	12.02
Nic-7	2,750	44.66	17.86
Nic-8	3,800	61.71	24.68
Nic-9	5,500	89.32	35.73
Nic-10	6,400	105.56	42.22

iii. Use TL-METHOD.035.F06, Nicotine Calibration Standard Preparation Calculator, to calculate the actual concentration of the standards used by the instrument quantification software.

iv. Alternatively, nicotine category 1 calibration standards may be used. Suggested concentrations for purchased standards are in the table below.

Nicotine analytical standard	Standard Solution Concentration (µg/uL)	Nicotine Added to Sample (mg) in 400 µl spike
Nic-1	0.25	.10
Nic-2	1.25	.50
Nic-3	3.75	1.50
Nic-4	10.0	4.00
Nic-5	18.75	7.50
Nic-6	30.15	12.06
Nic-7	45.07	18.03
Nic-8	62.50	25.00
Nic-9	80.09	32.04
Nic-10	105.04	42.02
Cal Check	50.04	20.02

v. Record the use of purchased standards by selecting the check box on TL-METHOD.035.F05, Nicotine Run Sheet. Enter the earliest expiration date of the standards used. Record the individual standard ID’s in the spaces provided under "Samples."

vi. Purchased standards can be stored at ambient temperatures in manufacturer-sealed ampoules for up to 3 years.

Method

1. pH measurement for free nicotine calculation

   a. Calibrate the pH meter using buffers 4.01 and 7.00 according to TL-EQUIP.075, pH Meter Monograph, or TL-EQUIP.200, Sirius Vinotrate Monograph.
   b. The Sirius Vinotrate uses a standing wash solution. Use the 50%

methanol/water solution for this wash.

c. Verify the calibration by measuring the buffer solutions at pH 4.01, 7.00, and 10.01. If the buffer pH measurements are not within ± 0.05, recalibrate the meter. If the meter fails more than twice, change the buffer and clean the electrode, if necessary, prior to re-calibrating. If the pH values continue to fall outside the range, replace the electrode. If poor system performance continues, remove the instrument from service according TL-EQUIP.010, Equipment, and contact the instrument vendor. Initiate corrective action, if needed, according toTL-ADMIN.050, Nonconformances and Corrective Action.
d. At the conclusion of each run, analyze a check sample using pH buffer 4.01. The pH must be within the acceptable range of 4.01 ± 0.05 for the run to be accepted.
    i. If the check sample fails criteria, determine if the pH measuring device is functioning properly. If it is not, remove the instrument from service as described above.
    ii. If the pH measuring device is functioning properly, samples may be reanalyzed one time.
    iii. If the check samples on re-analysis, initiate corrective action according to the TL-ADMIN.050, Nonconformances and Corrective Action.
e. Determine the appropriate volume for the pH measurement device used. For the Sirius Vinotrate, 10 ml deionized/distilled water should be used with the appropriate autosampler vial. For the Pinnacle Series pH meter, 20 ml deionized/distilled water should be used with a 40 ml sample vial.
f. Weigh 1.00 ± 0.01 g of tobacco product for every 10 ml of deionized/distilled water used. Record the weight on TL-METHOD.035.F04, pH for Nicotine.
g. For cigarette filler tobacco
    i. Cap the sample vial, place it on a mechanical mixer, and set the mixer to run for 30 minutes.
    ii. Place the sample in a dark environment for approximately one hour, until the tobacco particulates have visibly settled.
    iii. Decant the supernatant into another vial.
    iv. Measure the pH of the solution in the sample vial at 5, 15, 30, and 60 minutes.
    v. For the Pinnacles Series pH meter, record the pH measurements on TL-METHOD.035.F04, pH for Nicotine.
h. For smokeless tobacco
    i. Place the sample vial on a stir plate with a magnetic stir bar, and set the speed such that the tobacco is suspended in the deionized/distilled water.
    ii. For the Pinnacle Series pH meter, record the pH of the solution on TL-METHOD.035.F04, pH for Nicotine.

2. Blank Preparation

a. Record lot information and expiration dates for materials used in this preparation on TL-METHOD.035.F07, Nicotine Blank Preparation Log.
b. The blank ID is NGB-mmddyy-#, where "NGB" stands for Nicotiana glauca blank, "mmddyy" is the date, and # is a sequential number designating different batches received on the same day.
c. Grind the Nicotiana glauca for at least 30 seconds, until the sample appears visually uniform.
d. This blank material is used for preparation of analytical blanks and calibration standards.
e. The authenticity of the material is confirmed by full scan GC/MS analysis. The Nicotiana glauca samples contain high levels of anabasine and no appreciable nicotine peak.
f. The analyst records confirmation by circling and labeling the two regions, as well as initialing and dating the spectrum. The resulting record is stored in the same location as TL-METHOD.035.F07, Nicotine Blank Preparation Log.
g. Quantitatively verify the blank material by evaluating the y-intercept of the first calibration curve. Check the value to make sure it is below the most recently established limit of detection.

3. Sample Preparation

a. Sample material is stored in -20°C freezer if analysis is planned within 6 months of receipt. For longer-term storage, material is stored in a 70°C freezer.
b. Prior to analysis, remove samples from the freezer and allow them to equilibrate to room temperature for at least 30 minutes.
c. Prepare two QC samples and at least two tobacco blanks for every analytical run. For the calibration curve, two QC samples, two tobacco blanks (at the beginning of the sequence and after the highest calibrator), and the calibration standards are analyzed in each batch. One QC sample may consist of reference tobacco CRP2, and the other may consist of CRP3.
d. Weigh 1.00 ± 0.01 g of tobacco, and record the weight on TL-METHOD.035.F05, Nicotine Run Sheet. Transfer the sample to an amber bottle.
e. Add 5 ml of 2N NaOH to the amber bottle. Wait 15 to 20 minutes, then add 50 ml of MTBE extraction solution to the bottle.
f. Cap the bottle and place it horizontal on an orbital shaker set for 120 min at 160 rpm.
g. Transfer 1.5 ml of the extract from the amber bottle into a 2.0 ml autosampler vial.
h. Prepared samples can be stored in a 4°C refrigerator for up to two weeks prior to analysis.

4. Preliminary System Setup

a. Ensure that the valve on the helium cylinder is open and that the pressure on the tank regulator is greater than 500 psi.
b. Use the autotune function in ChemStation to tune the mass spectrometer using the instrument's internal perfluorotributylamine (PFTBA) standard.
c. Further manually tune the instrument to set the mass resolution and the abundance for the ion at mass 69. The Mass Spectrometer Set Up Parameters are found in work instruction METHOD.035.W01, Instrumentation for Nicotine Analysis.

5. Calibration Curve

a. Calibration curves are created each time a new batch of MTBE solution is prepared or a major instrument repair is performed, such as installation of a new ion source or column.
b. To create calibration samples, spike 400 µl of each calibration standard into a corresponding 1 g sample of blank tobacco.
c. Extract the calibration sample by adding 5 ml of 2N NaOH to the amber bottle. Wait 15 minutes, and then add 50ml of MTBE extraction solution to the bottle.
d. Cap the bottle and place it horizontal on an orbital shaker set for 120 min at 160 rpm.
e. Transfer 1.5 ml of the extract from the amber bottle into a 2.0ml autosampler vial.
f. Run a blank sample to check for peaks that interfere with either nicotine (133,161, and 162 amu) or quinoline (102 and 129 amu).
g. Calculate the slope, intercept, and R-squared value using an unweighted linear regression with a linear least squares fit. This analysis can be performed using ChemStation.

6. Run Analysis

a. Record run specific information on TL-METHOD.035.F05, Nicotine Run Sheet.
b. An analytical run typically consists of a tobacco blank, two QC samples, tobacco samples, and a final blank. Calibration samples may be included before the QC samples if a new batch of MTBE solution is being used.
c. Place the prepared samples in the sample tray of the autosampler.
d. Enter the sample sequence into ChemStation software.
e. For a PAL autosampler, on the LEAP hand held controller, select the correct method specified in TL-METHOD.035.W01, Instrumentation for Nicotine Analysis, and the appropriate tray. Enter the range of vials to be analyzed.
f. Click "Run Sequence" to start the GC run in ChemStation, and press "Start" on the hand-held controller.

7. Calculations

a. Process raw data files using the instrument's quantification file listed in TL-

METHOD.035.W01, Instrumentation for Nicotine Analysis.

b. Peaks are automatically integrated using the ChemStation RTE integrator.

8. Quality assessment

a. The following experimental checks are made on the stability of the analytical system with each run.
b. Analyze the initial blank for visible peaks that may signify contamination in the system, spiking solutions, or reagents.
c. Analyze the QC standards.
    i. The internal standard responses should be within ± 50% of the response from the previous calibration.
    ii. The signal to noise ratios should be > 100, and the retention time should be ± 0.5 minutes from the previous QC run.
d. Visually check to see if any single standard appears to be an outlier
    i. In order to discard an outlier, calculate the average response ratio of mass 133 divided by mass 102 for the 10 calibration standards. Use a relative 20% setting for the acceptance criteria in the Calibrate-Edit Compounds menu.
    ii. If removal of a point changes the slope or intercept by more than 10%, it is considered an outlier.
    iii. No more than one calibration standard may be discarded.
    iv. If either the high or low standard is removed, adjust the reporting limits to reflect the new reporting ranges.
e. Check the regression analysis of the calibration curve to confirm that R^2 is ≥ 0.98.
f. Review automated peak integration and record manual integrations according to TL-LABOP.015, Chromatographic Peak Integration. Use the QEdit function to manually integrate peaks, if needed. No more than 10% of peaks within a run should require manual integration.

Estimate of measurement uncertainty

Estimates of measurement uncertainty are calculated according to TL-QC.020, Measurement Uncertainty, based on QC sample data analyzed according to TL-QC.015, Control Charting and Trend Analysis.

QC Sample ID	Description	Analyte	Mean (mg/g)	Standard Deviation (mg/g)	Expanded Uncertainty (mg/g)
2504	3R4F	Nicotine	17.664	0.500	1.000
2505	CRP1	Nicotine	9.604	0.593	1.186
none	CRP2	Nicotine	12.774	0.426	0.852
none	CRP3	Nicotine	21.357	0.632	1.263

Protocol source: https://www.phenxtoolkit.org/protocols/view/730301