4.0 LABORATORY OPERATIONS

Written by Nancy Lance

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Concentrations of the chemicals in precipitation are low and frequently at or near the detection limits of sensitive analytical instruments. High-quality accurate measurements can only be obtained with adequate analytical methods, sensitive instrumentation and strict quality assurance and quality control (QA/QC) procedures throughout the analytical system. This section identifies the precipitation chemistry analytes recommended by GAW and the preferred methods for their analyses. It describes laboratory QA/QC objectives and activities, methods for sample handling and chemical analyses, and laboratory data verification and reporting.

4.1 Overview of Laboratory Measurements

GAW recommends analysis of the following species in precipitation samples: sulfate, nitrate, chloride, sodium, potassium, magnesium, calcium, ammonium, pH, and conductivity. Hydrogen carbonate (formerly bicarbonate ion) either must be analyzed or calculated. Total nitrogen and total phosphorus also may be important in certain regions, although their analyses are not required by GAW at this time. Analyses of formate and acetate are recommended for areas suspected of having high organic acid concentrations and oxalate measurements can be used to trace biomass burning. (Gardrat, 2016). Preferred analytical methods and alternate methods are listed in Table 4.1.

Table 4.1. Measurement Guide

Analyte	Status	Preferred Methods	Alternate Methods
Cl^-, NO_3^-, $SO_4^=$	Required	IC	
NH_4^+	Required	FIA	IC
Ca^{2+}, Mg^{2+}, Na^+, K^+	Required	ICP-AES	IC, FAA
pH	Required	Electrode	
Conductivity	Required	Conductivity Cell	
Hydrogen carbonate[1]	Required	IC	Calculated
Total Nitrogen	Optional	FIA-digestion	
Total Phosphorus	Optional	FIA-digestion	
Organic Acids	Optional	IC	IEC

IC = ion chromatography; ICP-AES = Inductively Coupled Plasma Atomic Emission Spectrometry; FIA= Flow Injection Analysis colorimetry, IEC= Ion Exclusion Chromatography, FAA= Flame Atomic Absorption

[1]Hydrogen carbonate by IC is recommended for areas where precipitation pH is greater than 6. Hydrogen carbonate may be calculated for areas where precipitation pH is less than 6.

The laboratory is also responsible for QA/QC measurements and procedures that ensure the quality of the analytical results. Laboratories should identify potential problems that may affect these results. Sources of potential problems are illustrated in Figure 4.1a. Figure 4.1b. illustrates factors that address these problems.

Some potential problem sources are:

i. Poor sample quality
ii. Poor laboratory conditions
iii. Poor reagent quality
iv. Measurement system out of control
v. Operator inexperience

Figure 4.1a. Sources of potential problems that may affect the quality of analytical results

Reducing or eliminating potential problems and implementing good quality control and quality assurance programs are keys to the production of good data.

Figure 4.1b. Examples of factors that reduce problems and produce good results

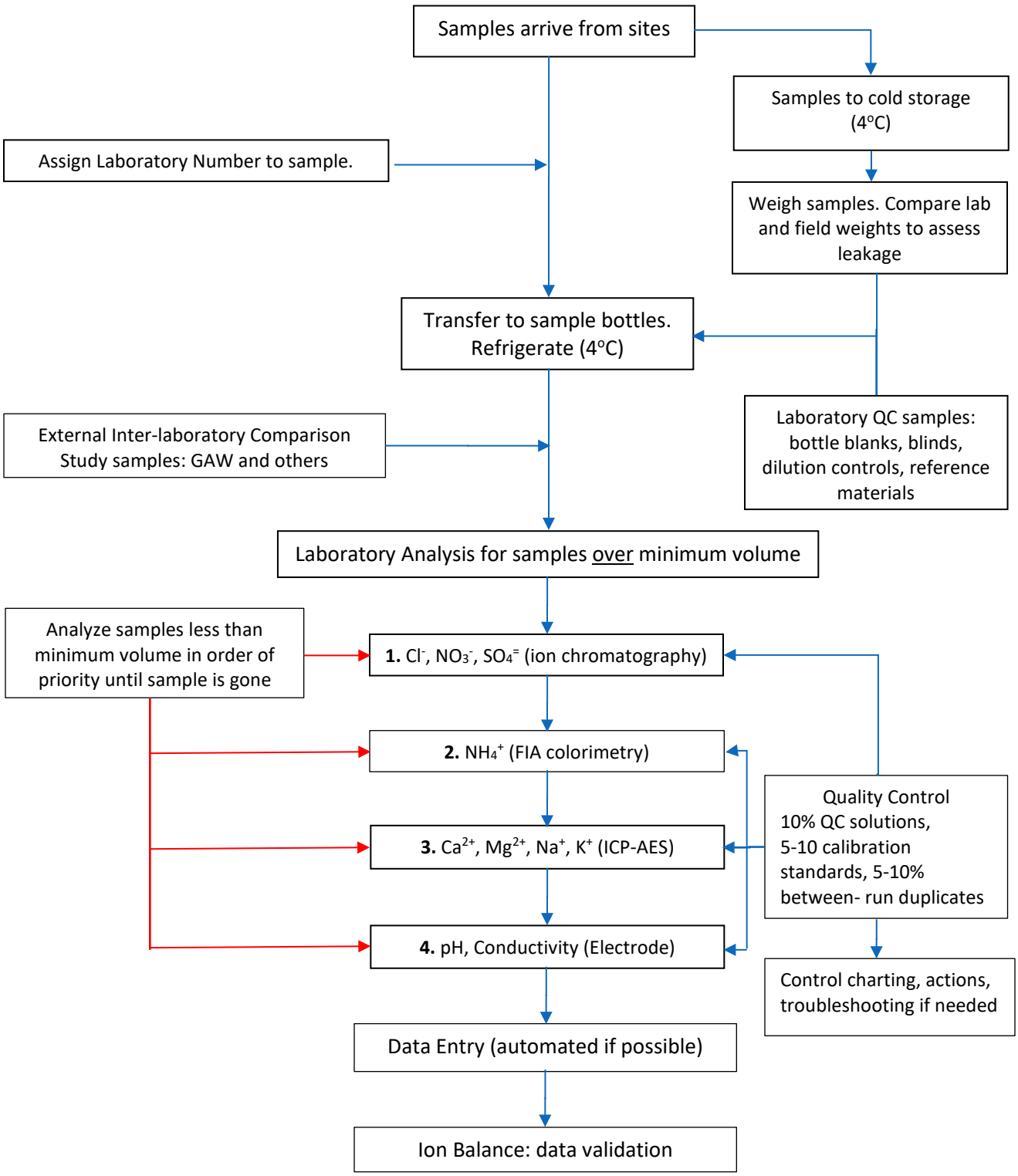
The goal in the laboratory is to measure all the required analytes for each sample. It is necessary first to determine the minimum volume required to measure the complete suite of analytes. For samples that have a volume less than this minimum, the laboratory must follow a set of measurement priorities, completing the analyses in order of importance. These priorities may be set by precipitation chemistry network policies. In the absence of such policies, here is a recommended order of measurements: (1) sulfate, nitrate, and chloride by ion chromatography; (2) ammonium by flow injection analysis; (3) calcium, magnesium, sodium, and potassium by inductively coupled plasma atomic emission spectrometry; (4) pH, to facilitate the calculation of an ion balance calculation; and (5) conductivity, to compare with calculated conductivity as a complementary check of the ion balance calculation.

Where equipment and personnel allow, analyses should be completed without long delays between measurements, minimizing the potential for chemical change and sample deterioration. Ideally, anions and ammonium would be measured on the same day, followed closely by pH. Where this is not possible, ammonium is typically the least stable of the required measurements and should be analyzed as soon as practical. Where the metals can only be analyzed one at a time, the suggested order is calcium, magnesium, sodium, and potassium.

In general, adding deionized water and diluting samples to complete all required measurements is discouraged. Where laboratory or precipitation chemistry networks require a dilution protocol, it is essential to follow strict standard operating procedures for adding deionized water. Deionized water must be tested in each analytical run before dilutions are performed. Use only deionized water where tests show the absence of the species of interest. Special care is needed to ensure that all labware is clean. Pipettes must be calibrated (See Appendix C for details.). Dilution changes sample free acidity, so pH must be determined before the sample is diluted.

Figure 4.2 outlines the priorities for analyzing samples.

Figure 4.2. Example of laboratory prioritization flow chart



4.2 Laboratory Data Quality Objectives (DQOs)

The DQOs for the GAW Precipitation Chemistry Programme are discussed further in Chapter 6 and Appendix A. It is the responsibility of each laboratory to implement an appropriate set of operational and QA/QC activities to ensure that these objectives are met or exceeded.

Every laboratory should have a QA Manager who is responsible for implementing and managing a laboratory quality assurance program.

4.3 Quality Assurance and Quality Control Activities

QA and QC activities for the laboratory can be divided into three distinct pillars:

i. Setting laboratory DQOs
ii. Adopting and implementing good QA/QC practices and procedures
iii. Reporting QA/QC data and information.

Details for how a laboratory conducts its QC procedures must be described in the laboratory Standard Operating Procedures (SOPs). Details of QA activities are best described in a Quality Assurance Project Plan (QAPP). Details on writing laboratory QA plans are available from the U.S. Environmental Protection Agency; for an example, see the U.S National Atmospheric Program website. Both SOPs and QAPPs must be reviewed and updated annually and made readily available to the laboratory staff. Laboratory staff are active participants in compliance and maintenance of these documents and in the laboratory QA/QC system.

QC procedures include but are not limited to the following activities. Details are provided in Figure 4.3. Additional references include (STP-867, 1985), (ASTM D6328-12), (ISO/IEC-17025, 2005):

i. Performing QC checks in every analytical run
ii. Charting QC results
iii. Implementing a QC action plan that specifies rules for corrective actions when QC measurements indicate something is wrong with the analytical system. This is termed a multi-rule system.
iv. Making precision checks (within-run and between-run duplicates).

QA procedures include but are not limited to:

i. Periodically analyzing Standard Reference Materials (SRM) or Certified Reference Materials (CRM)
ii. Participating in Inter-laboratory Comparison Studies, such as the semiannual studies conducted by the Quality Assurance/Science Activity Centre – Americas (QA/SAC-Americas)
iii. Conducting internal audits and/or reviews
iv. Ensuring traceability of calibration standards
v. Documenting and complying with SOPs
vi. Pursuing procurement procedures that ensure instrumentation and materials meet standards
vii. Having protocols for method development and documenting method development studies
viii. Following protocols for the smooth implementation of changes to analytical procedures
ix. Practicing preventive maintenance and documenting maintenance of laboratory equipment and instrumentation
x. Documenting analyst training
xi. Providing facilities appropriate to laboratory work
xii. Implementing and complying with safety protocols
xiii. Ensuring a well-defined laboratory chain-of-custody

Figure 4.3. Example of laboratory QA/QC activities (suggested frequency)

Daily

- Calibrate instruments and verify calibration curves using quality control solutions (QCS). These solutions should have concentrations that correspond to the 10th, 50th and 90th percentiles of the expected or historic sample concentrations.
  - Analyze a QCS for every 10 samples.
  - Record and plot QCS data on QC charts that are inspected during and after every run.
  - Perform replicate analyses on approximately 5% to 10% of samples.
  - Repeat instrument calibration and analysis run if control results violate QC action limits.
- Analyze deionized water and reagent blanks in each analysis run.
- Record standard preparation weights and calculations.
- Perform instrument maintenance and update maintenance records.

Weekly

- Analyze blanks from
  - all sample containers, including specimen bottles and vials.
  - sample containers that are sent to the field sites.
  - container blanks received from the field sites.
- Insert SRM or CRM into the sample stream for analysis. The identity of these samples must be unknown to the analyst to ensure CRMs and SRMs are analyzed in the same way as all other samples.
- Plot SRM and CRM data on control charts. Check for systematic errors and take corrective actions as required.
- Perform dilution checks and plot the results.
- Perform ion balance checks on precipitation samples and repeat the analyses as required.
- Evaluate repeated sample analysis data and amend data as required.
- Evaluate internal blind audit sample data and take corrective actions as required.

Semi Annually

- Participate in GAW Inter-laboratory Comparison Study.
- Participate in other available laboratory comparison studies.
- Prepare laboratory QA report for publication.
- Review all SOPs and revise as necessary.
- Perform internal laboratory audit of procedures.

4.3.1 Chemical Analyses QA/QC

Chemical laboratories involved in the analysis of GAW precipitation samples will achieve high quality performance through adherence to QA/QC procedures in the analytical system. The following QA/QC activities are discussed in this section:

1) Calibration of analytical instruments using standards to ensure accuracy.

2) Application of QC procedures
   a) QCS – to assess instrument function during the analytical run.
   b) Replicate analyses – to evaluate within-run and between-run analytical precision.
   c) Blank samples – to check background contamination, including laboratory deionized water sources.
   d) Blind samples – to check systematic error, including inadvertent protocol errors.
   e) Dilution checks – to assess the accuracy of dilution tools for high concentration samples and to ensure that diluted samples do not suffer from matrix effects or poor precision.

3) Use of SRMs and CRMs – to assess laboratory accuracy.

4) Inter-laboratory Comparisons – to assess laboratory accuracy and bias relative to other laboratories and compliance with GAW DQOs.

4.3.2 Instrument Calibration Control and Verification

Instrument calibration is an integral element of every laboratory's SOPs. Each laboratory must implement QC procedures that ensure accurate calibrations.

Log all information about the preparation of calibration standards in a laboratory logbook.

Document: Preparation dates of calibration standards
Calculations related to calibration standard preparation
Weights and volumes used in calibration standard preparation
Description of new materials and when they were used
Name of operator who prepared the calibration standards and solutions

Maintain laboratory logbooks permanently.

Recommended procedures include:

1) Prepare stock calibration solutions using traceable ultra-pure reagents that have a certificate of purity.

2) Prepare calibration solutions by measuring constituents by weight. Use calibrated analytical balances and ultra-clean tools. Ensure weigh boats are tested for impurities. For more information, see "Calibration and Operational Tests and Procedures for Laboratory Apparatus and Tools" in Appendix C. See also "Quality Control - Stock Consumables" in section 4.3.14.

3) Use an optimum number of calibration standards (typically 5) to describe the analytical range without exceeding instrument limits. Ensure that the concentrations of the calibration standards cover a range from 2% to 98% of expected precipitation sample concentrations.

4) Use extra calibration standards in non-linear portions of calibration curves. Reduce non-linearity by preparing separate calibration curves for different portions of the analytical range.

5) Do not extrapolate a calibration curve for a sample measurement that exceeds the highest calibration standard. Instead, dilute the sample into the calibration range. When using an ion chromatograph, very high concentration samples can overload the column. Avoid this by reducing the length of the injection loop, then prepare a high-concentration calibration curve.

6) Correct for changes in instrument response over time. Room temperature changes and other factors can cause instrument instability or drift. Run calibration standards frequently to correct for these changes.

7) Perform a calibration at the beginning of every analytical run (batch of samples) and verify that the calibration curve is not skewed and that the instrument responses are comparable to previous, valid analytical runs. Recalibrate at the end of the run and compare this end-of-run calibration with the beginning-of-run calibration. Peak areas, absorbencies and other instrument responses should be similar between and within analytical runs.

8) Check that the calibration curve is within acceptable limits for each individual analyte and that the correlation coefficient (R^2) of the curve is greater than or equal to 0.995.

9) As a minimum, analyze one QCS in each batch of 10 samples. Analytes that are unstable, such as hydrogen carbonate and organic acids, may require more QCS per batch of 10 samples.

4.3.3 Application of QC Procedures

QC is a system of maintaining data quality by testing a sample of the data output against expected results. QC differs from QA in that QC procedures are applied during sample analysis, while QA occurs after data collection.

Ideally, QCS are analyzed along with precipitation samples throughout the analytical run. This ensures that the QCS and precipitation samples are analyzed under the same conditions. QCS results are plotted on control charts with warning and control limits clearly identified. These charts are used as guidance to initiate corrective actions when QCS results violate rules set by the QA manager. Corrective action protocols are based on the statistical probability that a result or set of results is unlikely if the analytical system is in control. This set of rules is termed a multi-rule system. Multi-rule systems may be customized and developed for each analytical system or for the entire laboratory. Special software packages that apply multi-rule systems are commercially available.

4.3.4 QC Solutions

Natural precipitation may be used to make QCS. This approach requires a large batch of precipitation that must be sterilized and stored at 4°C. Stability is dependent upon sterilization, equilibration, and proper storage conditions. Preparation of synthetic QCS also may start with natural rain, which helps to ensure that the analytical response to QCS is like the response to precipitation. However, using natural rain for QCS for unstable species, such as organic acids, is not practical. For unstable species, QCS must be prepared from stock solutions different from the ones used to prepare calibration standards. Refer to Appendix C for details.

The mean of the QCS measurements is established from the measurement of at least 20 individual QCS analyses from separate analytical runs. New QCS must be analyzed prior to routine use to ensure that results fall within the calibration range and expected concentration percentiles. Validate new QCS by analyzing them along with the old QCS and making sure that the measurements are comparable and within control.

Usually three or more QCS are prepared, approximating the 10th, 50th and 90th percentiles of all sample concentrations. QCS are analyzed in each analytical run to check analytical performance.

4.3.5 Quality Control Charting

Quality Control Charts are described in detail in statistical reference books. A suggested reference is the U.S. National Institute of Standards and Technology/SEMATECH e-Handbook of Statistical Methods.

QC measurements are plotted during the analytical run and are monitored for values that violate laboratory QC rules. Manual plotting is acceptable and encourages the analyst to look at results closely; however, computer programs may be purchased that will plot results, flag errant data, and perform automatic calculations on the cumulative set of QC data. These programs use multi-rule systems, described below, which apply rules that flag data. An example is provided in Figure 4.4.

Whether manual or automated charting is used, the data should be audited periodically to ensure that instrument operators remain in full compliance with QC rules.

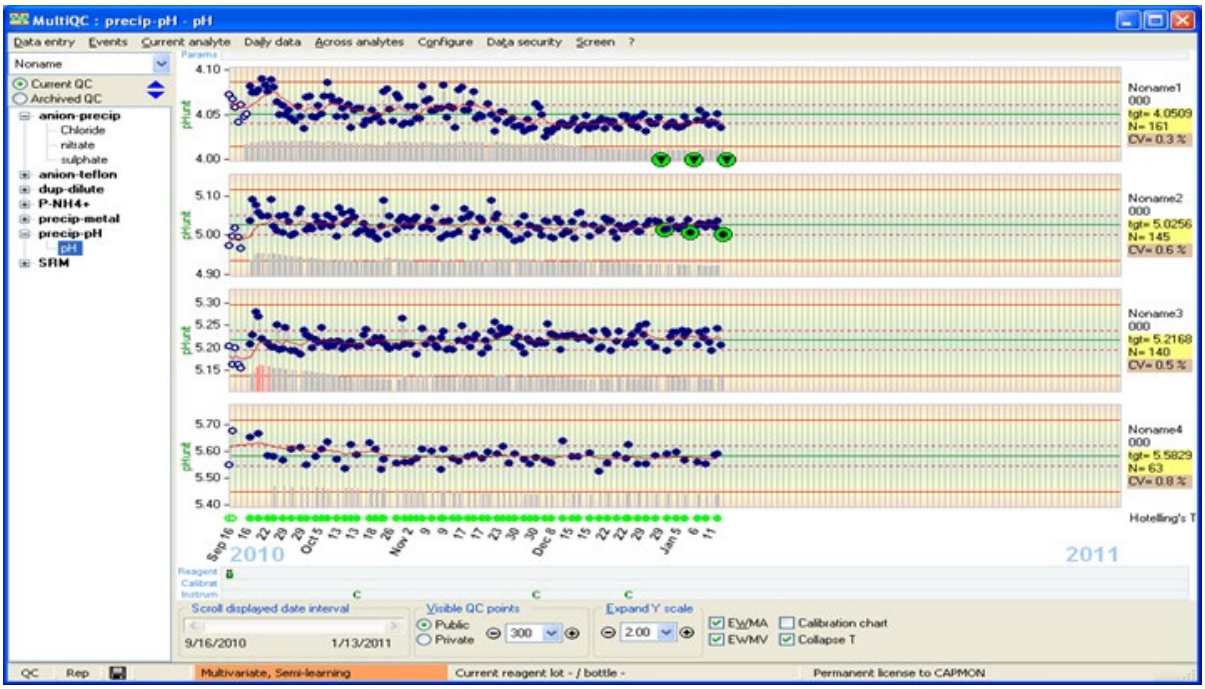


Figure 4.4. Example of Output from an Automated QC System

Multi-rule Systems

Multi-rule systems use a combination of decision criteria to determine if an analytical system or run is in or out of control.

Control charts typically assume a normal distribution of the analytical data and the control limits are based on multiples of the standard deviation (S) of the results. An example of a multi-rule system follows (Westgard, 2010) with illustrations in Figures 4.5 through 4.10.

Rule one (3S): Reject the run if a QC measurement is above or below the mean by 3S or more. The 3S value is the corrective action limit, which means the instrument must be recalibrated and the analytical run repeated.

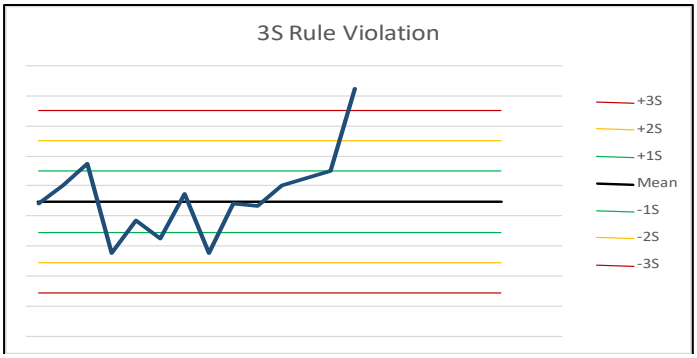


Figure 4.5. 3S rule violation

Rule two (2S): Reject the run if two consecutive QC analyses are more than 2S above or below mean. Recalibrate and repeat the analytical run.

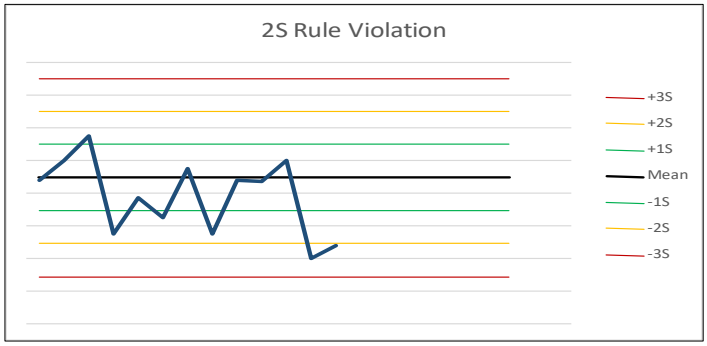


Figure 4.6. 2S rule violation

Rule three (4S): Reject the run when 1 QC value is above the mean +2S and a consecutive value is below the mean – 2S, or conversely. This rule should only be applied within-run, not between-run. Recalibrate and repeat the analysis.

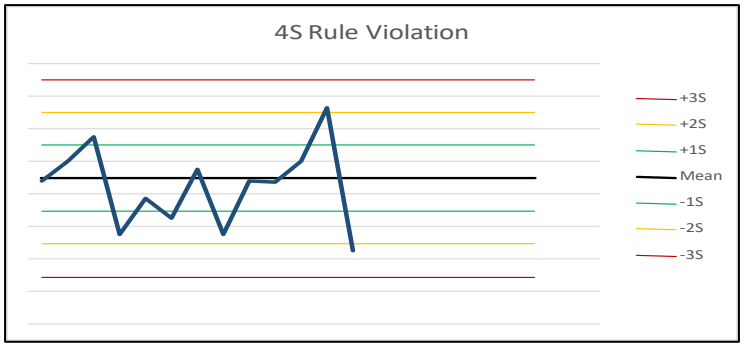


Figure 4.7. 4S rule violation

Rule four (10S): Investigate the analytical system when 10 QC results in a row fall on one side of the mean. Check the calibration log to see if new calibration standards were introduced and the maintenance log to see if maintenance was performed on the instrument. These actions may produce a bias or shift in the QC data. Another cause for a shift in the data could be systematic

laboratory contamination, for example bacteria in a deionized water reservoir, requiring corrective actions be taken to eliminate the problem.

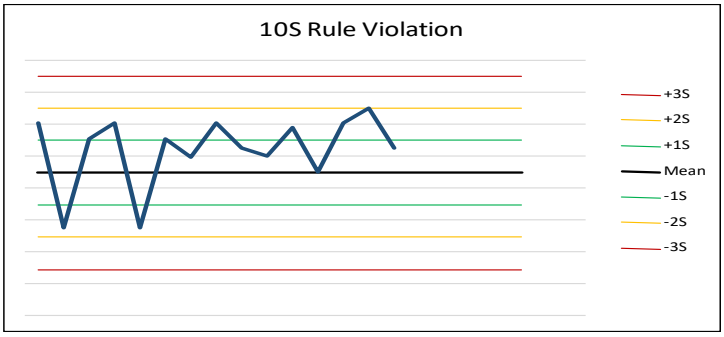


Figure 4.8. 10S rule violation

Rule five (7S): Investigate the analytical system when seven QC results are trending in one direction either high or low. Upward trending results may indicate that contamination is building in the analytical instrument and a maintenance period is required. Downward trends may indicate a sampler probe that is increasingly plugged so that smaller and smaller amounts of sample are entering the analytical system. Investigate and take corrective action as required.

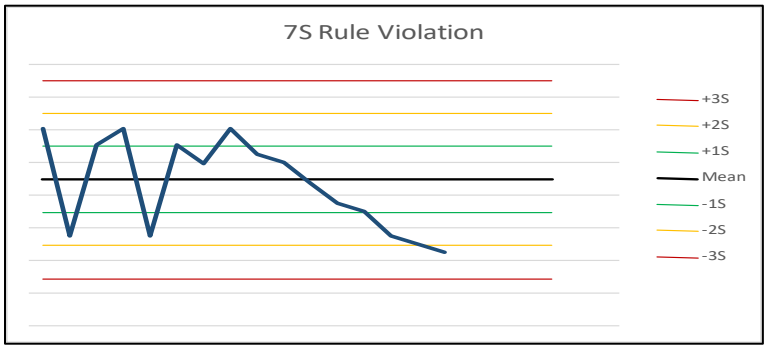


Figure 4.9. 7S rule violation

Mark maintenance periods and events on the control charts so that trends and shifts in the QC data can be attributed to a particular activity. This information should also be recorded in laboratory logbooks.

Mark start dates for new standards or reagents directly onto the chart for a visual indication of the impact of the new solution on the data quality.

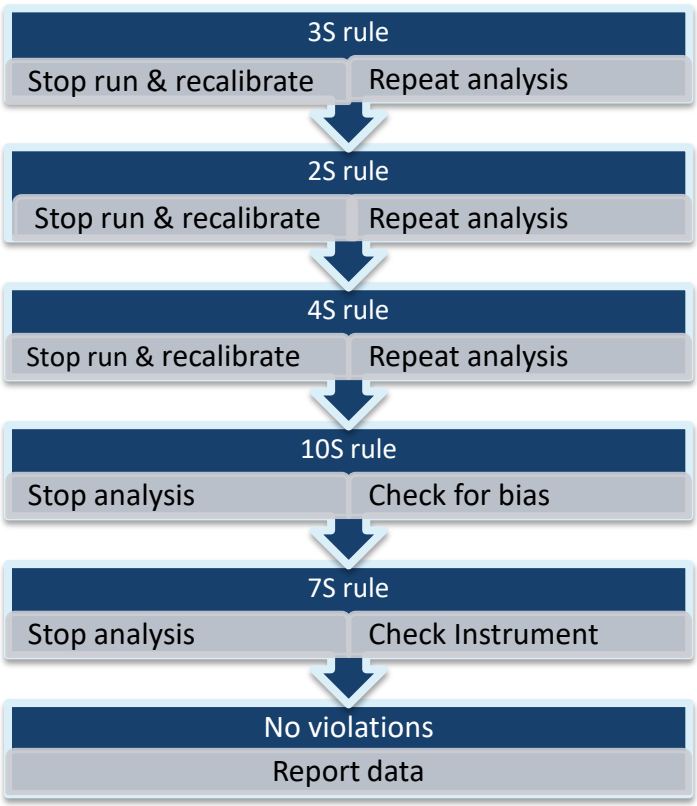


Figure 4.10. QC Decision Chart

Control charts should be readily available and maintained for a minimum of two years.

4.3.6 Replicate Analyses

Replicate analyses, defined as two aliquots of the same sample treated identically throughout the laboratory procedures, provide a measure of analytical precision. There are two types of replicate analyses: within-run and between-run. Between-run replicates capture the day-to-day variability of the analyses and are the basis for estimating GAW laboratory precision.

At least 2% to 5% of the sample load should be analyzed as between-run replicates. Select the samples for replicate analysis randomly from the sample stream with the condition that they have adequate volume for two analyses. Replicate analysis data may be tabulated by percent or absolute difference.

There are several ways of charting the data. High concentration values will give a large absolute difference but a small percent difference. Conversely low concentration values will give a small absolute difference but a large percent difference. Plotting replicate values next to each other on the same axis, as in Figure 4.11, provides a visual means of identifying values that are poorly correlated.

Where differences in replicate analyses suggest that the results may be from two different samples, check the sample labels and analysis order for possible mix-ups. Check also for copy-paste errors. Repeat the analysis of both samples if the differences cannot be resolved. It may be necessary to repeat a series of samples to determine whether samples have been mixed up.

Contamination by organic material can cause a sample to deteriorate and change chemically. Choosing samples randomly for replicate analysis may result in selection of a contaminated sample and the chemical deterioration may result in large differences unrepresentative of laboratory precision. To avoid this, select only samples for which field and laboratory observations show no signs of contamination.

Replicate data are used to estimate laboratory precision. The recommended method for calculating laboratory precision is given in Appendix A, Section A.3.

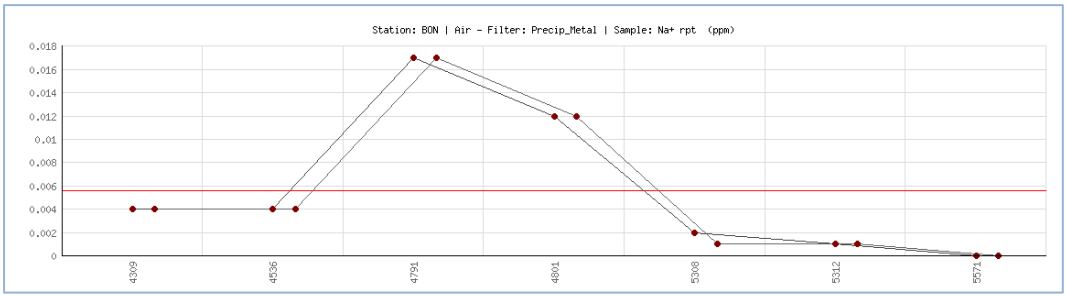


Figure 4.11. Example of Charting Replicate Analysis Data

Deionized water is integral to precipitation chemistry laboratory operations. It is used for cleaning, solution preparation and, most importantly, as a base for preparing calibration standards.

4.3.7 Deionized Water Quality

Background

Deionized (DI) water is water that has been treated to remove minerals and dissolved ions. Precipitation laboratories require ASTM D1193 Type I deionized water described in Table 4.2 (D1193-06, 2011).

Table 4.2. DI Water Quality Guide (TM Associates, 2009)

Constituent	Parameter	Type I character
Ions	Resistivity at 25°C	>18.0 MΩ•cm
Ions	Conductivity at 25°C	<0.056 µS/cm *
Organics	TOC	<10 ppb
Particulates	Size	<0.2 µm
Colloids	Silica	<10 ppb
Bacteria	Colony forming units	<1 CFU/mL
***Resistivity and conductivity represent reciprocal values**		

Apparatus

Figure 4.12 illustrates a DI water system. These systems may consist of, but are not limited to, the following components:

1) Water softener (optional). All deionized water systems work best with softeners which remove calcium and magnesium in exchange for sodium ions. Unlike calcium and magnesium, sodium does not collect and calcify on pre-filters and reverse osmosis (RO) membranes.

   Caution: Water softener salt is a potential source of contamination in the analytical laboratory. Store bags of salt away from instrumentation. If possible, isolate the water softener in a separate area.

2) Pre-filter package
   a. 25 µm particle filter
   b. charcoal filter
   c. pressure gauges before and after pre-filters

3) Main filtration system
   a. RO System
   b. 5 µm particulate filter (pre and post RO system)
   c. charcoal block filter (optional)
   d. pressure gauge

4) Holding tanks
   a. ultraviolet (UV) light
   b. number of tanks depends on system configuration and usage.

5) Polishing Loop
   a. resin filters (3-4)
   b. continual readout of water quality (optional)

6) Point of Use (POU) System
   a. dispenser
   b. 0.1 µm filter

Each laboratory must determine the best DI system for its purposes. Manufacturer specifications vary. The following are considerations (TM Associates, 2009)*:*

1) How much DI water is needed?

2) How many locations need access to DI? What kind of DI delivery is needed (POU dispenser, tank, continuous supply)?

3) How far are POUs from the DI system? Pumps may be needed.

4) What is the quality of the tap water and is the pressure in the tap water line at least 345 kPa (50 PSI) or more? Pump may be needed.

5) Is there adequate space for a DI system and can the water softener salt be isolated from the analytical laboratory, especially where sodium or chloride are analyzed?

6) Are there plans for future analyses that may require special DI water needs?

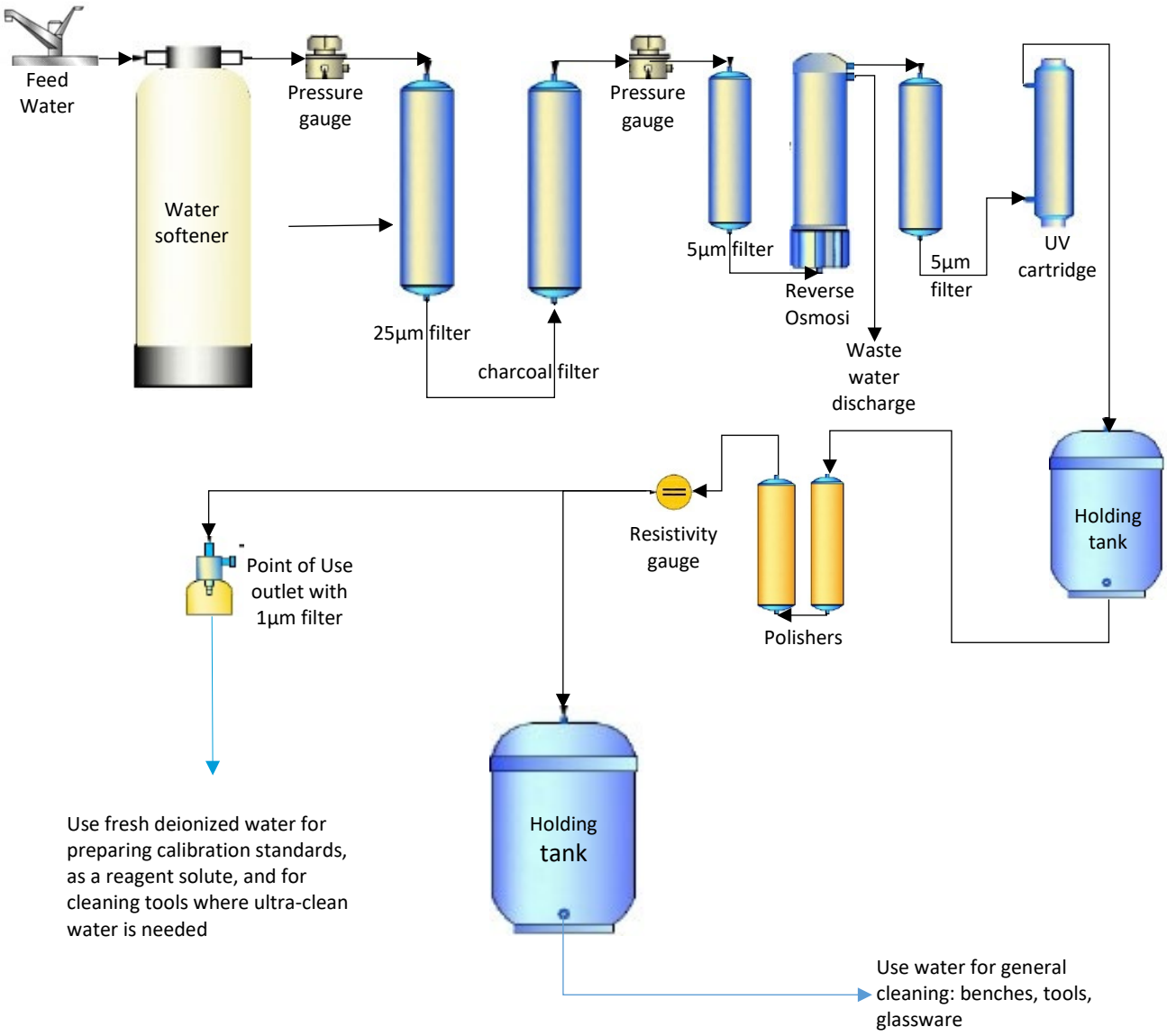


Figure 4.12. Example of a Deionized Water System

Calibration

The DI water system will have a readout that indicates the resistivity of the output. This readout must be verified annually against an alternate measurement that has been validated. Generally, the service provider for the system will do this and issue a certificate of quality. Alternatively, the laboratory may purchase a resistivity measurement device and perform an annual check. The measurement device must be calibrated and certified by an outside agency that is qualified to issue a certificate of performance. Calibration should occur during annual maintenance periods.

Maintenance

DI systems are as important as any instrumentation in the laboratory and require care, maintenance and documentation of readouts, procedures, and service.

Systems require the following maintenance:

1) Produce DI water every day to keep the water moving through the pre-filters, if by design water is not circulated by internal pumps.

2) Check softener salt levels weekly or more often if usage increases. Add salt as needed. Use only salt that is specified for water softeners. Check that the salt does not 'bridge' inside the storage tank leaving an air pocket under a solid salt overlay. Use a strong rod to break up salt bridges and ensure the softener salt has not hardened into a solid block.

3) Keep a logbook that records the readings of the gauges on the pre-filter system. Look for an acceptable range of pressure differences between the filters. Gauge pressures that begin to approach each other indicate that the filters are becoming too permeable and require changing. In addition, when the pressure differences between the filters is too high, one of the filters might be clogged and should be replaced.

4) Change the charcoal filter and RO membrane annually or when readouts and indicators suggest the membrane is deteriorating. New membranes ideally reject 98% to 99% of all feed water. Membranes become more permeable with age and reject less water. If the percent rejection drops below 90%, change the RO membrane. If chlorine breaks through the charcoal bed, it quickly will degrade the RO membranes. The amount of DI water used and local tap water quality must be considered in determining how often maintenance is required.

5) Check the resistivity meter on the polisher with every use and change the filters when the resistivity is either unstable or drops below 18 MΩ.

6) Have a service person change all tubing in the system annually to avoid bacterial growth or crimping. Flush or disinfect the distribution system approximately once every year to remove growth in the system.

7) Change UV lamps annually.

8) Change POU filters when polishing resins are changed.

Deionized Water Uses

Blanks

1) "Blanks" or blank samples are QC samples taken from DI water supplies and rinses of glassware and apparatus. Test the blanks from all materials that have direct contact with the samples, including vials, test tubes, bottles, and collection vessels. Also test blanks from tube caps, gloves, weigh boats and other materials that have incidental contact with the samples or the sample stream. See "Quality Control - Stock Consumables" in section 4.3.14.

2) Material blanks must be tested when either a supplier changes, a product changes or a new batch of materials is received. New materials must be tested before they are put into use. Document these test results in a logbook. Reject materials that contribute contamination to the samples. Laboratory managers and material suppliers should work together to develop a plan in which materials can be tested prior to their purchase. Materials that are rejected should never be available for use by laboratory personnel and should be returned to the supplier or discarded. See "Quality Control - Stock Consumables" in section 4.3.14.

3) One DI water blank and one reagent blank (where possible) should be analyzed per analytical batch. There should be no detectable analyte in either the DI water or reagent blank. Instrument noise may account for a signal near the detection limit. Plotting blank results on a timeline chart will indicate to the analyst and QA manager if analytical system

background levels are increasing over time. Figure 4.12 demonstrates an example of a DI water blank increasing over time.

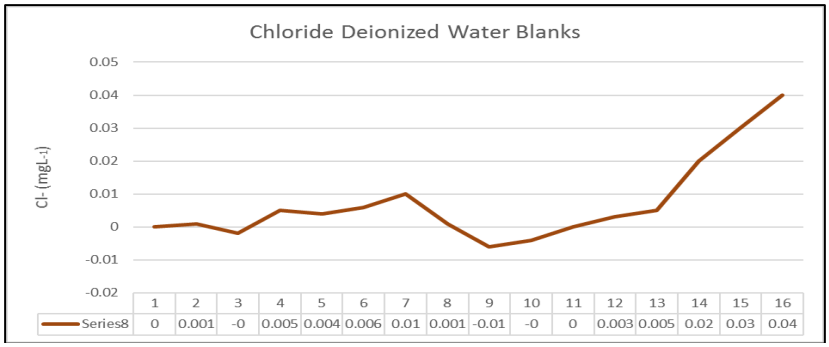


Figure 4.13. Example of DI water blank plot

Standards and Reagents

1) Calibration standards must be made with DI water directly from the POU dispenser.

2) Each method should specify if the reagent can be made from a holding reservoir or from the POU dispenser.

Cleaning

1) Rinse all tubes, sample bottles and tools that come into direct contact with precipitation samples three times with deionized water drawn directly from the polishing loop of the DI system. See Figure 4.12.

2) Use DI water to clean laboratory containers, glassware, surfaces, equipment, work benches and floors. **Caution**: Do not use chemical cleaning agents and soaps which can contaminate samples and supplies, especially ammonia-containing cleansers.

3) Where possible, use DI water in ultrasonic baths to clean instrument parts, frits and other apparatus with small crevices that are inaccessible to scrubbing.

4) Do not use detergents or acid solutions to clean field sample containers. If detergents or acids are used, only the minimum effective amount should be used. Special care must be taken to rinse away residues. The preferred approach is to allow the containers to soak in DI water for 24 to 48 hours, which will leach away soluble and exchangeable residues that may contaminate precipitation. Remove visible residues from sample container surfaces by wiping these surfaces with a Kimwipe®. Wear pretested clean disposable gloves when cleaning and handling sample containers and rinse surfaces repeatedly.

5) Clean all sample containers with DI water until the conductivity of the final rinse water (i.e., the rinsate) does not exceed 1.5 µS cm^{-1}, whether cleaned in the field or the laboratory. Routinely check that the rinsate conductivity does not exceed 1.5 µS cm^{-1}. After cleaning, shake the water from the sample containers and seal or cover them in plastic and store in a clean area. **Caution**: Air drying should be avoided, as many contaminants are soluble in water and stick to dry surfaces.

Blind samples are treated no differently than normal samples. Blind samples are used to encourage the trust of laboratory personnel and are not intended to find fault.

4.3.8 Blind Samples

Blind sample checks provide another means of evaluating the quality of laboratory data. Only the QA manager knows the concentrations of the blind samples. These checks assess the various effects of laboratory handling and analysis. They are used to test for bias and precision of the various analytes or to detect system contamination. Blind samples are useful to evaluate the performance of the analytical system, not to evaluate laboratory analysts or personnel.

Blind samples may be DI water, acidified DI water, SRMs or CRMs. It is recommended that blind sample checks be conducted on a weekly basis. Use samples with known values so that the analysis results can be plotted against the true value. Use the mean as the true SRM or CRM value and the range of true values as the upper and lower control limits. See Figure 4.14 for an example of a CRM plot.

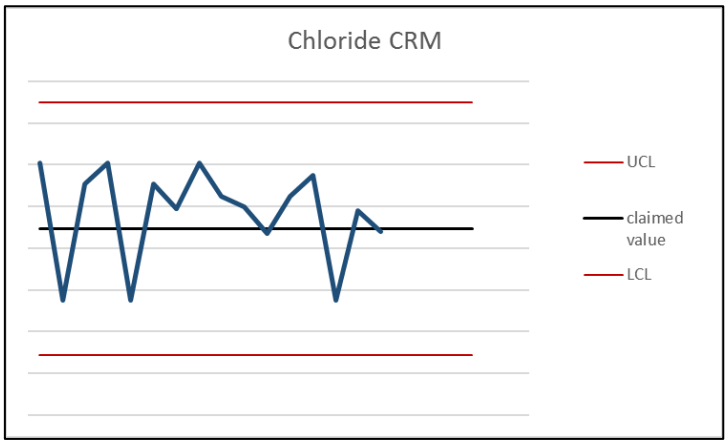


Figure 4.14. CRM plot for chloride. The claimed value is the mean. The UCL and LCL are the upper and lower control limits.

Blind samples are poured into a sample container and assigned a laboratory number so that they are indistinguishable from all other samples to the analyst. There must be adequate volume to perform all analyses. Following analysis, the QA manager identifies the blind sample and plots the data on QC charts. The same rules that apply to the QCS also apply to this data except that the corrective action is initiated by the QA manager and not the instrument operator.

4.3.9 Dilution Checks

Samples with a concentration above the calibration range of the analytical instrument are diluted until the concentration is within the calibration range. It is important to check the sample dilution procedures periodically to make sure that the diluent has no effect on the sample matrix and to check the accuracy of the dilution itself.

One accepted method for testing dilutions is to compare the measurements of sample aliquots before and after dilution. Unfortunately, most samples with concentrations that exceed the calibration limits have too little volume to do this. Instead, CRMs may be used so long as they are composed of a precipitation matrix, thereby canceling the potential for matrix effects. Measurements before and after dilution should be charted and compared as in Figure 4.14a. Generally, the diluted values are

less precise. The decrease in diluted results with time in Figure 4.14a illustrates a problem with the procedure or equipment. It could indicate a pipette that is leaking diluent into the sample. Charting the data in this way lets the analyst spot and investigate problems of this type.

Performing dilution checks does not negate the need for the regular calibration of pipettes, balances and other tools used to perform the dilution. (See Appendix C)

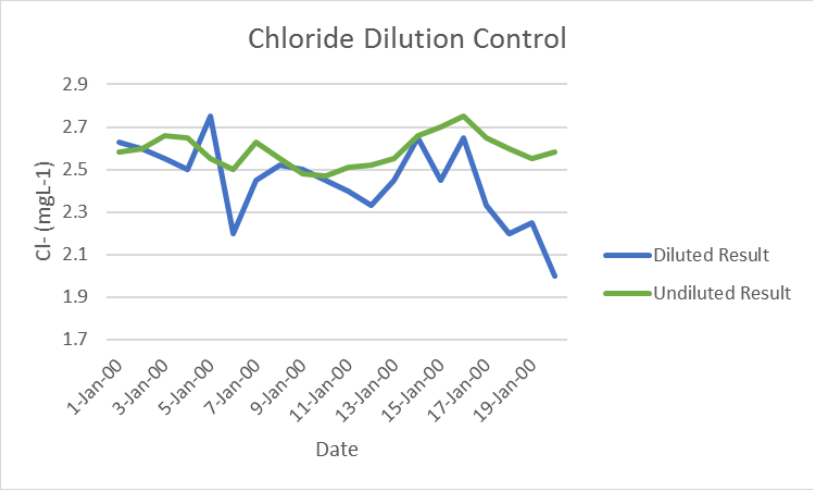


Figure 4.14a. Diluted versus undiluted sample measurements.

An alternative for assessing the dilution process parallels the blind sample tests described in the previous section. Test samples are prepared by the QA manager by adding a known concentrated stock solution to a precipitation matrix. Start with the analysis of the precipitation matrix. Add the concentrate so that all analytes have concentrations above the calibration ranges of all the procedures. The expected concentrations of the mixture can be calculated from the volume and concentrations of the precipitation matrix and volume and concentrations of the concentrated stock solution.

As in blind sample tests, this concentrated sample is added to a sample container and assigned a laboratory number so that it is indistinguishable to the analyst. Following analysis, the QA manager can plot the reported concentrations against the expected concentrations See Figure 4.14b where the reported values are biased low relative to the calculated value. The results are inconsistent, suggesting problems with the equipment or procedures. Examples include pipettes that leak or a bottle top dispenser that dispenses inconsistently because of a plunger that is not secured properly.

If differences in measured and calculated concentrations exceed ±10%, it is necessary to review the dilution system. Dilutions of precipitation for analysis should be suspended until further checks have proven that the method can meet this ±10% criterion.

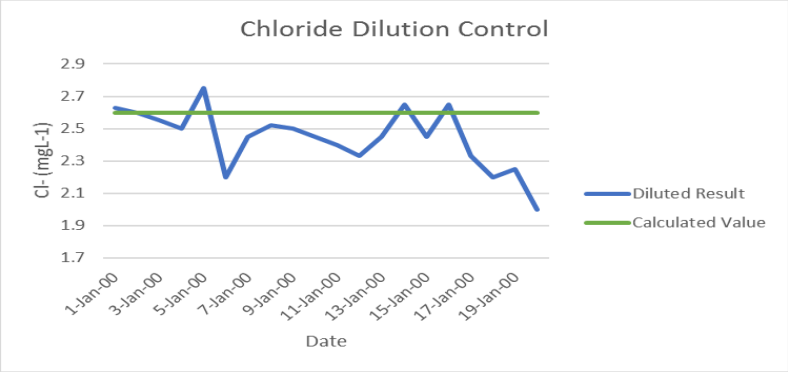


Figure 4.14b. Concentration reported following dilution protocol versus calculated concentration.

4.3.10 Standard Reference Materials (SRM) and Certified ReferenceMaterials (CRM)

Standard Reference Material is Certified Reference Material issued by the National Institute of Standards and Technology (NIST) and meets NIST-specific certification criteria. This material is issued with a certificate of analysis that reports the results of its characterizations. SRMs have constituent values established by consensus standard methods (NIST, 2015).

CRMs are characterized by metrologically valid procedures for one or more properties. The material is accompanied by a certificate that provides the value of the specified property, uncertainty, and a statement of metrological traceability (NIST, 2015). CRMs may be analyzed by more than one laboratory over time and the results are collated to establish a true value. CRMs used in conjunction with precipitation analysis must contain the analytes typical of precipitation and generally no others; that is, the CRM matrix should be analogous to the precipitation matrix.

The laboratory must obtain a volume of CRM or SRM material sufficient to allow the laboratory to build a data base of results. Contact the supplier and request a single batch or lot of SRM or CRM in quantities that will last one year. The solutions should come in small aliquots of approximately 100mL. Using small aliquots ensures that the batch or lot does not become contaminated with repeated use from opening and closing the container.

The QA manager should place CRMs or SRMs into the analytical queue weekly. The CRM (or SRM) sample should be placed in a laboratory container and labeled as a sample; thus, its identity is blind to the analysts. Transferring the sample to a laboratory specimen container tests the quality of the container cleaning and operator sample handling procedures. The CRM or SRM is analyzed on all systems giving the lab manager an overall picture of laboratory operations.

Bias is defined as the systematic difference between the measured and expected values due to laboratory sample handling or analytical procedures. Potential bias can be evaluated by plotting results of CRMs. Appendix D lists several sources for obtaining CRMs.

4.3.11 Inter-laboratory Comparison Studies

It is mandatory that all GAW precipitation chemistry laboratories participate in the WMO GAW Inter-laboratory Comparison Studies. GAW personnel in every country must register their laboratory (or laboratories) with the manager of the Quality Assurance Science Activity Centre - Americas (QA/SAC-Americas).

Inter-laboratory comparison samples are an indicator of a laboratory's performance metrics. Place these samples in the sample stream and treat them as precipitation samples.

Twice per year, the QA/SAC sends a set of samples to each laboratory for analysis. The analytical results must be reported to the QA/SAC within the prescribed time interval. Each laboratory also must report the names of the GAW stations for which they perform chemical analysis. Results of these studies are posted on the QA/SAC web site.

Precipitation chemistry data from countries that do not participate in the WMO GAW Inter-laboratory Comparison Studies are sequestered by the World Data Centre for Precipitation Chemistry (WDCPC).

Do not analyze GAW Inter-laboratory Comparison Study samples multiple times unless it is normal to analyze precipitation samples multiple times. Analyzing these samples multiple times will skew the final analysis of data and tabulation of results.

The WDCPC maintains on-line links between precipitation chemistry data sets and laboratory performance in the GAW Inter-laboratory Comparison Studies. These links enable users to evaluate whether the WDCPC data meet their needs for accuracy and comparability. See Chapter 6 for further discussions on assessing data quality.

Corrective Action for Unsatisfactory Performance

Unsatisfactory performance in one or more of the WMO GAW Inter-laboratory Comparison Studies indicates the need to take corrective actions to upgrade performance and improve data quality. Start by reviewing laboratory QA/QC procedures, especially Chapter 6. Suggested corrective actions include but are not limited to the following:

1) Request additional samples from the QA/SAC for repeat analysis.

2) Arrange for an external audit or expert visit by another GAW laboratory manager.

3) Upgrade the internal laboratory QC program.

4) Implement a control chart and multi-rule QC system.

5) Arrange to analyze split samples and compare the results with a GAW laboratory whose performance is satisfactory. Do this after corrective actions have been taken to ensure the success of these actions.

It is strongly recommended that GAW laboratories participate in other inter-laboratory studies to provide additional evidence of analytical comparability. Such studies include:

1) The European Monitoring and Evaluation Programme (EMEP) Laboratory Inter-comparison Studies operated by the Chemical Coordinating Centre of EMEP at the Norwegian Institute of Air Research.

2) The National Water Research Institute (NWRI) Inter-comparison Studies from Environment and Climate Change Canada.

3) The Acid Deposition Monitoring Network in East Asia (EANET) Inter-comparison Studies.

4) The United States Geological Survey (USGS) Inter-comparison Studies.

Details of these studies can be obtained by contacting the WMO QA/SAC-Americas.

4.3.12 Laboratory QA Reporting

Laboratories should prepare internal QA reports annually and make them available to GAW data users upon request (see (NADP, 2014)). These reports document the quality of the data generated by the laboratory and may include all types of QA information (e.g., statistical summaries of the various quality control samples, laboratory data verification checks, and laboratory inter-comparison study results).

At a minimum, annual laboratory QA reports should include the following information in summary form:

- laboratory QA/QC objectives
- laboratory procedures (list of laboratory SOPs with revision dates)
- results from laboratory water, reagent, and supply blank checks
- SRM and CRM analysis result data
- control chart results and analytical issues
- reanalysis results
- laboratory reference standard data
- WMO/GAW Inter-laboratory Comparison Study performance
- inter-comparison results from other providers
- internal audits and resulting changes
- equipment maintenance and repair
- significant analytical equipment and supplier changes during period
- certifications
- personnel changes

4.3.13 Laboratory Data Verification and Reporting

Manual Data Entry Validation

The GAW Precipitation Chemistry Programme recommends that data be transferred electronically between instrument and computer, minimizing the need for manual data entry.

Computerized programs are best used to verify that all records (i.e., names, dates, times, locations, identification numbers, and analytical results) are free of typographical errors. Data entry procedures must include error checking. The following error-checking procedure is recommended for data that must be entered manually: (1) a data clerk enters the data; (2) a second data clerk enters the data; (3) the two entries are compared and differences, if any, are corrected.

As an alternative, have a person who did not enter the data spot check 5% to 10% of the data entries for correctness. As laboratories differ in the type of computer systems and amount of manually entered versus computer-captured data, it is beyond the purview of this document to specify data merging procedures and basic statistical checks. However, if errors are found, the data entry process should be re-assessed. Any data error encountered must be returned to the originating party and be checked against laboratory and/or site records. Changes to the database must be authorized and confirmed by a designated quality control person.

Ion Balance Checks

The ion balance of an individual sample is used to check and flag data. The principle of electro-neutrality in solution requires that total chemical equivalents of anions equal the total chemical equivalents of cations; however, there can be a poor ion balance but the data can still be valid. For example, a forest fire near the sample collection site may increase ammonium and organic acids in the precipitation samples thereby skewing the pH; however, the measurements might still be valid.

A sample is flagged for reanalysis if the ion balance exceeds set limits. (Lockard, 1978-1983) (NADP/CAL, 2012). Below is a stepwise description of how to evaluate the ion balance.

Step 1: Use the factors in Table 4.3 to convert the concentrations from analytical measurements (i.e., the measured concentrations) of each ion to chemical equivalent concentrations:

$$\text{Chemical equivalent concentration } (\mu eq\ L^{-1}) = \frac{[\text{measured concentration in mg } L^{-1}] \times 1000 \times \text{Valence}}{[\text{gram molecular weight in g } mol^{-1}]}$$

OR

Chemical equivalent concentration (µeq L^{-1}) = [measured concentration in mg L^{-1}] X [chemical equivalent conversion factor in µeq mg^{-1}]

Table 4.3. Convert measured concentrations (mg L^{-1}) to chemical equivalent concentrations (µeq L^{-1})

Ion	†Gram Molecular Weight (g mol^{-1})	Valence	Chemical Equivalent Conversion Factor (µeq mg^{-1})
Cl^-	35.452	1	28.2072
NO_3^-	62.00506	1	16.12770
SO_4^{2-}	96.0651	2	20.81922
NH_4^+	18.03876	1	55.4362
Na^+	22.98977	1	43.49761
K^+	39.0983	1	25.57656
Mg^{2+}	24.305	2	82.2876
Ca^{2+}	40.078	2	49.90269
††HCO_3^-	61.01678	1	16.38894
***$HCOO^-$**	45.0174	1	22.2136
***CH_3COO^-**	59.0440	1	16.9365
***$C_2O_4^{2-}$**	87.9974	2	22.7279

* Included in the calculation if measured.
† Molecular weights (Standard Atomic Weights 2009, 2012)
††Use only if analyzing for HCO_3^- , otherwise use calculated value from step 2.

The influence of hydrogen carbonate on ion balance is most significant for samples with pH >5.6.

Step 2: Calculate the hydrogen carbonate (formerly bicarbonate) concentration from pH if hydrogen carbonate is not analyzed.

HCO_3^- is calculated for each sample based on pH values and the annual mean mixing ratio of carbon dioxide (CO_2) in the atmosphere. Using the partial pressure of CO_2 as a measure of the mixing ratio, this relationship is expressed as follows:

$$HCO_3^- \ (\mu eq\ L^{-1}) = \frac{K_1 K_H P_{CO_2} \times (10^6)}{10^{-pH}}$$

Substituting for the constants gives:

$$= \frac{(4.467 \times 10^{-7} \text{mol L}^{-1}) \times (0.0341 \text{ mol L}^{-1}\text{atm}^{-1}) \times (P_{CO_2} \text{ atm}) \times 10^6 \text{ (µeq) (mol}^{-1})}{10^{-pH} \text{ mol L}^{-1}}$$

Replacing the constants with a single variable gives:

$$= \frac{\text{Annual Hydrogen Carbonate Constant (µeq L}^{-1})}{10^{(6-pH)}}$$

Where: K_1= acid dissociation constant for carbonic acid = $10^{-6.35}$ or 4.467×10^{-7} (mol L^{-1})
K_H= Henry's Law constant for CO_2=0.0341 mol $L^{-1}atm^{-1}$
P_{CO_2} = annual average partial pressure of CO_2 (atm)

K_1: (Dissociation Constants of Inorganic Acids and Bases, 2011-2012)
K_H: (Aqueous Solubility and Henry's Law Constants of Organic Compounds, 2011-2012)
P_{CO2}: (Trends in Atmospheric Carbon Dioxide, 2018)

It is important to note that the variable, Annual Hydrogen Carbonate Constant, accounts for the annual average partial pressure of CO_2. This is a variable, since atmospheric partial pressures of CO_2 are rising and this increase means that the concentrations of hydrogen carbonate in precipitation are rising, as well. Table 4.4 lists the changes in CO_2 and the Hydrogen Carbonate Constant in 5-year increments. These values can be used in the equations, above, to calculate the hydrogen carbonate concentrations in precipitation in equilibrium with atmospheric CO_2. A more accurate approach would be to use annual data, which can be accessed here: Annual CO_2 Data.

Table 4.4: Annual average partial pressure of CO_2 and Hydrogen Carbonate Constant

Year	CO_2 Partial Pressure (atm)	Hydrogen Carbonate Constant
1975	$331.12*10^{-6}$	5.044
1980	$338.76*10^{-6}$	5.160
1985	$346.35*10^{-6}$	5.276
1990	$354.45*10^{-6}$	5.399
1995	$360.97*10^{-6}$	5.498
2000	$369.71*10^{-6}$	5.632
2005	$379.98*10^{-6}$	5.788
2010	$390.10*10^{-6}$	5.942
2015	$401.01*10^{-6}$	6.108
2020	$414.24*10^{-6}$	6.310

For routine laboratory QC or a retrospective determination of ion balances, use the appropriate annual CO_2 partial pressure.

Step 3: Calculate Hydrogen and Hydroxyl ion concentrations from pH as follows:

Hydrogen ion = H^+ (µeq L^{-1}) = $10^{(6-pH}$

Hydroxyl ion = OH^- (µeq L^{-1}) = $10^{(pH-8)}$

Step 4: Calculate the anion and cation concentration sums, using the results from Steps 1 to 3.

Where:
Cations = $(Ca^{2+}) + (Mg^{2+}) + (K^{+}) + (Na^{+}) + (NH_4^{+}) + (H^{+})$
Anions = $(Cl^{-}) + (NO_3^{-}) + (SO_4^{2-}) + (HCO_3^{-}) + (OH^{-})$

Step 5: Calculate the Ion Percent (%) Difference, as follows:

$$\text{Ion Percent (\%) Difference} = \frac{\text{Total Cation Equivalents} - \text{Total Anion Equivalents}}{\text{Total Cation Equivalents} + \text{Total Anion Equivalents}} \times 100$$

Step 6: Apply an action plan to flagged data

The GAW ion balance criteria are given in Table 4.5. If the required criteria are not met, repeat all the chemical analyses (volume permitting). If the second result improves the ion balance, use the second result. If the first and second results exceed the expected measurement precision and the ion balance does not improve, flag the data as having a poor ion balance. Do not invalidate data based on the ion balance test alone. Figure 4.15 outlines an action plan using ion balance results from sample data in Table 4.6.

Table 4.5: Required criteria for ion balance (Lockard, 1978-1983)

Anions + Cations ($\mu eq\ L^{-1}$)	Acceptable Ion % Difference (I%D)
≤ 50	–60 ≤ I%D ≤ +60
> 50 ≤ 100	–30 ≤ I%D ≤ +30
> 100 ≤ 500	–15 ≤ I%D ≤ +15
> 500	–10 ≤ I%D ≤ +10

Note: Some areas of the world are characterized by rain with pH values above 6, where the ion balance calculations are more affected by hydrogen carbonate. In these areas, it is best to analyze hydrogen carbonate rather than calculating its concentration.

Table 4.6: Ion balance results requiring Action Plan in Figure 4.15

Analyte	Measured Concentration (mg L^{-1})	Step number to evaluate	Chemical Equivalents (µeq L^{-1})
Cl^-	0.036	1	28.2072
NO_3^-	0.635	1	16.1277
SO_4^{2-}	1.821	1	20.81922
Ca^{2+}	0.132	1	49.90269
Mg^{2+}	0.040	1	82.2876
Na^+	0.015	1	43.49761
K^+	0.349	1	25.57656
NH_4^+	0.668	1	55.4362
pH = 4.72			
H^+ from pH		3	19.05
OH^- from pH		3	0.0005
HCO_3^- from pH & P_{CO2}		2	0.323
Total Concentration		4	**124.71**
Ion Percent Difference (I%D) from Step 5 = 20.9%			
Sample contains pollen			
See Table 4.5: I%D > 15% and 100 < 124.71 µeq L^{-1} ≤ 500. Action Plan required.			

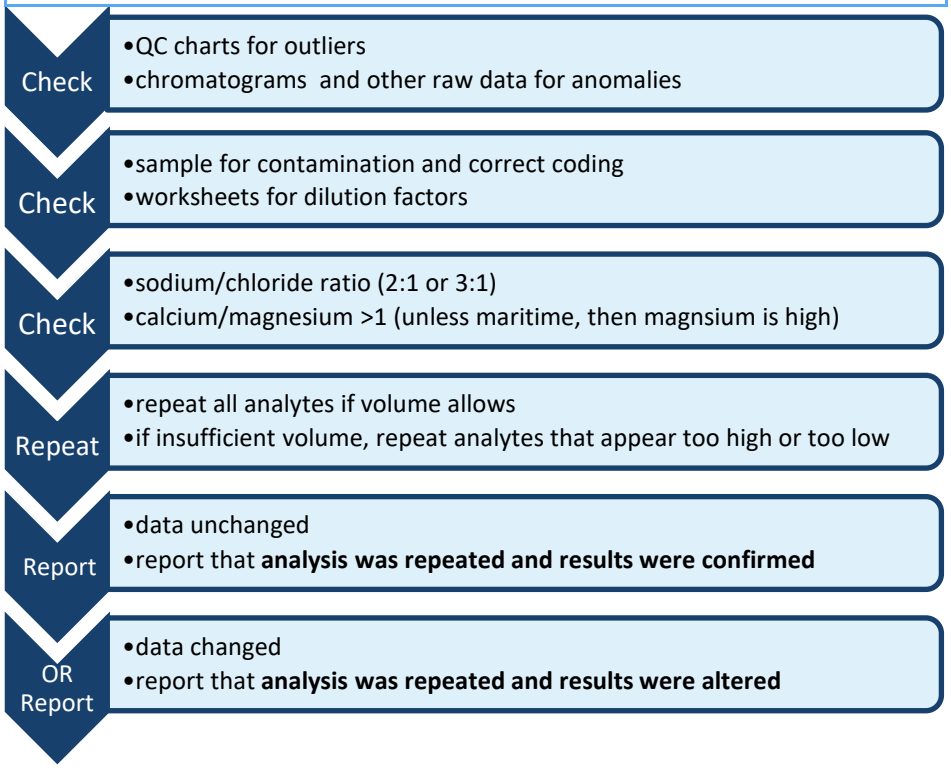


Figure 4.15. Example of decision action plan using ion balance results

Comparison of Measured and Calculated Conductivity

For dilute solutions (e.g., below 10^{-3} M), the total conductivity (or specific conductance) can be calculated in $\mu S\ cm^{-1}$ from the molar concentrations and molar ionic conductance (at infinite dilution) of the individual ions (NADP/CAL, 2012). The calculation is as follows:

Calculated conductance each ion= (ion concentration, $\mu eq\ L^{-1}$) X $\Lambda_{\pm}$ X 10^{-3}

Total Calculated Conductance=$\sum$ (Calculated conductance each ion)

Where:

Ion Concentration= [lab result mg L^{-1}] X hydrogen microequivalents (from Table 4.3)

$\Lambda_{\pm}$= ionic conductivity, S $cm^2\ mol^{-1}$ (Table 4.7)

Table 4.7. Molar ionic conductance at infinite dilution and 25°C (Haynes, 2015).

Anion or Cation	Molar Ionic Conductance, $\Lambda_{\pm}$ (S $cm^2 mol^{-1}$)
H^+	349.7
Cl^-	76.3
NO_3^-	71.4
½ SO_4^{2-}	80.0
NH_4^+	73.5
Na^+	50.1
K^+	73.5
½ Mg^{2+}	53.0
½ Ca^{2+}	59.47
*$HCOO^-$	54.6
*CH_3COO^-	40.9
* $C_2O_4^{2-}$	74.1
*HCO_3^-	44.5

* Included in the calculation only when measured in significant concentrations

Compare the calculated conductivity to the measured conductivity (or specific conductance) values for precipitation samples using the relation:

$$\text{Conductance Percent Difference} = \frac{(\text{Calculated Conductance} - \text{Measured Conductance})}{\text{Measured Conductance}} \times 100$$

Acceptable conductivity (or conductance) percent differences are given in Table 4.8.

Table 4.8. Required Conductivity Balance Criteria (Lockard, 1978-1983)

Measured Conductivity ($\mu S\ cm^{-1}$)	Acceptable Conductivity Difference (%)
≤5	≤±50
>5≤30	≤±30
>30	≤±20

If the criteria in Table 4.8 are not met, the analysis should be repeated or the sample concentrations should be flagged in the database.

4.3.14 Inventory Management and Control

Inventory management is important for sustained laboratory operations. Processes will come to an abrupt halt if tools or reagents are depleted or a known supplier suddenly no longer exists. Some considerations are listed below.

Stock Control

Procurement and inventory control are the responsibility of the lab manager. Expiration dates, refrigeration and storage space must be monitored to maintain adequate laboratory stock. Establish minimum quantities of supplies and replacement items needed to maintain laboratory operations without interruption. Place a purchase order when a minimum is reached. Account for delays in supply deliveries and the possibility that the item may no longer be available. Evaluate new supplies by performing blank checks (See Section 4.3.7, above, Table 4.9, below and Figure 4.16, below).

Use software or a spreadsheet system to manage stores and flag items that require ordering. Categorize items for easy sorting and lookup.

Account for items when they are received. Count items and ensure volumes ordered are volumes received. Return damaged items for credit or replacement. Items that do not expire can be stocked in large quantities. Items that do expire must not be over-stocked. As a general guideline, materials should not be stored more than one year.

Ensure items such as columns or pH electrodes are in sufficient quantity to maintain operations but not in such supply that they deteriorate over time. Consider a maintenance plan where some items are changed on a schedule before they fail or become depleted. For example, annually change separator columns on ion chromatography systems.

The quality of packaging is important. Sample bottles packed in a plastic bag or liner will be cleaner than bottles packed loosely in a cardboard box.

Quality Control – Stock Consumables

Should a supplier discontinue providing an essential item, a replacement of comparable quality both physically and chemically must be found. The purpose of testing these materials is to ensure that background contamination does not exist in the analytical process causing spurious results or systematic bias. Items such as tubes, specimen bottles, caps and pipette tips all have direct contact with the sample. Test these items under conditions like actual usage. For example, sample bottles may contain a sample for several weeks while a pipette tip may be in contact with the sample for only a few seconds. Thus, sample bottles will be allowed to rest with test solutions in them for the same amount of time that samples normally would be held in them.

Testing must have controls to ensure that new items are either better than the old items or the same. For example, set up a test for 100 mL sample bottles as in table 4.9. Test old and new containers using deionized water in three different volumes: 25 mL, 50 mL, and 100 mL. Perform the same kind of test using a CRM solution. With the DI water tests, look for chemicals that may be leached or desorbed from

the walls of the bottle. With the CRM solution tests, look for chemicals that may be leached from the bottle as well as chemicals that may be lost or adsorbed to the walls of the bottle.

Table 4.9. Example of a test design for new sample bottles.

Bottle Number	Test solution	Container	Test Solution Volume (mL)
1	DI water	old	25
2	DI water	old	50
3	DI water	old	100
4	DI water	new	25
5	DI water	new	50
6	DI water	new	100
7	CRM	old	25
8	CRM	old	50
9	CRM	old	100
10	CRM	new	25
11	CRM	new	50
12	CRM	new	100

When an item is no longer available, contact as many suppliers as possible and ask for samples that can be tested before placing an order. Ensure that the samples come in a clean, sealed package.

Test all supplies for chemicals entering the sample (desorption from the vessel) and for loss of chemicals from the sample (adsorption to the vessel). Ensure that the vessels are tested with sample preservatives, if used. For example, acids used as a preservative could change the adsorption or desorption characteristics of the container walls.

Pipette tips must also be tested under conditions like use. See Appendix C for pipette leak test and calibration instructions. Before doing any chemical tests on the pipette tips, first test them for leakage. Next see if the tips provide an accurate and precise aliquot by weighing ten separately dispensed aliquots. If the pipettes pass these physical tests, test for contamination, as follows:

i. Get Type I DI water.
ii. Using a fresh pipette tip each time, draw and dispense enough aliquots of DI water into a test tube to perform a chemical analysis.
iii. Add the same amount of DI water to another test tube as a control.
iv. Analyze the water dispensed from the pipette tip and the water from the control.

Be aware of lot numbers and that the quality of a supply item may change from lot to lot. Record all new lots in laboratory logbooks, including the date of first use and laboratory sample ID, as appropriate. Test all supplies frequently. Report all significant changes in suppliers or supply quality in the laboratory's annual QA report. Figure 4.16 summarizes the tests that should be performed on supplies.

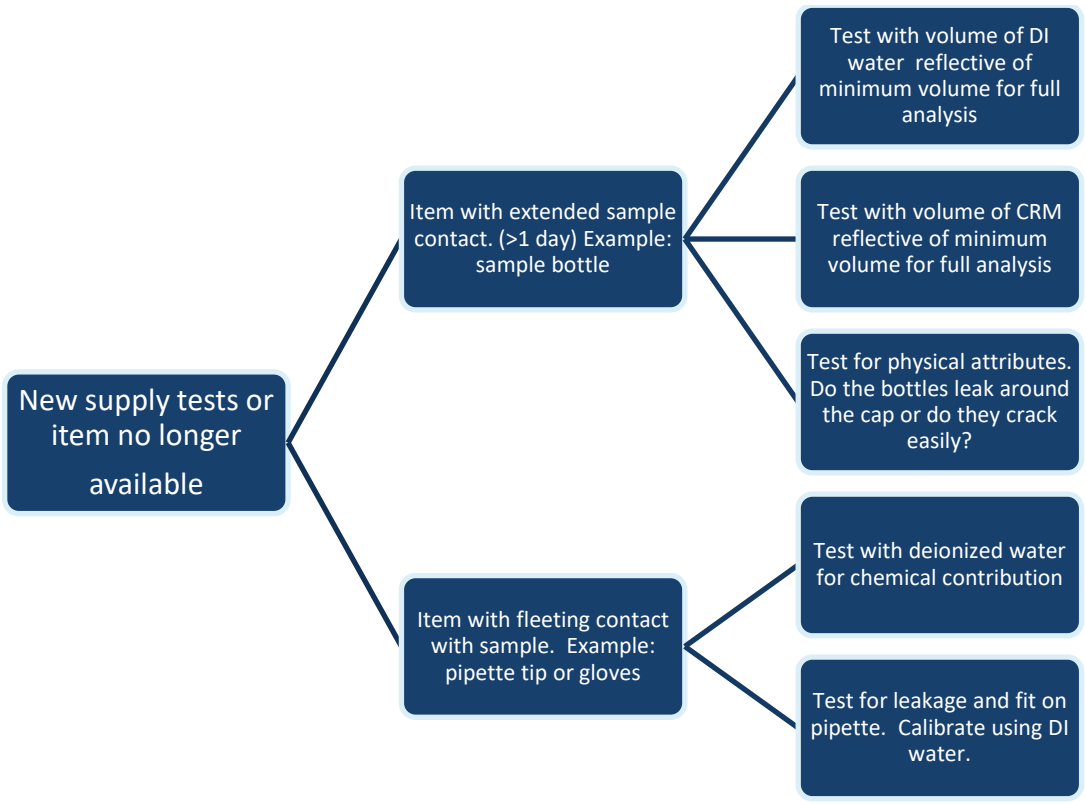


Figure 4.16. Inventory action plan

Examples of laboratory consumables that have extended contact with the sample:

- sample collection containers (buckets, funnels and bottles, bags, lids, lid liners and gaskets)
- laboratory sample bottles and caps
- tubes and caps

Items that have cursory contact with the sample may include:

- pipette tips
- weigh boats
- gloves
- instrument tubing for sample loops and transmission
- instrument probes

It is important to check the physical dimensions of supplies. Sample tubes must fit into the sample changer tray. Sampling containers must fit the field equipment and not change calculations that are dependent upon collection area.

Quality Control - Reagents

Decisions about the procurement of reagents can be influenced by storage requirements, purity of powder stock, cost, and waste disposal. Reagents and stock calibration standards may be

purchased or customized ready to use but all solutions need to be tested to ensure accuracy and quality. In general, precipitation chemistry laboratories do not purchase large quantities of chemicals and other materials. Most suppliers are interested in large volume consumers, so the range of suppliers for precipitation chemistry laboratories may be limited. If a supplier suddenly is bought out by another company or stops supplying an important item, it can be difficult finding a replacement supplier.

Establish a plan for testing calibration solutions or high purity salts used to prepare calibration standards (Figure 4.17). Establish another plan for testing chemical reagents used for laboratory methodologies (Figure 4.18). Consider waste disposal when purchasing new chemicals.

The purity and quality of compressed gases, including laboratory compressed air and vacuum systems, can affect the quality of data. Consider compressed gases as reagents.

Stock that is used primarily as a calibration solution or standard material must be of the highest purity available. These items should have a certificate of analysis stating impurities. Standards that are used for instrumentation where multiple elements are analyzed must not contain any of the other elements. For example, a sodium chloride stock must not contain sulfate or nitrate if the intended use is for anion analysis for ion chromatography.

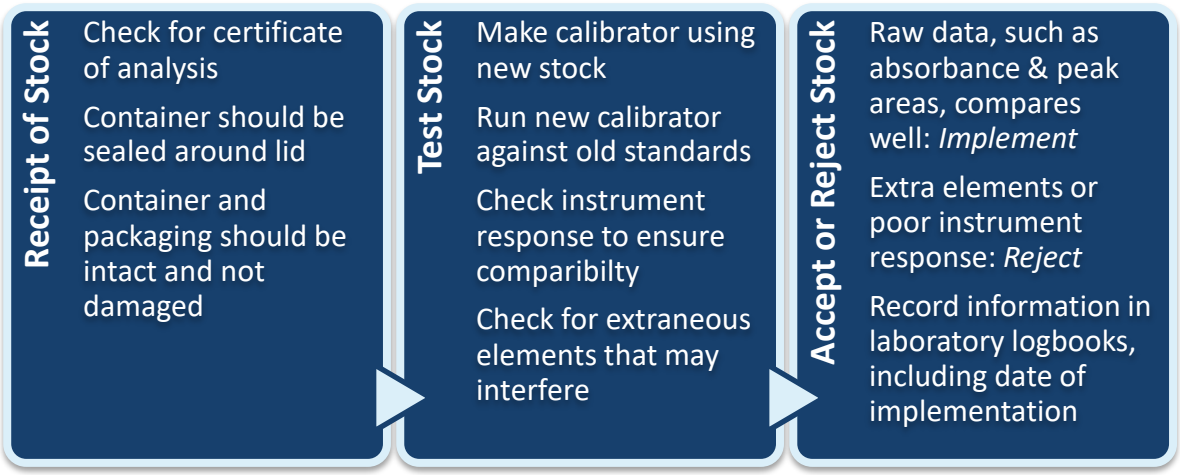


Figure 4.17. Check sequence for calibration or stock solutions

Reagent testing (Figure 4.18) may include:

- powders used to prepare reagents for colorimetric methods
- reagents prepared off-site
- compressed gasses
- columns
- tubing

When accepting or rejecting reagents, examine the raw instrument responses especially zero standards or blanks.

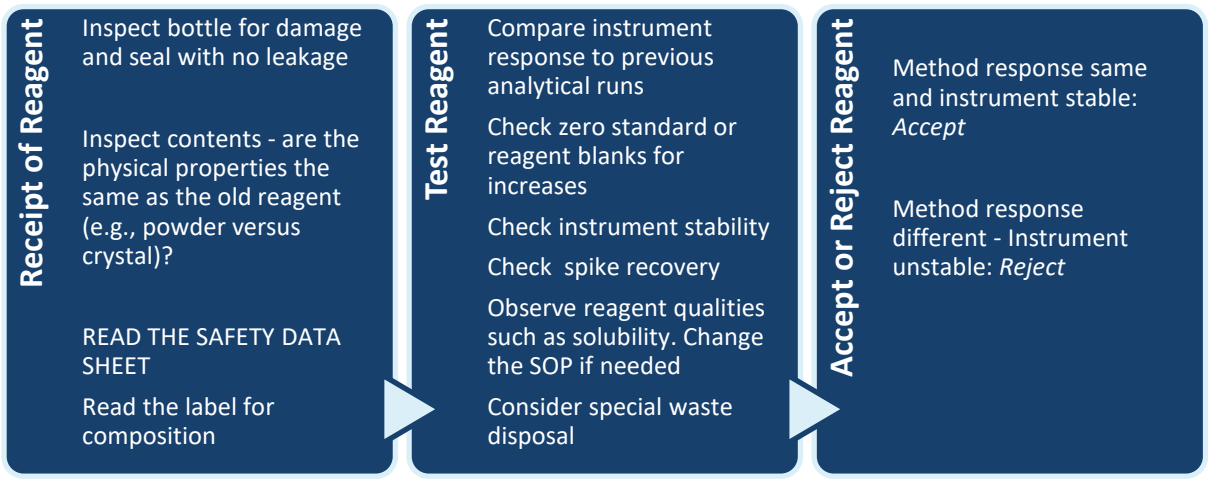


Figure 4.18. Check sequence for new reagents

4.3.15 Personnel Selection and Analyst Training

Personnel Selection

Laboratory operations and quality can depend on having an engaged and responsible staff. An ongoing training and staff development program can stimulate interest and elevate the data quality.

Establish minimum qualifications for the hiring process. Analyst qualifications may include:

- minimum educational requirement
- minimum experience in analytical setting
- good references
- specific previous training or experience using current laboratory equipment
- proven team participation
- proven communication skills
- proven organizational skills

Training

Select experienced personnel to train new analysts. Have a list of skills that are checked against basic standards. Precipitation samples are easily contaminated. New personnel must learn to avoid touching container rims or openings of tubes. They must learn to don gloves when needed.

The trainer should document the trainee's progress until the trainer certifies that the trainee is competent and able to work alone.

Guidelines for bench level training are described in Figure 4.19.

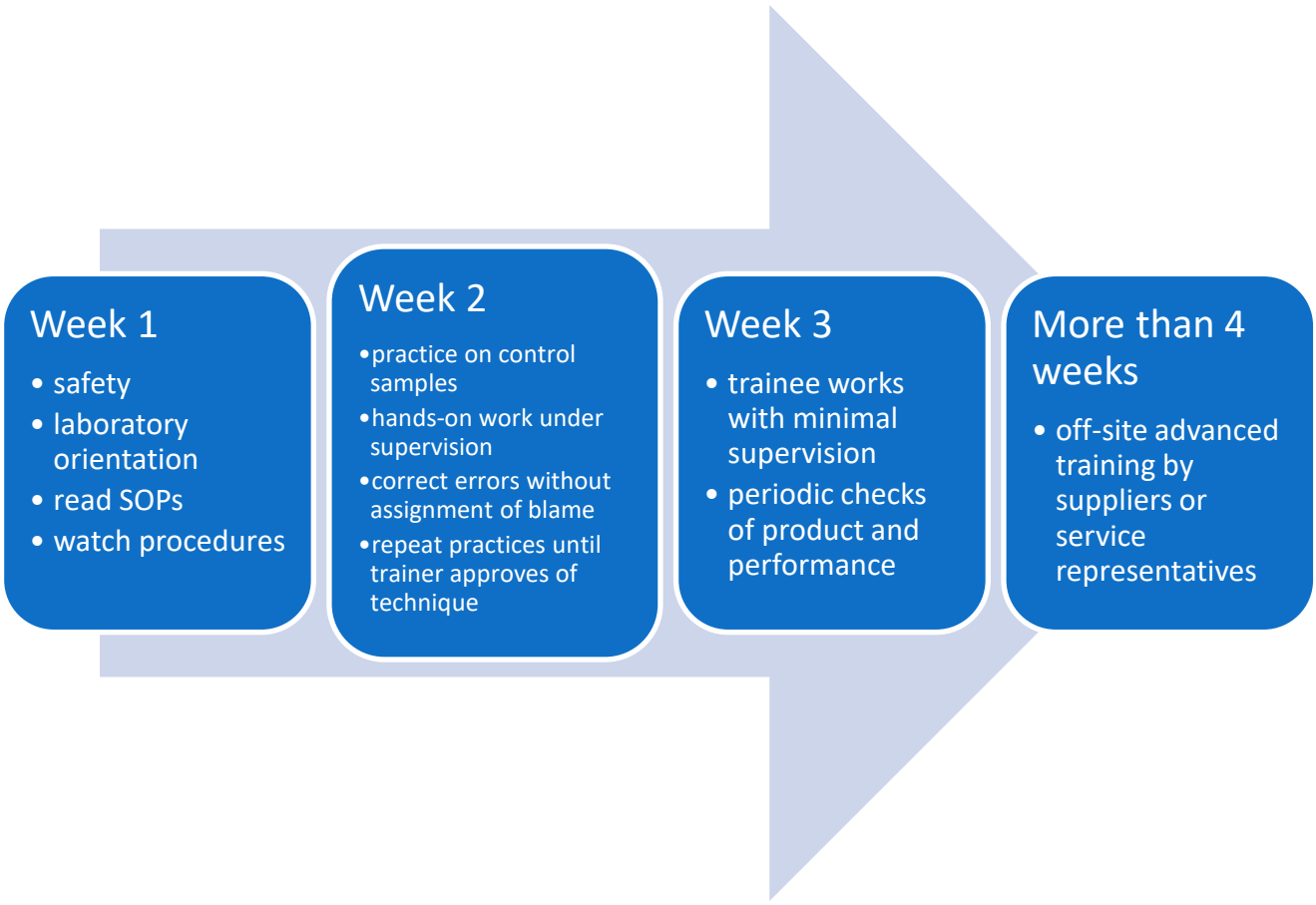


Figure 4.19. Example of training guide

4.3.16 Laboratory Safety

Laboratory safety is of paramount importance and safety precautions must be always followed. Safety training of all staff is mandatory. Always adhere to local and regional laws.

An overview of safety practices is available on the U.S. Occupational Safety and Health Administration website.

The following safety practices do not address all safety concerns, as this is beyond the scope of this manual. Everyone in the laboratory, including visitors, must always follow these practices:

- Protective eye-ware must be worn in the laboratory.
- Visitors must be escorted by trained laboratory personnel.
- Food and drinks should never be stored or consumed in laboratory areas.
- Cosmetics and contact lenses must not be applied in laboratory areas.
- Contact lenses must not be worn in the laboratory, especially when working with organics.
- Protective gloves and a lab coat should be worn when handling samples or reagents.
- All laboratory SOPs must address specific safety concerns and chemical hazards related to the method.
- Use fume hoods and avoid inhalation of vapors.
- Laboratory fume hoods must be maintained regularly and tested at a minimum of once per year to ensure face velocity is within specifications.

- Only minimal chemical waste should be stored in the laboratory in an appropriate container (or secondary container, as needed). Dispose of all chemical waste according to Safety Data Sheets.
- A current laboratory chemical inventory must be maintained in all laboratory areas and updated at a minimum of once per month.
- Chemical Safety Data Sheets (SDS) should be maintained and within easy access of staff and visitors for all stored and in-use chemicals.
- All laboratories should have a Chemical Hygiene Plan (CHP) to address facility-specific concerns. An adequate CHP would include, but not be limited to, personal protective equipment requirements, equipment inventory and operational requirements (such as vented hoods), employee training programs, medical programs, and safety.
- Develop an Emergency Response Plan (ERP) in the case of evacuation.
- Safety training of all personnel is essential even those who are not laboratory workers. Personnel such as office administrators who work in the laboratory vicinity must have basic safety training as well.
- Inspect all chemical containers, when received, for damage to the container itself or to the label. Do not accept damaged items.
- All laboratories should have a safety officer, who maintains the CHP and conducts regular internal safety audits (no less frequently than once per year.)
- Safety training may include:
    - basic familiarization of trainee with evacuation protocols and exits and fire alarms
    - how to read Safety Data Sheets
    - fire safety, including when and how to use extinguishers
    - correct transportation and use of compressed gases
    - safety symbols and what they mean
    - handling and disposal of waste chemicals
    - use of fume hoods
    - types of gloves and their correct use
    - when to clean up a chemical spill and when to call in Hazmat
    - how to clean up small spills
    - basic first aid
    - basic cardio-pulmonary resuscitation (CPR)

4.3.17 Internal Audit

Good laboratory practice (GLP) refers to a system of controls that ensures the uniformity, consistency, reliability, reproducibility, quality, and integrity of laboratory testing. Laboratories should conduct a periodic internal audit to assure that the principles of good laboratory practice are adhered to and that laboratory analyses provide continuous, reliable data.

Information from the internal audit provides both the lab manager and staff with feedback on how well the laboratory is following QA practices and procedures and if the quality systems are providing researchers and clients with high quality data.

Some basic steps to internal auditing are as follows:

1. Define the purpose of the audit. Audits are not intended to be adversarial nor to find fault with staff. Audits analyze the effectiveness of the programs that provide data to the client.

2. Define the scope of the audit. Will only analytical processes be audited or will the audit include data management, programming, web sites and field operations?

3. Select members of the audit team best suited for the scope of the audit. What special knowledge or skills are needed for the job?

4. Identify the authority for the audit. This may be the QA manager or a third party, such as a client.

5. Identify performance standards. Identify components of the quality system that are implemented and measure them against known standards.

6. Audit team members must fully understand the processes to be audited. In advance of the audit, team members must have access to manuals, previous audit reports and QC and QA documents.

7. The audit must be carefully scheduled since laboratories are busy and staff members need to accommodate observers while answering questions. Auditors need to see the laboratory working yet minimize interference with laboratory operations.

8. The audit team must develop written checklists of laboratory and data requirements and list specific issues to be examined. The main function is to gather data to provide feedback to the laboratory manager and staff.

9. While observing the laboratory at work, audit team members may ask questions to seek clarity but must not admonish or criticize staff members. The focus is on compliance with established processes and procedures, not on finding fault with staff members.

10. Once the audit has been completed, the audit team debriefs the laboratory manager and staff on key findings. Positive feedback is important. The laboratory should be thanked for allowing the team members access to the work and laboratory documents.

11. The final activity of the audit team is preparation of a report that remains positive while describing any operational or safety deficiencies. This report should supply references where corrective actions can be found and require that these actions be taken within a certain timeframe.

4.4 Laboratory Sample Handling

4.4.1 Sample Reception, Logging and Custody

Sample logging and chain-of-custody procedures must be implemented in the laboratory to ensure that samples are not lost or mixed up. Upon receiving samples, document the following:

i. date samples received
ii. site name
iii. contents of shipping container, ensuring the description on the field log matches the sample
iv. sample condition and quality (cold, contaminated, leaking)
v. name of laboratory person who received the samples.

Weigh the samples and compare the field and laboratory weights to determine whether the sample has leaked in transit. Document inconsistencies in the weights, dates, times, or sample quality and send this information to the QA manager.

Chemical species in precipitation are unstable, especially ammonium, orthophosphate, and organic acids. Chemical change or biodegradation of samples can be slowed during shipping and laboratory storage by refrigerating samples at 4ºC or freezing at <-20ºC. Place samples in a refrigerator at 4°C as soon as possible until they enter the analysis stream. Freeze the samples at -20ºC if analysis will be delayed longer than one week.

Each analytical section must account for every sample. Samples could be misplaced or inter-mixed at any step, so procedures must be put in place to account for all samples, including those lost through spillage in the laboratory or through some other mishap. Analysts should analyze the samples in numerical order. Sample bottle numbers, test tube numbers and analyzer numbers must be scrupulously matched through every analytical step.

Samples must be refrigerated, but not frozen or re-frozen, between analyses. For example, if anions and ammonium are analyzed on Monday and no additional analyses will be completed that day, the samples must be returned to the refrigerator until analysis can resume for alkaline metals.

Samples that do not have sufficient volume for a complete set of analyses must be coded or annotated in the database. Analyses should be done in priority order as outlined in Figure 4.2 or as specified in precipitation chemistry network policies. For low-volume samples, continue the analysis if there is sufficient volume for the next step. As each analysis is completed and there is no need for a repeat measurement, the sample bottle should be marked so that the next analyst can see that the sample is ready for the next step in the analytical process.

Samples that cannot be analyzed because of gross contamination must be coded or annotated in the database. Samples lost due to mishap such as leakage, spillage, etc., also must be coded or annotated in the database.

4.4.2 Sample Labeling, Transferring and Storage

Background

Establish and adhere to minimum holding times. Keep the time from the receipt of the sample to the completion of the analysis as short as possible. This is especially important for ammonium and other unstable species.

The point at which the sample is transferred from the field container to the sample bottle or tube is an important and requires special care to ensure that the sample goes into the right container. Errors during this procedure are difficult to trace and mixing up the samples invalidates the data. Keep a log that records the field identification number that goes with a uniquely assigned laboratory identification number. This record is how researchers connect the field and analytical laboratory data. Double check this record before transferring the sample from the field container to the laboratory sample bottle or tube.

QA samples are inserted into the sample analysis queue during the sample check-in. This includes blank tests of all kinds and blind QA samples. Label QA samples appropriately and log them into the analysis queue. Sample bottle and tube blanks must be prepared and placed into the analysis queue weekly to provide an ongoing test of container cleaning procedures. Do not wash then store sample containers weeks in advance of their use unless the containers are tested again for cleanliness before they are used. Washed and stored containers can become contaminated during storage, especially from volatile gases such as ammonia. Always test sample containers before they are used to ensure cleanliness.

Here is a recommended weekly procedure for preparing and testing sample bottle blanks: Add 25mL of Type I (resistivity >18.0 MΩ) DI water to each of three bottles, allow to stand for a minimum of 24 hours, then analyze the water for all constituents.

Here is a recommended procedure for preparing and testing tube blanks: With each analytical run, add 3 to 5 mL of Type I DI water to each of three tubes (volume should be typical of the volume used during sample analysis), then analyze the water for all constituents.

Sample bottles and analysis tubes may be identified with adhesive-backed labels. Test to make sure the labels do not fall off, especially when condensation forms on the outside of bottles removed from the refrigerator. Make sure that moisture does not allow the ink to smear, wipe or wash off. Also, make sure that marker ink does not contaminate samples. For example, ammonium analysis is sensitive to aromatic contaminants.

Guidance for Transferring Samples from Field Containers to Laboratory Bottles

1) Label all sample bottles, tubes or analytical vials with a unique laboratory identification number that can be cross-referenced directly to the unique field identifier. To avoid mix-ups when preparing the labels, line up the field and laboratory containers side-by-side in order.

2) Sample handlers must wear gloves to protect the sample from human exposure. Gloves must not contain powders nor be made from latex and they must be tested for chemical constituents (see Figure 4.16). Gloves used for sample handling should never be used to handle chemicals. Do not let gloves be a source of cross-contamination. With the gloves on, avoid touching bench surfaces, lab coats, etc. If the gloves contact a sample, rinse them with deionized water. It is a good practice to change gloves often, which can be made easier by wearing a thin clear plastic glove over a pre-tested, clean, laboratory glove. Caution is required since gloves can be slippery and make holding or opening bottles more difficult.

3) Allow frozen, snow or ice samples to melt completely. Then, thoroughly mix the liquid to ensure that no stratification remains before transferring the liquid to a sample bottle.

4) Be diligent when transferring a sample to a laboratory container. Ensure that the sample is transferred to the correct, labeled container and avoid spills. If a container is knocked over and the sample spills, the sample has contacted the laboratory bench and is contaminated.

5) Seal laboratory sample containers tightly. Store at 4^{o}C until analysis.

4.4.3 Sample Filtering and Chemical Additives

Precipitation samples collected over a one-week period often contain more soil and other particles and debris than samples collected daily. Longer exposure under field conditions increases the chance for contaminants to enter these samples. One method to reduce the effects of particles on sample chemistry is to filter the samples. Filtering samples eliminates the potential interferences from particles and protects analytical systems. However, filtration devices and membranes can absorb or desorb species of interest, so they must be tested routinely to ensure that the chemistry is not altered during filtration.

For measuring unstable species such as organic acids and orthophosphate, addition of a chemical preservative is recommended. Consider using a biocide such as thymol or chloroform. A small amount of chloroform can be added to samples immediately upon collection. Thymol is a solid and does not dissolve in water so it can be placed in the sample container prior to sample collection. Thymol has an unpleasant odor and it may interfere with some analytical procedures. When using these biocides, rigorously follow special handling precautions on Safety Data Sheets and ensure the safety of field and laboratory personnel. Both chloroform and thymol may cause respiratory irritation and should be handled in a fume hood. Prolonged exposure can have harmful health effects.

Before using biocides, it is necessary to fully research their potential for interfering with sensitive analyses and for potential effects on existing laboratory equipment. A suggested resource is ASTM D3694-96, Standard Practices for Preparation of Sample Containers and for Preservation of Organic Constituents (ASTM International, 1996). It also is necessary to check the purity of biocides and to test sample containers for degradation or alteration from the preservative.

Biocides may work well for some measurements but not others. For example, thymol interferes with the baselines of an ion chromatograph (Gardrat, Collection techniques, 2016). One option to preserve samples is to split the sample at the sampling site, volume permitting, promptly freeze one portion and add biocide to the other portion. Use the frozen portion for IC, pH, and conductivity measurements, where biocides would otherwise interfere, and the other portion for organic acid and orthophosphate analyses. Greiner centrifugation tubes are ideal containers for both portions. These polystyrene tubes are fitted with a screw cap, are sterile and are available in several sizes. (Gardrat, Collection techniques, 2016).

While biocides can be used to slow sample degradation, particle dissolution and inorganic chemical reactions can continue to alter cation and anion concentrations and free acidity. It is important to note that **biocides should not be used so that an analysis can be delayed**. **Sample refrigeration is necessary even if biocides are used.**

Avoid sample loss from cracked tubes. Field site SOPs should specify that a fill line be marked on sealed (Greiner) tubes that are to be frozen. The fill line should allow adequate space to prevent the frozen sample from cracking the tube.

4.5 Analytical Measurements

The GAW Precipitation Chemistry Programme recommends certain analytical approaches but does not specify step-by-step analytical procedures.

The following sections describe basic calibration standard and reagent preparations. Common problems and solutions for problems are described in minimal detail. All methods require validation in the laboratory where they are used.

4.5.1 Instrument Procurement

Procuring laboratory instruments is costly. Laboratories should implement a procurement plan which requires that instruments pass a performance test. The test should require that measurements of certified reference materials, blind to the vendor, meet certain performance criteria prior to purchase.

Consider these criteria, issues and questions when procuring laboratory instruments:

i. range of sample concentrations
ii. required sensitivity and detection limits
iii. purchase price limits and operating costs
iv. Is a sample changer included or an added feature that increases cost?
v. constraints on laboratory conditions (temperature, humidity fluctuations)
vi. electrical, ventilation and fume hood requirements
vii. space requirements (Will it fit through the door? Are renovations required in the laboratory?)
viii. potential for cross-contamination between analytical systems. Reagents from one system may contaminate or interfere with other systems.
ix. operational and maintenance requirements (Time needed for operations and maintenance?)
x. operator training requirements
xi. vendor history and referrals. When possible, ask other laboratories about the vendor and if they are satisfied with the product and service.
xii. expected instrument life (Will the instrument need to be replaced in five years, ten years?)
xiii. Does the instrument require tubes or reagents available only from the vendor?
xiv. Warrantee and service agreement (What is the service response time?)
xv. availability of repair or replacement
xvi. waste disposal requirements, especially if depleted reagents are toxic

Selection of instrumentation may completely depend on workstation software.

Consider the following when selecting instrument software:

i. software compatibility with in-house data bases and operating systems of existing workstations
ii. ease of data handling and export
iii. software limitations such as inability to handle numeric and non-numeric characters side-by-side or limitations to the number of calibrators
iv. capacity to read and import old data (for example, archived chromatograms) into new software
v. impact of internal algorithms that integrate peak areas on final data
vi. capacity of software to track instrument performance over the course of the analysis generating an audit trail
vii. software compatibility with the sample changer, if from a different vendor
viii. cost of software upgrades

4.5.2 Method Development

A new instrument must be evaluated against the existing instrument for differences. Newer instrumentation is not always better instrumentation.

Differences between a new and old method must be carefully evaluated. If the differences are small, then the two methods have the same relative accuracy. If the differences are large, it is necessary to identify which method is inaccurate. If the current (old) method is the inaccurate one, it will be necessary to quantify the inaccuracy and annotate the old data.

New methods can be verified by analyzing CRMs, SRMs or inter-comparison samples of known concentrations. Another good practice is to send samples to another laboratory for analysis and to compare results.

New additions to existing instrumentation such as software upgrades also must be assessed. Be very careful when upgrading software that includes integration of peak areas. A new algorithm can change results significantly. Compare results from both software packages at all concentration levels.

Method development studies must be kept indefinitely. Document all measurements from the new and old instruments and new and old methods.

Here are some suggestions of things to do when changing instrumentation.

1) Receive training, especially if the instrument is new. Vendors may offer free operator training.

2) Ask the vendor to perform instrument set-up. Often software will have method templates that the vendor can help adapt to your needs.

3) Ask a colleague or another GAW laboratory for their SOP.

4) Prepare calibration standards and reagents and use them for both the old and new instruments.

5) Use the same QCS for both instruments. Plot and compare QC charts for both instruments.

6) Select a set of samples that span the typical concentration range and that have sufficient volume for measurements by the old and new instruments. If possible, complete the sample analyses by both instruments on the same day.

7) Using both instruments (or methods) analyze at least 20 samples every day for 10 days. Plot and compare the results daily and reanalyze any samples with large differences.

    a. Perform a regression analysis and plot the results. See Figure 4.20a for an example of a regression plot with poor correlation at higher concentrations. There is also an outlier that is further skewing the results.
    b. An alternative to a regression is to plot the new method minus old method results against the old method results as in Figure 4.20b. Both plots show how the two methods begin to differ at higher concentrations.
    c. Where there are outliers, reanalyze the samples using both instruments. Also, investigate the cause of poor linearity or curvature in the instrumental responses. In the example in Figure 4.20a, the new instrument may be more sensitive. If this is the case, try injecting less sample to improve the linearity.
    d. Inspect the comparison plots daily and if the results are poor, stop the analyses and investigate the cause of the problem. Ensure that both old and new instruments are working well. Inspect the QC charts to see which system produces results that are closer to the SRM or CRM target values.
    e. Accept and adopt the new instrument, method, or procedure if R^2 is 0.95 or greater.

8) If possible, run both instruments together for a period of one month before using the new instrument for routine analyses. Make sure that the new instrument runs smoothly and has a linear response and adequate detection limits.

    a. Linear response can be determined by inspecting the correlation results in the high-concentration range. Typically, this is where the response tends to flatten or curve. Slight curvature at high concentrations can be addressed by adding more high-concentration standards to the calibration procedure, although this can lead to greater imprecision at low concentrations. Linearity studies can be discontinued when there are no longer signs of flattening or curvature in the calibration curve.
    b. A simple method for determining detection limits is to analyze a blank solution containing none of the analyte of interest and then analyzing a second solution spiked to near the instrument manufacturer's claimed detection limit with the analyte of interest. The lowest calibration standard is a suitable alternative for the spiked solution. Analyze both samples up to 10 times in the same run. Calculate the blank sample mean and the standard deviation of the spiked solution. The lower limit of detection (LLD) is the blank sample mean + standard deviation of the spiked sample. The below detection limit (BDL) is the LLD + standard deviation of the spiked sample.

9) Write a SOP and implement the new method.

10) Comment or flag the point in the data set where the new method or instrumentation was implemented. Monitor the data for shifts.

11) Train other operators and get advanced training for the operator who originally did the workup.

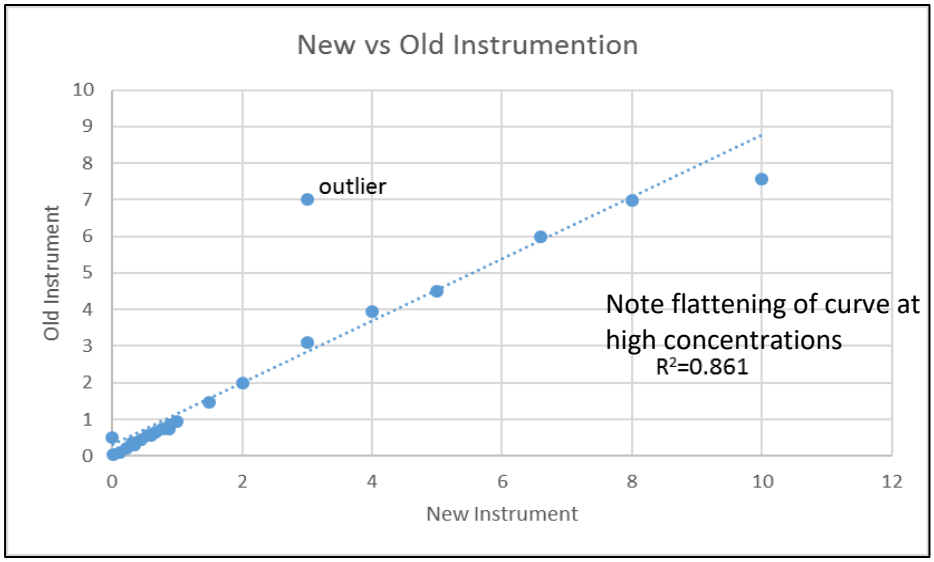


Figure 4.20a. Regression plot comparing old and new instrumentation

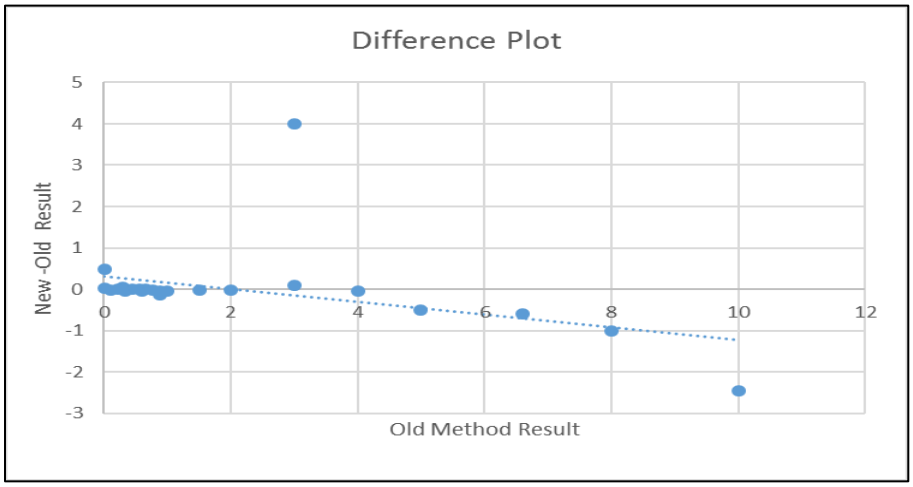


Figure 4.20b. Difference plot comparing method differences against the old method values

4.5.3 pH

Background

pH is a measure of the acidity or alkalinity of a substance or solution. Measurements are performed with a meter and associated pH electrode. It is typical to calibrate this system using a two-point calibration method, though many pH meters can accommodate three calibration standards. For a two-point calibration, the meter and electrode are calibrated against two standards that bracket the anticipated pH of the samples to be measured. These standards are reference solutions, also called pH buffers, because they are formulated to resist, or be buffered against, changes in pH. The pH of precipitation varies between about 3.0 and 7.5 pH units, which corresponds to a hydrogen ion concentration of 1000 μeq L^{-1} to <0.1 μeq L^{-1}. Lower and higher values are possible.

By definition, pH measures the free acid activity of a solution and is expressed as the negative logarithm of the hydrogen ion activity by the equation:

$$pH = - \log [H^+],$$

where $[H^+]$ is the chemical activity of free hydrogen ions in mol L^{-1}. For dilute solutions that approach ideal behavior, such as precipitation, concentration $\underset{\sim}{}$ activity and $[H^+]$ is the free hydrogen ion concentration. Also, $[H^+]$ in mol L^{-1} is the same as $[H^+]$ in eq L^{-1}.

A pH electrode is an electrochemical cell consisting of three components:

i. indicating electrode with an electrical potential that is directly proportional to pH
ii. reference electrode with an electrical potential that is constant and independent of pH
iii. ionic fill solution that provides an electrolytic bridge between the indicating and reference electrodes.

When immersed in a sample, the electrical potential between the indicating and reference electrodes is measured. This potential is dependent on the pH and temperature of the sample. Many pH electrodes include a temperature sensor that compensates for temperature, allowing for a temperature-corrected pH measurement.

Most pH measurements are made with a combination electrode. This consists of a glass electrode in combination with a silver/silver chloride reference element. A liquid junction, typically potassium chloride (KCl), provides the electrolytic bridge between the indicating glass and reference electrodes. The specially formulated pH-sensitive glass provides a partition between the precipitation sample and the internal reference solution. The difference in electrical potential between these two liquids produces a millivolt signal, which the accompanying meter converts to pH units.

Electrodes specific to low ionic strength samples, such as precipitation, are available and may be described as “designed for testing high purity samples or pure water”. When selecting an electrode, it is necessary to confirm that it accurately measures pH in low ionic strength samples. This is best done by measuring real precipitation samples, CRMs, SRMs, or comparison samples of known pH. The time for the pH reading to become stable should be less than about one minute.

Note that pH electrodes used for precipitation measurements should be used exclusively for these measurements. Use of pH probes for tap, surface, or other water analyses will quickly make them insensitive to the low ionic strengths of precipitation samples. Always store pH probes according to manufacturer’s recommendations. Soak the probes in DI water before use.

Apparatus

i. pH meter
ii. combination pH electrode

A pH meter should have both intercept and slope adjustments and must be capable of reporting a resolution of ±0.01 pH unit. For ease of use, combination electrodes containing measuring and reference electrodes and a temperature sensor are preferred (see Figure 4.21). When selecting a pH meter, consider one that enables export of the data to a spreadsheet or other data capture application.

Reagents and Solutions

The following reagents and solutions are required for measuring pH:

i. pH 4.01 buffer solution
ii. pH 6.86 (7.0) buffer solution
iii. electrode storage solution (typically KCl)
iv. Type I DI water: resistivity >18.0 MΩ.

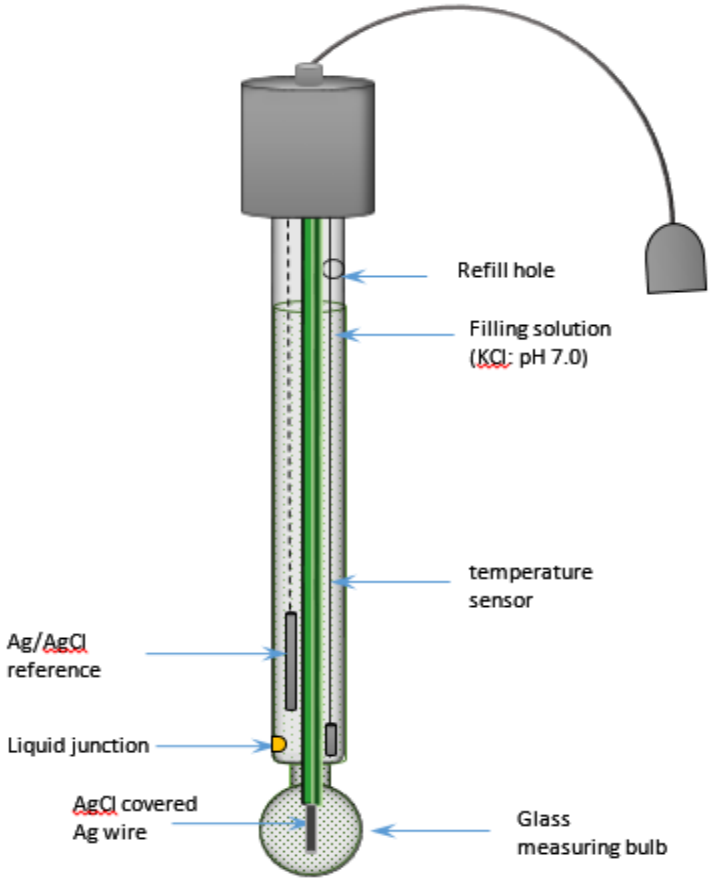


Figure 4.21. pH electrode

Calibration

Calibrate as specified in the manual for the pH meter. At a minimum calibrate at two points in the expected pH range. Calibrate before and after measuring each set of precipitation samples.

1) Fill the electrode with a filling solution (if required) then rinse the outside of the electrode with DI water. Check the room temperature and set the temperature of the meter to the current room temperature. If the pH instrument is equipped with a temperature sensor, the temperature correction occurs automatically and this adjustment is not needed. It is best if all calibration buffers and samples to be measured are stabilized at room temperature.

2) Place the 6.86 buffer solution in a clean polyethylene or borosilicate glass vessel with enough liquid to immerse the measuring bulb of the electrode. If using a glass beaker or vessel, use it only for the 6.86 buffer. Use a separate glass vessel for the 4.01 buffer. Buffers are highly concentrated (some are dyed) and the glass beaker may become “seasoned” with residual salts. If the same beaker were used for both buffers, this residual would carry over and contaminate the other buffer. Measure the pH of the 6.86 standard. It should be within 0.02 pH units of the certified buffer value. Set the meter to read this calibration value. Thoroughly rinse the electrode with deionized water.

3) Place the 4.01 buffer solution in a clean polyethylene or borosilicate glass vessel with enough liquid to cover the glass measuring bulb of the electrode. Measure the pH of this second standard. It should be within 0.02 pH units of the certified buffer value. Set the meter to read this calibration point. Thoroughly rinse the electrode with DI water.

4) Confirm the accuracy of the calibration by measuring the pH 6.86 buffer again, followed by the pH 4.01 buffer. Make sure that the results are within ±0.02 pH units of the certified values. If not, repeat steps (2) and (3), until the ±0.02 pH unit criterion is met. Calibration is completed only when this criterion is met.

5) Once the calibration is completed, rinse the electrode thoroughly with DI water. Precipitation is not buffered and has much lower ion strength than the buffered calibration standards. Incomplete rinsing will allow the buffers to contaminate precipitation samples and bias the readings.

Quality Control

The following are QCS and other control solutions for pH measurements:

1) QCS-1 (4.01 pH unit) solution: Dilute 1.0 g 0.1N H_2SO_4 in 1 L of DI water. QCS-1 is prepared in 10 L amounts and stored in a HDPE container. Stable for up to one year at 4^{o}C.

2) QCS-2 (5.05 pH unit) solution: Dilute 100 mL of QCS-1 in 1 L of DI water. Prepare QCS-2 in 10 L amounts and store in a HDPE container. Stable for up to one year at 4^{o}C.

3) A check solution comprised of a real precipitation matrix may be prepared by pooling precipitation samples. The pooled sample may be analyzed 20 times over 20 runs to establish a mean and preliminary standard deviation. This solution will not be stable for longer than six months unless sterilized.

4) Check the pH of DI water after the meter and electrode have been calibrated with buffers. Water in equilibrium with atmospheric CO_2 has a pH of about 5.65. DI water measurements are slower to stabilize than buffer measurements because they are very dilute (low ion strength) and typically read between 5.58 and 5.75 pH units.

5) The pH value of the QC solutions including DI water should agree within ±3 standard deviations (in pH units) of the expected value. If larger differences are observed, the system must be calibrated anew. If the QC values are outside acceptable limits, the electrode must be cleaned (see manual) or replaced.

6) Analyze a QCS of other control solution every 10 samples to ensure the system remains in control throughout the analytical run.

Analytical Procedure

The procedure for measuring pH is as follows:

1) Maintain all solutions to be measured at the same temperature. Allow samples and control solutions to come to room temperature.

2) Calibrate the pH meter and electrode according to the procedure outlined in the previous section.

3) Rinse the electrode with DI water. If sample volume allows, rinse the electrode with an aliquot of the sample by placing the sample solution in a clean polyethylene or borosilicate glass vessel with enough liquid to cover the glass measuring bulb (Figure 4.21) of the electrode.

4) Immerse the electrode in the sample vessel and swirl the sample gently for a few seconds to condition the electrode with the sample. Do not stir the sample with a rod or magnetic stirrer. Discard this sample aliquot. Do not rinse off the electrode.

5) Place a fresh aliquot of the same sample in a clean polyethylene or borosilicate glass vessel again with enough liquid to cover the glass measuring bulb of the electrode. Immerse the electrode in the sample vessel and swirl the sample gently for a few seconds. Do not stir the sample with a rod or magnetic stirrer.

6) Allow the electrode to equilibrate until a stable pH value is obtained. Samples with pH in the range of 6 to 7are slower to stabilize. Observe the reading on the meter. The reading may appear to be increasing even though the meter signals a stable reading. Press *Read* again until the value stops climbing. Record the pH value (to 0.01 unit).

7) Enter data into the data set. See section 4.3.14 for manual data verification if the pH meter does not have a data export function. Report values to the required decimal places. Account for missing samples and samples too contaminated to be analyzed. Add comments or codes as needed to account for all samples.

Troubleshooting

Problem 1: Measuring pH in precipitation is difficult due to the low ionic strength of the samples.
Solution 1: Some pH systems allow for a 1 µL injection of KCL to add ionic strength to the sample without changing the pH. It is not an ideal solution and the laboratory should conduct a study to see if pH results with KCl injections are the same as those without. Purchase an electrode specifically designed for low ion strength or pure water samples.

Problem 2: pH is affected by temperature.
Solution 2: Samples refrigerated at 4^{o}C must be brought to room temperature for analysis. Enter the room temperature into the pH meter to ensure an internal default temperature is not applied to the result or use a system equipped with a temperature sensor. Ensure buffers, QCS and samples are all at the same temperature during analysis.

Problem 3: Two main problems with pH measurements is aging of the electrode and using the electrode for samples other than precipitation. Electrodes tend to become less sensitive with age and may give incorrect measurements for precipitation samples, while giving correct readings for the calibration buffers. Probes used to analyze other water samples, such as stream water or drinking water, can also become insensitive and unresponsive to the low ion strength of precipitation samples.
Solution 3: Confirm electrode calibration and function by using real precipitation samples to make QC checks. Real precipitation has the advantage of being a low ion-strength solution. Use pH electrodes **only** for analyzing precipitation samples.

Problem 4: Bubbles in the glass measuring bulb cause unstable readings.
Solution 4: Electrode care and maintenance is important. Replace the electrode fill solution per manufacturer specifications. Inspect the bulb for bubbles and if present swing the electrode down to get the bubbles to rise out of the bulb.

Problem 5: Static electricity builds up on the glass bulb and causes erroneous measurements. Using a magnetic stirrer may produce the same effect.
Solution 5: Never dry the outside of the electrode by rubbing or wiping it with a Kimwipe®. Instead, rinse the electrode with sample solution but do not dry it. Do not allow the fill solution or the glass measuring bulb to dry out. When not in use, store the electrode in deionized water or electrode storage solution. Do not use stirring systems that continuously agitate the sample.

Problem 6: Poor calibration and control results. Recalibration does not solve the problem and response is slow.
Solution 6: A periodic cleaning procedure is recommended to remove built up organic material and to rejuvenate the electrode. Empty the electrode fill solution and soak the probe in a pH 4.0 solution for a minimum of 24 hours, then soak it in DI water for 24 hours. Refill the electrode with fill solution and performance test the electrode. If this does not solve the problem, replace the electrode. Cleaning

procedures vary with the type of electrode but usually involve soaking the electrode in a weak basic solution followed by a weak acidic solution. The acidic solution rejuvenates the glass membrane.

Tips to Improve Performance

1. Document maintenance procedures in a logbook.
2. Mark QC charts to document when maintenance is performed and to see the potential impact of maintenance on measurements.
3. Clean electrodes on a weekly basis or every 150 samples.
4. Use an electrode specifically configured for low ionic strength or pure water samples.
5. Set aside samples containing contaminants and measure them separately **after** measuring the clean and clear samples. Clean the electrode after analyzing contaminated samples.
6. Use a sample aliquot to rinse and condition the electrode prior to taking the reading.
7. Do not rush the analysis.
8. Do not measure hard water (lake, river, or tap water) samples using the same electrode that is used for measuring precipitation samples.
9. Routinely check internal fluid levels and refill as necessary.

4.5.4 Conductivity

Background

Conductivity measures the ability of a substance or solution to conduct an electric current. Conductivity varies with temperature and with the mobility and valence of ions, and it is proportional to the concentration of free ions in solution.

Conductivity may be measured by applying an alternating electrical current to two opposing electrodes immersed in solution. The voltage across the electrodes is measured. Cations migrate to the negative electrode (cathode), anions to the positive electrode (anode) and the solution acts as an electrical conductor. (Radiometer Analytical, 2004)

Conductivity

The conductivity of a solution is the reciprocal of its specific resistance and can be measured directly using a conductivity bridge connected to a cell that has two or more electrodes. The conductivity measurement depends on the area of the electrodes and spacing between them. These dimensions define the cell constant, which can be determined by calibrating the conductivity measurement system. A KCl solution of known concentration and conductivity is used to calibrate the system and determine the cell constant, K, defined as follows:

$$K = \frac{d}{a},$$

where K = the cell constant (cm^{-1}),
a = area of the electrodes (cm^2), and
d = distance between the electrodes (cm).

Conductivity is measured and expressed in units of microsiemens per centimeter ($\mu S\ cm^{-1}$), where microsiemens is a reciprocal measure of resistance. It is standard practice to report conductivity corrected to 25°C.

$$\varkappa = G \bullet K,$$

where $\varkappa$ = conductivity ($\mu S/cm^{-1}$),
G = conductance (µS), and
K = cell constant (cm^{-1}).

The conductivity range of precipitation samples is low, typically 5 to 1000 µS cm^{-1}. There are several types of conductivity cells available: 2-pole, 3-pole, 4-pole and 4-pole platinized. A 2-pole cell is recommended for precipitation samples. Use a conductivity cell that is dedicated solely for precipitation sample measurements. Avoid a cell that is used for tap water or surface water samples. For low-conductivity precipitation samples (Radiometer Analytical, 2004):

- Use a flow-through cell to avoid atmospheric contamination from carbon dioxide.
- Use a cell with a low cell constant, 1 cm^{-1} or lower.
- Use non-platinized cells for easier cleaning and faster response.

Conductivity meters measure the actual conductivity and temperature using built-in temperature sensors. This measurement is converted to the reference temperature (25°C) using an internal temperature correction function and the meter displays the temperature-corrected conductivity. If a temperature correction is not applied, the reported conductivity is the value at the temperature of the solution being measured. Always report the temperature with the conductivity result.

If there is not enough sample to measure both conductivity and pH, the aliquot that is used for the conductivity measurement can also be used for a pH determination. Conductivity must be measured before the pH to avoid KCl contamination from the pH electrode. Do not use the sample for pH if a flow-through cell is used for conductivity.

Apparatus

i. conductivity meter with a recommended operating range of 0.01 to 1000 µS cm^{-1}. Precision must be within 0.5% of the range and the accuracy at 1% of the range
ii. conductivity cell (see Figure 4.22)
iii. thermometer (0 to 40°C / 0.1°C)
iv. water bath 25°C
v. polyethylene or glass vessel corresponding to the diameter of the cell
vi. flow-through vessel (optional)

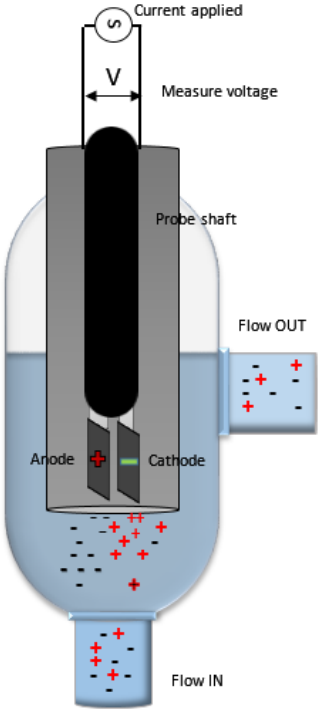


Figure 4.22. 2-pole cell with flow-through vessel

Reagents and Solutions

i. DI water, resistivity >18.0 MΩ
ii. potassium chloride, KCl, pro analysis (p.a.) quality
iii. stock solution A, 0.1M KCl: 7.456 g of pre-dried (2 hours at 105°C) KCl dissolved in DI water and diluted with DI water to 1000 mL at 25°C
iv. stock solution B, 0.01M KCl: 10 mL of 0.1M KCl (stock solution A) diluted with DI water to 100 mL at 25°C

Keep stock solutions in tightly closed high density polyethylene (HDPE) bottles. Stable for one year.

Calibration

1) Calibrate the conductivity meter and cell using more than one standard solution, i.e., perform a multipoint calibration. With each set of precipitation samples, prepare a set of 0.0001M, 0.0005M, and 0.001M KCl standard solutions from the 0.01M KCl stock solution B by diluting with DI water.

2) Measure the conductivity of the DI water used in preparing the standard solutions.

3) Measure the conductivity of the KCl standard solutions and plot them on a graph. If these measurements are outside the limits given in Table 4.10, consult the instrument manufacturer's manual for corrective actions. Repeat the calibration.

4) Read the conductivity of precipitation samples directly from this calibration plot.

Table 4.10: Calibration standards for conductivity (KCl solutions at 25°C)

Concentration KCl (M)	Theoretical (µS cm^{-1})	Conductivity (µS cm^{-1}) Upper limit	Conductivity (µS cm^{-1}) Lower limit
0.0001	14.9	16.5	**13.5**
0.0005	73.9	77.8	**70.2**
0.0010	**147.0**	**149.0**	**145.0**

Quality Control

Natural precipitation deteriorates with time, even if refrigerated, and should be avoided for use as a QCS for conductivity measurements. Here is a list of recommended QC solutions:

- Certified Reference Material
- pooled inter-comparison samples
- simulated samples made from an alternate standard material

Prepare at least three QC solutions that span the standard range. These solutions should test the low, middle, and high range of the calibration curve.

Analytical Procedures

1) Thoroughly rinse the outside of the cell and internal poles with DI water.

2) Rinse the outside of the cell and internal poles with the sample, whether a QCS or precipitation sample. Discard this sample. Do not wipe the probe dry. Repeat this step if volume permits.

3) Fill the cell with fresh sample to be measured.

4) Ensure that the poles are covered by the sample and that all air bubbles are eliminated by tapping the outside of the cell.

Note: The conductivity of a solution depends on temperature. When the temperature of a solution rises one degree, the conductivity rises about 2% to 2.5%, (depending on ionic composition) because of decreasing ion hydration and decreasing solution viscosity. To ensure consistent laboratory conductivity measurements, the GAW recommends either of the following procedures:

5) a) Measure the conductivity at 25°C using a water bath to equilibrate and stabilize the temperature. Care must be taken to avoid sample contamination from the water bath.

- OR -

5) b) Measure the conductivity at room temperature so long as the temperature ranges from 18°C to 30°C. Use the built-in temperature sensors and algorithms to correct the readout to 25°C. Check the readout against Table 4.11 to ensure that the result of the internal algorithm is consistent.

- OR -

5) c) Measure the conductivity at room temperature and then correct the measured value to 25°C using the coefficients given in Table 4.11.

Example:

$$\kappa_{25^oC} = \kappa_{RT} \text{ X Correction Coefficient}$$

where: κ_{RT} = conductivity at room temperature

κ_{25^oC} = conductivity at 25°C

- OR -

5) d) Measure a standard solution at the same temperature as the samples to calculate a correction factor which accounts for both cell constant and temperature effects. This method is a one-point calibration method that assumes the calibration curve is linear throughout. Using three calibrators describes the calibration curve more accurately accounting for non-linearity.

6) Enter data into the data set. See section 4.3.13 for manual data verification if the conductivity meter does not have a data export function. Report values to the required decimal places and account for missing samples and contamination by adding comments or codes to the sample result.

Table 4.11. Correction coefficients to adjust conductivity values to 25°C

Temperature (°C)	Correction coefficient
18	0.865
19	0.884
20	0.904
21	0.923
22	0.940
23	0.961
24	0.980
25	1.000
26	1.020
27	1.041
28	1.061
29	1.080
30	1.100

7) Follow the manufacturer's directions for operation, maintenance, and storage of the measurement cell.

8) Between each sample, rinse the cell thoroughly with DI water and then rinse with the sample solution 2 or 3 times before taking a measurement. (If it is possible that the cell has become contaminated, see Problem 1 in the section on Troubleshooting for corrective actions.)

9) Store the probe per manufacturer's recommendations.

Troubleshooting

Problem 1: An accumulation of ionic species or an organic film forms on or near the electrode surface causing polarization and contamination.
Solution 1: Clean the cell often. Carefully remove any accumulation by gently wiping the cell with a cotton swab soaked in a mild detergent. Do not use harsh or abrasive materials for cleaning and never touch the probe with bare hands. Thoroughly rinse the probe and poles with DI water to remove all traces of detergent.

Problem 2: Measurements are frequently biased and it is difficult to get correct results.
Solution 2: Calibrate every 30 to 50 samples. Be aware that the cell constant may change. Make sure that there is adequate sample to completely cover the poles. A two-pole cell must be centered in the measuring vessel. Avoid rubbing the probe; pat it dry to avoid static buildup or damage to the probe.

Problem 3: Measurements are affected by temperature differences between samples and standards.
Solution 3: Ensure that the samples and calibration standards are measured at the same temperature and that stirring is the same for both samples and calibration standards.

Problem 4: The cell surface can be sufficiently porous to adsorb ions from solution leading to a carry-over effect from one sample to the next and resulting in a slower response.
Solution 4: A conductivity sensor with an epoxy body and graphite electrodes is recommended for its durability and superior chemical resistance. Also consider a cell with a steel or titanium body.

Problem 5: Air bubbles in the cell result in unstable readings.
Solution 5: Even a tiny air bubble adhering to the electrode surface increases the resistance, which lowers the conductivity reading. Inspect the cell contents and remove air bubbles before every measurement, whether a precipitation sample, a calibration solution, or a QC sample. Tap the probe lightly to dislodge bubbles.

Problem 6: Calibrating the cell yields inconsistent results.
Solution 6: Conductivity calibration standards are not as robust as pH buffer solutions and are more prone to contamination from dilution and from carbon dioxide entering from atmospheric exposure. Use freshly prepared solutions. Never pour used solutions back into the original containers. Use containers with a tight seal and store solutions at room temperature.

4.5.5 Chloride, Nitrate, and Sulfate Determination by Ion Chromatography

Background

Ion chromatography (IC) is recommended for measurements of chloride, nitrate, and sulfate. In general, IC systems are robust, require low maintenance and can be adjusted to optimize results. Optimizing the system for sensitivity and range can be done by changing the columns, altering the eluent concentration, increasing the injection loop size to increase the volume, and by using the proper range of calibration standards. It is recommended that the IC system be equipped with a programmable sample changer, which makes it unnecessary for an operator to be in constant attendance to inject a sample aliquot.

In an IC, a sample aliquot is pumped through a separator column where the ions of interest are separated because of their differential affinities for the ion exchange material in the column. When anions are the species of interest, the ion exchange material is positively charged. The strength of the interaction between the oppositely charged ionic groups in the molecules of the sample and the ion exchange material depends on the number, valence, and location of the charges on these molecules.

Anions in the sample are carried through the separator column by a salt solution called an eluent. The composition and concentration of the eluent affects the rate at which the anions are loosed from their bond with the ion exchange material and continue to pass through the column. Molecules with the weakest ionic interactions elute from the column first. Molecules with a stronger ionic interaction elute later. As anions elute from the column, they pass through a suppressor and ultimately are sensed by an electrolytic conductivity detector. The conductivity of the eluate is recorded in a chromatogram.

There are two types of eluents commonly used for inorganic anion chromatography: potassium hydroxide and sodium carbonate/bicarbonate. Carbonate eluents produce a high background conductivity. As a result, a sudden drop in conductivity occurs when the sample water passes out of the column. This sudden drop results in a “water dip” in the chromatogram. This dip occurs just before the chloride peak at the beginning of the chromatogram. The elution order for anions separated by carbonate eluents is chloride, nitrate then sulfate. Carbonate eluents require higher currents in the suppressor. With carbonate eluents, the separator columns can withstand large injection volumes and are not easily overloaded.

Hydroxyl eluents have a low background conductivity thus chromatograms have no water dip. The elution order for anions separated by hydroxyl eluents is chloride, sulfate then nitrate. The sulfate peak occurs next to the carbonate peak.

Self-regenerating systems come with concentrated eluent contained in a cartridge that is installed in the system. High purity Type I DI water is the only additive. The cartridges are self-contained and do not require any reagent preparation, eliminating batch to batch differences.

After the eluent passes through the separator column, it enters the suppressor, which decreases the conductivity of the eluent. It does this by changing the eluent salts to acids, thereby suppressing the background conductivity of the eluent effectively enhancing the conductivity of the ions being measured. In short, the suppressor improves sensitivity by increasing the signal strength and reducing the background noise. In a regenerating suppressor, this process is then reversed by restoring the original salt solution of the eluent.

Two types of pumps are available for IC systems. Isocratic pumps deliver eluent at one flow rate and concentration with no variability. Gradient pumps can alter the flow and concentration of eluent to enhance peak separation. Gradient pumps are easily programmable through accompanying software and are recommended for organic acids. For simple inorganic ions, isocratic pumps are robust and reliable.

The time required for analysis and the quality and separation of the signal peaks are dependent on the type of column, the type of eluent, and the concentration and flow rate of the eluent. Figure 4.24 depicts a typical IC system. The circular valve in the figure has two positions, LOAD and INJECT. The caption describes the LOAD position. In the INJECT position, eluent is pumped through the sample loop carrying the sample through the columns, where ion separation occurs. Table 4.12 lists the concentration range for each anion using the method described later in this section.

Table 4.12. Operating range of anions in precipitation using standards described in Section 4.5.5-Standards

Analyte	Concentration Range (mg L^{-1})
Chloride	0.005 to 7.00
Nitrate	0.005 to 12.50
Sulfate	0.005 to 12.50

There are three types of IC columns: standard-bore (4mm), micro-bore (2mm) and capillary (0.4mm). (Dionex Corp, Thermo Scientific, 2012).
Although smaller bore columns can clog more easily, they offer several advantages:

i. smaller injection loops require less sample volume for analysis
ii. lower volumes of reagents needed
iii. sharper peaks
iv. better peak separation

Apparatus

In general, an IC system consists of:

i. IC with isocratic pump, conductivity detector, sample loop and injection system
ii. automatic sample changer with software
iii. desktop computer
iv. chromatography workstation (integration software)
v. self-regenerating suppressor – anions
vi. analytical column (anions)
vii. guard column (anions)
viii. eluent generator cartridge (potassium hydroxide or carbonate)

Use low pressure inline filters (Figure 4.22) to keep bacteria and contaminants out of the IC system. 2 µm pore frits (Figure 4.23) can be cleaned by sonication and reused up to twenty times. Upchurch products supplied through Millipore Sigma-Aldrich is a suggested manufacturer.

Place the filter between the sample changer and the injection valve.

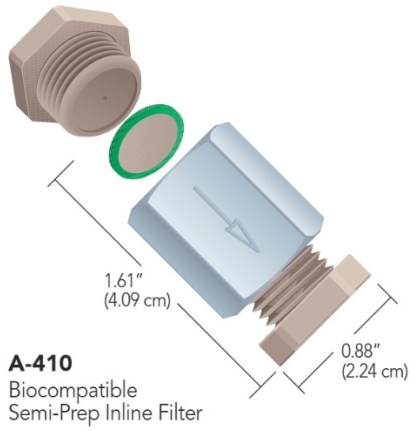


Figure 4.22. Example of an inline filter assembly (Gilson Scientific Limited, 2013)

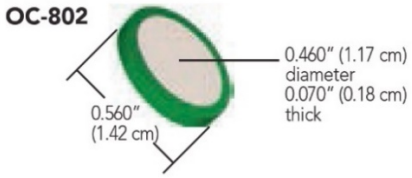


Figure 4.23. Example of in-line 2 um frit (Gilson Scientific Limited, 2013)

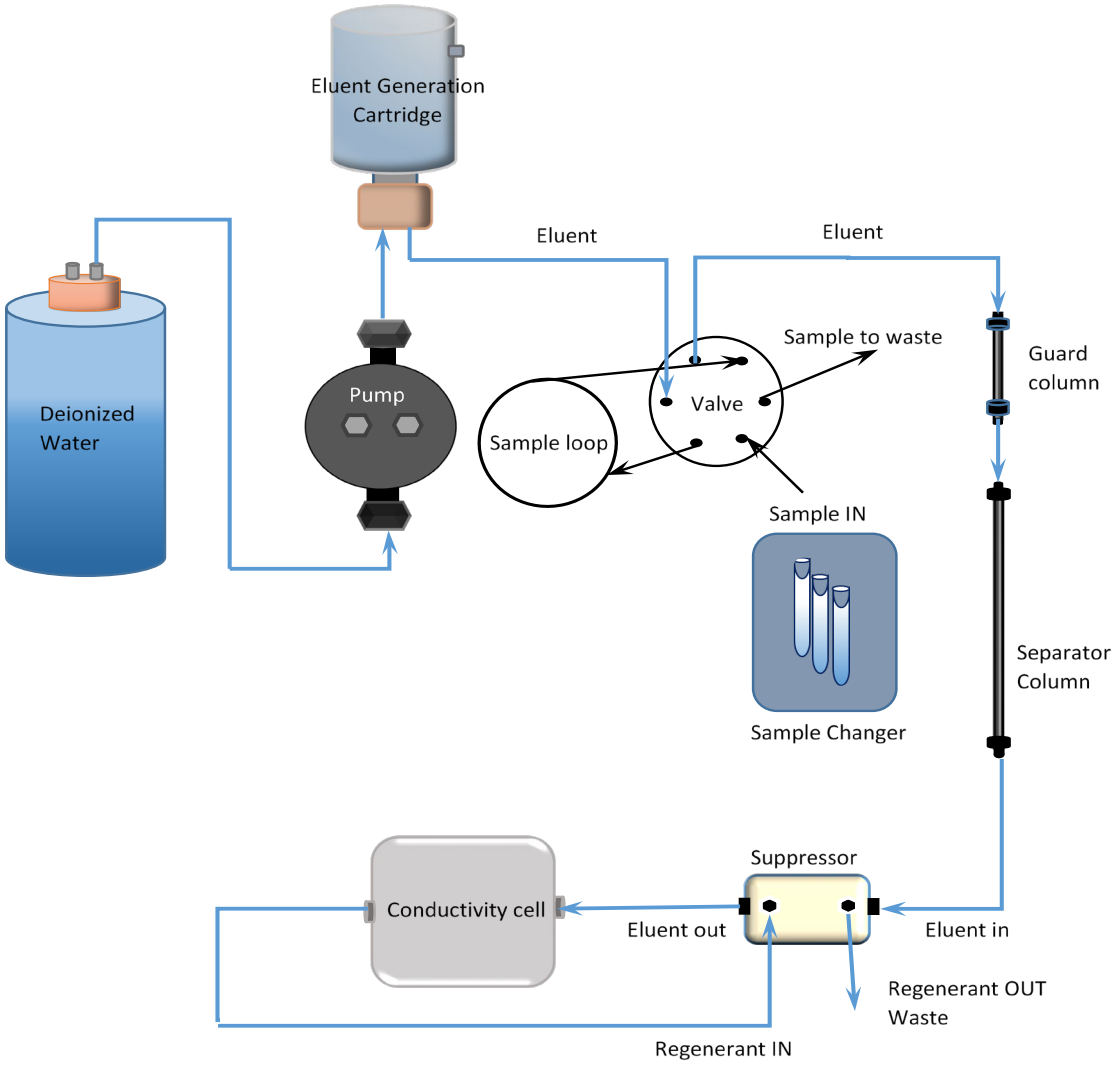


Figure 4.24. Typical IC system with injection valve in LOAD position. Sample enters sample loop while eluent is pumped through columns.

Reagents and Solutions

i. Self-regenerating systems only require an eluent generation cartridge.
ii. Ultra-pure Type I DI water (resistivity >18MΩ).

Calibration

Stock Standard Solutions

Stock standard solutions each containing 1000 mg L^{-1} of chloride, nitrate and sulfate either may be purchased as certified solutions or prepared from high purity salts. When preparing the standard solutions from salts, be sure to dry the salts at 105°C for an hour before dissolving them in DI water and diluting to 1000 mL. Table 4.13 lists the masses of dried salts to use in preparing stock standard solutions.

New flasks and bottles used as containers for stock standard solutions need to be conditioned. This is done by soaking them in DI water over night, then rinsing them three times with DI water and drying them in a warm oven. This conditioning only needs to be performed the first time that new containers are put into service. See Appendix C for calibration procedures for flasks and analytical balances.

1) Weigh all volumes using an analytical balance. Rinse all weigh boats thoroughly. Use conditioned HDPE bottles to store stock standard solutions. Use containers that are dedicated solely to standard solution preparation and storage and not for other procedures.

2) Prepare standard solutions by weighing the DI water volume. Calibrate the receiving flasks by dispensing DI water by weight into the flask and then marking the flask at the fluid line. See Appendix C for details.

3) Make three stock solutions. Weigh each salt carefully into a calibrated and conditioned 1 L volumetric flask. Mix and store in designated, conditioned HDPE bottles. Stable for one year.

4) To ensure consistency between old and new stock standard solutions, prepare a dilution of the new stock standard solution and analyze it as an unknown, using old calibration standards to calibrate the instrument. Here is a step-by-step procedure:

i. Into a rinsed weigh boat dispense 1 gm of new stock standard solution.
ii. Pour this solution into a clean, rinsed and calibrated 1 L volumetric flask.
iii. Using Type I DI water, rinse the weigh boat into the flask and fill the flask to the 1 L mark.
iv. Mix well then allow the solution to stand and equilibrate for at least one hour.
v. Analyze this new stock standard solution but calibrate the IC using the old calibration standards.
vi. Measurement should fall within the expected range of precision around 1.00 mg L^{-1}.
vii. If this diluted stock standard solution meets the 1.00 mg L^{-1} QC specification, transfer the full strength (1000 mg L^{-1}) new stock standard solution to an HDPE flask and store at 4° C. If this specification is not met, discard the solution and start the preparation again. Remember to allow the solution to stand (equilibrate) for one hour before analysis.

Table 4.13. Anion Stock Standard Solutions, Standard 1. The masses specified in the table result in 1000 mg L^{-1} of Cl^-, $NO3^-$ and $SO_4^=$. (CAPMoN, 2013)

Salt	Weight (g)
NaCl	1.648
KNO_3	1.628
$(NH_4)_2SO_4$	1.375

Dispensing large volumes of stock solution to make working calibration standards is a more accurate procedure than dispensing concentrated stock solutions in small volumes.

Low Working Standard1

1) Prepare Low Working Standard 1 (L-Std 1) by dispensing each stock standard solution by weight into a calibrated, conditioned 1 L volumetric flask. The volumes are specified in table 4.14. Dilute to 1 L with DI water.

Table 4.14. Preparation of L-Std 1

Low Std. #	Solution	Cl^- (mL)	NO_3^- (mL)	SO_4^{2-} (mL)	Final Volume (mL)
1	Each stock standard	0.500	1.250	1.250	1000

2) Use L-Std 1 to prepare low-range calibration standards 2 through 6, listed in table 4.16. All flasks are conditioned, calibrated and designated for storing L-Std 1 solution.

High Working Standard1

1) Prepare High Working Standard 1 (H-Std 1) by dispensing each stock standard solution by weight into a calibrated, conditioned 1 L volumetric flask. The volumes are specified in table 4.15. Dilute to 1 L with DI water.

Table 4.15. Preparation of H-Std 1

High Std. #	Solution	Cl^- (mL)	NO_3^- (mL)	SO_4^{2-} (mL)	Final Volume (mL)
1	Each stock Standard	7.000	12.500	12.500	1000

2) Use H-Std 1 to prepare high-range calibration standards 2 through 5, listed in able 4.17. All flasks are conditioned, calibrated and designated for storing H-Std 1.

IC system software should be capable of addressing two calibration ranges (low and high) in one analytical run.

Run all samples in the low calibration range. For values above the low range, use the high calibration range. Only dilute samples with concentrations above the high range.

Table 4.16. Example of low range anion calibration standards (CAPMoN, 2013)

Low Std. #	Solution	Volume (mL)	Final Volume (mL)	Cl^- (mg L^{-1})	NO_3^- (mg L^{-1})	SO_4^{2-} (mg L^{-1})
1	stock			0.500	1.250	1.250
2	L Std. 1	175	250	0.350	0.875	0.875
3	L Std. 1	125	250	0.250	0.625	0.625
4	L Std. 1	75	250	0.150	0.375	0.375
5	L Std. 1	40	250	0.080	0.200	0.200
6	L Std. 1	12.5	250	0.025	0.063	0.063

Table 4.17. Example of high range anion calibration standards (CAPMoN, 2013)

High Std. #	Solution	Volume (mL)	Final Volume (mL)	Cl^- (mgL^{-1})	NO_3^- (mgL^{-1})	SO_4^{2-} (mgL^{-1})
1	Stock			7.000	12.500	12.500
2	H Std. 1	175	250	4.900	8.750	8.750
3	H Std. 1	90	250	2.520	4.500	4.500
4	H Std. 1	45	250	1.260	2.250	2.250
5	H Std. 1	25	250	0.700	1.250	1.250

Working standard Solutions

A minimum of five calibration standards per calibration curve is recommended. IC curves are not linear and often do not go through zero. Most IC workstations allow for an unlimited number of standards. To minimize the biases due to this nonlinearity, prepare IC curves in two sections: a low calibration range and a high calibration range (see figures 4.25 and 4.26). This is very important so that the calibration curves do not extend to concentrations where the results become skewed due to nonlinearity.

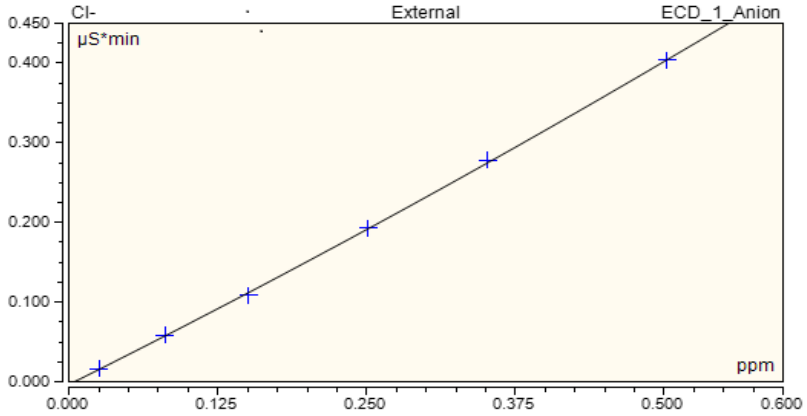


Figure 4.25. Low calibration curve for chloride

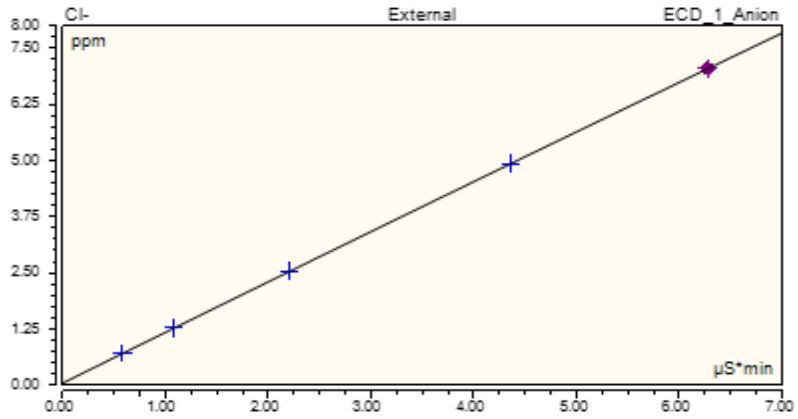


Figure 4.26. High calibration curve for chloride

Integrate chromatography data using peak area. Most IC curves are not linear and are best described by a quadratic fit.

Measure all samples against the low calibration standards. Results that exceed the low calibration range are read using the high calibration range. Sample concentrations that exceed high calibration limits must be diluted and reanalyzed. **Never extrapolate the calibration curve to estimate results.** The ranges of measured anion concentrations must be established by each laboratory and may vary over time.

Calibration standards may be stored in clean HDPE containers at room temperature and are stable for up to six weeks.

Quality Control

Preparing QC Solutions

Prepare two QC solutions, one for the low calibration range and one for the high calibration range. Analyze a low QC sample immediately after the IC is calibrated in the low range. Do the same in the high calibration range using the high QC solution. See Appendix C for details on sterilization and preparation of QC solutions.

Low QC Solutions – Precipitation Matrix

1) Save the excess volume from low-concentration precipitation samples that have been analyzed and reported. Pool the excess precipitation from some of these samples into a 10 L HDPE container and the excess from other samples into a second 10 L HDPE container.

2) Analyze the pooled samples from each container. Examine the results and designate the pooled sample with the lower concentration for each analyte as QC-A and the other pooled sample as QC-B.

3) Add DI water as needed to bring the concentration of QC-A near the detection limit. Add 1000 mg L^{-1} stock solution as needed to bring the concentration of QC-B to the mid to high range of the low calibration curve.

4) See Appendix C for sterilization and further details.

High QC Solutions – Precipitation Matrix

1) Save the excess volume from high-concentration precipitation samples that have been analyzed and reported. Pool the excess precipitation from some of these samples into a 10 L HDPE container and the excess from other samples into a second 10 L HDPE container.

2) Analyze the pooled samples from each container. Designate one of the pooled samples as QC-C and the other as QC-D.

3) Add 1000 mg L^{-1} stock solution as needed to bring the concentration of QC-C to the low to mid-range of the high calibration curve and QC-D to the mid to high range of the high calibration curve. Avoid a concentration that is higher than the highest calibration standard.

4) See Appendix C for sterilization and further details.

Analytical Procedures

1) Do not power down an IC system when not in use. Always leave the power on.

2) Check reagent levels. Check the fluid and ion percent in the eluent cartridge and ensure there is adequate eluent for a full run. Change the DI water in the flush reservoir of the sample changer every day. Inline filters may be used to minimize the introduction of particulate matter into the system. Change inline filters daily.

3) Run DI water samples until the system is stable and equilibrated.

4) Label each tube. Prepare a schedule of analysis in the workstation software. Enter sample identification numbers into the software in the same order as the tubes will be installed in the sample changer rack.

5) Prepare samples for analysis. Make sure that each tube has a minimum volume. Minimum volumes will vary according to injection loop size and loop rinse. Cover each tube opening with Parafilm® or with a cap that can be pierced. Place the tubes in order in the sample changer rack.

6) Check for a stable pump pressure and conductivity.

7) Check the DI water chromatograms for the correct shape. The shape of the chromatogram depends on the eluent type. (a) When a carbonate eluent is used, the chromatogram will have a water dip usually two to three minutes into the chromatogram. The water dip occurs right before the chloride peak. See Figure 4.27 for the DI water chromatogram using carbonate eluent. Note that the scale on the Y-axis is -4.50 to 0.800. (b) When the KOH eluent is used, the water dip is much smaller. See Figure 4.28 for the DI water chromatogram using KOH eluent. Note that the scale on the Y-axis is -0.300 to 0.800. Note also that the DI water chromatogram has a carbonate peak positioned after chloride and before sulfate. The height of the carbonate peak will depend on water quality, the age of the water and ambient room carbon dioxide levels. **DI water chromatograms must be free of the anions of interest before starting the analytical run.**

8) Start the run. Run calibration standards first. The injection should start with the highest concentration standard followed by decreasing concentrations.

9) Run a low QCS directly after completing the low calibration curve and a high QCS after completing the high calibration curve. Inject a QCS (randomly selected, high or low) every ten samples thereafter. Plot the QCS results on control charts.

10) Calibrate every 30 to 50 samples.

11) Following the run, check all calibration curves and QCS results before reporting, collating or tabulating sample results. Use only the peak area, not the peak height, for calculating results.

12) Examine each chromatogram individually for correct shape and integration. The baseline must not drift up or down and must not be bumpy. All carbonate chromatograms should have a water dip and all hydroxyl chromatograms should have a carbonate peak. The peaks should all have a typical Gaussian shape and show good separation from each other. Comment on all anomalies and flag data accordingly. Repeat samples that have drifting or bumpy baselines after resolving the cause. See Troubleshooting, below.

    Note that IC software uses peak 'windows'. The software expects the peak for each analyte to elute in a certain window of time. Peaks that fall outside this window will not be integrated. Also, very large peaks that fill the window may not be recognized and thus produce a zero result. Make note of these exceptions and repeat the analysis.

13) Calculate the results against the appropriate calibration curve. Use the correct decimal places. Apply detection limit notations as needed. Mark all samples that exceed the upper calibration ranges. Dilute these samples and repeat the analysis. Account for any missing samples and ensure contamination codes are applied as needed.

14) Export the data from the IC system and archive all parameters associated with the analysis, including calibration data, integration data, and instrument audit trails. Audit trails include instrument parameters (e.g., pump pressure) that may be useful in diagnosing a problem, such as a chromatogram with a drifting baseline. It may be necessary to repeat the analysis at the point where the problem began.

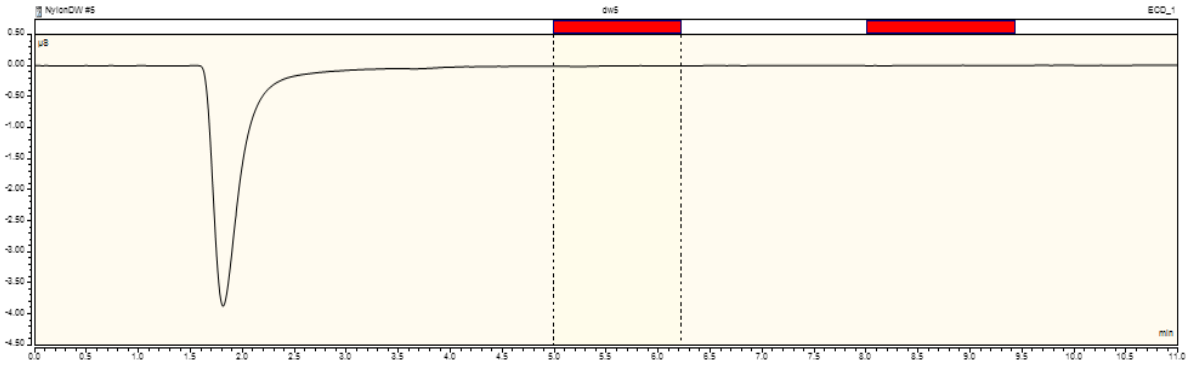


Figure 4.27. Deionized water chromatogram using carbonate eluent

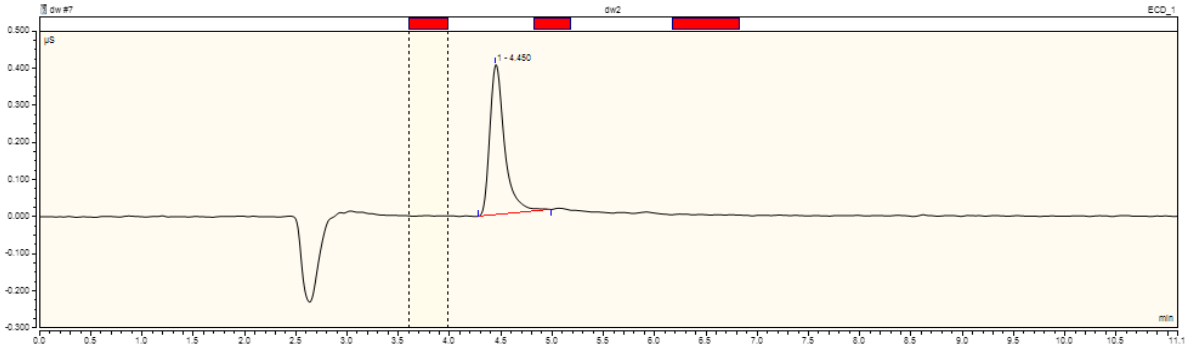


Figure 4.28. Deionized water chromatogram using KOH eluent

Troubleshooting

Problem 1: Pump loses pressure or prime.
Solution 1: Check the EluGen® cartridge for leaks. Change the cartridge if required. Prime the pump. Check the system for leakage. Re-prime the pump and run DI water to check the system. If the pump is still unstable, disconnect the column and pump methanol through the system. Flush with water. If these steps do not eliminate the problem, change the piston seals (provided the operator has been trained to do so). Soak the piston seals in methanol for a few minutes. This ensures a better seal around the piston.

Problem 2: Rough or drifting baseline (Figure 4.29)
Solution 2: Microbore systems take longer to stabilize. Wait several hours before attempting to resolve the problem. If the baseline doesn't' stabilize, recondition the suppressor. The reconditioning procedure is the same as the procedure used to condition new suppressors prior to installation. Instructions are in the suppressor packaging. Replace the suppressor if reconditioning does not work.

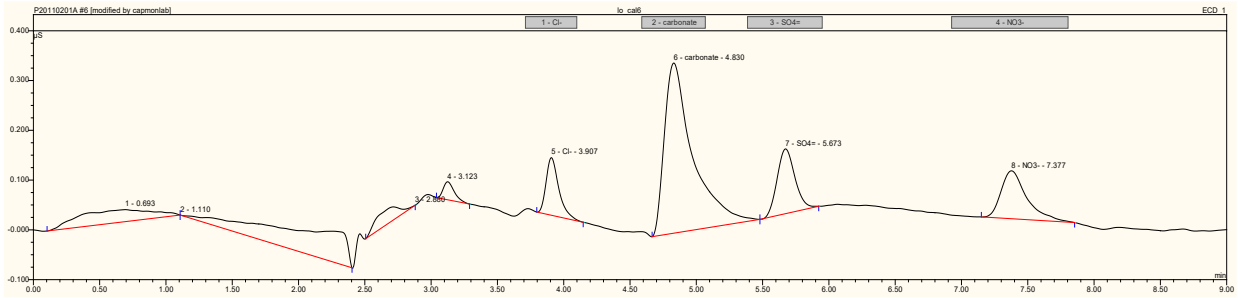


Figure 4.29. Rough baseline

Problem 3: Precision not meeting QC specifications.
Solution 3: Check the injection valve for leaks or blocks. Make sure the sample loop is filling with each injection. The sample loop should rinse about ten times with sample to ensure that there is absolutely no mixing of a sample with the previous sample or with rinsate. Check this by placing the sample loop waste line from the injection valve into a graduated cylinder. Introduce a sample from the sample changer. The volume collected in the cylinder should be about ten times the injection loop volume.

Problem 4: Loss of precision.
Solution 4: Check the injection valve for leaks or blocks. Check the probe and sample lines for plugs or leaks. Change the sample loop and clean the injection valve. A plug can be found by disconnecting each length of tubing one section at a time. The pump pressure will increase significantly if the bed supports are fouled or the guard column is fouled.

Problem 5: Low sulfate values but other species in same sample are within expected range.
Solution 5: Change the sample changer probe and all sample delivery lines.

Problem 6: Anion that elutes with a retention time like chloride, sulfate, or nitrate, potentially interfering with the signal for one of these anions. See example in Figure 4.30.
Solution 6: Resolve the peak using the IC integration software, if possible, otherwise try running the sample with less concentrated eluent. Protocols describing a corrective action should be described in the standard operating procedure.

Problem 7: Retention times get shorter and the resolution is poor. Peaks fall short of their expected windows and retention times. Pump pressure increases.
Solution 7: Clean or change the guard column. If there is no improvement clean or change the separator column.

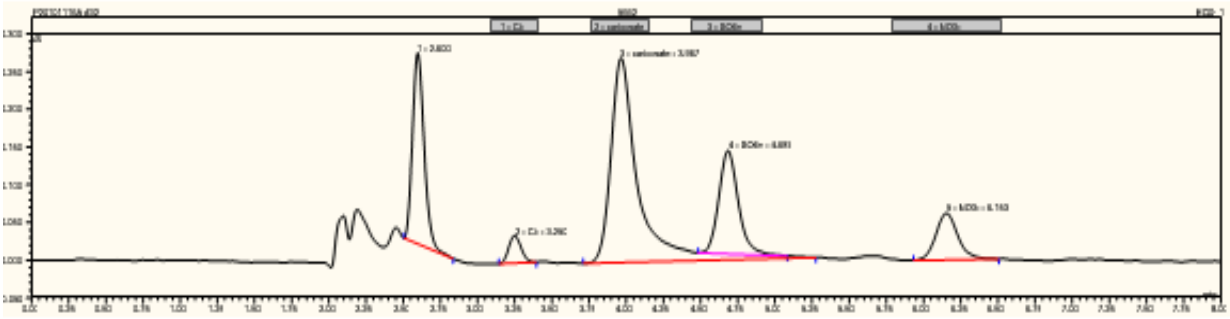


Figure 4.30. Chromatogram from KOH eluent system. Note crowding of carbonate and sulfate peaks, where the sulfate is lifted off the baseline by the shoulder on the carbonate peak. Note that the organic acids eluting early in the chromatogram are also crowded together and unresolved.

Problem 8: Several chromatograms in a row have no water dip (carbonate eluent) or carbonate peak (KOH eluent). Chromatograms have no peaks for any anions.
Solution 8: Check the probe for plugs. Check the sample lines for plugs right up to the injection valve and then check the injection loop for blocks.

Problem 9: Species elute too late and are seen eluting in the following chromatogram.
Solution 9: Extend the run time. Sometimes the run times and retention times can lengthen significantly when new columns are put into service. It is important to test the system with calibration standards to sort out where the new retentions occur.

Tips to Improve IC Performance

1. Develop a maintenance program and keep a logbook that records maintenance procedures. Keep track of instrument breakdowns and actions taken to resolve the problem.
2. Perform a complete system preventative maintenance once a year. Change interconnecting tubing, change pump and piston seals, and calibrate the conductivity cell. Injection valve stators should be inspected for wear and changed as required.
3. Use smaller bore columns to increase system efficiency. Modern IC pump systems can be converted to micro-bore without changing the pump.
4. Do not reuse ferrules when changing connecting tubing. Ferrules tighten and restrict flow in PEEK tubing, increasing the system pressure. Keep a logbook of calibration standard details. Record dates that standards are prepared. Show calculations, weights, and volumes. Record the name of the person who prepared the solutions.
5. Calibrate glassware by mass. See Appendix C for details.
6. Mark maintenance periods, eluent cartridge changes, and new calibration solutions on quality control charts and in logbooks to track potential shifts in control chart data.
7. Calibration solutions require dedicated glassware and containers. Do not use these containers for any other purpose. Condition containers by soaking them overnight in deionized water or in the solution that the container will hold.
8. Do not run contaminated or hard water samples using the same columns as precipitation.
9. Cover samples using caps that can be pierced or use Parafilm®. Keep the area clean.
10. Use calibration ranges typical of sample concentrations.
11. If very concentrated samples must be analyzed, reduce the length of the sample loop to minimize column overload or use a higher standard range.
12. Increase sample loop length to increase sensitivity. This increases the injection volume. Experiment with different lengths to optimize the system without overloading the columns.
13. Use inline filters to keep bacteria out of the system.
14. Change bed supports and guard columns as a first step when system pressures increase.

4.5.6 Hydrogen Carbonate by Ion Exclusion Chromatography

Bicarbonate ion is now termed hydrogen carbonate per IUPAC nomenclature.

Background

Hydrogen carbonate is a weak acid that is present in precipitation as a result of dissolved carbon dioxide and dissolved carbonate minerals that are captured by precipitation as tiny airborne particles. The extent to which hydrogen carbonate affects the ion balance of precipitation samples is highly dependent on pH. Hydrogen carbonate concentrations increase as the inverse of free acidity (H^+). Above a pH of ~5.6 hydrogen carbonate concentrations exceed H^+ concentrations. For this reason, it is important for laboratories to have the capability to measure hydrogen carbonate if the precipitation pH in their region is frequently above pH 6. This occurs in regions unaffected by substantial acidic sulfate or nitrate and where airborne carbonate dust particles are prevalent.

The methodology for the determination of hydrogen carbonate needs to be fast and easy. Automated digital titrators for alkalinity are available but suffer from interferences from anions such as carboxylic acids that contribute to systematic errors in titrimetric determinations. (Gros & Nemarnik, 2007) Hydrogen carbonate levels in rainwater are usually less than 10.22 mg L^{-1} and a sensitive system is needed to make accurate quantification of this weak acid. Generally, anion chromatographic systems require a strong basic carbonate or hydroxyl eluent. These eluents result in a high background conductivity that must be suppressed if the anion peaks are to appear sufficiently above the background to be quantified accurately. These systems work well for strong acids (hydrochloric, sulfuric, nitric) but not for weak carboxylic acids.

Ion exclusion chromatography offers an alternative but the acidic eluents used in this method suppress ionization of the carbonate fraction thereby decreasing sensitivity. The solution is non-suppressed ion exclusion chromatography which uses deionized water as the eluent. This method uses an ICE-AS1 column from Dionex Corporation; however, comparable products from other companies can provide similar results. A dedicated IC system is required for this method. (Gardrat, 2016)

Hydrogen carbonate analysis requires scrupulous attention to sources of carbonate and carbon dioxide contamination. Laboratory air may contain carbon dioxide levels that are above outside ambient levels. Testing the laboratory ambient air may be necessary to determine if hydrogen carbonate analysis can yield representative measurements under these conditions.

Hydrogen carbonate can also be estimated by calculation from pH and airborne carbon dioxide concentrations. See ion balance calculations in section 4.3.14 for a description of how to do this.

Apparatus

Carbon dioxide in the eluent can affect the linearity of the calibration and skew the results. Removing and keeping carbon dioxide out of the eluent is essential.

There are several approaches for removing gases from solutions, such as eluents:

Offline Degassing

i. Boil DI water (eluent) for 15 minutes.
ii. Sparge DI water using helium for 30 minutes (Gardrat, 2016).

iii. Sonicate DI water under vacuum for 15 minutes (Gros & Nemarnik, 2007).

Offline methods do not maintain the degassed condition. Within a few hours, gases will diffuse back into solution. It is necessary to prepare fresh eluent daily and to maintain its purity by immediately pressurizing the eluent container at 2 to 5 psi with helium.

Online Degassing

Displace the air in solution by bubbling helium through the eluent reservoir before and during the analytical run. Helium replaces the air. (Waters Corperation, 2001)

Inline Degassing

Eluent moves through a vacuum chamber where the vacuum pulls gases through a permeable membrane and the pump expels them. These degassers operate between the eluent reservoir and the pump, minimizing gas reabsorption into the eluent. Flow rate determines the degassing efficiency. Efficiency can be enhanced by sparging the eluent and rinse from the sample changer before introducing them into the IC system. On starting up the system, allow ample time to flush the lines and for the system to stabilize. It also is good to minimize the length of transmission tubing as much as possible throughout the IC system.

In general, the ion exclusion chromatography system (figures 4.31 and 4.32) consists of:

i. IC with isocratic pump, conductivity cell, sample loop and injection system
ii. eluent degasser (optional)
iii. sample changer with software
iv. desktop computer
v. chromatography workstation (integration software)
vi. separator column (9X250mm ICE-AS1 – Dionex)

Reagents and Solutions

i. DI water. It is essential that the water used for eluent be Type I (resistivity of >18.0 MΩ) water from the point of use outlet. It is extremely important that the source of DI water or DI water reservoirs be completely free of bacteria. Use a DI water system equipped with a UV lamp and a 1 µm pore-size filter on the point of use dispensing gun.
ii. Analytical grade helium. Use for sparging eluent and sample changer flush. Use to pressurize the eluent container.

Keep a reservoir of degassed ultra-pure water under a few psi of helium for preparing the matrix of calibration standards. For short term storage of this calibration matrix, use a dedicated, conditioned HDPE container equipped with a stopcock.

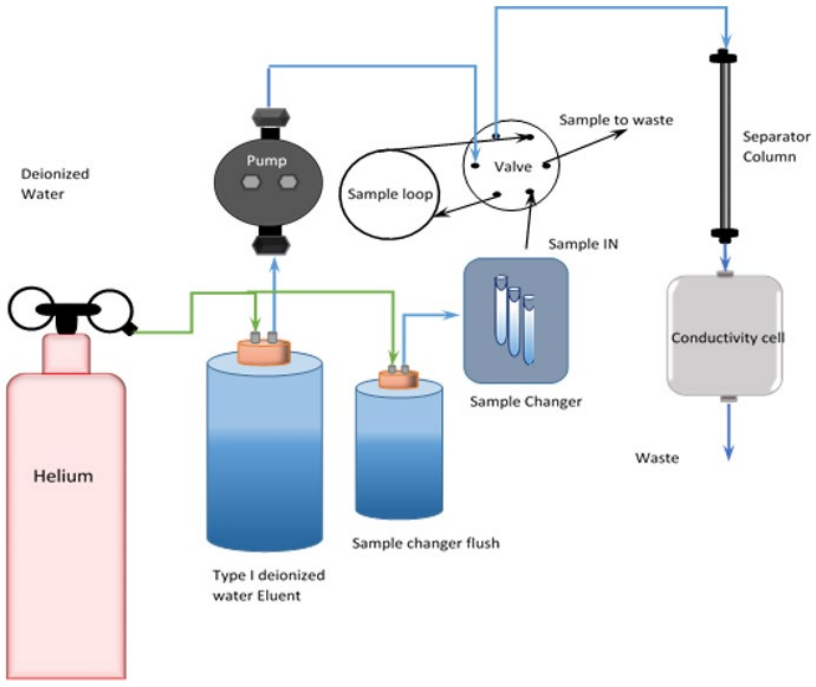


Figure 4.31. Ion Exclusion Chromatography System using pressurized eluent

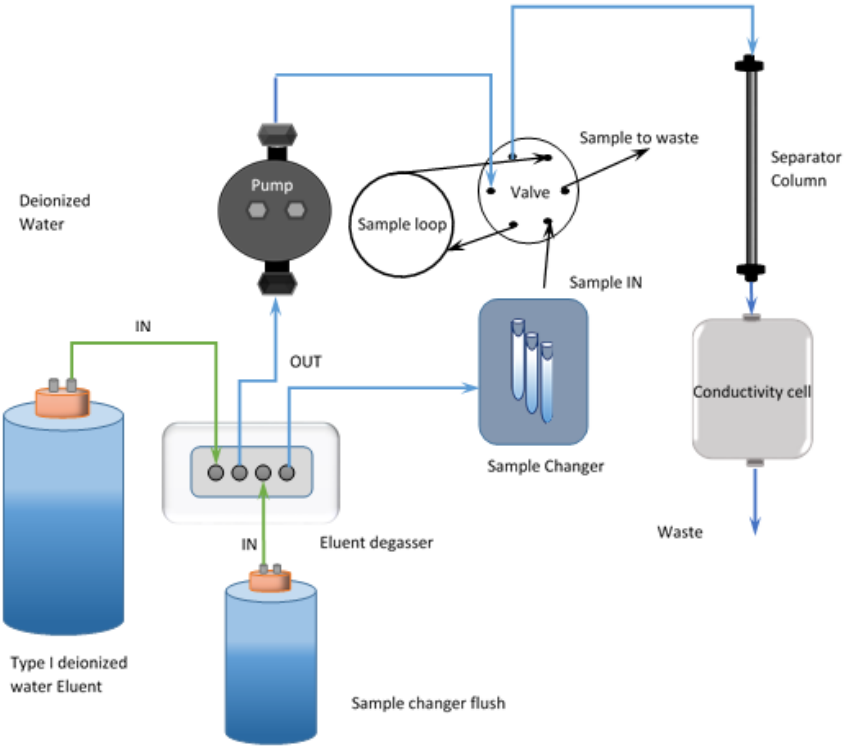


Figure 4.32. Ion Exclusion Chromatography System with inline degasser

Important: Keep sample introduction lines clean. Bacteria from rainwater samples can generate carbon dioxide. Place an inline filter after the sample changer and before the injection valve to prevent bacterial entry into the system. Change sample lines often. See section 4.5.3 for details.

If sample lines must be cleaned, use solutions that do not impact the analysis. When cleaning, disconnect the column from the system. Rinse thoroughly.

Calibration

Use at least five calibration standards per calibration curve. IC curves are quadratic and often do not go through zero. Most chromatography software workstations allow for an unlimited number of standards. To minimize the biases due to this nonlinearity, prepare IC curves in two sections: a low calibration range and a high calibration range. This is very important so that the calibration curves do not extend to concentrations where the results become skewed due to nonlinearity.

Measure all samples against the low calibration standards. Results that exceed the low calibration range are read using the high calibration range. Sample concentrations that exceed high calibration limits must be diluted and reanalyzed. Never extrapolate the calibration curve to estimate results.

Stock Standard Solution

Stock standard solution containing 1000 mg L^{-1} of hydrogen carbonate may be purchased as a certified solution from different manufacturers or prepared from high purity salts. Dry the salts at 105° C for an hour before they are dissolved and diluted to 1000 mL. Table 4.18 lists the mass of dried salts to use in preparing a stock standard solution.

New flasks and bottles used as containers for stock standard solutions need to be conditioned. This is done by soaking them in DI water overnight, then rinsing them three times with DI water and drying them in a warm oven. This conditioning only needs to be performed the first time that new containers are put into service. See Appendix C for calibration procedures for flasks and analytical balances.

It is highly recommended that standard solutions be prepared by weighing the DI water volume. Calibrate the receiving flask by dispensing DI water by weight into the flask and then marking the flask at the fluid line. **Use only degassed DI water as diluent for all calibration standards.** See Appendix C for calibration of flasks and balances and storage details.

Table 4.18. Weight for 1000 mgL^{-1} of stock standard solution of HCO_3

Salt	Weight (g)
$NaHCO_3$	1.377

High Working Standard 1

1) Prepare High Working Standard 1 (H-Std 1) by dispensing the standard stock solution by weight into a calibrated, conditioned 1 L volumetric flask. The volume is specified in table 4.19. Dilute to 1 L with degassed DI water.

2) To ensure consistency between old and new stock standard solutions, prepare a dilution of the new stock standard solution and analyze it as an unknown, using old calibration standards to calibrate the instrument.

i. Into a rinsed weigh boat dispense 1 gm of new stock standard solution.
ii. Pour this solution into a clean, rinsed and calibrated 1 L volumetric flask.
iii. Using Type I DI water, rinse the weigh boat into the flask and fill the flask to the 1 L mark.
iv. Mix well and allow the solution to equilibrate for at least one hour.
v. Analyze this diluted new stock standard solution using the old calibration set.
vi. Measurements should fall within the expected range of precision around 1.00 mgL^{-1}.
vii. If this diluted stock standard solution meets the 1.00 mgL^{-1} QC specification, transfer the full strength (1000 mgL^{-1}) new stock standard to an HDPE flask and store at 4°C. If this specification is not met, discard the solution and start the preparation again.

Table 4.19. Preparation of H-Std 1

High Std. #	Solution	HCO_3^- (mL)	Final Volume (mL)
1	stock	12.500	1000

3) Use H-Std 1 to prepare all calibration standards in both the high and low ranges. All flasks must be conditioned, calibrated, and specially designated for standard solutions.

Calibration Standards

The ranges of hydrogen carbonate concentration must be established by each individual laboratory and may vary over time (see tables 4.20 and 4.21).

Weigh all volumes using an analytical balance. Rinse all weigh boats thoroughly. Use only conditioned HDPE bottles to store calibration standards. Use containers that are dedicated to calibration standard preparation and storage. Do not use these containers for any other procedures. Use bottles and tubes that can be filled to the top with little or no head space and seal tightly.

Prepare working standards fresh every day. Use only degassed DI water as diluent for all standards.

Table 4.20. Example of low-range hydrogen carbonate calibration standards.

Low Std. #	Volume H-Std 1 (mL)	Final Volume (mL)	HCO_3^- (mgL^{-1})
1	10.000	100	1.250
2	7.500	100	0.938
3	5.000	100	0.625
4	2.500	100	0.313
5	1.000	100	0.125
6	0.500	100	0.063

Table 4.21. Example of high-range hydrogen carbonate calibration standards

High Std. #	Volume H-Std 1 (mL)	Final Volume (mL)	HCO_3^- (mgL^{-1})
1			12.500
2	64.000	100	8.000
3	36.000	100	4.500
4	18.000	100	2.250
5	10.000	100	1.250

Quality Control

Do not use natural precipitation for QC purposes. Natural precipitation will deteriorate with time, even if refrigerated. Here are some acceptable QC solutions.

- Prepare simulated precipitation samples at hydrogen carbonate concentrations different than the calibration standards. Prepare these samples fresh every day.
- Use a recovery spike to determine if the analysis has yielded good data.
- Run ultra-pure DI water as a control sample.

Prepare at least two QC solutions, one for each calibration range.

Pay scrupulous attention to sources of contamination. Never top up an old reagent with new reagent. Ensure all systems are free of microbial growth. Use only sterile tubes and sample containers.

Analytical Procedure

Sample integrity is important. Care for the samples as follows:

i. Collect samples in a clean, sterile bottle with a tight seal.
ii. Completely fill and immediately cap the bottle.
iii. Prevent agitation and exposure to air.
iv. Analyze as soon as possible, ideally within 24 hours of receiving the sample.
v. If analysis cannot be performed within 24 hours, store the sample in a tightly sealed container at 4°C. Alternatively, transfer an aliquot specifically held for hydrogen carbonate analysis into a tightly sealed tube or container and either refrigerate the aliquot at 4°C or freeze it at -20°C. If freezing is the storage method of choice, be sure to leave head space for the liquid to expand when frozen. Also, frozen samples must be brought to room temperature and mixed well prior to analysis.

1) Do not power down the IC system during off times. Leave the power on according to manufacturer recommendations.

2) Laboratory carbon dioxide increases during work hours. Prepare fresh standards and start the run in the late afternoon so that the analysis occurs overnight.

Consider running two IC systems using one sample changer and one workstation. Use a T-junction to split the sample into two streams before the injection valve of each system.

Running a dual system saves time and sample volume. It is convenient to run systems together that have similar run times.

3) Fill the eluent container and sample changer flush reservoir with ultra-pure DI water and sparge both for 15 minutes using helium. Connect the container and reservoir to their respective systems and pressurize to 2 to 5 psi with helium. By using a T-junction after the pressure gauge, the helium tank can be used to pressurize both the eluent container and sample changer flush reservoir at the same time.

4) Check the sample changer flush reservoir for green bacterial growth. If there is evidence of bacterial growth, replace the reservoir with a new one. Bacteria can invade even tiny plastic crevices, making it impractical to clean and re-use the reservoir, once it is contaminated.

5) Inline filters may be used to minimize the introduction of particles into the system. Change inline filters daily. If inline filters are not used, inspect lines for bacterial growth and change or clean the lines as needed. Bacterial growth may be green or black and is most often seen in connectors, bed supports and sometimes on the injection valve stator faces. If black mold is found on the injection valve stator face, the face must be changed. It cannot be adequately cleaned of bacteria.

6) Run samples of ultrapure, sparged DI water to ensure the system is stable and equilibrated. The chromatogram for DI water should be a flat line with no water dip (because water is the eluent) and no carbonate peak.

7) Label each tube. Prepare a schedule of analysis in the workstation software. Enter sample identification numbers into the software in the same order as the tubes will be installed in the sample changer rack.

The samples can exchange carbon dioxide with the air. Keep analytical runs short to minimize exposure

8) Prepare samples for analysis. Make sure that each tube has the minimum volume needed for analysis. Minimum volumes will vary according to injection loop size and volume of loop rinse. Cover each tube opening with a cap that can be pierced or with Parafilm®.

9) Initiate the run. Run calibration solutions first. Begin with the highest concentration standard followed by decreasing concentrations.

10) If there is more than one calibration curve, run a low QCS after the low calibration curve and a high QCS after the high calibration curve. Run a QCS chosen at random every five samples thereafter. Plot the results on control charts.

11) Re-calibrate after every 30 samples.

12) Following the run, check all calibration curves and QC results before reporting, collating or tabulating sample results. Use only the peak area parameter, not the peak height, for calculation of results.

13) Examine each chromatogram individually for correct shape and integration. Comment on all anomalies and flag data accordingly. See typical chromatogram in Figure 4.33.

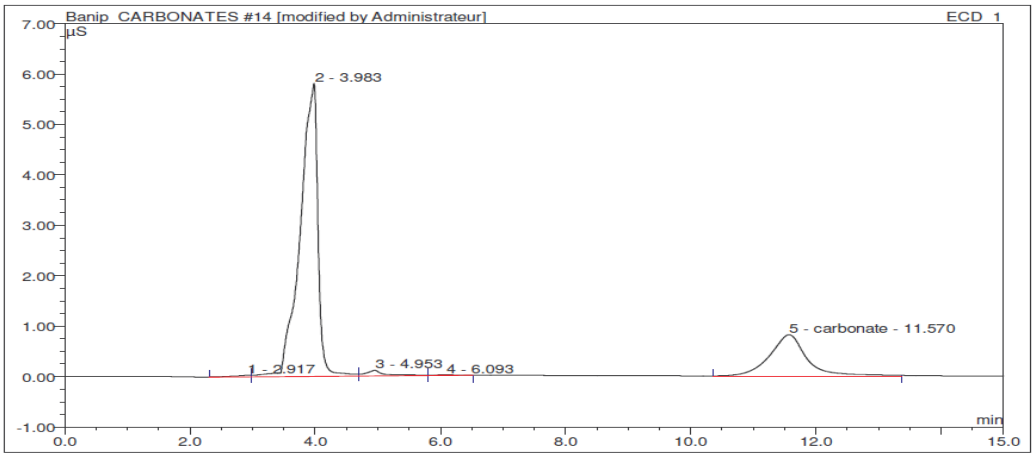


Figure 4.33. Hydrogen carbonate chromatogram using DI water as eluent (Gardrat, 2016)

14) Calculate results against the appropriate calibration curve and apply detection limits and correct decimal places as required. Mark all samples that exceed upper calibration ranges for dilution and repeat analysis. Ensure missing samples are accounted for and contamination codes are applied as needed.

15) Export data from the IC and archive all parameters associated with the analysis including calibration data, integration data and instrument audit trails. Audit trails include instrument parameters (e.g., pump pressure) that may be useful in diagnosing a problem, such as a chromatogram with a drifting baseline. It may be necessary to repeat the analysis at the point where the problem began.

Troubleshooting

Problem 1: Rough baseline. Pump loses prime.
Solution 1: Re-prime the pump and restart the system. Place the DI water eluent container above the chromatography system to improve flow. Check to see if the eluent container is pressurized and make sure the helium tank is not empty. Check for leaks in the system, then re-prime the pump and run DI water to check the system. If the pump is still unstable, disconnect the column and pump methanol through the system. Flush with water. If these steps do not eliminate the problem, change the piston seals (provided the operator has been trained to do so). Soak the piston seals in methanol for a few minutes. This ensures a better seal around the piston.

Problem 2: Results are higher than expected.
Solution 2: Check for microbial contamination in all sources, including the DI water system, reservoirs, inline filters, tubing, and columns. Check the ambient air in the laboratory for carbon dioxide (CO_2) levels. Building intake vents could be near parking lots or other sources of CO_2. If the problem is bacterial contamination, the results will continue to climb, so if the same sample is analyzed three times in a row, each result will be higher than the previous result. It may be necessary to replace tubing with new polyether ketone tubing in the entire system. All connectors should be replaced. Even columns and suppressors can become contaminated with bacteria.

Problem 3: Results are lower than expected.
Solution 3: Standards are old. Prepare fresh standards and rerun. Check the injection loop for plugs. Change the loop if necessary. Also check the sample changer probe for blocks along with any connecting tubing between the sample changer and IC column. Make sure that the sample loop is filling completely.

The sample loop should flush ten times with sample to ensure that there is absolutely no rinse water mixed with the sample injected onto the column.

Problem 4: Retention times get shorter, poor resolution and decreased peak height.
Solution 4: Column may be contaminated or aged. Clean the column with the recommended cleaning solution. If retention times and peak heights do not improve, change the columns. (Gardrat, 2016). Note that cleaning the column can result in significant downtime to purge the cleaning solution from the column. Changing the column may be faster.

Problem 5: Species elute too late and are seen eluting in the following chromatogram.
Solution 5: Extend the run time. Sometimes the run times and retention times can lengthen significantly when new columns are put into service. It is important to test the system with calibration standards to sort out where the new retention times will be.

Problem 6: Precision not meeting QC specifications.
Solution 6: Check the injection valve for leaks or blocks. Ensure that the sample loop is filling with each injection. The sample loop should rinse about ten times with sample to ensure that there is absolutely no mixing of a sample with the previous sample or with rinse water. Check this by placing the sample loop waste line from the injection valve into a graduated cylinder. Introduce a sample from the sample changer. The volume collected in the cylinder should be about ten times the injection loop volume.

Check the probe and sample lines for plugs or leaks. Change the sample loop and clean the injection valve. A plug can be found by disconnecting each length of tubing one section at a time. The pump pressure will increase significantly if the bed supports are fouled or the guard column is fouled.

4.5.7 Carboxylic (Organic) Acid Determination Using Ion Chromatography

Background

Formic acid (HCOOH) and acetic acid (CH_3COOH) are generally the most important carboxylic acids in precipitation. See (Keene, et al., 2015), for more on the many sources of these organic acids in rainfall in both continental and marine regions. Oxalic acid ($H_2C_2O_4$) is useful as a tracer for biomass burning. (Gardrat, 2016). These acids are highly unstable and rapidly disappear from unpreserved precipitation samples. To generate reliable data, precipitation must be sampled on a daily or event basis and immediately preserved with the addition of a biocide such as chloroform ($CHCl_3$). Samples are treated with $CHCl_3$ at a ratio of 500:1. For example, add 0.5 mL of $CHCl_3$ to a 250 mL sample. Seal tightly and refrigerate until analysis.

Carboxylic anions can be analyzed by both ion chromatography (IC) and ion exclusion chromatography (ICE). Current generation anion separator columns have greatly improved the peak resolution for carboxylic anions such that IC is now the preferred method in most laboratories.

IC is a liquid chromatographic technique that combines ion exchange chromatography, eluent suppression, and conductimetric detection. The sample is injected into the system and along with a KOH eluent is pumped through guard and separator columns containing anion exchange resins. Organic and inorganic ions are separated based on their relative affinities for the resin exchange sites. The sample stream then passes through a suppressor column that reduces the conductivity of the eluent and converts the anions to their acid forms. The anions are then measured using a conductivity detector. Anions are identified based on retention times and are quantified by comparing peak areas to a calibration curve generated with known standards that bracket the concentration ranges of anions in the sample. Formate and acetate have a weak affinity for the stationary phase of the column and typically elute early whereas the stronger inorganic acids have a stronger affinity and elute later. As a result, the formate and acetate will crowd near the beginning of the chromatogram, depending on the type of resin in the columns. Oxalate is a stronger acid and elutes with the inorganic acids between sulfate and nitrate.

Both IC and ICE quantify the sum of dissociated (or ionized) and undissociated species. For example, the measured total formate ($HCOO_t$) includes both formate ion ($HCOO^-$) and undissociated formic acid (HCOOH).

The dissociated fraction contributes to the sample's ionic strength and, therefore, must be included in ion-balance calculations. This fraction also corresponds to the portion of each acid that contributes to the free acidity of the sample.

Detection limits (DLs) for IC vary as a function of analytical ranges. At typical analytical ranges for precipitation, detection limits for $HCOO_t$, CH_3COO_t and C_2O_{4t} are approximately 0.01 mg L^{-1} each but, if greater resolution is needed, lower detection limits can be achieved by expanding the analytical ranges into the lower end of the calibration curve.

Due to the nature of organic acid analysis, some research may be required. Several suitable columns for organic acid analysis are available. It is necessary to identify the combination of columns and instrumentation that is best for the expected organic acids in precipitation. This may require method development, optimization, and subsequent documentation.

Optimal operating conditions vary between different columns. During initial setup, adjustments in flow rate or the ionic strength of the eluent may be required to adequately separate $HCOO_t$ and CH_3COO_t peaks.

Contamination from laboratory air poses a problem especially for analyzing CH_3COO_t. Minimize sample exposure to air and ensure reagents and cleaning solutions that contain acetic acid are not open before or during analysis. (Keene and Maben, 2016)

Oxalate elutes much later. It follows nitrate when using the AS18 (4 mm) column and sulfate when using the AS11 (4 mm) column. Note that there may be variability between runs and optimization can be a daily requirement. Tables 4.23 and 4.24 give examples of IC operating conditions (other possibilities exist).

Apparatus

There are three types of IC columns: standard-bore (4mm), micro-bore (2mm) and capillary (0.4mm). For example: Dionex IonPac AS11 Separator Column (4X250mm, 2X250mm or 0.4X250mm). (Dionex Corporation, Thermo Scientific, 2012). These are advantages of using smaller bore systems:

i. Less sample volume is required for analysis because smaller injection loops are used.
ii. Less volume of reagents is needed.
iii. Peaks are sharper.
iv. There is better peak separation.

Use guard and separator columns that are of the same diameter and type. AG11 (4mm) and AS11 (4mm) go together. Suppressors and system connective tubing also need to match the column diameters.

Two methods are described below as a guideline, both are based on the use of the Dionex IonPac columns. Note that other suitable columns are available.

Gradient IC System (Gardrat, 2016)

In general, a gradient IC system consists of:

i. IC with gradient pump capability, injection loop system and conductivity detector
ii. sample changer with software
iii. desktop computer
iv. chromatography workstation (integration software)
v. anion self-regenerating suppressor – Dionex ASRS300
vi. analytical column Dionex AS11 (4mm)
vii. guard column Dionex AG11 (4mm)
viii. generator cartridge sodium hydroxide (Dionex EGC III NaOH)

Isocratic IC System (Keene and Maben, 2016)

In general, an isocratic IC system consists of:

i. IC with isocratic pump and conductivity detector
ii. sample changer with software
iii. desktop computer
iv. chromatography workstation (integration software)
v. anion electrolytically-regenerating suppressor - Dionex AERS 500
vi. analytical column (Dionex AS18 4mm)
vii. guard column (Dionex AG18 4mm)
viii. generator cartridge potassium hydroxide (Dionex EGC III KOH)

Reagents and Solutions

i. Self-regenerating systems only require an eluent generation cartridge.
ii. The purity of water used for dilution is highly important. Use only filtered, DI water (resistivity >18.0 MΩ).
iii. Use ACS grade chloroform for addition to the standards and to match the matrix of the preserved samples.

Calibration (Keene and Maben, 2016) (Gardrat, 2016)

1000 mgL^{-1} Stock Standard Solution

Prepare three stock standard solutions, each containing 1000 mg L^{-1} of $HCOO_t$, CH_3COO_t and C_2O_{4t} from high purity salts that are dried at 105°C for a minimum of one hour. Allow the salts to cool in a desiccator. Store the bottles containing the salts in a desiccator, using a desiccant that contains an indicator dye that changes color when the desiccant needs to be re-dried. Replace and re-dry the reagent salts every 6 months or as needed. The dried salts are weighed, then dissolved in DI water and diluted to 1000 mL.

New flasks and bottles used as containers for stock standard solutions need to be conditioned. This is done by soaking them in DI water over night, then rinsing them three times with DI water and drying them in a warm oven. This conditioning only needs to be performed the first time that new containers are put into service.

Table 4.22 lists the masses of dried salts to use in preparing stock standard solutions.

1) It is highly recommended that standard solutions be prepared by weighing the DI water volumes. Calibrate the receiving flasks by dispensing DI water by weight into the flask and then marking the flask at the fluid line. See Appendix C for calibration of flasks and balances and glassware storage.

2) Make three stock solutions. Weigh each salt carefully into a calibrated and conditioned 1L volumetric flask. Add DI water to the fluid line marked on the flask.

3) Flush the chloroform dispenser a few times then add 0.50 mL chloroform to each flask. Mix and store in designated, conditioned HDPE bottles at 4°C. Replace stock standard solutions every three months or as needed.

4) To ensure consistency between old and new stock standard solutions, prepare a dilution of the new stock standard solution and analyze it as an unknown, using old calibration standards to calibrate the instrument.

i. Into a rinsed weigh boat dispense 1 gm of new stock standard solution.
ii. Pour this solution into a clean, rinsed and calibrated 1 L volumetric flask
iii. Using Type I DI water, rinse the weigh boat into the flask and fill the flask to the 1 L mark.
iv. Mix well and allow solution to equilibrate for at least one hour.
v. Analyze this diluted new stock standard solution using the old calibration set.
vi. Measurement should fall within the expected range of precision around 1.00 mg L^{-1}.
vii. If this diluted stock standard solution meets the 1.00 mg L^{-1} QC specification, transfer the full strength (1000 mg L^{-1}) new stock standard solution to an HDPE flask and store at 4°C. If the specification is not met, discard the solution and start the preparation again.

Table 4.22. Organic acid stock standard solutions (1000 mg L^{-1} $HCOO_t$, CH_3COO_t and C_2O_{4t})

Salt	Weight (g)
NaHCOO	1.5107
$NaCH_3COO$	1.3894
$Na_2C_2O_4$	1.2583

Intermediate stock solutions

Prepare intermediate stock solutions fresh daily. Allow stock standard solutions to come to room temperature before proceeding. Recommendations for intermediate stock solutions depend on the range of organic acid concentrations observed at individual locations. Tables 4.23 and 4.24 list analytical ranges typical of measurements in two different locations. Stocks A and B are offered as guidelines in preparing intermediate stock solutions for these locations. These may need to be adjusted if the range of actual measurements differs from these guidelines.

Stock A: Use for low latitude, mid latitude and continental locations: 20 mg L^{-1} $HCOO_t$; 10 mg L^{-1} CH_3COO_t; 10mg L^{-1} C_2O_{4t}

Add 20 mL of 1000 mg L^{-1} $HCOO_t$ stock standard solution, 10 mL of 1000 mg L^{-1} CH_3COO_t stock standard solution and 10 mL of 1000 mg L^{-1} of C_2O_{4t} stock standard solution to a 1000 mL calibrated volumetric flask, then fill to the mark with fresh DI water.

Table 4.23. Operating range for low latitude, mid latitude, and continental locations

Species	Analytical Range (mg L^{-1})
$HCOO_t$	0.01 to 2.00
$CH3COO_t$	0.01 to 1.00
C_2O_{4t}	0.01 to 1.00

Stock B: Use for marine and high latitude locations: 10 mg L^{-1} $HCOO_t$; 5 mg L^{-1} $CH3COO_t$; and 5 mg L^{1} C_2O_{4t}

Add 20 mL of 1000 mg L^{-1} $HCOO_t$ stock standard solution, 10 mL of 1000 mg L^{-1} CH_3COO_t stock standard solution and 10 mL of 1000 mg L^{-1} of C_2O_{4t} stock standard solution to a calibrated 2000 mL flask, then fill to the mark with fresh DI water.

Table 4.24. Operating range for marine and high latitude locations

Species	Analytical Range (mg L^{-1})
HCOOt	0.01 to 1.00
$CH3COO_t$	0.01 to 0.50
C_2O_{4t}	0.01 to 0.050

Calibration standards

Prepare fresh daily.

Add specified volumes of intermediate stock solutions A or B, as listed in Tables 4.25 and 4.26, to labelled, 250 mL volumetric flasks and dilute to mark with fresh DI water. Add 0.5 mL $CHCl_3$ to each flask. Place stoppers on flasks and mix thoroughly.

Table 4.25. Calibration standard concentrations: low latitude, mid latitude, and continental locations

Volume of Stock A (mL) to 250 mL DW	Final concentrations (mg L^{-1})		
	C_2O_{4t}	CH_3COO_t	$HCOO_t$
25	1.00	1.00	2.00
20	0.80	0.80	1.60
15	0.60	0.60	1.20
10	0.40	0.40	0.80
5	0.20	0.20	0.40

Table 4.26. Calibration standard concentrations: marine and high latitude locations

Volume of Stock B (mL) to 250 mL DW	Final concentrations (mg L^{-1})		
	C_2O_{4t}	CH_3COO_t	$HCOO_t$
25	0.500	0.500	1.00
20	0.400	0.400	0.80
15	0.300	0.300	0.60
10	0.200	0.200	0.40
5	0.100	0.100	0.20

Quality Control

Carboxylic acids in natural precipitation samples deteriorate too rapidly, even if refrigerated, to use for QC solutions. Simulated samples provide an alternate standard material to use in preparing QC solutions.

One vendor, Inorganic Ventures, markets 1000 mg L^{-1} NIST-traceable stock solutions for acetate, formate, and oxalate. See Appendix D for details. Prepare at least three QC solutions, one each in the low, middle, and high range of the calibration curve, by diluting these NIST-traceable stock solutions (Keene and Maben, 2016). Use fresh Type I DI water. Prepare QC solutions fresh with each analytical run.

Analytical Procedure

Two analytical approaches are described in this section: (A) an isocratic IC system that measures all inorganic and organic anions in a single run and (B) a gradient system for only carboxylic acids. Analyses of inorganic anions are described separately in section 4.5.5. Selecting an approach depends on the instrumentation available, as well as the workload and staffing resources. For lab managers who are planning purchases, some advantages and disadvantages of each approach are listed here.

Approach A: Analyze all anions using an isocratic IC system.

Advantage:

- less expensive, since only one instrument and one setup are needed for all anion measurements (carboxylic acids, sulfate, nitrate, and chloride)

Disadvantages

- Slow – waiting for all anions to elute requires longer run times.
- Instrument breakdown means delays in all anion measurements, not just carboxylic acids.
- Calibration solutions ideally should contain all analytes, inorganic and organic. The organic acid solutions must be prepared daily while inorganic acids are more stable. If organic and inorganic calibration solutions are prepared and analyzed separately, instrument calibration could take a full working day. It is important to have an operator always present so that if the calibration fails, the run can be stopped before precipitation samples are compromised or consumed.
- Calibration and QC procedures must be optimized for all anions.
- Samples for organic acid analysis are treated with $CHCl_3$ and must be allowed to sit while the $CHCl_3$ settles to the bottom of the sample tube. If the sample changer has a probe that goes to the bottom of the tube to draw sample, the sample must be transferred to another tube, leaving behind the $CHCl_3$. **$CHCl_3$ must not enter the IC column**.
- This method uses a 500 µL loop. Generally, sample loops should be rinsed ten times with sample to preclude any chance that the sample will mix with the previous sample or with rinsate. Ten rinses at 500 µL per rinse consumes 5 mL, a very large minimum volume. To reduce the minimum volume, use microbore (2 mm) AS18 columns, which require only about 20 µL of sample.

Table 4.27 lists examples of an analytical profile, that is, a set of parameters to accommodate all species using AS18 and AG18 4 mm columns with an AERS 500 suppressor.

Table 4.27. Isocratic system parameters using 4mm columns (Keene and Maben, 2016)

Parameter	Condition
Eluent concentration	18.0 mM KOH
Injection volume	500 µL
Flow Rate	1 mL/min

Run time	25 minutes

Monocarboxylic acids (such as formate and acetate) separate within the first ten minutes, while the inorganic anions (sulfate, nitrate, and chloride) and oxalate follow in the remaining 15 minutes.

Approach B: Analyze inorganic anions on IC isocratic system and organic acids on separate gradient IC system.

Advantages:

- Each system can be optimized - inorganic acids can be analyzed quickly with good sensitivity on one type of column while the gradient system uses a column specifically for organic acids.
- The column described in this method uses a 200 µL loop, significantly reducing the sample volume. AS11 columns are available in microbore (2 mm) and capillary (AS11-HC) sizes and would reduce sample volume even further. High capacity (HC) columns may be used with a concentrator and are packed with the same resins as 2- and 4-mm columns. HC columns use about one-hundredth of the amount of eluent and sample that are used for a 4 mm column.
- Greater laboratory flexibility - if one system is down, the laboratory can continue analyzing samples with the other system. If necessary, the gradient system can be used for inorganic acids but with no savings in time. Chromatograms from the inorganic IC system can be screened for organic acid peaks, reserving the gradient IC system for organic acid analysis only.
- Limiting $CHCl_3$ addition to samples that require organic acid analysis saves time. First, it is only necessary to prepare new calibration standards daily for organic acid analysis. Second, $CHCl_3$ is not added to the samples for inorganic acid analysis, eliminating the need to transfer samples to a new tube to prevent $CHCl_3$ from being drawn up by the probe in the sample changer. Keeping $CHCl_3$ out of the inorganic analysis eliminates the risk of interferences or damage to the columns.

Disadvantages

- More expensive - two complete systems are needed, one for inorganic acids and one for organic acids, each with a sample changer and separator columns. Though the sample can be split into organic acid and inorganic acid streams from a single sample changer, differences in run times and sample preparation requirements make this a very cumbersome process, especially since inorganic anion columns are generally intolerant of $CHCl_3$.

- Gradient systems produce a step in the middle of the chromatogram that can make integration of the inorganic species problematic. See Figure 4.34 for a typical gradient chromatogram.

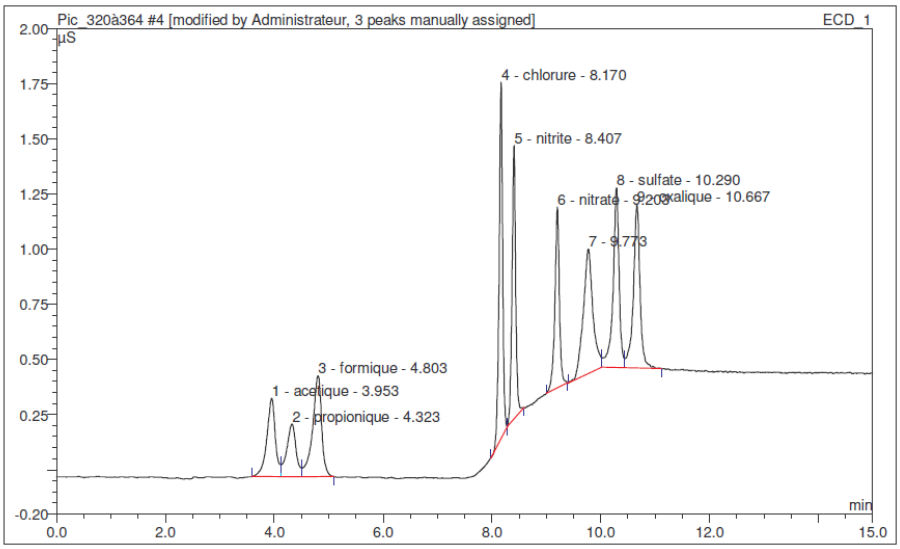


Figure 4.34. Chromatogram from gradient system with both organic and inorganic ions (Gardrat, 2016)

Table 4.28 lists a gradient system profile for organic acids.

Table 4.28. Gradient system profile for elution of organic acid species using AS11, AG11 and ASRS300. Note that times will vary with column age and operating conditions. (Gardrat 2015)

Parameter	Condition
Eluent Concentration	90% H_2O; 10% 5 mM NaOH (2.7 min) 89% H_2O; 11% 100 mM NaOH (9.8min) 90% H_2O; 10% 5 mM NaOH (6.5 min)
Injection Volume	200 µL
Flow Rate	1.0 ml/min
Run time	19 min

A concentrated eluent may be used to cleanse the column between injections, preventing late eluting species from carrying over into the next chromatogram. Alternatively, the eluent concentration may be returned to a low concentration at the end of a chromatogram to allow the system to equilibrate for the next sample in sequence. See Figure 4.35 for an example of an eluent concentration gradient.

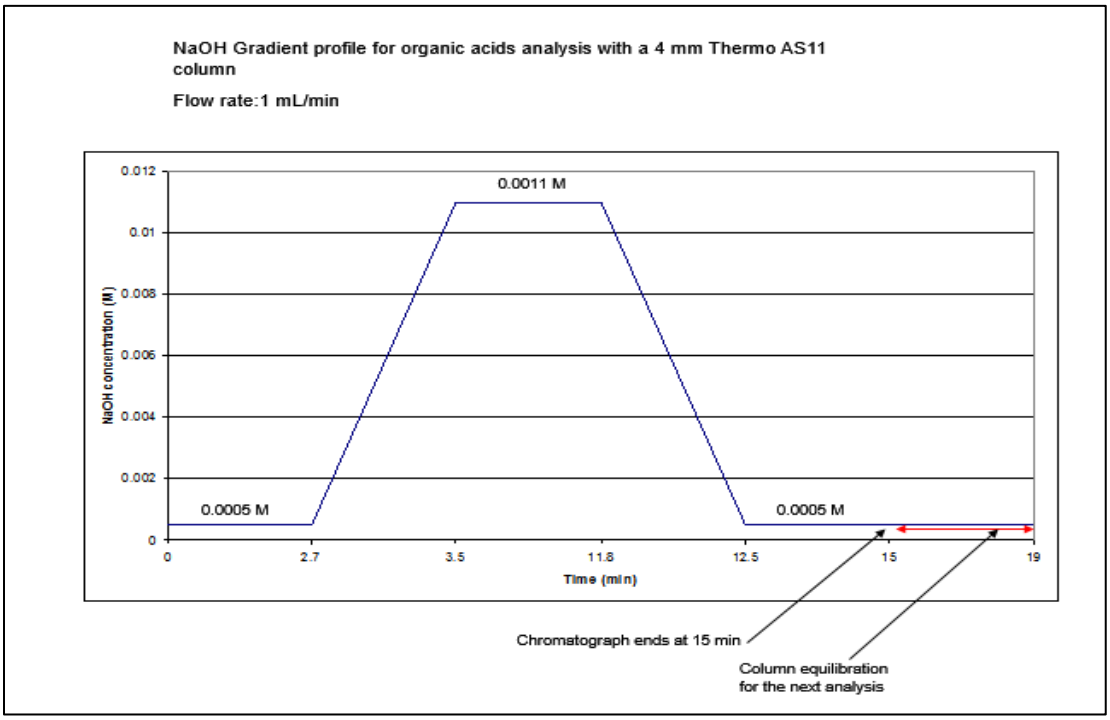


Figure 4.35. Example of concentration gradient using AS11 4 mm columns (Gardrat, 2016)

Chloroform can damage IC columns but is immiscible in and denser than water. It sinks to the bottom of the container.

To prevent damage to IC columns, allow samples and standards to settle for a few minutes after $CHCl_3$ is added. Only after the $CHCl_3$ has separated completely from the water is it safe to pour supernatant from the top of the container into the sample

changer vial.

Step-by-step Procedures (Approaches A and B)

1) Do not power down an IC system when the system is not in use. Always leave the power on.

2) Check reagent levels. Check the fluid and ion percent in the eluent cartridge and ensure there is enough for a full run. Change the DI water in the flush reservoir of the sample changer daily. Inline filters may be used to minimize introduction of particulate matter into the system. Change inline filters daily.

3) Run DI water samples until the system is stable and equilibrated.

4) Label each tube. Prepare a schedule of analysis in the workstation software. Enter sample identification numbers into the software in the same order as the tubes will be installed in the sample changer rack.

5) Prepare samples for analysis. If the samples have been preserved with chloroform, allow the samples to sit for about half an hour and check the bottom of the tube for an immiscible yellow fluid. Decant or pipette the supernatant into another identically labeled tube. Immediately cover each tube with a cap that can be pierced or with Parafilm®.

6) Ensure that each tube has a minimum volume. Minimum volumes will vary according to injection loop size and loop rinse.

7) Check the instrument operation for a stable pump pressure and conductivity. Check the DI water chromatograms for the correct shape (described below). Begin the run. Calibration standards are run first. The injection should start with the highest concentration standard followed by decreasing concentrations.

   a. Chromatogram shape for DI water depends on the eluent and pump type.
   b. Hydroxyl chromatograms have a small water dip. The DI water chromatogram will have a carbonate peak positioned after chloride and before sulfate. The height of the peak will depend on water quality, the age of the water and ambient room carbon dioxide levels. See Figure 4.36 for a deionized water chromatogram for a system using KOH eluent.

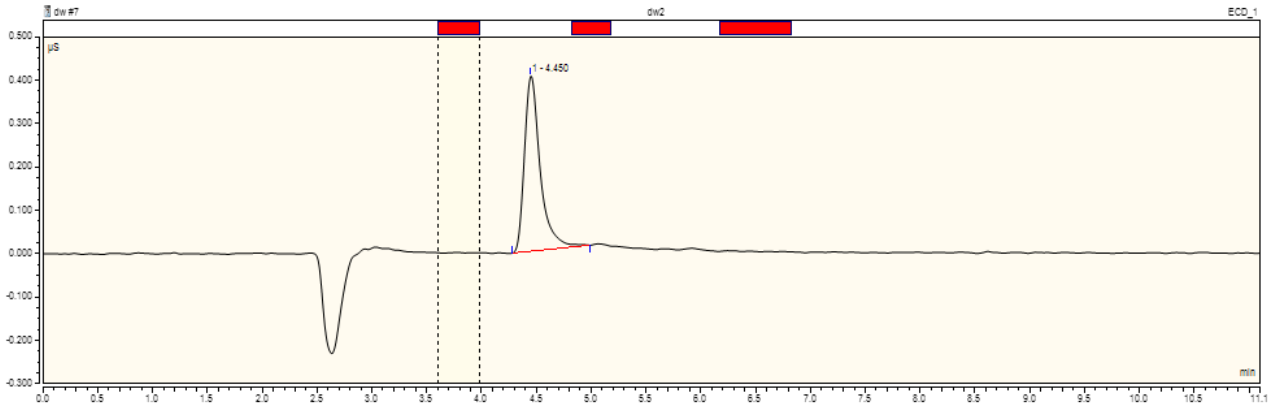


Figure 4.36. DI water chromatogram using KOH eluent and isocratic pump

   c. Gradient pump DI water chromatograms will be similar until the eluent concentration increases and then the chromatogram baseline will climb and level off.
   d. DI water chromatograms **must be free of the anions of interest** before starting the

analytical run.

8) Run a low QCS directly after completing the low calibration curve and a high QCS after completing the high calibration curve. Inject a randomly selected QCS every five samples thereafter. Plot the QCS results on control charts.

9) Calibrate every 20 samples.

10) Following the run, check all calibration curves and QC results before reporting, collating or tabulating sample results. Use only the peak area parameter, not the peak height, for calculation of results.

11) Examine each chromatogram individually for correct shape and integration. The baseline must not drift up or down for isocratic systems and must not be bumpy. The peaks should all have a typical Gaussian shape and show good separation from one other. Comment all anomalies and flag data accordingly. Repeat analyzing samples where there is a drifting or bumpy baseline.

    Note that chromatography software uses peak 'windows'. The software expects the peak for each analyte to elute in a certain window of time. Peaks that fall outside this window will not be integrated. Also, large peaks that fill the window may not be recognized and thus produce a zero result. Make note of these exceptions and repeat the analysis.

12) Calculate results against the appropriate calibration curve and apply detection limits and correct decimal places as required. Mark all samples that exceed upper calibration ranges for dilution and repeat the analysis. Ensure missing samples are accounted for and contamination codes are applied as needed.

13) Export data from the IC and archive all parameters associated with the analysis including calibration data, integration data and instrument audit trails. Audit trails include instrument parameters (e.g., pump pressure) that may be useful in diagnosing a problem, such as a chromatogram with a drifting baseline. It may be necessary to repeat the analysis at the point where the problem began.

Troubleshooting

Problem 1: Uneven or drifting baseline. Pump loses prime.
Solution 1: Check the EluGen® cartridge for leaks. If necessary, change the cartridge then re-prime the pump and restart the system. Place the DI water container above the IC system to improve flow. Check the system for leakage. Re-prime the pump and run DI water to check the system. If the pump is still unstable, disconnect the column and pump methanol through the system. Flush with water. If these steps do not eliminate the problem, change the piston seals, provided the operator has been trained to do so. Soak the piston seals in methanol for a few minutes. This ensures a better seal around the piston.

Problem 2: Measurements of DI water exhibit carboxylic acid peaks.
Solution 2: Draw DI water directly from the point of use gun. Replace and freshen the DI water in the flush rinse reservoir.

Problem 3: Retention times grow shorter and the resolution is poor. Peak heights are decreased.
Solution 3: The column may be contaminated or aged. Clean it with the recommended cleaning solution. If retention times and peak heights do not improve, change the columns. (Gardrat, 2016). Sometimes cleaning a column can result in significant downtimes because the cleaning solution needs to be purged from the system. Changing the column may take less time than cleaning the column.

Problem 4: Precision does not meet QC specifications.
Solution 4: Check the injection valve for leaks or blocks. Ensure that the sample loop is filling with each injection. The sample loop should rinse about ten times with sample to ensure that there is absolutely no

mixing of a sample with the previous sample or with rinsate. Check this by placing the sample loop waste line from the injection valve into a graduated cylinder. Introduce a sample from the sample changer. The volume collected in the graduated cylinder should be about ten times the injection loop volume. Check the probe and sample lines for plugs or leaks. Change the sample loop and clean the injection valve. A plug can be found by disconnecting each length of tubing one section at a time. The pump pressure will increase significantly if the bed supports are fouled or the guard column is fouled.

4.5.8 Calcium, Magnesium, Sodium and Potassium by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES)

Background

Inductively coupled plasma - atomic emission spectroscopy (ICP-AES) is the preferred method for determining the concentrations of alkali (sodium and potassium) and alkaline earth (calcium and magnesium) metals in precipitation. The advantages of ICP-AES include its linear response over a wide range of concentrations and detection limits that are an order of magnitude below the detection limits that can be obtained with flame atomic absorption (FAA) spectrometry or IC. ICP-AES has the capacity to measure multiple analytes simultaneously. Although ICP-AES is an extremely sensitive instrument, especially in the laboratory environment, newer models are not as sensitive to laboratory conditions as older models. Operators must be trained though operation of the instrument is not complex.

ICP-AES systems use argon as fuel whereas FAA uses acetylene or nitrous oxide. Acetylene and nitrous oxide are highly flammable and require special handling and storage precautions. Bottles of compressed gas must be secured in place and protected from impact. Storage must be limited to short-term use. Alternatively, argon has few safety concerns, though in a small, enclosed area it can displace oxygen.

Disadvantages of ICP-AES are the cost to purchase and operate the system as well as the potential for interferences. Interferences are more common in complex matrices where the presence of many elements produce many spectral lines. The ICP-AES system allows the user to choose one of many spectral lines for each element of interest. The choice of the spectral line may be specific to the concentration of the sample. Examine table 4.29 and note that different concentrations are read at different spectral lines.

Table 4.29. Typical wavelength selection for ICP-AES

Analyte	Typical Wavelength (nm)	Concentration Range ($mg\ L^{-1}$)	Typical Wavelength (nm)	Concentration Range (mgL^{-1})
Calcium	393.366	<1.000	315.887	> 1.000
Magnesium	279.553	All		
Potassium	766.491	<0.500	769.897	>0.500
Sodium	589.592	<0.500	588.995	>0.500

Figure 4.37 illustrates the components of the ICP-AES system. Samples are drawn by a vacuum pump into a T-junction, where a carrier gas with an internal standard solution containing yttrium and cesium chloride are mixed into the sample stream. Next, using argon this mixture is nebulized to form an aerosol that enters a cyclonic spray chamber. In this chamber the largest, macro-sized aerosol particles are removed by gravity, leaving relatively uniformly distributed micro-sized aerosol particles, which are then aspirated into the plasma.

The plasma is created by heating argon gas inductively in a radio-frequency electromagnetic field. Plasma temperatures range from about 6000K to 10,000K. At these temperatures, the plasma initiates ionization of the chemical species of interest, as well as some incidental ionization. Isolating the ionization

to only the analytes of interest is enhanced by the presence of an incidental ionization suppressor, cesium, which is added to the sample at the T-junction.

All alkali and alkaline earth metals of interest are measured simultaneously. These elements become thermally excited, emitting photons of light at their characteristic wavelengths. The photons are collected by an array detector and pass through a diffraction grating that resolves the light into its wavelength spectrum. At this point the light signal is detected and amplified to yield an intensity that is proportional to concentration. This signal intensity is converted to concentration by comparison with the signal intensity generated by calibration standards. Selecting the wavelength to read depends on the concentration range. Table 4.29 lists typical wavelength selections for different concentration ranges. Table 4.32 lists the recommended ICP-AES range of operation for precipitation analyses.

Yttrium is used as an internal standard in ICP-AES measurements. Its role is to correct for drift and improve accuracy and precision. The internal standard must be chemically compatible with but not naturally occurring in the samples being analyzed. The internal standard must behave similarly to the species of interest and it must not produce spectral interferences. This internal standard is measured along with the samples but at a spectral line that is different from any of the species of interest. At 242.220 nm, the spectral line of yttrium does not interfere with any of the alkali or alkaline earth metals of interest. *(Note that laboratories should consider verifying the best wavelength for yttrium measurements, as another laboratory has 371.03 nm to be the best wavelength.)* Yttrium is introduced into the ICP-AES using the same system as the sample, thus correcting for any variation or drift. ICP-AES software uses the response of the internal standard to make corrections in the final data output.

Apparatus

ICP-AES instruments feature either an axially or radially-viewed plasma. Axial systems focus on the center of the plasma and are preferred for typical precipitation analyses. Radial systems look at a slice of the plasma from the side. Radial systems add range to the higher end of the calibration curve.
Dual systems have both radial and axial options and offer both sensitivity and a wide range of measurements. (Figure 4.37)

i. ICP-AES
ii. peristaltic pump for delivery of sample and internal standard
iii. sample changer
iv. desktop computer with workstation and data acquisition software
v. radio frequency (RF) generator
vi. conical nebulizer: dissolved solids up to 5%, particulates up to 75 μm
vii. spray chamber: 50 ml cyclonic, borosilicate glass
viii. dual view (DV) torch with tubing
ix. FAST™ sample introduction system (optional)
x. internal standard mixing T

FAST™ sample changer systems introduce the sample to the nebulizer through vacuum and are not subject to potential problems experienced with peristaltic pump systems. Other advantages are faster throughput times, improved precision, and cleaner and

faster rinses and cleanups.

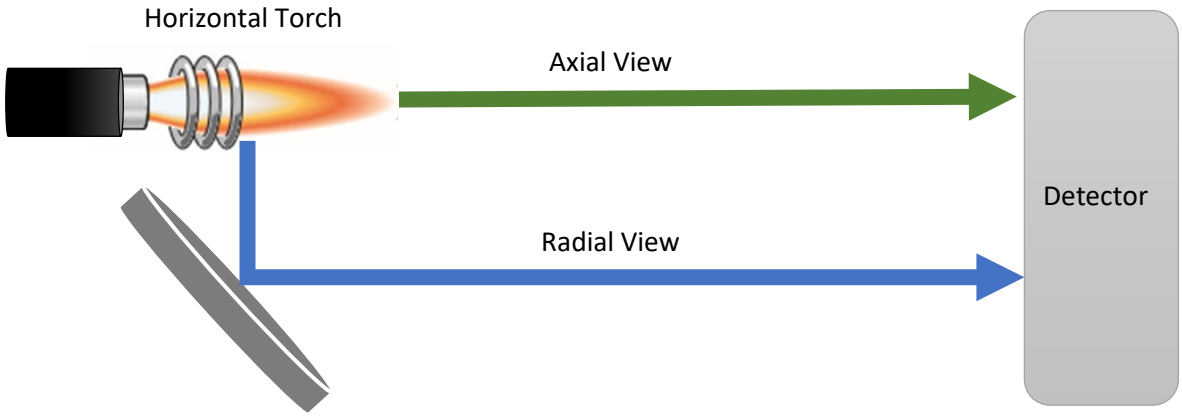


Figure 4.37. Dual View ICP-AES

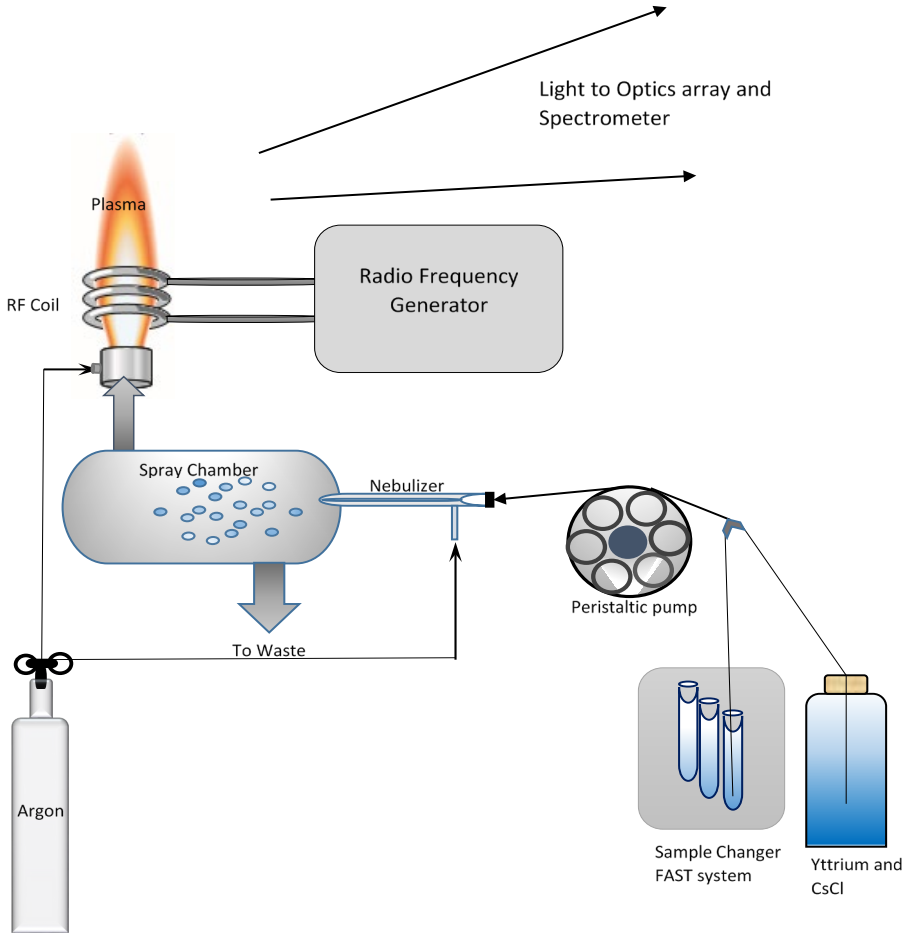


Figure 4.38. ICP-AES schematic

Reagents and Solutions

i. Water purity (used for dilution) is especially important. Use only filtered DI water (resistivity >18.0 MΩ).
ii. liquid argon

Argon may be used as a compressed gas but expect to change the tank often. Liquid argon is cheaper in the long run and requires less maintenance.

iii. nitric acid at various concentrations for cleaning the torch
iv. Triton-X 100 for cleaning the spray chamber

Calibration

Stock standard solutions generally are purchased commercially:

i. yttrium (1000 mg L^{-1}, 250 mL in 2% HNO3)
ii. cesium chloride (100 g)
iii. calcium (1000 mg L^{-1}, 250 mL in 2% HNO3)
iv. magnesium (1000 mg L^{-1}, 250 mL in 2% HNO3)
v. sodium (1000 mg L^{-1} ppm, 250 mL in 2% HNO3)
vi. potassium (1000 mg L^{-1}, 250 mL in 2% HNO3)

Stock standard solutions must come with a certificate of analysis. Each one must be pre-tested to ensure purity. For example, magnesium stock standard solution must not contain calcium, sodium, or potassium. The presence of these other elements will contaminate the intermediate stock solution, where all the elements are combined from measured portions of stock standard solutions.

Prepare a dilution of the new stock standard solution and analyze it as an unknown by using old calibration standards to calibrate the instrument.

i. Into a rinsed weigh boat dispense 1 gm of new stock standard solution.
ii. Pour this solution into a clean, rinsed and calibrated 1 L volumetric flask. See Appendix C for details concerning calibration of glassware.
iii. Using Type I DI water, rinse weigh boat into the flask and fill the flask to the 1 L mark.
iv. Mix well and allow the solution to equilibrate for at least one hour.
v. Analyze this diluted new stock standard solution using the old calibration set.
vi. Measurements should fall within the expected range of precision around 1.000 mg L^{-1}. There must be no other alkali or alkaline earth metals evident in each stock standard solution.
vii. If this diluted stock standard solution fails to meet the 1.000 mg L^{-1} QC specification, discard the solution and start the preparation again. Ensure that the solution has rested for one hour before analysis. If a second preparation fails or if other elements are present, do not use this stock standard solution. Discard it or, if possible, return it to the vendor.

Intermediate Stock Solutions

New flasks and bottles used as containers for intermediate stock solutions must be conditioned. This is done by soaking them in DI water over night, then rinsing them three times with DI water and drying them in a warm oven. This conditioning only needs to be done the first time that new containers are put into service. See Appendix C for calibration procedures for flasks and procedures

for storing glassware. Also see Appendix C for analytical balance calibration details.

1) Start with a 1 L volumetric flask that has been calibrated and is to be used for this purpose only. Calibrate this flask by dispensing DI water by weight into the flask and then marking the flask at the fluid line.
2) Next, weigh a stock standard solution in the amount listed in Table 4.30 (to the 4th decimal place) in a weigh boat that has been rinsed with DI water. Transfer the weighed stock standard solution from the weigh boat into the calibrated 1 L volumetric flask.

3) Rinse the weigh boat three times with DI water and transfer each of the three rinses into the volumetric flask.

4) Repeat this procedure for each of the four stock standard solutions, combining the solutions into the same calibrated 1 L volumetric flask.

5) Record the exact weights of stock solutions used in the ICP-AES Intermediate Stock and Calibration Standards logbook. Add DI water to dilute the contents of the flask to the 1 L fluid line.

*Table 4.30. ICP Intermediate Stock Requirement and Final Concentrations in a **single 1 L** flask (CAPMoN, 2013)*

Analyte	Required Stock Volume (mL)	Examples of Actual Volume used (mL)	Concentration in final 1 L solution (mg L^{-1})
Mg^{2+}	50	50.0009	50.0009
Ca^{2+}	50	50.0005	50.0005
Na^{+}	50	50.0007	50.0007
K^{+}	50	50.0004	50.0004

Calibration Standards

1) Weigh all volumes using an analytical balance. Rinse all weigh boats thoroughly. Use conditioned HDPE bottles that are dedicated for use in storing calibration standards. Do not use bottles from other procedures.

2) Starting with Standard 9 in Table 4.31, prepare the calibration standards by diluting the required volumes listed in the table to 500 mL with DI water. Weigh the required volumes as close as possible to the amounts listed in the table. Record the exact weights in the ICP-AES Intermediate Stock and Calibration Standards logbook.

3) Prepare fresh calibration standards every four months or as required.

Table 4.31. ICP Calibration Standards Prepared by Diluting the Required Volumes to 500 mL for each standard (CAPMoN, 2013)

Standard	Concentration (mg L^{-1})	Required Volume (mL)	Stock Used
Standard 9	5.000	50	Intermediate stock
Standard 8	3.000	30	Intermediate stock
Standard 7	1.500	15	Intermediate stock
Standard 6	1.000	10	Intermediate stock
Standard 5	0.500	5	Intermediate stock
Standard 4	0.250	2.5	Intermediate stock
Standard 3	0.100	1	Intermediate stock
Standard 2	0.050	25	Standard 6

Standard 1	0.015	7.5	Standard 6

Table 4.32. ICP Range of Operation

Analyte		Concentration Range (mg L^{-1})
Calcium		0.005 to 5.000
Magnesium		0.005 to 3.000*
Potassium		0.005 to 3.000*
Sodium		0.005 to 5.000

*Magnesium and potassium standard curves may be extended to 5.000 ppm. These two analytes tend to have low concentrations in precipitation. Magnesium concentrations may be high in marine locations due to sea salt and potassium concentrations may be high at some continental locations due to organic matter. Adding the 5.000 ppm standards for magnesium or potassium may result in greater imprecision at lower concentrations. Most software will allow the user to select which standards are needed for the calibration curve.

Yttrium (Y) and Cesium Chloride (CsCl) Solutions (CAPMoN, 2013)

1) Weigh out 10.1 g of CsCl powder and transfer it into a calibrated 1 L volumetric flask that is not used for any other purpose. Note the exact weight of CsCl to the first decimal place in the ICP-AES Intermediate Stock and Calibration Standards logbook. Rinse the weigh boat three times with DI water and transfer the rinse into the flask. Note the lot number of CsCl in the logbook.

2) Weigh out 8.8000 g of Y solution and transfer it into the same volumetric flask that contains the CsCl powder. Note the exact weight of Y solution to the 4th decimal place in the logbook. Rinse the weigh boat three times with DI water and transfer each rinse into the flask. Note the lot number of Y stock solution in the logbook.

3) Mix the solution until all CsCl is dissolved. Bring the final volume to the calibration line while swirling the flask. Stopper the flask and mix by inversion.

4) Transfer the solution into a 1 L HDPE bottle reserved for this solution. The solution is kept at room temperature and is stable for six months.

Quality Control

Prepare a minimum of three QC solutions to test the calibration curve and validate sample results. It is best if one of these solutions is in the low end of the concentration range, one in the middle of the range, and one in the high end of the range. When possible, it is best to use a natural precipitation matrix; however, stock standard solutions also may be used.

QC Solutions – Precipitation Matrix

1) Save low-concentration precipitation samples that have been analyzed and the results reported. Pool this “excess precipitation” in one 10 L HDPE container.

2) Save mid-concentration precipitation samples that have been analyzed and the results reported. Pool this “excess precipitation” in one 10 L HDPE container.

3) Save high-concentration precipitation samples that have been analyzed and the results reported. Pool this “excess precipitation” in one 10 L HDPE container.

4) Analyze the pooled samples to determine the initial alkali and alkaline earth metal concentrations.

5) Adjust the concentrations of the mid- and high-concentration pooled samples, if necessary, to ensure that the concentrations are approximately in the mid to high range of the calibration curve. Adjustments can be made by adding measured portions of the 1000 mg L^{-1} stock standard solutions. Similarly, it may be necessary to dilute the low-concentration pooled sample with DI water to achieve the low range of the calibration curve.

6) See Appendix C for sterilization of QC solutions and additional details.

Run QC solutions promptly after completing calibration of the ICP-AES and every ten samples thereafter. Plot the results on a control chart.

Analytical Procedure

1) Turn on the sample changer, the pump and the ICP-AES. Turn on the personal computer and open the software for the instrument and for the sample changer.

2) Perform daily maintenance. Check the argon levels and ensure there is enough for a full run. Check the yttrium/cesium solution levels. Change the DI flush water in the sample changer daily.

3) Turn on the Radio Frequency (RF) Oscillator Circulator.

4) Ensure that the coolant gas, auxiliary (Aux) gas, nebulizer gas, pump speed and RF parameters are set according to the manufacturer's recommendations. Here are some suggested settings:

   a. coolant gas: 19 LPM
   b. Aux gas: 0.3 LPM for ignition, Aux gas 0.00 for analysis
   c. nebulizer gas: 1.2 LPM
   d. pump speed: 1.2 mL min^{-1}
   e. plasma control: 0.9 kW

5) Check that all interlocks and safety features are met and secure.

6) Light the plasma. The instrument must stabilize for 20 minutes (or to manufacturer recommendations) with the plasma on.

7) Run a sequence of water blanks until the response for Y, the internal standard, has stabilized. If the Y response does not stabilize, the Y solution (internal standard) may need to be replaced. Another corrective action to stabilize the Y response is to rinse and drain the peristaltic pump tubing.

8) Run a complete calibration sequence followed by all three QCSs. Continue with precipitation samples only after the instrument is stable and it has been successfully calibrated.

9) Pour 4 to 5 mL aliquots of all QC and precipitation samples into sample tubes and fill in the sampler racks. Determine the minimum volume the system needs for a successful analysis and ensure that the volume in each tube meets this minimum.

10) After all standards have been run, re-cap respective lids on all standards.

11) Following the run, check all calibration curves and QC results before reporting, collating or tabulating results.

12) Check all raw data individually for correct integration and output. Comment on all anomalies in the laboratory notebook and flag data accordingly.

13) Calculate results against the appropriate calibration curve and apply detection limits and correct decimal places as required. Mark all samples that exceed upper calibration ranges for dilution and repeat analysis. Ensure missing samples are accounted for and contamination codes are applied as needed.

14) Export data from the ICP-AES system software and archive all parameters associated with the analysis, including calibration data, raw data, and instrument audit trails. Audit trails include instrument performance throughout the analytical run and any information that may be useful in diagnosing a problem, such as a drifting baseline. It may be necessary to repeat the analysis at the point where the problem began.

Troubleshooting

Problem 1: Output and results are unstable.
Solution 1: Increase the plasma stabilization time. Also, check for fluctuations in room temperature. The instrumental response of the ICP-AES is affected by swings in room temperature. The ICP-AES laboratory may need supplemental air conditioning to stabilize the room temperature.

Problem 2: Contamination of the sample and/or signal
Solution 2: ICP-AES is much more sensitive than flame atomic absorption spectrophotometry, thus laboratory cleanliness is very important. Be aware of possible contamination from overhead ventilation or dirt from shoes or clothing. The instrument operator must wear a lab coat and shoes that are worn only in the ICP-AES lab. If the operator also performs other analyses, e.g., flow injection analyses, the lab coat worn for other analyses must not be worn to perform ICP-AES analysis. Also, it is important to clean the torch and spray chamber regularly.

Problem 3: The quality of the results declines throughout the analytical run.
Solution 3: The tubing in systems equipped with a peristaltic pump is susceptible to wear. Consider purchasing a FAST™ system if this is a recurring problem. FAST™ systems use a vacuum to move the sample from the sample tube into the injection loop. Several generic models of the FAST™ system are available but make sure the system is compatible with the instrumentation and software.

Problem 4: Sudden drop in concentration or poor agreement with duplicate or triplicate measurements
Solution 4: Check the sample lines for blockage.

Problem 5: Deterioration of internal standard readings
Solution 5: Check the peristaltic pump tubing for wear. Change the tubing. Establish a maintenance program that prevents problems due to excess wear from ever occurring.

Tips to Improve Performance

1) Keep a logbook that documents maintenance procedures.
2) Maintain a record of the preparation of calibration solutions. Record details, such as the dates of preparation, weights, volumes, calculations, and the person who prepared the solutions.
3) Use only dedicated glassware and containers for calibration solutions. Do not use these containers for any other purpose.
4) Clean the ICP-AES spray chamber and torch weekly or according to the workload.
5) Track the potential impact of instrument maintenance, changes in calibration solutions or the use of new reagents by specially marking these events on quality control charts.
6) Do not operate other instruments in the ICP-AES area. ICs that have KOH and carbonate eluents can be sources of contamination. Flow injection analysis systems must be kept in a room separate from the ICP-AES.
7) Keep the area clean. Dust and residue are serious contaminants for this analysis. Make sure the ICP-AES is installed in an area where there is minimal foot traffic.

8) Place tack mats on the floor in the doorway to the ICP-AES laboratory. Keep dirt from other areas out of the ICP-AES lab space.
9) Wear a lab coat and don't wear the lab coat used in the ICP-AES laboratory in other laboratories.
10) Wear a pair of shoes that have not been worn outside. Be careful not to track soil into the ICP-AES laboratory.
11) During an analytical run, cover the sample tray with a dust shield.
12) Do not run other samples, such as hard water samples or lake or stream water samples, before running precipitation samples without thoroughly cleaning the system.
13) If the ambient laboratory temperature is not stable, use a dedicated air conditioner to stabilize the temperature in the ICP-AES laboratory; however, be sure to avoid drafts near the instrument.
14) Avoid finger contact with the rims of test tubes. Wear clean, pre-tested gloves if necessary.
15) Use liquid argon. Two tanks can be set side by side. Install an inline switch so that when one tank is empty, the next tank can be used.

4.5.9 Calcium, Magnesium, Sodium, Potassium, and Ammonium by Ion Chromatography

Background

Ion Chromatography (IC) is an alternative to ICP-AES for determining the concentrations of alkali (sodium and potassium) and alkaline earth (calcium and magnesium) metals in precipitation. In addition to these metals, the IC also can be used for ammonium measurements.

An aliquot of sample is pumped through the IC separator column where the ions of interest are separated because of their different affinities for the ion exchange material in the column. Since cations are the species of interest in this analysis, the ion exchange material is oppositely (i.e., negatively) charged. The strength of the interaction between the cationic groups in the sample molecules and the ion exchange material depends on the number, valence, and location of the charges on the sample molecules. The ions in the sample are carried through the separator column by an eluent, which for this analysis is methanesulfonic acid (MSA). Molecules with the weakest ionic interactions elute from the column first. Molecules that have a stronger ionic interaction elute later.

Following separation of the cations, the eluent carries the solution through a self-regenerating suppressor, where hydroxyl ions are exchanged for methanesulfonate ions. This exchange lowers, or suppresses, the conductivity of the eluent, effectively enhancing the conductivity of the species of interest, the alkali and alkaline earth cations.

Self-regenerating systems come with concentrated eluent contained in a cartridge that is installed in the system. High purity Type I DI water is the only additive. The cartridges are self-contained and no reagent preparation is needed. This eliminates the potential for batch-to-batch differences.

In addition to the alkali and alkaline earth metals, the IC may be used for ammonium determination. It is important to keep in mind that ammonium is in an equilibrium state:

$$NH_3 + H^+ \rightleftarrows NH_4^+ .$$

The equilibrium state changes as the pH of the mobile or eluent phase changes. Under acidic conditions, the equilibrium moves to the right and the amount of NH_4^+ increases. As the concentration of ammonium increases, the pH of the eluent increases, the dissociation is suppressed, and the concentration of NH_4^+ decreases. In short, these pH changes disproportionately affect the relationship between the concentration and the instrumental response, resulting in a non-linear calibration curve. As the concentration of ammonium increases in the sample, the calibration curve tends to flatten. This curvature at high concentrations as well as the tendency of the curve to lift from zero at low concentrations limits the usefulness of IC to determine ammonium concentrations, unless the method can be validated against an alternate colorimetric method, such as Flow Injection Analysis. Concentrations to about 1.000 mg L^{-1} fall into a range that is approximately linear.

Note: The high ammonium concentration range produces a calibration curve with a high-order polynomial fit and gives a better correlation with colorimetric data when the curve is reversed. Dionex software does not allow the reversal of the curve to apply only to ammonium. Instead, the curve will be reversed for all cations including sodium, potassium, magnesium and calcium. The impact of curve reversal on each of these cations must be investigated and determined to be minimal. See Figure 4.39 for reversed low and high ammonium calibration curves.

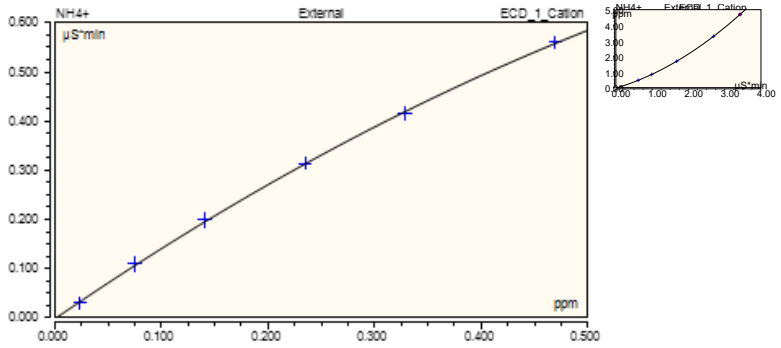


Figure 4.39. Typical reversed calibration curve for low level ammonium (left – concentration on X-axis) and high-level ammonium (right – concentration on Y-axis). Note the greater curvature for high level ammonium concentrations.

The IC operating range for cations is listed in table 4.33.

Table 4.33. IC Operating Range for Cations in Precipitation.

Analyte	Concentration Range (mg L^{-1})
Sodium	0.005 to 5.00
Potassium	0.010 to 3.00
Calcium	0.020 to 5.00
Magnesium	0.010 to 3.00
Ammonium	0.010 to 5.00

Apparatus

There are two types of IC columns for cation analysis: standard-bore (4mm) and micro-bore (2mm). (Capillary columns (0.4 mm) are available for some cation systems.) Although smaller bore columns can clog, they offer several advantages:

i. smaller injection loops require less sample volume for analysis
ii. lower volumes of reagents needed
iii. sharper and more well-defined peaks
iv. higher resolution

In general, an IC system consists of:

i. IC with isocratic pump, conductivity detector, sample loop and injection system

ii. automatic sample changer with software
iii. desktop computer
iv. chromatography workstation (integration software)
v. self-regenerating suppressor – (Cation Atlas Electrolytic Suppressor)
vi. analytical column (CS12-Dionex)
vii. guard column (CG12-Dionex)
viii. eluent generator cartridge (Methanesulfonic Acid-Dionex)

Place a low-pressure inline filter after the sample changer and before the injection valve to keep bacteria and contaminants out of the IC. 2µm pore frits can be cleaned by sonication and reused. See section 4.5.5 for a more complete description.

Reagents and Solutions

i. ultra-pure Type I DI water (resistivity >18MΩ).
ii. Eluent Generator Cartridge Methanesulfonic Acid (MSA)

Calibration

Stock standard solutions

Stock standard solutions containing 1000 mg L^{-1} of each sodium, potassium and ammonium ion may be purchased as certified solutions from different manufacturers or prepared from high purity salts that are dried at 105°C for an hour, dissolved and diluted to 1000 mL as listed in Table 4.34.

Calcium and magnesium 1000 mg L^{-1} solutions are available in 2% nitric acid. See ICP-AES section 4.5.8 for details.

New flasks and bottles for storing stock standard solution must first be conditioned by soaking in DI water over night, then rinsed with DI water three times and dried in a warm oven. Conditioning of flasks and bottles only needs to be done once when the containers are new and before putting them into service. See Appendix C for calibration of flasks and balances and for glassware storage conditions.

1) It is highly recommended that standard solutions be prepared by dispensing the volumes by weight and calibrating all receiving flasks. Flasks may be calibrated by dispensing DI water by weight into the flask and then marking the flask at the fluid line.

2) Weigh all volumes using an analytical balance. Rinse all weigh boats thoroughly. Use conditioned HDPE bottles to store stock standard solutions. Use glassware and bottles that are dedicated solely to standard solution preparation and storage.

3) Make three stock standard solutions: NaCl, $(NH_4)_2SO_4$ and KNO_3, each with a concentration of 1000 mg L^{-1}. Table 4.34 lists the required salt masses. Weigh each salt carefully into a calibrated and conditioned 1 L volumetric flask. Mix and store in designated, conditioned HDPE flasks. Solutions are stable for one year.

4) To ensure consistency between old and new stock standard solutions, prepare a dilution of the new stock standard solution and analyze it as an unknown by using old calibration standards as calibrators. Here is a step-by-step procedure:

   i. Into a rinsed weigh boat dispense 1 gm of new stock standard solution.
   ii. Pour this solution into a clean, rinsed and calibrated 1 L volumetric flask.

iii. Using Type I DI water, rinse the weigh boat into the flask and fill the flask to the 1 L mark.
iv. Mix well and allow solution to equilibrate for at least one hour.
v. Analyze this diluted stock standard solution but use the old standard solutions to calibrate the instrument.
vi. Measurements should fall within the expected range of precision around 1.00 mg L^{-1}. If so, transfer the full strength 1000 mg L^{-1} stock standard solution into an HDPE flask and store at 4°C. If this QC specification is not met, discard this stock standard solution and begin the preparation again. Be sure to allow the solution to equilibrate for an hour before analysis.

Dispensing large volumes of stock solution to make working calibration standards is a more accurate procedure than dispensing more concentrated stock solutions in small volumes.

Table 4.34. Cation Stock Standard Solutions. The salt masses specified result in 1000 mg L^{-1} of Na^+, K^+ and NH_4^+). Final volume is 1 L.

Salt	Weight (g)
NaCl	2.542
KNO3	2.586
$(NH_4)_2SO_4$	3.663

Low Working Standard1

1) Prepare Low Working Standard 1 (L-Std 1) by dispensing each stock standard solution by weight into a calibrated, conditioned 1 L volumetric flask. The volumes are specified in table 4.35. Dilute to 1 L with DI water.

Table 4.35. Preparation of L-Std 1

Low Std. #	Solution	Na^+ (mL)	K^+ (mL)	NH_4^+ (mL)	Ca^{2+} (mL)	Mg^{2+} (mL)	Final Volume (mL)
1	Each stock standard	0.500	0.500	0.500	0.500	0.500	1000

2) Use L-Std 1 to prepare low-range working standards 2 through 5 (Table 4.37). Flasks must be conditioned, calibrated, and designated for L-Std 1.

High Working Standard1

1) Prepare High Working Standard 1 (H-Std 1) by dispensing each stock standard solution by weight into a calibrated, conditioned 1 L volumetric flask. The volumes are specified in table 4.36. Dilute to 1 L with DI water.

Table 4.36. Preparation of H-Std 1

High Std. #	Solution	Na^+ (mL)	K^+ (mL)	NH_4^+ (mL)	Ca^{2+} (mL)	Mg^{2+} (mL)	Final Volume (mL)

1	Each stock standard	5.000	3.000	5.000	5.000	3.000	1000

2) Use H-Std 1 to prepare high-range working standards 2 through 5 (Table 4.38). Flasks must be conditioned, calibrated, and designated for H-Std 1.

Table 4.37. Low-Range Cation Working Calibration Standards

Low Std. #	Solution	Volume (mL)	Final Volume (mL)	Na^+ (mg L^{-1})	K^+ (mg L^{-1})	NH_4^+ (mg L^{-1})	Ca^{2+} (mg L^{-1})	Mg^{2+} (mg L^{-1})
1	stock			0.500	0.500	0.500	0.500	0.500
2	L Std. 1	125	250	0.250	0.250	0.250	0.250	0.250
3	L Std. 1	50	250	0.100	0.100	0.100	0.100	0.100
4	L Std. 1	25	250	0.050	0.050	0.050	0.050	0.050
5	L Std. 1	10	250	0.020	0.020	0.020	0.020	0.020

Table 4.38. High-Range Cation Working Calibration Standards

High Std. #	Solution	Volume (mL)	Final Volume (mL)	Na^+ (mg L^{-1})	K^+ (mg L^{-1})	NH_4^+ (mg L^{-1})	Ca^{2+} (mg L^{-1})	Mg^{2+} (mg L^{-1})
1	Stock			5.000	3.000	5.000	5.000	3.000
2	H Std. 1	200	250	4.000	2.400	4.000	4.000	2.400
3	H Std. 1	100	250	2.000	1.200	2.000	2.000	1.200
4	H Std. 1	50	250	1.000	0.600	1.000	1.000	0.600
5	H Std. 1	25	250	0.500	0.300	0.500	0.500	0.300

IC system software should be capable of addressing low and high calibration ranges in one analytical run.

Run all samples in the low calibration range and for values above the low range, use the high calibration range. Dilute only those samples that have values above the high range.

Working Standard Solutions

A minimum of five working calibration standard solutions per curve is recommended. IC curves are not linear and often do not go through zero. Most IC workstations allow for an unlimited number of standards. By preparing low calibration (Table 4.37) and high calibration (Table 4.38) IC curves, the potential for biases due to this non-linearity is minimized. See Figure 4.40 for calibration curves for sodium.

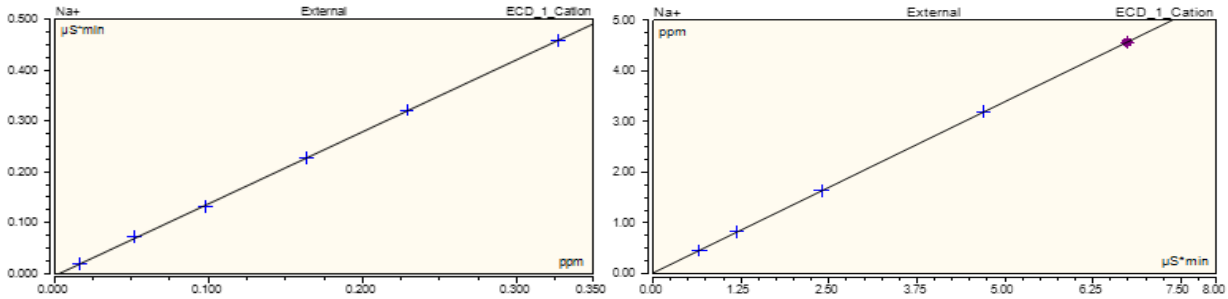


Figure 4.40. Calibration curves for low- and high-level sodium, resp. Linearity is good in both ranges.

Standard operating procedures call for first measuring all samples against the low calibration standards. Results above this range are read using the high calibration range. Concentrations that exceed the high-calibration limits must be diluted and reanalyzed. Never extrapolate the calibration curve to estimate results.

The two ranges of working standards illustrated in Tables 4.37 and 4.38 are typical; however, these ranges may not be best for every laboratory. In practice, ranges must be established by each laboratory and may vary over time.

Working calibration standards may be stored in clean HDPE containers at room temperature and are stable up to six weeks.

Quality Control

Preparing QC Solutions

Prepare two QC solutions, one for the low calibration range and one for the high calibration range. Analyze a low QC sample immediately after the IC is calibrated in the low range. Do the same in the high calibration range using the high QC solution. See Appendix C for details on sterilization and preparation of QC solutions.

Low QC Solutions – Precipitation Matrix

1) Save the excess volume from low-concentration precipitation samples that have been analyzed and reported. Pool the excess from some of these samples in a 10 L HDPE container and the excess from other low-concentration samples in a second 10 L HDPE container.

2) Analyze the pooled samples from each container. Designate the pooled sample with the lower concentrations as QC-A and the other as QC-B. QC-A will generally have the lowest concentration of each analyte.

3) Add DI water as needed to bring the cation concentrations of QC-A near the detection limit. Add stock standard solution as needed to bring the cation concentrations of QC-B to the mid to high range of the low calibration curve.

4) See Appendix C for sterilization and further details.

High QC Solutions – Precipitation Matrix

1) Save the excess volume from high-concentration precipitation samples that have been analyzed and reported. Pool the excess from some of these samples in a 10 L HDPE container and the excess from other high-concentration samples in a second 10 L HDPE container.

2) Analyze the pooled samples from each container. Designate the pooled sample with the lower concentrations as QC-C and the other as QC-D.

3) Adjust concentrations using stock standard solution, as needed, so that QC-C concentrations are near the low to mid-range of the high calibration curve and QC-D concentrations are near the mid to high range of the high calibration curve.

4) See Appendix C for sterilization and further details.

Analytical Procedures

1) Do not power down an IC system when not in use. Always leave the power on.

2) Check reagent levels. Check the eluent cartridge and ensure there is enough for a full run. Change the DI water in the flush reservoir and sample changer every day. Inline filters may be used to minimize particulate introduction into the system. Change inline filters daily.

3) Run DI water samples until the system is stable and equilibrated.

4) Label each tube. Prepare a schedule of analysis in the workstation software. Enter sample identification numbers into the software in the same order as the tubes will be installed in the sample changer rack.

5) Prepare samples for analysis. Ensure that each tube has a minimum volume. Minimum volumes will vary according to the size of the injection loop and loop rinse. Cover each tube opening with a pierceable cap or use Parafilm®. Place the tubes in order in the sample changer rack.

6) Check for a stable pump pressure and conductivity.

7) Check the DI water chromatogram for the correct shape. See Figure 4.41.

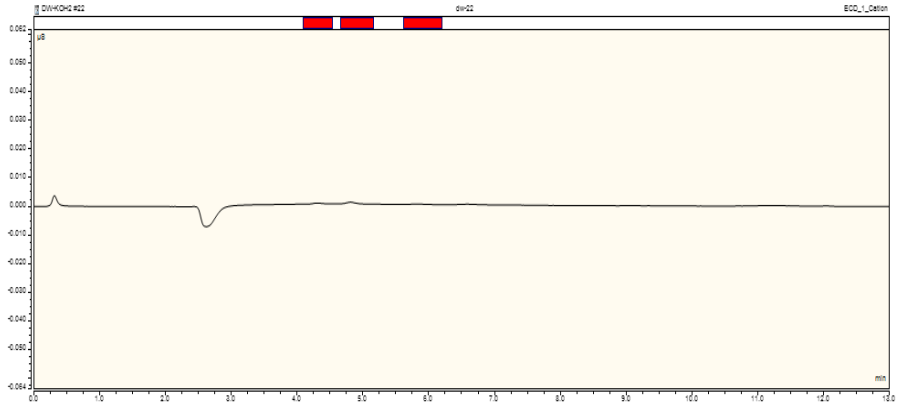


Figure 4.41. DI water chromatogram using MSA as eluent. Note the small water dip and small peak that marks a slight increase in pump pressure at the point of sample injection.

8) Initiate the run. Run calibration standards first. The injection should start with the highest concentration standard followed by decreasing concentrations.

9) Run a low QCS directly after completing the low calibration curve and a high QCS after completing the high calibration curve. Inject a QCS, selected at random, every ten samples thereafter. Plot the QCS results on control charts.

10) Calibrate every 30 to 50 samples.

11) Following the run, check all calibration curves and QC results before reporting, collating, or tabulating sample results. Use only the peak area, not the peak height, for calculating results.

12) Check each chromatogram individually for correct shape and integration. See Figure 4.42. Comment on all anomalies and flag data accordingly.

13) Calculate results against the appropriate calibration curve. Use the correct decimal places. Apply detection limit notations as needed. Mark all samples that exceed upper calibration ranges for dilution and repeat analysis. Account for missing samples and ensure that contamination codes are applied as needed.

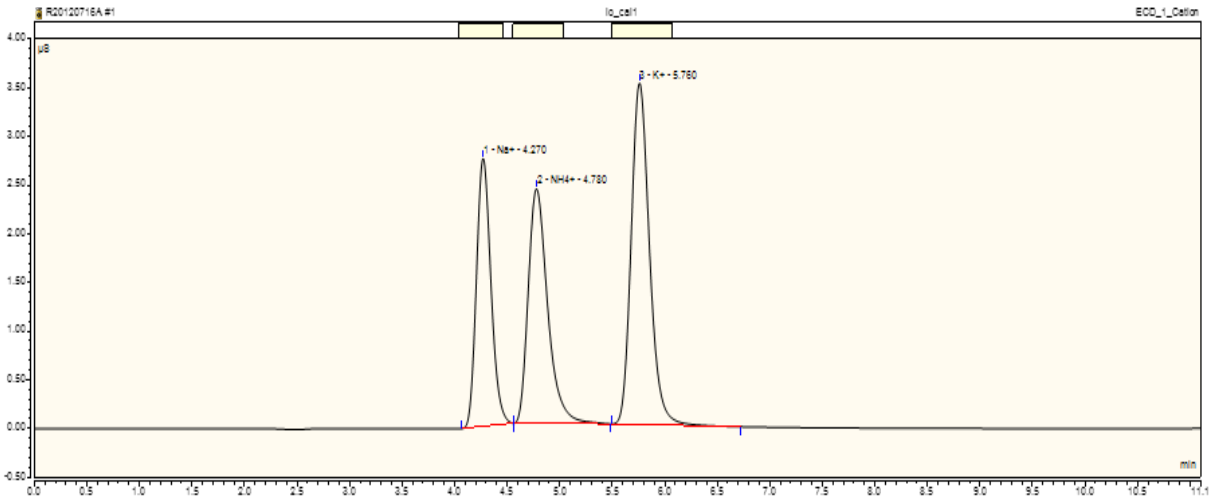


Figure 4.42. Typical cation chromatogram using MSA as eluent.

14) Export the data from the IC system and archive all parameters associated with the analysis, including calibration data, integration data, and instrument audit trails. Audit trails include instrument parameters that may be useful in diagnosing a problem, such as a chromatogram that exhibits a drifting baseline. It may be necessary to repeat the analysis at the point where a problem started.

Troubleshooting

Problem 1: Pump loses pressure or prime.
Solution 1: Check the EluGen® cartridge for leaks. Change the cartridge if required. Prime the pump. Check the system for leakage. Re-prime the pump and run DI water to check the system. If the pump is still unstable, disconnect the column and pump methanol through the system. Flush with water. If these steps do not eliminate the problem, change the piston seals (provided the operator has been trained to do so). Soak the piston seals in methanol for a few minutes. This ensures a better seal around the piston.

Problem 2: Precision not meeting QC specifications.
Solution 2: Check the injection valve for leaks or blocks. Make sure the sample loop is filling with each injection. Check the probe and sample lines for plugs or leaks. Change the sample loop and clean the injection valve. A plug can be found by disconnecting each length of tubing one section at a time. Pump pressure will increase significantly if the bed supports are fouled or the guard column is fouled.

Problem 3: Ammonium curve drops below zero at low concentrations but other cation curves are linear

and go through zero and behave as expected.
Solution 3: Prepare new standards and repeat the calibration. If this does not improve, change the suppressor.

Problem 4: Retention times shorten and resolution between ammonium and sodium is poor.
Solution 4: Clean or change the guard column. If there is no improvement clean or change the separator column.

Problem 5: Ammonium peak is very high and crowds the potassium peak into a 'rider' position. See Figure 4.43 for example.
Solution 5: Dilute the sample to reduce the ammonium peak and rerun the sample for potassium.

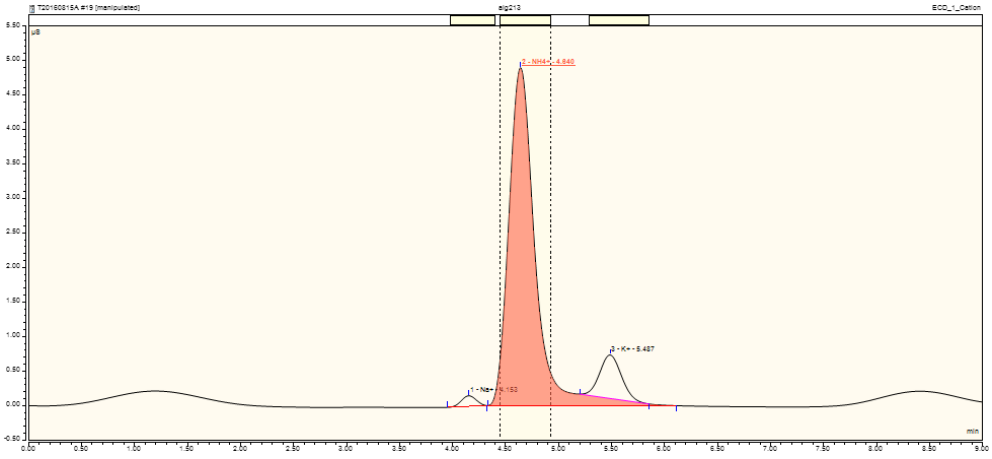


Figure 4.43. Potassium peak riding on the tail of a high ammonium peak. This chromatogram shows magnesium cut off from the chromatogram and the calcium from the previous sample eluting first (see Problem 6.).

Problem 6: Calcium and magnesium elute very late and are seen eluting in the following chromatogram (See peak at the beginning of Figure 4.43.).
Solution 6: Extend the run time. Sometimes run times and retention times can lengthen significantly when new columns are put into service. It is important to test the system with calibration standards to identify the retention times and adjust the run times accordingly.

4.5.10 Ammonium by Flow Injection Analysis Colorimetry

Background

Flow Injection Analysis (FIA) offers a precise colorimetric determination of ammonium concentrations. Here are some advantages of ammonium analysis by FIA compared with IC:

1) FIA instruments use injection loops so that each sample has the same volume injected into the reagent stream. FIA systems are robust, sensitive and versatile. Multiple systems can be linked to draw from a single sample tube. Most systems are modular so that a module can be replaced without replacing the entire system.

2) FIA systems offer fast run times. Up to 150 samples can be analyzed in duplicate in a single working day. By comparison, ammonium analysis by IC would take two days, running over night, to analyze 150 samples. Fast run times have another advantage; since ammonium is unstable, it is important to perform the analysis quickly.

3) Calibration curves for FIA colorimetry have very good linearity through the zero point. IC calibration curves for ammonium are non-linear and often do not go through zero. The detection limit can deteriorate with aging standards when the curve drops below the zero point. FIA can have detection limits as low as 0.005 mg L^{-1}.

Colorimetry is the measurement of light transmitted through a colored complex as a function of concentration. Colorimetry follows the Beer-Lambert law which states: Absorbance is linearly proportional to the thickness (or path length) of the sample, the concentration of the absorbing medium, and the absorptivity or measure of a given molecule to absorb light. The fundamental relationship between the amount of light entering or incident on a sample and the amount of light passing through or transmitted by that sample is described mathematically as (Clark, 2013):

$$\text{Log } (I_o/I) = abc = \text{Absorbance}$$

Where:
I_o = incident radiant power
I = transmitted radiant power
a = absorptivity (constant for a given system)
b = sample path length
c = concentration of absorbing species (ppm)

In FIA colorimetry, a sample is mixed with chemical reagents in precise proportions and the reagents and sample combine to yield a colored complex. A sample changer provides a sample tray from which the sample is introduced into the sample loop and injection valve. A peristaltic pump delivers sample, carrier and reagents to a manifold where the reagents and sample are mixed. Proportioning the amount of reagent is controlled by the inner diameter of the reagent pump tubing. The color complex absorbs light of a specific wavelength in proportion to the concentration of the analyte being measured. Figure 4.44 shows a typical FIA system for ammonium analysis.

Here's a brief description of how ammonium by FIA is measured. The sample is injected into the system along with ethylenediaminetretraacetate, or EDTA, which prevents the calcium and magnesium in the sample from precipitating. This solution is further mixed with alkaline phenol and with sodium hypochlorite to form an indophenol blue complex. Sodium nitroprusside is added to enhance the sensitivity of the measurement by intensifying the color of the blue complex. This solution enters a temperature-controlled heating block which at 60ºC increases the rate of color formation. After color development, the flow cell receives the solution with its colored complex and a light beam at a wavelength of 630 nm passes through the sample. The light transmitted through the sample is measured by the photodetector and the transmitted light is directly proportional to the ammonium ion concentration in the sample.

Note that the odor of phenol, one of the reagents, is unpleasant, though not harmful. Due to this odor, it is recommended that this dedicated FIA system be placed in a well-ventilated room.

The calibration curve is constructed using 6 to 10 calibration standards and a DI water zero standard. From this curve, the concentration of ammonium in the precipitation sample is determined.

Apparatus

FIA systems use injection loops to introduce the sample to the colorimeter, offering a more precise system than auto-analyzers.

i. FIA colorimeter
ii. sample changer
iii. reagent delivery system (peristaltic pump)
iv. manifold with flow cell

v. desktop computer and printer
vi. workstation software
vii. analytical balance

Reagents and Solutions (CAPMoN, 2013)

i. DI water – the carrier - purity is highly important. It should be filtered and deionized to a resistivity of >18.0 MΩ.
ii. 20% H_2SO_4
iii. helium (UHP grade)
iv. sodium phenolate
   a. liquid phenol (ASC grade)
   b. sodium hydroxide (ASC grade)
v. disodium ethylenediamine-tetraacetate of EDTA (ASC grade)
vi. sodium hypochlorite (ASC grade) Do not use household bleach.
vii. sodium nitroprusside (ASC grade)

Sparging with Helium

Bubble helium vigorously through the sodium hypochlorite, disodium EDTA and sodium nitroprusside for a minimum of 1 minute to remove ammonium. Reduce the gas flow if the solution foams up and threatens to spill over.

20% H_2SO_4 (Scrubber)

Dilute 200 mL concentrated H_2SO_4 (95%-97% GR grade) in 800 mL of DI water. Note in Figure 4.39 how this H_2SO_4 scrubbing solution is vented to all reagent containers to cleanse any air in these containers of incidental ammonia gas. Refresh the scrubber solution every three months.

Sodium Phenolate

1) In a 2 L volumetric flask dissolve 188 g liquid phenol (C_6H_5OH) (ASC grade) in approximately 1 L of DI water. While stirring, slowly add 64 g sodium hydroxide. Continue stirring until the sodium hydroxide (NaOH) pellets are dissolved.

2) Let the solution cool. Dilute to the 2 L mark with DI water. Cap the flask and invert it three times to mix the solution thoroughly. Do not sparge this solution.

3) Store at room temperature in two darkened 1 L Nalgene bottles. Stable for one month.

Sodium Hypochlorite

Attention: Prepare one day in advance of the day of analysis.

1) Dilute 300 mL 5% sodium hypochlorite (NaOCl) (reagent grade) to 500 mL with DI water in a volumetric flask. Cap and mix by inversion.

2) Sparge with helium. Store at room temperature in a 1 L Nalgene bottle. Stable for 24 hours.

Disodium Ethylenediamine-Tetraacetate (EDTA)

1) In a 2 L volumetric flask, dissolve 110.8 g Na_2EDTA and 11.0 g NaOH in 1 L of DI water. Dilute to the 2 L mark with DI water. Cap and mix by inversion.

2) Sonicate the flask for approximately 1 hour to dissolve the solutes or insert a stirring magnet and mix on a magnetic stirrer until the solution is clear.

3) Sparge with helium. Store the solution at room temperature in a 2 L Nalgene bottle. Stable for one month.

Sodium Nitroprusside

1) In a 2 L volumetric flask, dissolve 7.0 g sodium nitroprusside (sodium nitroferricyanide) $[Na_2[Fe(CN)_5NO]\cdot 2H_2O]$ in 1 L of DI water. Dilute to the 2 L mark with DI water. Cap and mix by inversion to dissolve the crystals.

2) Sparge with helium. Store the solution at room temperature in a 2 L Nalgene bottle. Stable for one month.

Reagent consistency is important. New materials should be tested for consistency of results. See section 4.3.15 for details.

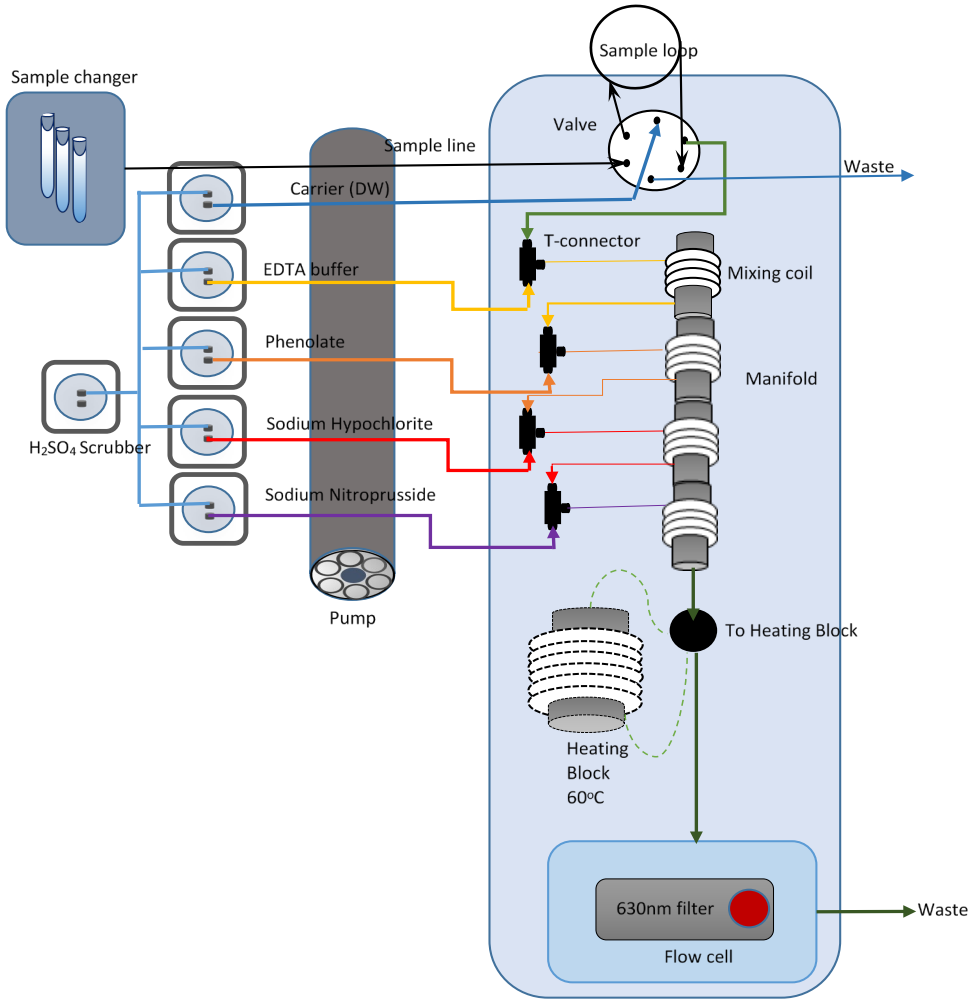


Figure 4.44. FIA Setup for Ammonium

Calibration

Calibrate the FIA after every 40 to 50 injections. FIA calibration curves generally are linear; however, the curves will tend to flatten at very high concentrations. Use only the linear portion of the curve. The expected analytical range for this method is 0.005 mg L^{-1} to 1.000 mg L^{-1}.

Stock Standard Solution (1000 mg L^{-1})

To prepare the ammonium chloride solution, place approximately 4 g of NH_4Cl in a glass crucible and dry it overnight at 110ºC.

New flasks and bottles are conditioned by soaking in DI water over night. Following this soak, rinse the flasks and bottles with DI water three times and dry in a warm oven. Flasks and bottles need only be conditioned once when they are new and before putting them into service. See Appendix C for calibration of flasks and analytical balances and for glassware storage details.

1) Weigh all volumes using an analytical balance. Rinse all weigh boats thoroughly. Use containers that are dedicated to the preparation and storage of stock standard solutions.

2) It is highly recommended that stock standard solutions be prepared by weighing the DI water

volume. Calibrate the receiving flasks by dispensing DI water by weight into the flask and then marking the flask at the fluid line.

3) Gently tap approximately 2.972 g of dry ammonium chloride into a weigh boat. Record the weight to 3 decimal places in your calibration logbook.

4) In a 1 L calibrated and conditioned volumetric flask dissolve the NH_4Cl powder in 800 mL of DI water. Dilute to the 1 L mark with DI water. Cap the flask and invert it three times.

5) Calculate the concentration of NH_4^+ to 3 decimal places. Record the concentration and preparation date in your calibration logbook. This solution contains 1000.000 mg L^{-1} of NH_4^+.

6) Record the concentration and preparation date on a label and affix it to the flask.

7) To ensure consistency between old and new stock standard solutions, prepare a dilution of the new stock standard solution and analyze it as an unknown.

   i. Into a rinsed weigh boat dispense 0.500 gm of new stock standard solution.
   ii. Pour this solution into a clean, rinsed and calibrated 1 L volumetric flask.
   iii. Rinse the weigh boat into the flask and fill the flask to the mark with Type I DI water.
   iv. Mix well and allow the solution to equilibrate at least one hour.
   v. Analyze as a sample, using the old calibration set.
   vi. Results should be 0.500 mg L^{-1} and fall within the QC precision guidelines at that concentration.
   vii. 1000 mg L^{-1} standard is best kept in its original glass flask sealed with Parafilm® around the stopper. Store at 4°C. Stable for six months.

8) If the concentration of this new diluted stock standard solution does not fall within the expected range, discard the 1000 mg L^{-1} solution and prepare a new stock standard solution. Make sure the newly prepared stock standard solution has equilibrated for one hour before analysis.

Intermediate Working Standard (1.000 mg L^{-1})

1) Prepare this working standard fresh with each analytical run. Use a calibrated glass volumetric flask dedicated for the preparation and storage of this solution. A best practice is to use the same flask that was used to store the working standard from the previous run. This flask is conditioned for an NH_4Cl solution of the same strength. Empty this seasoned flask and rinse it three times with DI water. You can use DI water from the holding tank for the first two rinses, but preform the final rinse using fresh DI water from the point of use dispenser. Fresh DI water should be completely free of any dissolved ammonium.

2) Weigh all volumes using an analytical balance. Rinse all weigh boats thoroughly.

3) Dispense 1.000 g of stock standard solution into a weigh boat. Measure to 3 decimal places. Record the weight in your calibration logbook.

4) Transfer this aliquot into the 1 L calibrated, conditioned, dedicated volumetric flask and dilute to the 1 L mark with **fresh DI water** from the point of use dispenser. Cap the flask and invert it three times to ensure the solution is thoroughly mixed.

5) Calculate the concentration of NH_4^+ to 3 decimal places. Record the concentration in your calibration logbook. This solution contains approximately 1.000 mg L^{-1} NH_4^+. Use this intermediate working standard to make the rest of the standards as in table 4.39.

Calibration Standards

1) Weigh all volumes using an analytical balance. Rinse all weigh boats thoroughly. Use calibrated, dedicated glass volumetric flasks as a final reservoir. Do not use vessels from other procedures.

2) Use DI water from the dispensing gun as the tenth or zero calibration standard.

3) Table 4.39 lists a suggested calibration series. Figure 4.45 illustrates a calibration curve using these standards.

Table 4.39. NH_4^+ Calibration Standard Preparations (CAPMoN, 2013)

Calibration Standard #	Intermediate Working Standard Volume (mL)	Final Volume (mL)	Concentration (NH_4^+) (mg L^{-1})
1	Intermediate Working Standard		1.000
2	80	100	0.800
3	50	100	0.500
4	30	100	0.300
5	10	100	0.100
6	8	100	0.080
7	5	100	0.050
8	3	100	0.030
9	1	100	0.010
10	0	100	0.000

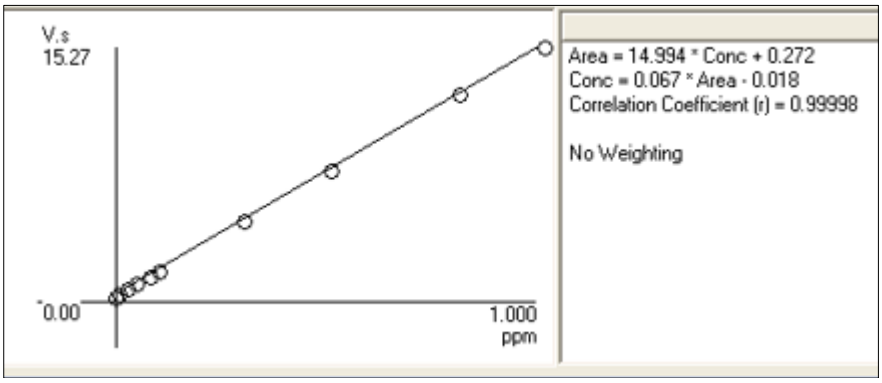


Figure 4.45. Ammonium Calibration Curve

Quality Control

Ammonium deteriorates too rapidly in natural precipitation, even if refrigerated, to use for QC solutions. Other sources of QC solutions include:

- Certified Reference Material
- simulated samples made from an alternate standard material such as ammonium sulfate
- Pooled inter-laboratory comparison samples may be used if they have been sterilized by filtration; however, do not use this solution if a pipette has been dipped into it or if the bottle openings have been touched with hands, allowing for bacterial contamination.

Prepare at least three QC solutions in the standard range, like standards 9, 7 and 4 in Table 4.39. QC solutions should test the low, mid, and high range of the calibration curve.

Analytical Procedure

1) Turn on the computer, sample changer, peristaltic pump and system unit that houses the heating block and light source.

2) Place the waste line from the flow cell into a waste container. Connect the transmission tubing to the reagents. Waste contains phenol and other reagents and must not be poured down the sink. A container equipped with a charcoal filter on the air vent will help lessen the phenol odor. Dispose of waste responsibly following local regulations.

3) Place the reagent pump tubing under tension as per the manufacturer's specifications.

4) Start the peristaltic pump and condition the transmission tubing with the reagents and establish a baseline. Periodically check the display for any deviation from the baseline. This may indicate that there are air pockets in the tubing. Conditioning usually takes about half an hour. The analyst can make up standards during this warm-up period.

5) Run a DI water sample to check instrument stability. Usually only one to three water injections are required.

6) Start the calibration after a good baseline has been achieved and the temperature of the heating unit has reached 60°C.

Do not dispense samples to be analyzed until the system demonstrates a stable baseline. This delay will help minimize exposure of the samples to laboratory air. Humans exhale ammonia and this gas has many other sources, including some cleaning solvents. It is important to isolate standards and samples from the ambient laboratory environment.

7) Begin instrument calibration by dispensing about a 5 mL volume of the calibration standards into each tube. This is enough for two analyses from the same tube. Be aware that when tubes are labeled some markers may have ammonia in the ink. Use a wax pencil or labels with an adhesive backing. Place Parafilm® over each tube promptly after adding liquid to the tube. Ensure each tube has the minimum volume required for duplicate readings.

8) Run the calibration sequence followed immediately by a QCS. After all standards have been run, re-cap all standards. If the system does not stabilize and a troubleshooting period is required, dispense fresh standards again.

9) Continue with precipitation samples only after instrument stability and successful calibration have been verified.

10) Fill the sample rack according to the specified sample sequence. Ensure there is enough volume in each tube to perform the analysis without drawing air into the FIA system. A run of fifty samples (in duplicate) takes about 1.5 hours. Standards that have been capped can be used for the next calibration. Dispense fresh standards from the flasks every three hours.

Note: Do not discard calibration standards after analysis. Leave the standards in the flasks to condition the flasks.

11) Following the run recheck all calibration curves and QC results before reporting, collating or tabulating results.

12) Check all raw data points individually for correct integration and output. Comment on all anomalies and flag data accordingly.

13) Calculate results against the appropriate calibration curve and report the correct number of decimal places. Apply detection limit notations as needed. Mark all samples that exceed upper calibration ranges for dilution and repeat analysis. Ensure missing samples are accounted for and contamination codes are applied as needed.

14) Export data from the FIA system and archive all parameters associated with the analysis, including calibration data, raw measurement data, sample identification and instrument audit trails. Audit trails include instrument parameter settings that may be useful when the baseline is found to have drifted. It may be necessary to repeat the analysis from the point where a problem was discovered in the FIA output. The signal trace from an FIA is called an FIAgram. Figure 4.46 is an example of an FIAgram.

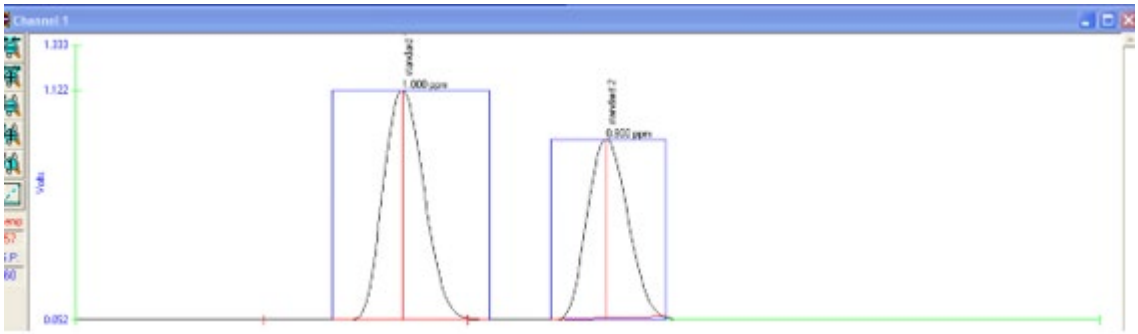


Figure 4.46. Example of FIAgram

Troubleshooting

Problem 1: Air spikes are observed on the real-time display as noisy irregular spikes in the baseline or sample peak.
Solution 1: Air bubbles will be introduced to the flow cell if reagents are not degassed or the transmission tubing is not securely connected. Ensure all reagents are sparged with helium in advance of analysis. Check the volume of sample in each tube to ensure there is enough for the analysis. If a tube cracks and the sample leaks out, the probe could draw in air causing air spikes.

Problem 2: Carry-over. Extremely high-concentration samples do not wash out of the sample lines completely, resulting in erroneously high readings for the following sample or samples.
Solution 2: Samples must be reanalyzed. Dilute the high-concentration sample and compare the result with the first analysis. Also, repeat the analyses of several samples that followed the high-concentration sample and by comparing the initial and re-analysis results ensure that any carry-over effects are eliminated. Do not analyze very contaminated samples. Samples contaminated with bird droppings will not produce a valid ammonium result and only contaminate the system. Report, code or comment that the sample was too contaminated for analysis.

Problem 3: Reagent line leakage.

Solution 3: Replace peristaltic pump lines frequently depending on use. Keep maintenance logs, review the logs and periodically check the condition of all of the lines. Replace transmission lines as needed. Sometimes the waste line can be blocked causing enough back pressure to loosen the reagent line connections or even cause them to become disconnected. Ensure the waste line is clear at all times. Look for crimps on the waste line and ensure lines are not tangled or constricted in any way.

Problem 4: Deterioration of QC measurements.
Solution 4: Check the age of the QCS. Prepare fresh QCS as needed. Prepare the ammonium hypochlorite one day before the day of analysis.

Problem 5: Low peak areas on calibration standards and controls compared to previous runs.
Solution 5: Check the age of all reagents and prepare fresh. Check sample lines for blockage.

Problem 6: Peaks show a small dip before each peak, i.e., a pre-peak dip.
Solution 6: If samples have been acidified as a preservation step, the pH in the reagent system will change. This pre-peak dip can be overcome by adding a proportional amount of acid to the DI water carrier, so that the pH of the mixture of reagents matches the pH of the sample matrix. See Figure 4.47 for an example of pre-peak dips.

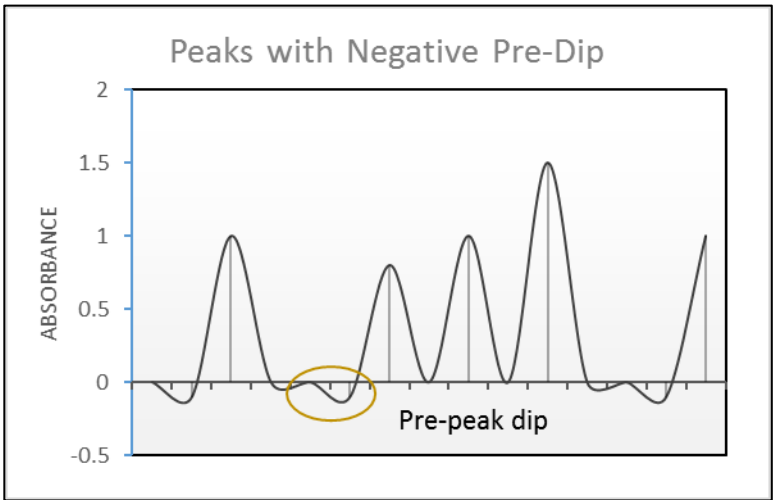


Figure 4.47. FIAgram with pre-peak dip

Tips to Improve Performance

1. Keep a logbook that records maintenance procedures.
2. Maintain a logbook for details of calibration solution preparations. Record dates of preparation, calculations, weights, volumes and the person who prepared the solutions.
3. Calibration solutions need dedicated glassware and containers. Do not use these containers for any other use.
4. Reserve an analytical balance for standard preparation and use another balance for reagents.
5. Cap samples immediately to minimize exposure to ambient air and minimize the head space in sample tubes.
6. Prepare standards fresh with each analytical run. Use calibrated flasks that have not been used for any other solutions. Weigh out calibration standard solutions.
7. Keep the area clean. Do not allow reagent spills to dry and form residue on laboratory benches.
8. Install the instrument in a low traffic area. Place in a well-ventilated room.
9. Change the peristaltic pump tubing every 400 injections.
10. Change interconnecting tubing every three months.
11. Do not top up reagent bottles. Empty reagent bottles completely and rinse the container before putting new reagent in the bottle.

12. Always check that the sample line and injection valve is free of bacterial growth. Check that the waste line flows freely. T-junctions are places for residue to collect so regularly check them.
13. Do not extrapolate standard curves under any circumstances. Colorimetric calibration curves flatten out when saturated.

4.5.11 Total Phosphorus Using Digestion and Flow Injection Analysis Colorimetry

Background

Phosphorus is present in water in three forms: orthophosphate, acid hydrolysable phosphate and organic phosphate. In this method organic phosphorus and other phosphates are converted to orthophosphate by UV persulfate digestion and by sulfuric acid digestion, respectively. Once converted to orthophosphate, the analysis proceeds by Flow Injection Analysis (FIA) – colorimetry. FIA technology is described in the background of section 4.5.10.

For total phosphorus, an added module is needed in the FIA system to digest the various forms of phosphorus and convert them to orthophosphate. Following digestion, the sample is mixed with ammonium molybdate and antimony tartrate to form phosphomolybdate, which is reduced under acidic conditions to form a blue complex. Colorimetry, following the Beer-Lambert Law, is employed to detect and quantify this blue complex at a wavelength of 880 nm. The transmitted light is proportional to the concentration of orthophosphate, which is a measure of the total phosphorus in solution.

Reagent and sample delivery are via a peristaltic pump. An exact amount of sample is introduced into the reagent stream using an injection valve. Figure 4.48 illustrates the FIA system for total phosphorus.

Apparatus

i. FIA Colorimeter
ii. sample changer
iii. sample digestion module with 254 nm UV lamp and 120^{o}C heater
iv. peristaltic pump
v. manifold with flow cell
vi. desktop computer and printer/workstation software
vii. analytical balance

FIA systems may be set up to run more than one method by splitting the sample stream. FIA systems use Rheodyne injection valves. The sample waste line from the first injection valve is connected to the in-port of the injection valve in the second system. Be aware that total analysis times are important to synchronize sampling times and data output.

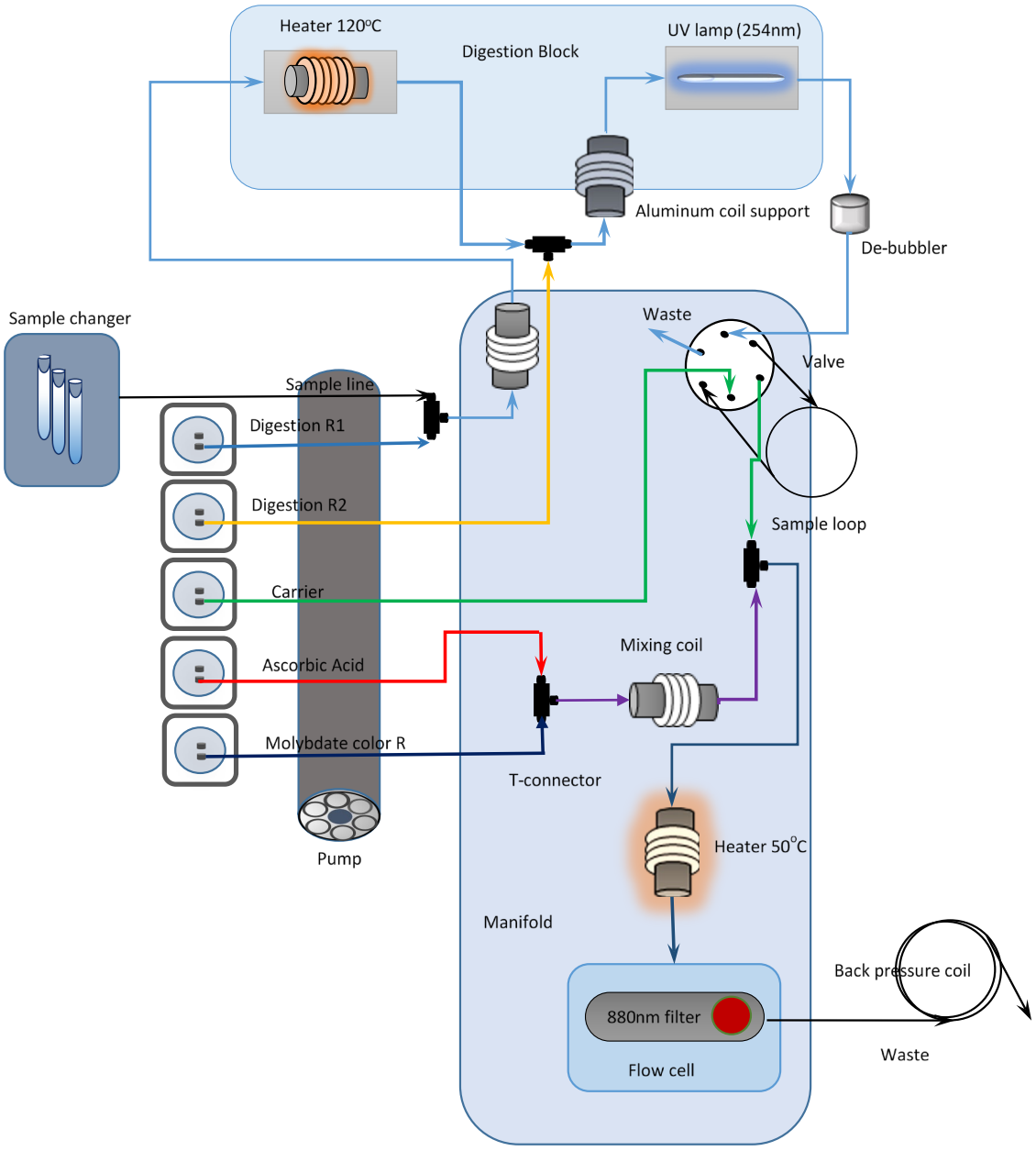


Figure 4.48. Schematic of FIA System for Total Phosphorus

Reagents and Solutions (Missouri State University, Ozarks Environmental and WaterResources Institute (OEWRI), 2007)

i. DI water – purity is especially important. It should be filtered and deionized to a resistivity >18.0 MΩ.
ii. ammonium molybdate (ASC grade)
iii. antimony potassium tartrate (ASC grade)
iv. molybdate color reagent
   a. sulfuric acid (H_2SO_4)
   b. ammonium molybdate (ii)

c. antimony potassium tartrate (iii)
v. ascorbic acid reducer
a. ascorbic acid
b. sodium dodecyl sulfate
vi. carrier
a. sulfuric acid (H_2SO_4)
b. potassium chloride (KCl)
vii. digestion reagent 1
a. sulfuric acid (H2SO4)
viii. digestion reagent 2
a. potassium persulfate ($K_2S_2O_8$)

Ammonium Molybdate Solution

Dissolve 40.0 g ammonium molybdate tetrahydrate [$(NH_4)_6Mo_7O_{24}$ - $4H_2O$] in 800 mL of DI water in a 1 L volumetric flask. Dilute to the mark and stir this mixture with a magnetic stirrer for at least four hours. Stable for two months at 4°C.

Antimony Potassium Tartrate

Dissolve 3.0 g potassium antimonyl tartrate hemihydrate [$K(SbO)C_4H_4O_6$.$1/2H_2O$] in 800 mL of DI water in a 1 L volumetric flask. Dilute to the mark and mix until dissolved. Store in a dark HDPE bottle at 4°C. Stable for two months.

Molybdate Color Reagent

Add 25 mL concentrated sulfuric acid to 500 mL DI water in a 1L volumetric flask. Add 213 mL of ammonium molybdate solution (above) and 72 mL of antimony potassium tartrate solution (above). Dilute to mark. Prepare fresh weekly. Discard if blue or yellow precipitate develops.

Ascorbic Acid Reducer

Dissolve 70.0 g granular ascorbic acid in approximately 700 mL of DI water in a 1L volumetric flask. Dilute to the mark and mix. Add 1.0 g sodium dodecyl sulfate and mix. Prepare fresh with every run.

Carrier

Add 30 mL sulfuric acid to 600 mL DI water in a 1L volumetric flask. Add 9.0 g potassium chloride and dilute to the mark with DI water. Mix. Stable for one week.

Digestion Reagent 1

Add 106.5 mL concentrated sulfuric acid to 500 mL DI water in a 1L volumetric flask. **Caution: This solution is highly exothermic**. Dilute to the mark and mix by inversion. Allow to cool to room temperature before using.

Digestion Reagent 2

Add 26 g potassium persulfate ($K_2S_2O_8$) to 800 mL DI water in a 1 L volumetric flask. Mix until dissolved. Dilute to the mark. Stable for one week.

Calibration

Calibrate the FIA every 40 to 50 injections. Since colorimetry follows Beer's Law, the calibration curve is linear, though the curve will flatten at the upper end if too-concentrated standards are used. Use only the straight linear portion of the curve. The expected analytical range for total P by FIA-colorimetry is 0.003 to

0.500 (mg L^{-1} P).

Stock Standard Solution (1,000 mg L^{-1} P)

To prepare the phosphate stock solution, place approximately 4 g of KH_2PO_4 in a glass crucible and dry it overnight at 110ºC.

New flasks and bottles are conditioned by soaking in DI water over night. Following this soak, rinse the flasks and bottles with DI water three times and dry in a warm oven. Flasks and bottles need only be conditioned once when they are new and before putting them into service. See Appendix C for calibration of flasks and analytical balances and for glassware storage details.

1) Weigh all volumes using an analytical balance. Rinse all weigh boats thoroughly. Use containers that are dedicated to the preparation and storage of stock standard solutions.

2) It is highly recommended that stock standard solutions be prepared by weighing the DI water volume. Calibrate the receiving flasks by dispensing DI water by weight into the flask and then marking the flask at the fluid line.

3) Gently tap approximately 2.197g of dry anhydrous KH_2PO_4 into a weigh boat. Record the weight to 3 decimal places in your calibration logbook.

4) In a 500 mL volumetric flask dissolve the KH_2PO_4 powder in 200 mL DI water. Dilute to the 500 mL mark with DI water. Cap, then invert the flask three times to thoroughly mix the solution.

5) Calculate the concentration of P to 3 decimal places. Record the concentration and preparation date on a label and affix it to the flask. Also record this information in your calibration logbook. This solution contains approximately 1000.000 mg L^{-1} P.

6) To ensure consistency between old and new stock standard solutions, prepare a dilution of the new stock standard solution and analyze it as an unknown,

   i. Into a rinsed weigh boat dispense 0.25 gm of new stock standard solution.
   ii. Pour this solution into a clean, rinsed and calibrated 1 L volumetric flask.
   iii. Rinse the weigh boat into the flask and fill the flask to the mark with Type I DI water.
   iv. Mix well and allow the solution to equilibrate for at least one hour.
   v. Analyze as a sample, using the old calibration set.
   vi. Results should be 0.250 mg L^{-1} and fall within the QC precision guidelines at that concentration. If the results do not fall within the expected range, discard this stock standard solution and prepare another 1000 mg L^{-1} P. Make sure that this solution, once prepared, has been allowed to equilibrate for one hour before analyzing it.

7) Store the newly prepared stock standard solution in its original flask and store the flask at 4 ºC. This solution is stable for 6 months.

Intermediate Working Standard (1 mg L^{-1} P)

1) Prepare this intermediate working standard fresh within 24 hours of the start of the analytical run. Weigh all volumes using an analytical balance. Rinse all weigh boats thoroughly. Use dedicated glass volumetric flasks as a final reservoir. Do not use vessels from other procedures.

2) Dispense 1.000 g of stock standard solution into a weigh boat. Measure to 3 decimal places. Record the weight in your calibration logbook.

3) Transfer this aliquot into a 1000 mL volumetric flask and dilute to the mark with **fresh DI water** from the point-of-use dispenser. Cap then invert the flask three times to ensure the solution is well-mixed.

4) Calculate the concentration of P to 3 decimal places. Record the concentration in your calibration logbook. This solution contains approximately 1.000 mg L^{-1} P. Prepare this solution fresh with each analytical run. Use this intermediate working standard to prepare all the other standards.

Calibration Standards

1) Prepare fresh calibration standards daily. Weigh all volumes using an analytical balance. Rinse all weigh boats thoroughly. Use dedicated glass volumetric flasks as a final reservoir. Do not use vessels from other procedures.

2) Use DI water from the point-of-use dispensing gun as the seventh or zero calibration standard.

3) Table 4.40 lists a suggested calibration series. Note the addition of sulfuric acid to the solutions.

Table 4.40. Phosphorus Calibration Standard Preparation

Standard #	H_2SO_4 (mL)	Intermediate Working Standard Volume (mL)	Final Volume (mL)	Concentration (mg L^{-1} P)
1	0.4	50	100	0.500
2	0.4	20	100	0.200
3	0.4	10	100	0.100
4	0.4	5	100	0.050
5	0.4	2	100	0.020
6	0.4	1	100	0.010
7	0.4	0	100	0.000

Digestion Check Control Standards

Digestion check control standards are introduced to ensure that the digestion process is working and that the organic P is completely converted to orthophosphate and accurately represented in the final total P value. Run digestion check controls every 10 to 15 samples.

Preparation of Stock Digestion Check Control Standard: 100 mg L^{-1} $P_{organic}$

Add 117.9 mg adenosine-5'-monophosphate monohydrate (AMP, MW = 363.24) to 100 mL DI water. Store refrigerated at 4°C. Stable for one month.

Digestion Check Control: 0.200 mg L^{-1} P

1) Prepare fresh with each analytical run. Dispense 0.500 ml of stock digestion check control standard (100 mg L^{-1} $P_{organic}$) into a weigh boat. Record the weight to 3 decimal places in your calibration logbook. Transfer this aliquot into a 250 mL volumetric flask.

2) Dilute to the 250 mL mark with DI water. Cap the flask and mix the solution by inverting the flask.

3) Calculate the concentration of P to 3 decimal places. Record the concentration in your calibration logbook. Prepare this solution fresh with each analytical run.

Quality Control

Phosphates deteriorate too rapidly in natural precipitation, even if refrigerated, to use precipitation for QC purposes. Other sources of QC solutions are listed, below.

- Certified Reference Material
- simulated samples made from an alternate standard material such as adenosine-5"-monophosphate monohydrate (AMP)
- Pooled inter-laboratory comparison samples may be used if they have been sterilized by filtration; however, do not use this solution if a pipette has been dipped into it or if the bottle openings have been touched with hands, allowing for bacterial contamination.

Prepare at least three QC solutions in the standard range, like calibration standards 6, 4, and 2 in table 4.40. Control solutions should test the low, mid, and high range of the calibration curve.

Note that large aliquots of any control material will deteriorate after repeated openings of the container. Store small control aliquots under refrigeration.

Analytical Procedure

1) Turn on the computer, sample changer, peristaltic pump, inline digestion units and system unit.

2) Place the waste line from the flow cell into a waste container. Connect the transmission tubing to the reagents. Waste contains toxic reagents and must not be poured down the sink. **Dispose of waste responsibly following local regulations.**

3) Place the reagent pump tubing under tension as per the manufacturer's specifications. Use only PVC tubing.

4) The inline digestion unit will take at least 20 to 30 minutes to reach the required temperature of 120°C. Start the reagent pump so reagents and samples will reach the same 120°C temperature. In addition, the heating block must reach 50°C. These temperatures must be met before starting any analysis**. Do not run heating blocks and digestion units without reagents pumping through the system. These units will overheat without fluid going through them**.

5) Establish a baseline. Periodically check the display for any deviation from the baseline that may indicate that there are air pockets in the tubing. Conditioning usually takes about half an hour. The analyst can prepare standards and samples during this warmup period.

6) Samples that are not collected and preserved with sulfuric acid must be pre-treated with sulfuric acid. This pre-treatment also applies to QCS and blank samples.

   a. Pipette 5.0 mL of each sample into labeled sample tubes. Place each tube in the correct position in the sample tray.
   b. Using a repeater pipette, add 20 µL of 10% H_2SO_4 solution into each 5 mL sample aliquot including blanks, QCS and digestion check control samples.
   c. Cap the tubes and invert three times to ensure they are well mixed.

7) Dispense 5 mL volumes of calibration standards into sample tubes. Do not add H_2SO_4 to the standards since it was added during preparation of the standards (see table 4.40). Promptly place Parafilm® over each tube and check that the minimum volume requirements are met for duplicate readings.

8) Run a DI water (i.e., a blank) sequence to check instrument stability. Usually only one to three water injections are required. Check the heating block and digestion block temperatures to ensure they have reached 50°C and 120°C, respectively. Once a stable baseline has been

achieved, begin instrument calibration.

9) Run a calibration sequence followed immediately by a QC solution. Continue with precipitation samples only after instrument stability and successful calibration have been verified. Samples that have been treated with sulfuric acid are stable for one day and may be refrigerated until analysis if necessary.

10) Calibrate every 50 samples and use a fresh aliquot of standard material each time. Run QCS and digestion check control samples every 10 samples.

11) If the system does not stabilize and a troubleshooting period is required, re-dispense fresh standards. A run of 50 samples takes about 2 hours. Dispense fresh calibration standards from the flasks every 50 samples. Use fresh standard solution to rinse the sample tube several times to eliminate any trace of the standard remaining from the previous test.

12) Following the run, recheck all calibration curves and QC results before reporting, collating or tabulating results.

13) Check all raw data points individually for correct integration and output. Comment on all anomalies and flag data accordingly.

14) Calculate results against the appropriate calibration curve and report the correct number of decimal places. Apply detection limit notations as needed. Mark all samples that exceed upper calibration ranges for dilution and repeat analysis. Ensure missing samples are accounted for and contamination codes are applied as needed.

15) Export data from the FIA system and archive all parameters associated with the analysis, including calibration data, raw measurement data, sample identification and instrument audit trails. Audit trails include instrument parameter settings that may be useful when the baseline is found to have drifted. It may be necessary to repeat the analysis from the point where the problem was discovered in the FIA output.

Troubleshooting

Problem 1: Air spikes are observed on the real-time display as noisy irregular spikes in the baseline or sample peak.
Solution 1: Air bubbles will be introduced to the flow cell if the transmission tubing is not securely connected. Ensure there is enough sample in each tube for analysis and all reagent lines are secure. If a tube cracks and the sample leaks out, the probe could draw in air resulting in air spikes.

Problem 2: Carry-over. Extremely high-concentration samples do not wash out of the sample lines completely, resulting in erroneously high concentration results for the next sample or several samples.
Solution 2: Samples must be re-analyzed. Dilute the high-concentration sample and compare the result with the first analysis. Also, repeat the analyses of several samples that followed the high-concentration sample and by comparing the initial and re-analysis results ensure that any carry-over effects are eliminated. Do not analyze samples that are contaminated, especially with insects or vegetative matter. The results are not representative of precipitation and will contaminate the FIA system. Report or code the sample as too contaminated for analysis.

Problem 3: Reagent line leakage.
Solution 3: Replace peristaltic pump lines frequently depending on use. Keep maintenance logs, review the logs, and periodically check the condition of all the lines. Replace transmission lines as needed. Sometimes the waste line can be blocked causing enough back pressure to loosen reagent line connections or even causing them to become disconnected. Ensure the waste line is always clear.

Problem 4: Deterioration of QC measurements.
Solution 4: Check the age of reagents and ensure there is a maintenance schedule for reagent

preparation. It is important to prepare reagents before deterioration occurs.
Problem 5: Only inorganic phosphorous species are being quantified. Loss of organic species.
Solution 5: Ensure the digestion block has the correct temperature. Ensure that digestion check control solutions are being run and that they correctly identify occasions when the digester is not working properly.

Problem 6: Peaks show a small dip before each peak, i.e., a pre-peak dip (see figure 4.47 in section 4.5.10).
Solution 6: If samples have been acidified as a preservation step, the pH in the reagent system will change. This pre-peak dip can be overcome by adding a proportional amount of acid to the DI water carrier, so that the pH of the mixture of reagents matches the pH of the sample matrix.

4.5.12 Total Nitrogen Using Digestion and Flow Injection Analysis Colorimetry

Background

Nitrogen is present in precipitation in both inorganic and organic forms. In this method all dissolved nitrogen compounds are converted to nitrate, a stable, oxidized, inorganic form of nitrogen. This conversion occurs in a digester that uses persulfate oxidation followed by an in-line ultraviolet (UV) light to oxidize organic and reduced forms of nitrogen to nitrate. This oxidation is achieved at 90^{o}C with additional energy supplied by the UV light.

After passing through the digester, the sample analysis proceeds by Flow Injection Analysis (FIA) – colorimetry. In the FIA, the sample is mixed with reagents that ultimately convert N to a colored dye that can be detected and quantified by a colorimeter, which is based on the Beer-Lambert Law relating light transmittal to chemical concentration. This technology is described more fully in the background of section 4.5.10.

Figure 4.49 illustrates the components of the digester and FIA-colorimetric system. In the FIA, the sample is sent through a copperized cadmium column where nitrate is reduced to the more reactive nitrite. Nitrite then undergoes diazotization with sulfanilamide under acidic conditions to form a diazonium ion. This ion couples with N-(1-naphthyl) ethylenediamine dihydrochloride, or NED, to form an azo-dye which is pink in color. The resulting dye absorbs light at 540 nm and is proportional to the total nitrogen concentration.

Apparatus

i. FIA Colorimeter
ii. sample changer
iii. sample digestion module with 254 nm UV lamp
iv. cadmium column
v. peristaltic pump
vi. manifold with flow cell
vii. desktop computer and printer/workstation software
viii. analytical balance

Reagents and Solutions

i. DI water – the carrier – purity is highly important. It should be filtered ad deionized to a resistivity of >18.0 MΩ.
ii. 10% H_2SO_4
iii. ammonium chloride buffer (ASC grade constituents)
   a. concentrated hydrochloric acid (HCl)
   b. ammonium hydroxide (NH_4OH)
   c. EDTA (Na_2EDTA)
iv. sulfanilamide color solution (ASC grade constituents)
   a. phosphoric acid (H_3PO_4)

b. sulfanilamide
c. N-(1-naphthyl)-ethylenediamine dihydrochloride (NED)

v. meta-bisulfite (ASC grade)

vi. persulfate and borate (ASC grade each)
a. potassium persulfate ($K_2S_2O_8$)
b. disodium tetraborate deca-hydrate ($Na_2B_4O_7.10H_2O$)

vii. water matrix
a. sulfuric acid (H_2SO_4)

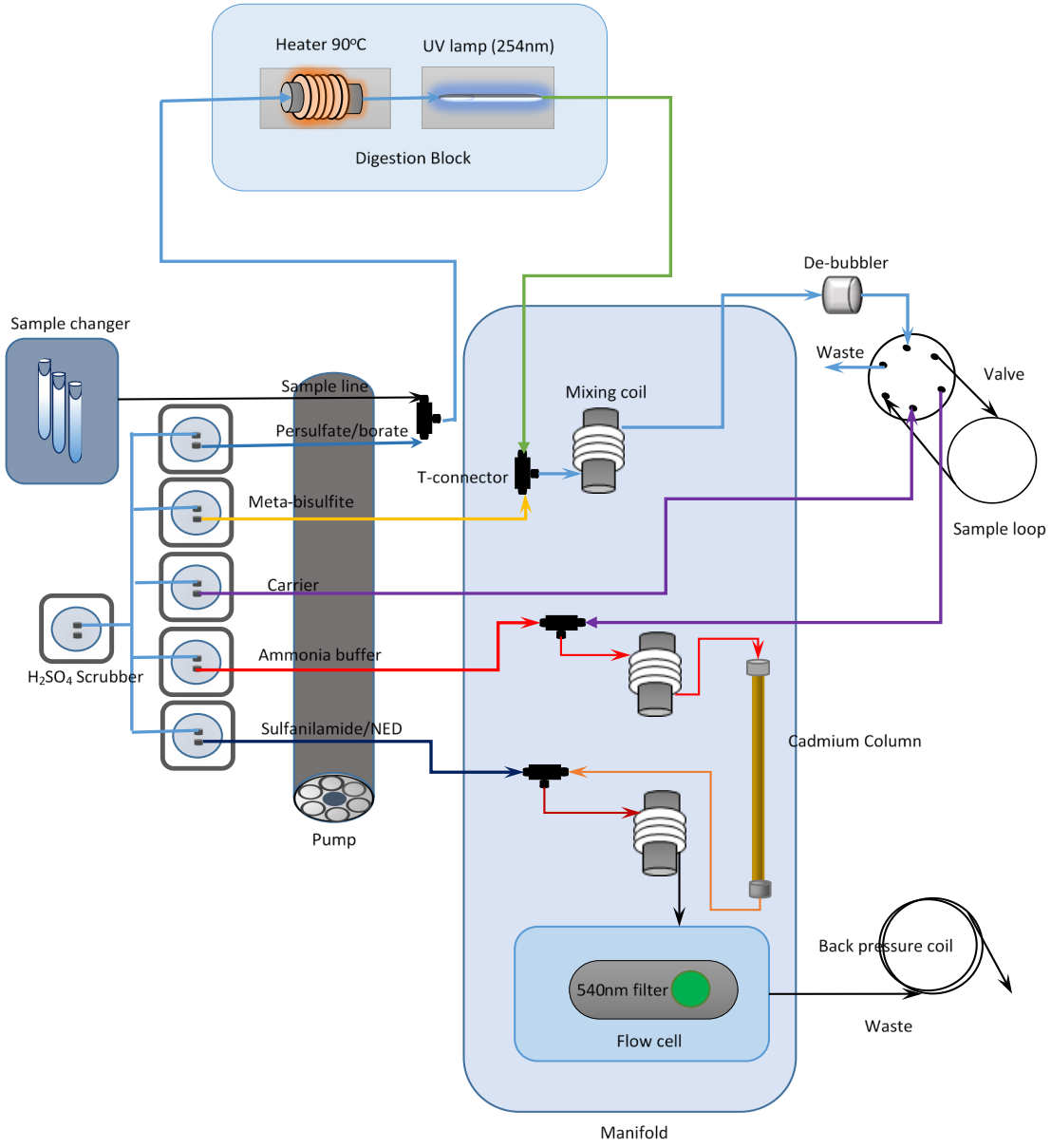


Figure 4.49. Schematic of FIA System for Total Nitrogen

Deionized Water

Use Type I DI water (> 18.1 MΩ) for sample changer rinse, carrier solution and reagent preparation. Take water from the point of use dispenser and sparge with helium to remove dissolved N gases such as

ammonia.

Sparging with Helium

Bubble helium vigorously through the solution for a minimum of 1 minute. Reduce the gas flow if the solution foams up and threatens to spill over.

10% H_2SO_4

This solution is used for scrubbing the reagent lines and for sample pre-treatment. Prepare as needed by adding 100 mL of concentrated H_2SO_4 (95%-97% GR grade) to 900 mL of DI water. Refresh every three months.

Note in Figure 4.49 how this sulfuric acid scrubbing solution is vented to each of the reagent containers via polyethylene or polyvinyl chloride (PVC) transmission tubing. To prevent cross-contamination, ensure that tubing inserted into the scrubbing container does not reach the acid solution.

Ammonium Chloride Buffer (Caution: Fumes)

1) Prepare using a 2 L volumetric flask in a fume hood. Caution: To prevent fumes add acid and base solutions to the flask slowly.

2) Add in order:

   i. 1000 ml DI water
   ii. 210 ml concentrated hydrochloric acid (HCl)
   iii. 190 ml ammonium hydroxide (NH_4OH)
   iv. 2.0 g disodium EDTA (Na_2EDTA)

3) Mix to dissolve EDTA then dilute with DI water to the mark.

4) Adjust solution to pH 8.5 with HCl or NaOH solution. Use litmus paper to estimate the pH. Stable for one month.

Sulfanilamide Color Reagent

1) To a 500 mL amber volumetric flask add in order:

   i. approximately 300mL DI water
   ii. 50 mL of 85% phosphoric acid (H_3PO_4)
   iii. 20.0g sulfanilamide
   iv. 0.5g N-(1-naphthyl)-ethylenediamine dihydrochloride (NED)

2) Shake and stir for 30 minutes to ensure complete dissolution. Dilute to the mark with DI water. Do not use the solution if it turns pink.

Meta-bisulfite

1) Add 2.5 g sodium meta-bisulfite to a 1 L volumetric flask, then add DI water to the 1 L mark. Cap the flask and mix this solution.

2) Sparge this solution with helium. Prepare fresh weekly.

Persulfate and Borate

1) In a 1 L volumetric flask, dissolve 10.0 g potassium persulfate ($K_2S_2O_8$) and 8.75g disodium tetraborate deca-hydrate ($Na_2B_4O_7.10H_2O$) in 500 mL of DI water.

2) Dilute to the 1 L mark with DI water. Cap and mix until dissolved. Stable for one month.

***Important**: Potassium persulfate is known to have nitrogen contamination. Ultra-pure forms are available but must be tested, nevertheless, to ensure purity. Traces of N will raise the detection limit.*

To reduce contamination and retain a lower detection limit, re-crystallize potassium persulfate by following the procedure below. Detection limits using re-crystallized potassium persulfate are near 0.02 mg $L^{-1}N$. Detection limits using unpurified potassium persulfate approach 0.200 mg $L^{-1}N$.

Procedure for Re-crystallizing Potassium Persulfate

1) Dissolve 100 g of potassium persulfate in approximately 600 ml of DI water previously heated to 60°C in a 1 L flask. Use a medium sized stir bar.

2) Filter the solution rapidly through a sintered glass funnel. Rinse the 1 L flask used in step 1 then pour the filtrate back into this flask.

3) Cool the (filtrate) solution to about 4°C by placing the flask in ice water. Swirl the flask continuously to prevent the solution from freezing.

4) Filter this solution, now at about 4°C, and wash the residual white solid on the filter with ice cold DI water. Save the white solid crystals and discard the solution.

5) Using the same flask that was used to cool the solution, add 450 ml of DI water and heat to 60°C.

6) Add the white crystals from step 4 to the DI water at 60°C and mix into solution.

7) Repeat steps 2 through 4.

8) Dry the crystals at room temperature under vacuum (28"Hg) over anhydrous calcium chloride for 24 hours. Rapid drying in a good vacuum and at low temperature is essential to minimize the sulfuric acid formation in the crystals. Stable for one month.

Water Matrix

Fill an 8 L bottle with DI water and add 4 ml of 98 % concentrated H_2SO_4. Shake well and store at room temperature. Stable for six months. Prepare as needed.

Note: If the FIA output shows a small dip in the baseline before the nitrogen peak occurs, use this water matrix as the carrier solution. Using this water matrix should eliminate the pre-peak dip. A pre-peak dip brings down the baseline and makes integration of the N peak difficult. (For an example of the pre-peak dip, see figure 4.47 in section 4.5.10.)

Calibration

The expected range for this analysis is 0.02 mg $L^{-1}N$ to 2.00 mg $L^{-1}N$.

Stock Standard Solution (1000 mg L^{-1} N)

New flasks and bottles are conditioned by soaking in DI water over night. Following this soak, rinse the flasks and bottles with DI water three times and dry in a warm oven. Flasks and bottles need only be conditioned once when they are new and before putting them into service. See Appendix C for calibration of flasks and analytical balances and for glassware storage details.

1) Weigh all volumes using an analytical balance. Rinse all weigh boats thoroughly. Use containers that are dedicated to the preparation and storage of stock standard solutions.

2) It is highly recommended that stock standard solutions be prepared by weighing the DI water volume. Calibrate the receiving flasks by dispensing DI water by weight into the flask and then marking the flask at the fluid line.

3) To prepare the potassium nitrate solution, place approximately 8 g of KNO_3 in a glass crucible and dry for several hours at 60ºC, then:

   i. Gently tap approximately 7.221 g of dry potassium nitrate into a clean weigh boat. Record the weight to 3 decimal places in your calibration logbook.
   ii. In a 1 L volumetric flask dissolve the KNO3 powder in 800 mL DI water. Dilute to the 1 L mark with DI water. Cap and mix the solution by inversion.
   iii. Calculate the concentration of N to 3 decimal places. Record the concentration and preparation date on a label and affix it to the flask. Also record this information in your calibration logbook. Store the solution at 4ºC. This solution is stable for 12 months.

4) To ensure consistency between old and new stock standard solutions, prepare a dilution of the new stock standard solution and analyze it as an unknown.

   i. Into a rinsed weigh boat dispense 1 gm of new stock standard solution.
   ii. Pour this solution into a clean, rinsed and calibrated 1 L volumetric flask.
   iii. Rinse the weigh boat into the flask and fill the flask to the mark with Type 1 DI water.
   iv. Mix well and allow the solution to equilibrate for at least one hour.
   v. Analyze as a sample while still using the old calibration set.
   vi. Results should be 1.00 mg L^{-1} and fall within the QC precision guidelines at that concentration. If the results do not fall within the expected range, discard this stock standard solution and prepare another 1000 mg L^{-1} N solution. Make sure that this solution, once prepared, has been allowed to equilibrate for one hour before analyzing it.

5) Store the newly prepared stock standard solution in its original flask and store the flask at 4º C.

Calibration Standards

Notes: All calibration standards are prepared using the water matrix described above.

1) Weigh all volumes using an analytical balance. Rinse all weigh boats thoroughly. Use dedicated, calibrated glass volumetric flasks as a final reservoir. Do not use vessels from other procedures.

2) Pipette 2 g of stock standard solution into a weigh boat. Record the weight to three decimal places in your calibration logbook.

3) Transfer this aliquot into a calibrated 1 L volumetric flask and dilute to the mark with water matrix. Cap and mix the solution.

4) Calculate the concentration of N to 3 decimal places. Record the concentration in your calibration logbook. Use this standard to make all the other working standards.

5) Use water matrix as the tenth or zero calibration standard. Table 4.41 lists a suggested set of calibration standards for total nitrogen analysis. Standards are stable for one week.

Table 4.41. Total Nitrogen Calibration Standard Preparation

Standard #	Volume Standard #1 (mL)	Final Volume (mL)	Concentration (mg L^{-1} N)
1			2.000
2	75	100	1.500
3	50	100	1.000
4	40	100	0.800
5	20	100	0.400
6	10	100	0.200
7	5	100	0.100
8	2	100	0.040
9	1	100	0.020
10 (water matrix)	0	10	0.00

Digestion Check Control Standards

Digestion check control standards are introduced to ensure that the digestion process is working and that the organic N is completely converted to nitrate and accurately represented in the total N measurement. Run digestion check controls every 10 to 15 samples.

Preparation of Stock Digestion Check Control Standard: 1000 mg L^{-1} N

In a 500 ml calibrated volumetric flask dissolve 1.072 g of urea in about 400 ml of water matrix. Dilute to the mark and invert three times to mix the solution thoroughly. Stable for three years.

Digestion Check Control: 2.000 mg L^{-1} N

1) Pour 1 ml of stock digestion check control standard (1000 mg L^{-1} N) into a weigh boat. Record the weight to 3 decimal places in your calibration logbook. Transfer the aliquot into a 500 mL volumetric flask.

2) Dilute to the 500 mL mark with water matrix. Cap and mix.

3) Calculate the concentration of N to 3 decimal places. Record the concentration in your calibration logbook. Stable for six months.

Quality Control

Nitrogen deteriorates too rapidly in natural precipitation, even if refrigerated, to use precipitation for QC purposes. Other sources of QC solutions are listed, below.

- Certified Reference Material
- simulated samples made from an alternate standard material such as urea
- Pooled inter-laboratory comparison samples may be used if they have been sterilized by filtration; however, do not use this solution if a pipette has been dipped into it or if hands have touched the bottle openings, allowing bacterial contamination.

Prepare at least three QC solutions in the standard range, like calibration standards 8, 6, and 3 in table 4.41. Control solutions should test the low, mid, and high range of the calibration curve.

Note that large aliquots of any control material will deteriorate after repeated openings of the container. Store small control aliquots under refrigeration.

Analytical Procedure

1) Turn on the computer, sample changer, peristaltic pump, inline digestion units and system unit.

2) Place the waste line from the flow cell into a waste container. Connect the transmission tubing to the reagents. Waste contains toxic reagents and must not be poured down the sink. Dispose of waste responsibly following local regulations. Cadmium columns contain a known carcinogen and can be returned to some suppliers, who will dispose of them responsibly. **The cadmium must be disposed of according to local and regional regulations.**

3) Place the reagent pump tubing under tension as per the manufacturer's specifications. Connect the transmission tubing to the reagents.

4) The inline digestion unit will take at least 20 to 30 minutes to reach the required temperature of 90°C. Start the reagent pump so reagents and samples will reach the same 90°C temperature. This temperature must be met before starting any analysis. **Do not run heating block and digestion unit without reagents pumping through the system. These units will overheat without fluid going through them**.

5) Condition the transmission tubing with the reagents until a stable baseline is maintained. Periodically check the display for any deviation from the baseline. This may indicate that there are air pockets in the tubing.

6) Samples that are not collected and preserved with sulfuric acid must be pre-treated with sulfuric acid. This pre-treatment also applies to QCS and blank samples.

    a. Pipette 5.0 mL of each sample into labeled sample tubes. Place each tube in the correct position in the sample tray.
    b. Using a repeater pipette, add 25 µL of 10% H_2SO_4 solution into each 5 mL sample aliquot including QCS and blanks. Calibration standards and digestion check control solutions are made up in the water matrix and do not require pre-treatment with H_2SO_4.
    c. Cap the tubes and invert three times to ensure they are well mixed.

7) Dispense 5 mL volumes of calibration standards into sample tubes. Do not add acid H_2SO_4 to the standards since it was added during preparation of the standards. Promptly place Parafilm® over each tube and check that each tube contains the minimum volume required for duplicate readings.

8) Run a DI water sequence to check instrument stability. Usually only one to three water injections are required. Check the temperature of the heating block to ensure it is at 90°C.

9) Once a stable baseline has been achieved, begin instrument calibration.

10) Run a calibration sequence followed immediately by a QCS and digestion check control solution. Continue with precipitation samples only after instrument stability and a successful calibration have been verified. Samples that have been treated with sulfuric acid are stable for one day and may be refrigerated until analysis if necessary.

11) Calibrate every 50 samples and use a fresh aliquot of standard material each time. Run QCS and

digestion check control solutions every 10 samples.

12) If the system does not stabilize and a troubleshooting period is required, re-dispense fresh standards. A run of 50 samples analyzed in duplicate takes about 2 hours. Dispense calibration standards fresh from the flasks every 50 samples. Use fresh standard solution to rinse the sample tube several times to eliminate any trace of the standard remaining from the previous test.

13) Following the run, recheck all calibration curves and QC results before reporting, collating or tabulating results.

14) Check all raw data points individually for correct integration and output. Comment on all anomalies and flag data accordingly.

15) Calculate results against the appropriate calibration curve and report the correct number of decimal places. Apply detection limit notations as needed. Mark all samples that exceed upper calibration ranges for dilution and repeat analysis. Ensure missing samples are accounted for and contamination codes are applied as needed.

16) Export data from the FIA system and archive all parameters associated with the analysis, including calibration data, raw measurement data, sample identification and instrument audit trails. Audit trails include instrument parameter settings that may be useful when the baseline is found to have drifted. It may be necessary to repeat the analysis from the point where the problem was discovered in the FIA output.

Troubleshooting

Problem 1: Air spikes are observed on the real-time display as noisy irregular spikes in the baseline or sample peak.
Solution 1: Air bubbles will be introduced to the flow cell if reagents are not degassed or the transmission tubing is not securely connected. Ensure all reagents have been sparged with helium in advance of analysis. Make sure there is adequate sample volume for analysis in each tube. If a tube cracks and the sample leaks out, the probe could draw in air resulting in air spikes.

Problem 2: Carry-over. Extremely high-concentration samples do not wash out of the sample lines completely resulting in erroneously high concentration results for the next sample or several samples.
Solution 2: Samples must be re-analyzed. Dilute the high-concentration sample and compare the result with the first analysis. Also, repeat the analyses of several samples that followed the high-concentration sample and by comparing the initial and re-analysis results ensure that any carry-over effects are eliminated. Do not run samples that have been contaminated with bird droppings or organic material such as leaves or grass. The results are not representative of precipitation and will contaminate or clog the FIA system. Report or code the sample as too contaminated for analysis.

Problem 3: Reagent line leakage.
Solution 3: Replace peristaltic pump lines frequently depending on use. Keep maintenance logs, review the logs and periodically check the condition of all lines. Replace transmission lines as needed. Sometimes the waste line can be blocked causing enough back pressure to loosen reagent line connections or even causing them to become disconnected. Ensure the waste line is always clear.

Problem 4: Deterioration of QC measurements.
Solution 4: Check the age of the cadmium column and ensure that there is a maintenance schedule for column changes. It is important to change the column before deterioration occurs. Cadmium columns may be used for about 500 sample analyses.

Problem 5: Detection limits increase and sensitivity decreases. For example, the usual detection limit of 0.020 mg L^{-1} N increases to 0.050 mg L^{-1} N.
Solution 5: The quality of potassium persulfate is very important. Ensure that the crystals have been purified and quality testing has occurred.

Problem 6: Only inorganic nitrogen species are being quantified. Loss of organic species.
Solution 6: Ensure the digestion block has the correct temperature and the UV lamp is lit. Ensure that digestion check control solutions are being analyzed and that they correctly identify when the digester is not working properly. Samples must be acidified for effective digestion to occur.

Problem 7: Output shows a small dip before each peak (see figure 4.47 in section 4.5.10).
Solution 7: Samples have been acidified as a preservation step. The sample acidity may change the pH in the reagent system. To overcome this pre-peak dip, add a proportional amount of acid to the DI water carrier, so that the pH of the mixture of reagents matches the pH of the sample matrix.

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